Encontro Investigação Jovem da Universidade do Porto

BOOK OF
ABSTRACTS

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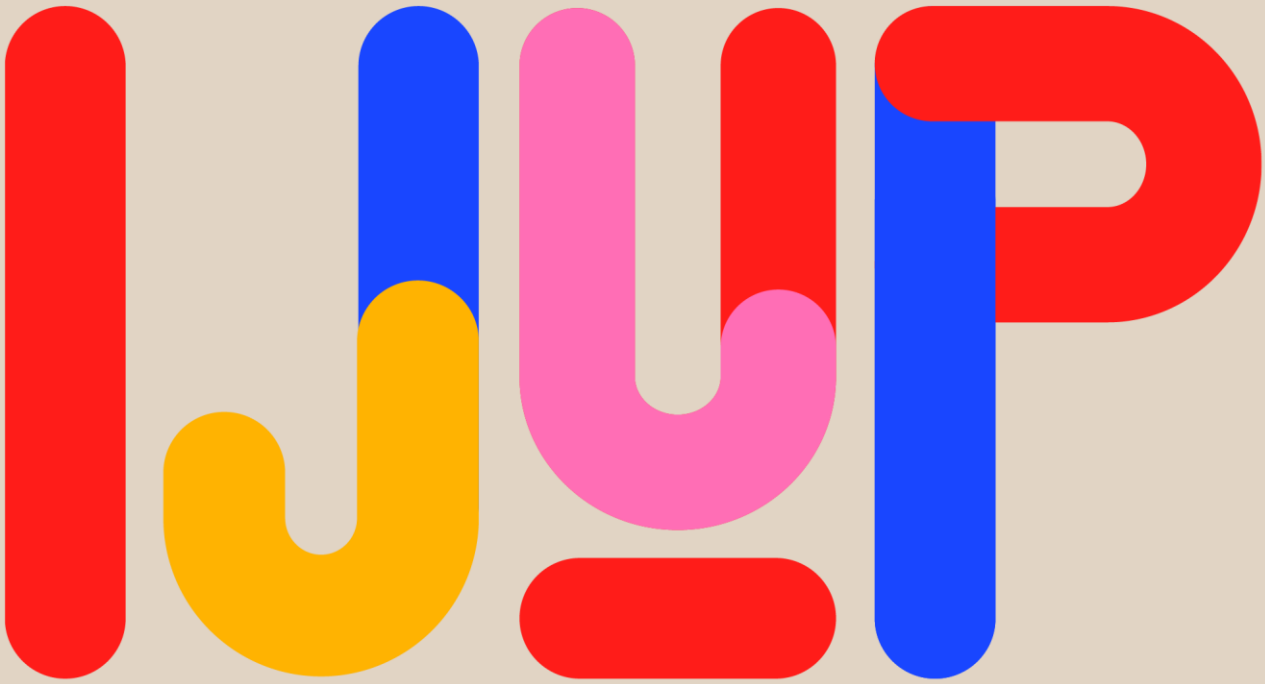

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IJUP INVESTIGAÇÃO JOVEM DA UNIVERSIDADE DO PORTO

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Encontro Investigação Jovem da Universidade do Porto

YOUNG RESEARCHERS MEETING

06 - 07 - 08 Maio 2026

19.ª Edição

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TÍTULO | *TITLE*

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PROGRAM

06 - 07 - 08 Maio 2026

19.ª EDIÇÃO

	May, 6th	May, 7th	May, 8th
8H	Opening of the secretariat for all participants		
8H30 > 10H30	**PARALLEL ORAL SESSIONS I** A1 – Criminology and Law I A2 – Health Sciences I A3 – Sport Sciences I A4 – Architecture I A5 – Engineering I A6 – Biological Sciences I	**PARALLEL ORAL SESSIONS IV** A1 – AgroFood I A2 – Health Sciences IV A3 – Sport Sciences III A4 – Astronomy A5 – Chemistry I A6 – Biological Sciences IV	**PARALLEL ORAL SESSIONS VII** A1 – Arts I A2 – Health Sciences VII A3 – HSS IV – History, Archeology, Cultural Studies and Communication Sciences A4 – Physics I A5 – Chemistry III A6 – Biological Sciences VII
10H30 > 11H	Coffee Break		
11H > 13H	**PARALLEL ORAL SESSIONS II** A1 – Criminology and Law II A2 – Health Sciences II A3 – Sport Sciences II A4 – Architecture II A5 – Engineering II A6 – Biological Sciences II	**PARALLEL ORAL SESSIONS V** A1 – AgroFood II A2 – Health Sciences V A3 – Sport Sciences IV A4 – Maths & Physics A5 – Engineering IV A6 – Biological Sciences V	**PARALLEL ORAL SESSIONS VIII** A1 – Environment II A2 – Health Sciences VIII A3 – Psychology & Education Sciences III A4 – Physics II A5 – Chemistry IV A6 – Economics & Management
13H > 14H30	Lunch Break		
14H30> 16H	**POSTER SESSION I** Architecture Biological Sciences I Criminology and Law Engineering I Health Sciences I Sport Sciences I	**POSTER SESSION II** AgroFood Biological Sciences II Chemistry Engineering II Health Sciences II Sport Sciences II	**POSTER SESSION III** Arts Astronomy, Physics & Maths Economics & Management Environment & Geology Health Sciences III History & Sociology Psychology & Education Sciences
16H > 16H30	Coffee Break		
16H30 > 18H30	**PARALLEL ORAL SESSIONS III** A1 – HSS I – Political Sciences A2 – Health Sciences III A3 – Psychology & Education Sciences I A4 – HSS II – Political Sciences, Sociology and Geography A5 – Engineering III A6 – Biological Sciences III	**PARALLEL ORAL SESSIONS VI** A1 – Environment I A2 – Health Sciences VI A3 – Psychology & Education Sciences II A4 – HSS III – History of Art A5 – Chemistry II A6 – Biological Sciences VI	**PARALLEL ORAL SESSIONS IX** A1 – Arts II A2 – Health Sciences IX A3 – Psychology & Education Sciences IV A4 – HSS V – History A5 – HSS VI – Literature and Philosophy A6 – Health Sciences X

HSS – Humanities and Social Sciences

APRESENTAÇÃO | *FOREWARD*

A Universidade do Porto tem procurado consolidar-se como a melhor instituição de ensino superior em Portugal, tornando-se capaz de captar os melhores alunos, proporcionar uma formação de excelência aos seus estudantes, promover a inovação, atrair talento internacional e criar conhecimento passível de alavancar o progresso e o bem-estar da comunidade. Tornámo-nos, deste modo, líderes da produção científica nacional, com mais de 600 patentes ativas e cerca de 30.000 artigos indexados na Web of Science (24% do total nacional), o que não seria possível sem o essencial estímulo à investigação, que procuramos aprofundar desde os primeiros anos de estudo.

Iniciativa pioneira em Portugal, o IJUP–Encontro de Investigação Jovem da Universidade do Porto constitui uma peça-chave desta estratégia, na medida em que procura impulsionar e dar visibilidade ao trabalho científico desenvolvido pelos estudantes nas mais diversas áreas do saber. Porventura mais importante, permite-lhes adquirir e desenvolver um conjunto de competências que serão decisivas para a sua futura vida profissional.

Este ano na sua 19ª edição, o IJUP voltou a suscitar o interesse de um grande número de estudantes, comprovando que a geração de talento científico depende, em grande medida, da nossa capacidade para suscitar e estimular a curiosidade dos nossos estudantes e o seu gosto pela investigação. Para tal contamos com o trabalho empenhado dos docentes e técnicos que asseguram a efetiva transposição da teoria das salas de aulas para a experiência concreta do trabalho de pesquisa

Cabe-me, por isso, expressar o reconhecimento da academia pelo esforço de todos os envolvidos no processo virtuoso que permite a investigação jovem na Universidade do Porto, esperando que esta edição do IJUP possa, para muitos estudantes, constituir um primeiro momento de efetiva integração no contínuo esforço que tem procurado estabelecer os fundamentos técnicos, científicos, culturais e éticos da civilização humana.

António de Sousa Pereira

Reitor da Universidade do Porto

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ORAL
SESSION

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AGROFOOD

24127 | Unveiling the potential of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace as a sustainable food resource for dogs

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Background & Aim: The food industry generates substantial processing waste that can be recovered for animal feeding within a circular economy framework. Portugal is the third-largest European producer of tomato, with industrial processing generating large amounts of tomato pomace (TP), whose disposal and stabilization are challenging due to its high moisture content. This study first evaluated the effects of enzymatic pre-treatment of vacuum paddle-dehydrated TP on its chemical composition, *in vitro* digestibility and antioxidant activity, showing improved digestibility. Secondly, the effects of increasing levels of enzymatic pre-treatment, vacuum paddle-dehydrated TP (ETP) in extruded diets for adult dogs were evaluated for the first time. **Methods:** Three iso-nitrogenous and iso-caloric diets containing 0%, 2% or 4% ETP were formulated, with minimal impact on chemical composition. Three two-bowl tests were conducted to assess palatability by pairwise comparison of all diets, and a feeding trial was performed according to four 3 × 3 Latin square design with 12 healthy adult Beagle dogs, three diets and three experimental 28-day periods. **Results:** Palatability parameters were not affected, although a tendency for a preference for the 2% diet over the 4% diet was observed. Body weight, coat quality, food intake, fecal consistency, output, and IgA concentration were unaffected. Fecal dry matter content and pH were lower, and butyrate and valerate proportions were higher, in dogs fed the 2% ETP diet. Effects on digestibility lack biological meaning. **Conclusions:** Overall, ETP represents a sustainable food resource for dogs, but further studies evaluating its effects on fecal microbiota and health-related parameters are needed to elucidate its functional potential and address current challenges in tailored companion animal nutrition.

Keywords: Antioxidant activity; chemical composition; circular economy framework; dog nutrition; tomato pomace.

24555 | Characterization of DNA and RNA viruses in Miranda donkeys

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Background & Aim: The Miranda donkey, an autochthonous asinine breed from Portugal, is currently classified as endangered (FAOSTAT, 2023), despite its recognized genetic, cultural, and socio-economic value. The Associação para o Estudo e Proteção do Gado Asinino (AEPGA), established in 2001, aims to preserve and promote this breed, addressing challenges related to breed decline and limited knowledge. This study aimed to characterize the DNA and RNA virome of Miranda donkeys, supporting assessment of their sanitary status, epidemiological monitoring, disease prevention, and conservation. **Methods:** A total of 53 fresh stool samples were collected from Miranda donkeys. Viral DNA and RNA were extracted using standardized molecular protocols, quantified, and amplified. Viral libraries were constructed and sequenced using high-throughput platforms. Bioinformatic analyses included quality control, read trimming, host sequence removal, *de novo* assembly, and taxonomic assignment to identify DNA and RNA viruses. **Results:** Preliminary results revealed that DNA viromes were predominantly composed of bacteriophages, including representatives of the *Microviridae* family, that often infect gut bacteria, giant eukaryotic viruses were also detected at lower abundance. Regarding RNA viromes, a virus belonging to the *Picornaviridae* family was identified, suggesting partial similarity to picornaviruses infecting horses, with BLAST analysis showing a low query coverage (~14%). Viruses most closely related to this sequence have been described in association with enteric infections in equine host. **Conclusions:** These findings provide a preliminary characterization of the DNA and RNA viromes of the Miranda donkey, indicating that most of the viral diversity is associated with the intestinal microbiota. However, the picornavirus identified warrants further investigation. This study contributes to virological knowledge of an endangered autochthonous breed and supports metagenomic surveillance and conservation.

Keywords: Autochthonous breed; Virome; Metagenomics; Sequencing; Epidemiological monitoring.

References:

[1] Food and Agriculture Organization of the United Nations (FAOSTAT). 2023. World Livestock: Domestic Animal Diversity Information System (DAD-IS). Online. Available from: Accessed: Feb 27, 2026.

24903 | Genomic Diversity of *Escherichia coli* and *Klebsiella pneumoniae* Species Complex in the Gut of Community-based Portuguese Adults

Lúcia Nova[1,2,3]; Joana Mourão[4]; Cátia Matos[1,2]; Beatriz Gonzalez[1,2]; Luísa Peixe[2]; Ana R. Freitas[2,5]; Carla Novais[2]; Patrícia Antunes[1,2]

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Background & Aim: *Escherichia coli* (Ec) and *Klebsiella pneumoniae* (Kp) species complex (KpSC) are human gut commensals/pathobionts that may include pathogenic and antimicrobial-resistant (AMR) strains [1,2]. However, their genomic diversity and links with clinical and food-chain isolates remain poorly understood [3]. We characterized the genomic diversity of gut-colonizing Ec and KpSC in Portuguese adults and assess their relatedness to strains from other sources. **Methods:** Fourteen Ec and 22 KpSC isolates from 27 colonized adults (41%women/18–73 years/2022) underwent Illumina WGS. Assemblies (SPAdes) were analysed for species/serotype/KL-type, MLST, AMR/virulence genes and plasmids (CGE, EnteroBase, Pathogenwatch, MOB-RECON). Comparative genomics used cgMLST clustering (Ec-HierCC; KpSC-≤10 alleles). **Results:** Ec (12 STs) included globally disseminated ST69, ST95 and ST131, while KpSC (3 species; 19 STs) included ST15-KL19 and ST35-KL22. ST69 and ST131 clustered with clinical and food-chain isolates (ST69:HC20,≤20 alleles; ST131:HC50≤50 alleles). Kp ST15-KL19 and ST35-KL22 were related to Portuguese clinical and livestock strains and other lineages to human, animal and environmental sources. Six Ec carried plasmid-borne AMR genes (aminoglycosides/β-lactams/sulphonamides/tetracyclines/trimethoprim) and resistant mutations to ciprofloxacin (*gyrA/parC/parE*-n=6) and colistin (*basR*-n=1). Only 3 KpSC isolates carried AMR genes (aminoglycosides/β-lactams/tetracyclines), one also with *gyrA/parC* mutations. ExPEC (7/14), aEPEC (2/14) and STEC (1/14) pathotypes were detected. Kp ST35-KL22 carried yersiniabactin (ybt9/ICEKp3). Metal decreased susceptibility genes (*ars/sil/pco*) were frequent (Ec-64%; KpSC-68%) and mainly plasmid-associated in KpSC. **Conclusions:** Portuguese adults harbor diverse and clinically relevant Ec and KpSC lineages, including globally disseminated clones genetically related to clinical and food-chain strains, highlighting the importance of community-based One Health genomic surveillance.

Keywords: Enterobacterales, Whole-genome sequencing, Genomic diversity; One-health; Human gut.

Acknowledgments: This work is funded by national funds from FCT- Fundação para a Ciência e a Tecnologia under the projects UIDP/04378/2020, and UIDB/04378/2020 of the Research Unit in Applied Molecular Biosciences–UCIBIO, and project LA/P/0140/2020 from the Associated Laboratory i4HB.

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24944 | Differential Kinetics of Reference Antileishmanial Drugs in Acute and Chronic Murine Visceral Leishmaniasis

Carolina Faria[1,2*]; Ricardo Monteiro[1,3*]; Joana Ribeiro[1,3]; Filipa Neves[1,3]; Sofia Libório-Ramos[1]; Nuno Santarém[1,3], Anabela Cordeiro-da-Silva[1,3] and Joana Tavares[1,4]

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*: equal contribution

Background & Aim: Leishmaniasis is a vector-borne disease caused by parasites belonging to the *Leishmania* genus. In the Mediterranean basin, *Leishmania infantum* is the causative agent of visceral leishmaniasis, the most severe form of the disease[1]. Although fatal when untreated, current options pose several limitations, including toxicity and the emergence of resistant strains[2]. Using whole-body bioluminescence imaging in a susceptible murine model, we aimed to evaluate the kinetics of parasite elimination using reference treatments in the acute and chronic phases of infection. To further elucidate their efficacy, we aimed to quantify parasite burdens in the target organs (spleen, liver, and bone marrow) as well as in the unconventional niche of the lungs. **Methods:** BALB/c mice were infected with *L. infantum* amastigotes expressing an optimized, red-shifted luciferase[3]. The animals were treated at day 14 or 56 post-infection with Miltefosine, Paromomycin, Glucantime or AmBisome, in regimens according to the literature[4], and bioluminescence was acquired during suitable timepoints. The kinetics of parasite elimination was determined through nonlinear regression analysis, using the one-phase decay model. Ultimately, the animals were sacrificed, and the target organs and lungs collected to quantify parasite burdens using the limiting dilution assay. **Results:** Miltefosine and AmBisome were the most effective drugs. Although none of the treatments fully eliminated parasite burdens in both phases of infection, the differences in the efficacy between the acute and chronic phases were treatment-dependent. Interestingly, Miltefosine was the only drug capable of significantly reducing the pulmonary burden in the acute phase, while in the chronic stage, Paromomycin and AmBisome also had a significant impact. **Conclusions:** We successfully evaluated the

efficacy of reference treatments administered in different phases of infection, highlighting the persistence of parasites in the lungs.

Keywords: *Leishmania infantum*; Visceral leishmaniasis; Bioluminescence imaging; Drug efficacy and kinetics.

Acknowledgments:

We thank Professor Guy Caljon (Laboratory of Microbiology, Parasitology and Hygiene, University of

Antwerp) for providing the pLEXSY-hyg2 plasmid necessary for the generation of the bioluminescent *Leishmania infantum* line. This work was funded by the NORTE 2030-FEDER-01777300 - SCALE-ImmunoHUB 2030, supported by Norte Portugal Regional Operational Program (NORTE 2030) and under the PORTUGAL 2030 Partnership Agreement, through the FEDER. This work also received National Funds through Fundação para a Ciência e a Tecnologia (FCT), I.P., through the Research Unit Nº 4293 under the project UID/4293/2025. Ricardo Monteiro was funded by an individual fellowship, reference 2021.09486.BD. RM, NS, ACS and JT are members of the COST Action CA21111: One Health Drugs against Parasitic Vector-Borne Diseases in Europe and Beyond (OneHealthDrugs).

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24562 | Recreational waters as reservoirs of multidrug-resistant Aeromonas: a One Health assessment

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Background & Aim: The number of reported outbreaks of bacterial pathogens linked to recreational bathing have been increasing in recent years, associated with the spread of autochthone waterborne bacteria under the current climatic change favorable conditions. *Aeromonas* spp. are ubiquitous aquatic bacteria recognized as emerging human pathogens with ability to acquire and disseminate antimicrobial resistance, representing a One Health concern. This study aimed to identify and characterize *Aeromonas* isolates from recreational waters of Northern Portugal to identify potential public health risks. **Methods:** Surface water was collected monthly from three year-round recreational sites, Matosinhos (coastal), Freixo and Gramido (estuarine). Presumptive *Aeromonas* were isolated on Ryan selective media (n=86), screened for oxidase activity, and confirmed by *gyrB* PCR gene. Species identification (*A. hydrophila*, *A. media*, *A. caviae, and A. veronii)* was performed by multiplex PCR. Antimicrobial susceptibility was assessed by the Kirby Bauer disk diffusion method using 24 antibiotics from 9 classes, and interpreted according to the CLSI/EUCAST guidelines. **Results:** Overall, 86% of the presumptive isolates were confirmed as belonging to the genus *Aeromonas*. Site specific patterns were observed. *A. caviae* predominated at the coastal site, *A. hydrophila* at Freixo (mid-estuary), and *A. media* at Gramido (upper estuary), suggesting distinct ecological niches along the coastal-estuarine gradient. Most isolates (92%) were multidrug-resistant (resistance ≥ 3 antibiotic classes). All isolates were penicillin resistant, whereas 84% of the isolates were resistant to cephalosporins, and 81% are resistant to rifamycin, all first-line antibiotics. **Conclusions:** This study highlighted the need for a routine currently non-existing integrated microbiological water quality assessment in recreational water within a One Heath framework, to ensure public health and the safety of users.

Keywords: *Aeromonas* spp.; Antimicrobial resistance; Recreational waters; Public health, One Health

Acknowledgments:

This work is a result of the project ATLANTIDA II (ref. NORTE2030-FEDER-01799200), co-financed by the European Union through the NORTE 2030 Regional Program and the European Regional Development Fund (ERDF). Scientific collaboration from FCT project UID/04423/2025, UID/PRR/04423/2025, and LA/P/0101/2020 is also acknowledged.

24240 | Whole-Genome Sequencing of a Bovine Mastitis-Associated Enterobacter oligotrophicus Isolate: Exploring Antimicrobial Resistance and Virulence Potential

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Background & Aim: Bovine clinical mastitis poses economic and health challenges in the dairy industry (1). While coliforms are recognized environmental pathogens, the genetic features of species like *Enterobacter oligotrophicus* remain underexplored. This study aimed to perform a pilot genomic characterization of an *E. oligotrophicus* isolate recovered from a bovine mastitis in Northwest Portugal, focusing on its antimicrobial resistance (AMR), virulence factors (VFs), and plasmid replicon patterns. **Methods:** A Gram-negative isolate was recovered from the milk of a cow with clinical signs of mastitis. Antimicrobial susceptibility testing was performed using the MicroScan WalkAway Plus system. Genomic DNA was sequenced using Oxford Nanopore Technology. The genome was assembled (Flye), annotated (Bakta), and the species was confirmed via FastANI; AMR genes (ResFinder, CARD, MEGARes), VFs (VFDB), and plasmids (PlasmidFinder, MOBscan) were screened. Phylogenomic inference was done using IQ-TREE 3 (2–5). **Results:** Taxonomic analysis confirmed the isolate as *E. oligotrophicus*. Phylogenomic reconstruction showed the isolate clustered with an *E. oligotrophicus* reference sequence (GCA_009176645), forming a distinct clade separate from relevant species like *E. cloacae*, *E. asburiae*, and *E. kobei*. Mapping of the complete circular genome detailed the distribution of Clusters of Orthologous Groups (COGs) associated with metabolism, cellular processes, and information storage. Genomic screening provided a high-resolution profile of the genetic traits of the isolate such as genetic resistance for several antibiotics. **Conclusions:** This pilot study successfully characterizes the complete genome of an *E. oligotrophicus* isolate causing bovine CM. Whole-Genome sequencing gave us clear information of the bacteria's strengths and risks, highlighting the importance of genomic surveillance in dairy herds.

Keywords: Bovine Mastitis; *Enterobacter oligotrophicus*; Whole-Genome Sequencing; Antimicrobial Resistance; Virulence Factors

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24274 | The role of *Viola Tricolor* polyphenol-enriched extracts in the modulation of Inflammatory Bowel Disease

Daniela Rocha; Wen Tao; Nuno Mateus; Hélder Oliveira

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Background & Aim: Inflammatory bowel disease involves epithelial barrier disruption and excessive inflammatory signaling. Polyphenol-rich plant extracts are promising modulators of intestinal oxidative stress. *Viola tricolor* contains bioactive compounds with antioxidant and anti-inflammatory properties. This study evaluated the safety of different *V. tricolor* extracts and their protective effects against inflammation-induced epithelial stress in vitro. **Methods:** Cytotoxicity of four *V. tricolor* extracts (12.5–200 µg/mL) was assessed in Caco-2 and HT-29 cells using the MTT assay to determine non-toxic ranges. Lipopolysaccharide (LPS) assays identified stress-inducing conditions without major viability loss, confirmed by reactive oxygen species (ROS) production. A transwell co-culture system (Caco-2/HT-29) was established to better mimic the intestinal environment. Cells were pre-treated with selected extract concentrations before LPS exposure. **Results:** All extracts showed low cytotoxicity (IC_{50} >200 µg/mL). Viability remained above 80% up to 100 µg/mL and above 50% at 200 µg/mL. LPS increased ROS levels, confirming stress induction. Pre-treatment with *V. tricolor* extracts reduced ROS and restored viability near basal levels. In the co-culture model, LPS compromised barrier integrity, while extract pre-treatment preserved barrier function, improved viability, and reduced stress-related effects. **Conclusions:** *Viola tricolor* extracts showed a favorable safety profile and counteracted LPS-induced oxidative stress. Reduced ROS, preserved viability, and maintained barrier integrity support their protective role in intestinal dysfunction, highlighting their potential for further study

Keywords: *Viola tricolor*; Inflammatory Bowel Disease; Oxidative stress.

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24373 | Dissecting female signaling during pollen tube growth under heat exposure

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Background & Aim: Fruits and seeds, which sustain the global food supply, arise from plant reproduction, one of the most heat-sensitive stages of plant development. Rising global temperatures threaten fertilization efficiency, crop yield and food security[1]. It all begins when a pollen grain (PG) lands on a stigma, and germinates a pollen tube (PT) which grows along the pistil tissues towards the ovule[2]. Most PT growth occurs along the transmitting tract (TT) a female tissue deeply embedded in the pistil. PT-pistil interactions are extensively studied, but a significant gap remains: how the TT contributes for this dialogue, especially under heat-stress (HS) conditions. **Methods:** The *ntt (no transmitting tract)* mutant was used as a genetic tool to dissect TT-specific signaling pathways[3,4]. RNA-seq is being performed on wild-type (WT) and *ntt* pistils under control and HS to identify differentially expressed genes (DEGs) associated with TT function and stress responses. Reproductive performance was evaluated in WT and *ntt* plants using phenotypic and molecular approaches. *Solanum lycopersicum* cv MicroTom was used as a crop validation system, and an *in silico* analysis identified candidate *NTT* orthologs in tomato, to be functionally validated using CRISPR/Cas9. **Results:** An optimized RNA extraction protocol for Arabidopsis pistils was established and RNA seq data is currently being analyzed to identify DEGs. HS assays revealed phenotypic differences between WT and *ntt* plants. Four putative orthologs were identified in tomato, with Solyc11g062060 emerging as the best candidate to proceed for CRISPR/Cas9 editing. **Conclusions:** This work will identify key regulators of PT-TT interactions, highlighting the contribution of female tissues to this process and revealing how HS disrupts this process. Functional analysis in tomato will further assess the role of NTT in crop species.

Keywords: Plant reproduction; Transmitting tract; *Arabidopsis thaliana*; *Solanum lycopersicum*, Heat stress

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24413 | Mineral Profile of Corn Silage from Dairy Farms in Mainland Portugal: Implications for Precision Mineral Nutrition in Dairy Cows

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Background & Aim: Precision nutrition involves formulating diets that meet but do not exceed animal nutrient requirements to improve feed efficiency, reduce nutrient losses, and limit environmental impact. Precision mineral nutrition can lower toxicity risk but may increase deficiency likelihood when the mineral composition of staple forages is uncertain. In mainland Portugal, dairy systems rely primarily on corn silage, typically ≥50% of dietary dry matter (DM). This study quantified the mineral composition of corn silages to support more accurate, cost-effective, and environmentally sustainable feeding strategies. **Methods:** Forty-five dried, ground corn silage samples from dairy farms across mainland Portugal were provided by ALIP in 2025. Samples were analyzed for macro- and microminerals and toxic trace elements. After acid mineralization using a high-performance microwave digestion system (MLS 1200 Mega, Milestone, Sorisole, Italy), solutions were analyzed by ICP-MS (iCAP Q, Thermo Fisher Scientific, Waltham, MA) and, for Na and K, by FAAS (Analyst 200, PerkinElmer, Shelton, CT). Summary statistics were calculated using SAS® OnDemand for Academics (SAS Institute Inc., 2026). **Results:** Among macrominerals, median P was 1.8 g/kg DM, indicating supplementation is required but should be adjusted to prevent P pollution, whereas low K benefits dry cows by reducing udder edema risk and supporting Ca balance. Microminerals, except Fe, were generally below recommended levels; although I was adequate in some samples, most required supplementation (Figure 1). A high Cu:Mo ratio indicates minimal risk of Mo interference, while Se was below detection in 64% of samples, compromising cow health and performance. Toxic trace elements were negligible, with Hg below detection in 89% of samples and Cd and Pb at very low levels. **Conclusions:** The results emphasize routine mineral analysis to enable precision nutrition, prevent antagonisms, lower environmental impact, and promote dairy cow health and productivity.

Keywords: corn silage; dairy cows; mineral profile; precision nutrition

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The authors thank Sílvia Azevedo (ICBAS-UP) for the valuable technical assistance.

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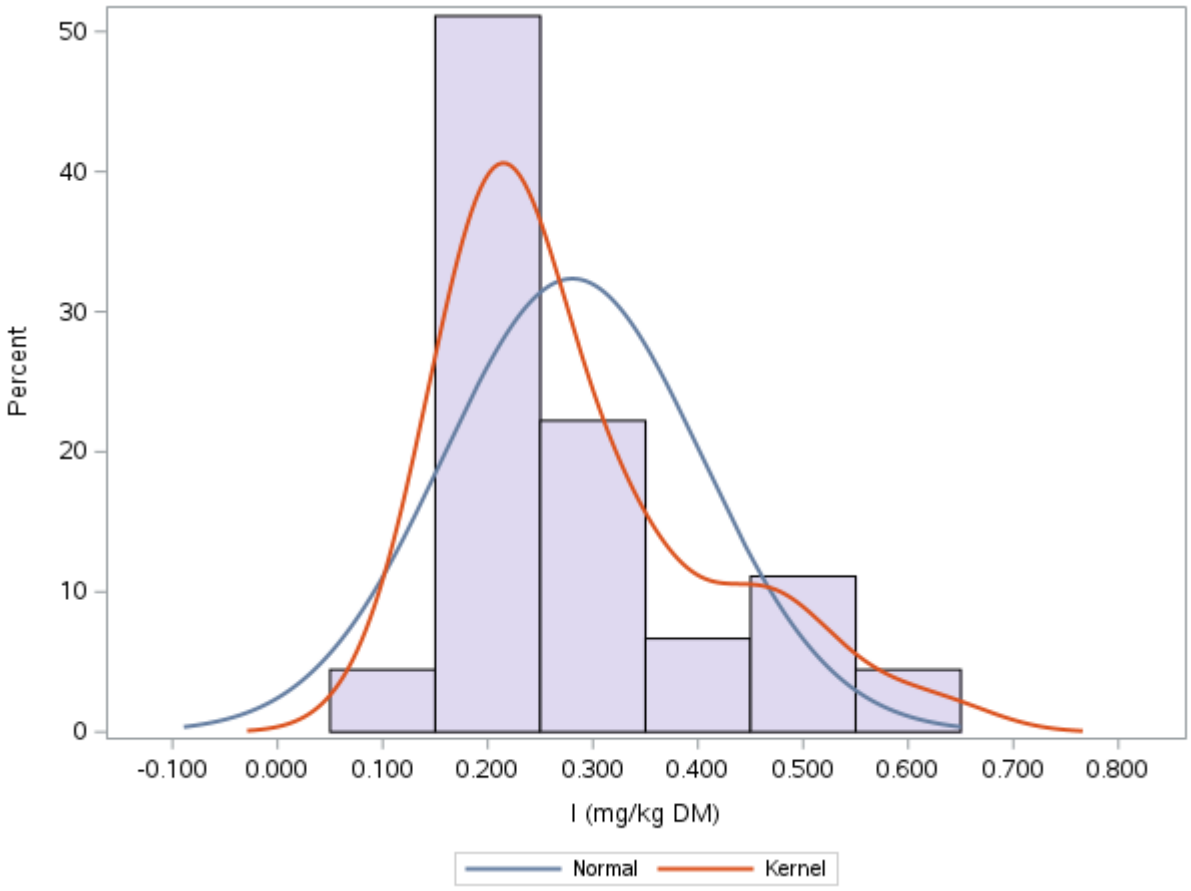

Figure 1: Distribution of I concentrations (mg/kg dry matter; DM) in corn silage grown throughout mainland Portugal. The smooth line indicates what the distribution would be under normality, while the bars represent the observed distribution. The adequate intake for lactating cows is 0.48 mg I/kg DM, and the maximum tolerable level is 50 mg I/kg DM (NASEM, 2021).

24572 | Pressurized Liquid Extraction of Bioactive Compounds from Coffee Silverskin: Evaluation of Anti-Inflammatory Activity

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Background & Aim: The growing demand for sustainable solutions in the agri-food sector has increased interest in valorizing coffee processing residues, particularly coffee silverskin (CSS), a major by-product with reported health-promoting properties [1]. This study aimed to optimize Pressurized Liquid Extraction (PLE) conditions using Response Surface Methodology (RSM) to maximize extraction yield, followed by the characterization of bioactive compounds by HPLC-DAD and the evaluation of anti-inflammatory activity. **Methods:** PLE optimization was performed using Response Surface Methodology (RSM with a Box-Behnken design). In each run, 1g of the extract was submitted to different combinations of temperature (40–120 °C) and ethanol concentration (10–50 %). Extracts were analyzed for caffeine, HMF, and total CQA (3-, 4-, and 5-O-caffeoylquinic acids) by HPLC-DAD. The anti-inflammatory activity was evaluated by measuring nitric oxide (NO) release in RAW 264.7 murine macrophages. Cells were cultured in 96-well plates (8×10^4 cells/well) for 24 h, pre-treated with CSS extract (1000 µg/mL), and then stimulated with lipopolysaccharide (LPS, 1 µg/mL). Non-treated cells served as basal control and cells exposed to LPS alone served as inflammatory control. NO levels were quantified at 550 nm using the Griess reagent [2]. Statistical significance ($p < 0.05$) was evaluated by ANOVA. **Results:** CSS extracts presented high caffeine content, substantial levels of CQAs, and relatively low HMF formation. Both temperature and ethanol concentration had a statistically significant positive effect on extraction yields. LPS increased NO production, confirming the induction of an inflammatory response. CSS extract significantly reduced NO levels in LPS-stimulated cells, restoring them to near basal values. **Conclusions:** CSS extracts obtained by optimized PLE are rich in caffeine and chlorogenic acids, show low HMF formation, and exhibit significant anti-inflammatory activity, supporting their valorization as a functional ingredient.

Keywords: Coffee Silverskin; Pressurized Liquid Extraction; Phenolic compounds; Anti-inflammatory activity; Sustainability.

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24579 | Characterization of phenotypical traits associated with wild grape introgression in Vinhos Verdes varieties

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Background & Aim: Domestication of the European grapevine (*Vitis vinifera* L.) has involved hybridization with the wild subspecies *V. v. sylvestris after the initial domestication effort*, and through genomic studies we have shown that Portuguese Vinhos Verdes varieties have strong signatures of this introgression [1-3]. We hypothesized that the presence of introgression in Vinhos Verdes varieties produces measurable morphological and physiological differentiation, possibly of an intermediate state between wild and fully domesticated grapes. **Methods:** Whole-genome resequencing data from domesticated and wild grapevines were analyzed with PCA, to help define a cohort of varieties that represented either Vinhos Verdes varieties with strong introgression potential, or non-introgressed Portuguese varieties. Sampling was supplemented with *sylvestris* genotypes. A subset of International Organization of Vine and Wine (OIV) morphological descriptors were used for quantitative and qualitative characterization of grapevine traits related to bunch and berry characteristics, among other developmental aspects. Seeds were collected and subjected to morphological analysis. Protocols were implemented at the lab for seed surface sterilization, stratification and germination. **Results:** We generated an important dataset for morphological comparison between 6 Vinhos Verdes and 6 additional (non-introgressed) Portuguese varieties, that suggest the presence of variation for traits such as bunch density and berry shape. We generated improved protocols for stratification, disinfection, and germination of grapevine seeds and cuttings. We next looked at germination rates under supplementation with the stress hormone ABA, that hints at different sensitivity to this treatment in Vinhos Verdes varieties. **Conclusions:** The combined genomic-phenotypic approach suggests that post-domestication introgression from *V. v. sylvestris* left a detectable imprint on the phenotype of Vinhos Verdes varieties.

Keywords *Vitis vinifera,* introgression, phenotypic variation, Vinhos Verdes, domestication

Acknowledgments:

Estação Vitivinícola Amândio Galhano/ Comissão de Viticultura da Região dos Vinhos Verdes (EVAG/CVRVV), and INIAV for support with accessing biological material.

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24642 | Optimisation of a methodology for the detection of antibiotics in feed for production animals

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Background & Aim: In recent decades, in addition to therapeutic use, antibiotics have been improperly added to feed as growth promoters for farm animals. Adding antibiotics to feed without a veterinary prescription is illegal, as it promotes selective pressure on bacteria and consequently facilitates the spread of antimicrobial-resistant bacteria (AMR) to the environment, humans and other animals [1]. The manufacturing process of medicated feed is equally concerning, as cross-contamination of antibiotics occurs in non-medicated feed intended solely for animal nutrition [2]. The aim of this study is to optimise a screening method using HPLC-TOF-MS for the multi-class detection of the most detected antibiotics in non-medicated feed. **Methods:** Fourteen different extraction methods were evaluated, including liquid-liquid extractions and dispersive solid-phase extractions (d-SPE), using different solvents such as: 1% acetic acid in methanol, 1% formic acid in methanol, methanol/formic acid/water (79/1/20), acetonitrile, 1% formic acid in acetonitrile, acetonitrile/formic acid/water (79/1/20) and McIlvaine-EDTA buffer (0.1M) with subsequent addition of acetonitrile. **Results:** The recoveries of the selected antibiotics were evaluated for each combination of extraction procedures and solvents. Throughout the procedure, filtration after centrifugation was found to be necessary due to the lighter components of the bovine feed. **Conclusions:** In summary, this study has enabled the optimisation of an accessible method that guarantees adequate extraction and cleaning, compatible with a highly sensitive technique such as HPLC-TOF-MS, allowing the simultaneous quantification of antibiotics from different classes in a single analytical method.

Keywords: antibiotics; feed; liquid chromatography; mass spectrometry; HPLC-TOF-MS

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24726 | Characterizing bacterial antagonists of *Erwinia amylovora* for sustainable fire blight control in pear

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Background & Aim: Biological control agents (BCAs) are sustainable alternatives to antibiotics and pesticides in crop protection[1,2]. In this regard, native microbial communities of crops may reveal potential BCAs associated with the crop's life cycle, environment, and agricultural management practices. In pear orchards, phytopathogens such as *Erwinia amylovora* pose a significant economic threat[3]. Therefore, this study aims to characterize bacterial isolates from pear orchards and identify antagonists against *E. amylovora*. **Methods:** Bacterial isolates from organic and conventional pear orchards were cultured, and genomic DNA was extracted. The 16S rRNA gene was amplified and sequenced for taxonomic identification. Antagonistic activity was evaluated by overlay assay against *E. amylovora*, and inhibition halos were quantified using ImageJ. Selected strains were further assessed through cell-free supernatant assays, comparing induced and non-induced conditions, followed by dual-layer assay to evaluate antimicrobial activity. The selected strains were prioritized for subsequent phenotypic characterization (e.g., Indole acetic acid (IAA), siderophores, etc.). **Results:** Taxonomic identification was achieved for all 141 isolates. Fifty-seven isolates (40.4%) were assigned to plant- and/or human-associated pathogenic taxa. Non-pathogenic bacteria were more frequent in organic orchards, with *Bacillus* spp. as the dominant group (~35%). Antagonism assays identified 18 candidates assigned to five taxa: *Bacillus* sp., *Bacillus siamensis*, *Bacillus velezensis*, *Staphylococcus epidermidis*, and *Bacillus licheniformis*. All showed consistent antibacterial activity against *E. amylovora*. **Conclusions:** This study provides a molecular characterization of the culturable orchard-associated endophytic bacteria. Moreover, this study revealed 4 (out of >10) pear-endophytic strains with high potential to control the fire blight. Further research should focus on the BCA application and validation of antagonistic efficacy under in planta conditions.

Keywords: Biological control agents; endophytes; *Erwinia amylovora*

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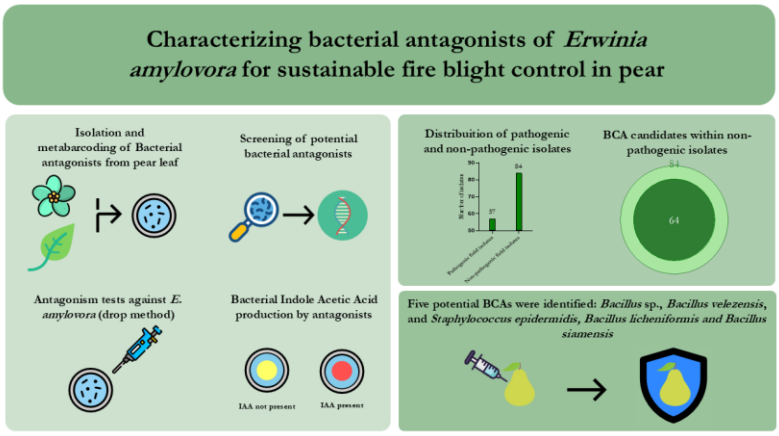

Figure 1: Workflow for identifying and characterizing bacterial antagonists of *E. amylovora* for sustainable fire blight control in pear. The process includes isolation and metabarcoding of bacterial isolates from pear leaves, screening for potential antagonistic activity, distribution analysis of pathogenic and non-pathogenic isolates, antagonism assays, and characterization of antagonists. Five promising bacterial candidates (*Bacillus sp.*, *B. velezensis*, *Staphylococcus epidermidis, B. licheniformis* and *B. siamensis.*) were identified for potential biocontrol application.

24819 | Combined heat and drought stress triggers organ-specific physiological and redox responses in pepper (*Capsicum annuum* L.)

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Background & Aim: Climate change is intensifying the occurrence of heat waves and droughts events, severely constraining crop productivity worldwide. Pepper, a key Mediterranean crop, is sensitive to water deficit and high temperatures, yet its response to their combined exposure remains poorly understood. Thus, this study aimed to evaluate the physiological responses of pepper plants to single and combined heat and drought stress. **Methods:** *Capsicum annuum* var. "California Wonder" seedlings were potted in an agricultural soil and grown under controlled conditions for 21 days. Plants were then divided and exposed for 14 days to drought [25% soil water holding capacity (WHC)], heat (42 °C, 4 h day^{-1}) or their combination. Control plants were kept under optimal conditions (60% WHC, 25 °C). Growth traits (leaf area, biomass, organ length), tissue water content and photosynthetic pigments were measured. In parallel, the oxidative status was assessed through the content of superoxide anion, hydrogen peroxide, lipid peroxidation, and antioxidant capacity. **Results:** Heat alone had minor effects on growth, whereas drought, alone or combined with heat, significantly reduced leaf area, biomass and organ length, with stronger inhibition observed in roots. Interestingly, pigments levels increased under heat, regardless of water availability, suggesting an adjustment of the photosynthetic system. Moreover, heat (both single and combined) enhanced superoxide accumulation in both organs, while water scarcity reduced hydrogen peroxide levels in roots. In leaves, drought increased lipid peroxidation regardless of heat co-exposure, while in roots this biomarker increased under heat treatment. In addition, antioxidant capacity remained stable in leaves but declined in roots, particularly under combined stress. **Conclusions:** Overall, drought was the main factor limiting growth, whereas heat primary modulated root redox metabolism, highlighting organ-specific responses to both single and combined stress exposure.

Keywords: Climate change; Abiotic stress; Stress physiology; Oxidative stress; Bell pepper

24834 | From real-time PCR to nanoplate digital PCR: Improving trace-level detection of hidden peanut allergens in complex foods matrices

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Background & Aim: Peanut (*Arachis hypogaea*) is a major allergen capable of triggering severe allergic reactions. Allergic consumers depend on reliable labeling, yet complex production chains and cross-contact can introduce undeclared peanut at levels that challenge current analytical methods [1]. This project aims to develop a highly sensitive peanut-specific real-time PCR assay and to transfer it to a nanoplate digital (nd)-PCR platform for absolute, trace-level quantification of peanut in complex foods [2]. **Methods:** Model food mixtures (pancakes) containing known amounts of peanut flour (10% to 0.000001% (w/w)) were prepared. DNA was extracted using the NucleoSpin Food kit and quantified by UV spectrophotometry. Peanut-specific mitochondrial (*Cytc oxidase subunit 1*, *CO1*) and nuclear (*ITS*) regions were selected for *in silico* primer design, which were experimentally tested for specificity against other species. The primer pair presenting the best amplification performance and specificity was used to establish a quantitative real-time PCR method using a specifically designed MGB probe. **Results:** Results demonstrated that the *CO1* gene provided the highest sensitivity while retaining its specificity, justifying its selection for peanut detection at residual concentration. Preliminary real-time PCR results achieved an absolute limit of detection of 0.2 pg of peanut DNA with acceptable performance parameters of coefficient of correlation, slope and PCR efficiency. Based on these findings, a real-time PCR assay is currently being optimized for the quantification of residual peanut levels in complex foods, which will be further validated with blind model samples and applied to analyze commercial products. Implementation of ndPCR should further improve sensitivity and precision, while providing absolute copy-number quantification. **Conclusions:** The proposed work will deliver a specific, sensitive and operationally straightforward DNA-based tool to monitor peanut as hidden allergen in processed foods, supporting improved food safety and consumer protection.

Keywords: Peanut; Food allergy; Real-time PCR; Nanoplate digital PCR; Allergen risk management

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24840 | Modulating Peanut Protein IgE-Binding by Food Processing: Insights from Spiked Foods and Commercial Peanut Flours

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Background: The consumption of legume-derived protein flours, concentrates and isolates incorporated into a wide range of food formulations has increased substantially in recent years. Driven by the pursuit of healthier lifestyles or the desire to improve nutritional intake, the demand for protein-enriched foods continues to grow. Among these, peanut-based products are particularly popular due to their wide availability as protein flours to be incorporated into a variety of homemade recipes. However, peanut consumption poses a significant health risk for individuals with peanut allergies, considering the severity of the elicited immune responses that can be triggered [1]. **Aim:** This study intends to investigate the effects of food processing treatments on the solubility/IgE-reactivity of peanut proteins from different commercial peanut materials. **Methods:** Peanut materials, namely roasted peanut flour (RPF), defatted roasted peanut flour (DRPF) and defatted peanut butter powder (DPBP), and model foods spiked with DRPF were submitted to distinct thermal or enzymatic treatments, including frying, boiling, baking, microwaving, and enzymatic hydrolysis. Proteins were extracted with Tris-HCl 0.1 M (pH 8.0) and quantified by UV-Vis spectrophotometry. Protein profiles were analyzed by SDS-PAGE, followed by immunoblotting with anti-peanut Ara h 1 IgG and sera from peanut-allergic patients to test IgG/IgE-reactivities, respectively. **Results:** Thermal and enzymatic processing significantly affect protein extractability and its IgG/IgE-reactivity, with some treatments reducing/mitigating their IgE-binding capacity, while others preserved or even enhanced immunoreactivity. Digestibility is currently being carried out to test its effect on thermal processed foods like brownies. **Conclusions:** These findings improve our understanding of how processing and gastrointestinal digestion affect the IgE-binding capacity of peanut proteins. This knowledge can support the development of strategies to produce safer food products for allergic individuals.

Keywords:

Peanut; Food allergy; protein IgG/IgE-reactivity, thermal processing, enzymatic hydrolysis.

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24887 | Proline heats the stage: linking plant reproduction and stress physiology in a warming climate

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Background & Aim: Climate change favours the occurrence of heatwaves, threatening global food security by reducing crop productivity. In flowering plants, yield depends on reproductive success, a highly sensitive stage to high temperatures that impair fertilisation and seed formation. Heat stress triggers reactive oxygen species overaccumulation, leading to oxidative stress. To overcome this, plants can accumulate proline, a multifunctional amino acid with osmoprotectant, antioxidant (AOX) and signalling roles, usually associated with stress tolerance [1]. Although well studied in vegetative tissues, its contribution to reproductive thermotolerance remains unclear [2]. Thus, this study explores how proline contributes to heat stress resilience in Arabidopsis, focusing on both vegetative and reproductive tissues. **Methods:** Wild-type (WT) plants and T-DNA mutant lines defective in proline biosynthesis (*p5cs1-4*) and catabolism (*pdh1-2*) were exposed to heatwave-like conditions (38 °C for 4h) at vegetative (24-day-old) and reproductive (40-day-old) stages. Leaves, flowers, and siliques were harvested for subsequent reproductive and physiological analyses. **Results:** Genotype-dependent differences were evident under control conditions: both mutants developed siliques shorter than WT, and proline deficiency is associated with reduced shoot biomass. Aniline-blue staining showed less fertilised ovules in *p5cs1-4* mutants comparing to WT, indicating that adequate proline levels are required for reproductive success. At vegetative stage, heat increased lipid peroxidation (LP) in WT but not in mutant plants. Conversely, at reproductive stage, heat reduced ROS and LP in leaves of *pdh1-2* mutants, supporting a protective AOX effect of proline accumulation. Heat did not markedly increase oxidative damage in inflorescences. **Conclusions:** This work highlights that proline is a key regulator of redox homeostasis, mainly under heat, while also controlling the reproductive performance under optimal and stress conditions.

Keywords: Heat stress resilience; *Arabidopsis thaliana*; oxidative stress; proline metabolism

Acknowledgments:

This project was supported by Fidelidade - Companhia de Seguros, S.A., which provided a scholarship to Clara Konrad within the scope of the Impact Center for Climate Change initiative. AMP received funding from Fundação para a Ciência e Tecnologia through the 2023.07134.CEECIND.

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ARCHITECTURE

24270 | From the Human Modulor to the Horse Scale: A Non-Anthropocentric Proportional System for Architecture

Sara Salta[1]; Filipa Guerreiro[1]; Pedro Baía[1]

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Background & Aim: Through history, architectural proportion systems have been derived from the human body. DaVinci drew the "Vitruvian Man" and LeCorbusier developed the "Modulor I & II" both contributing to the consolidation of human-based proportional systems in architecture. These systems positioned the human body as the primary dimensional reference for architectural design. However, when designed for animals, a project is still conceived according to human references. This research therefore proposes the development of a proportional system derived from the horse's body, grounded in geometric and mathematical principles, to support non-anthropocentric approaches to architectural design. **Methods:** Historical and bibliographic research was conducted on anatomical and morphometric studies of the horse, complemented with photographic documentation and measurements of a single horse, observed in conditions both with and without human interaction. The collected data were analyzed in comparison with canonical human proportional systems. Geometric relationships were tested through circle-square constructions and explored in relation to the Golden Ratio and the Fibonacci sequence, leading to the development of a geometric proportional framework applicable to architectural spatial design. **Results: T**wo proportional series were established based on the horse in a neutral stance and with the head raised. In correlation with the *Modulor* logic, these configurations resulted in a structured system of equine proportions. The resulting system operates as a proportional tool for architectural design, supporting the definition of spatial clearances, enclosing volumes and circulation parameters in horse-related facilities. **Conclusions:** This research introduces a non-human anatomical reference into architectural proportional systems, advancing their formulation for design practice in ways that transcend strict anthropocentrism and enable more coherent spatial integration between human and non-human scales.

Keywords: Horse; Scale; Golden ratio; Fibonacci sequence; Proportions

References:

[1] DaVinci, *Vitruvian Man*

[2] Le Corbusier, *The Modulor I &II*

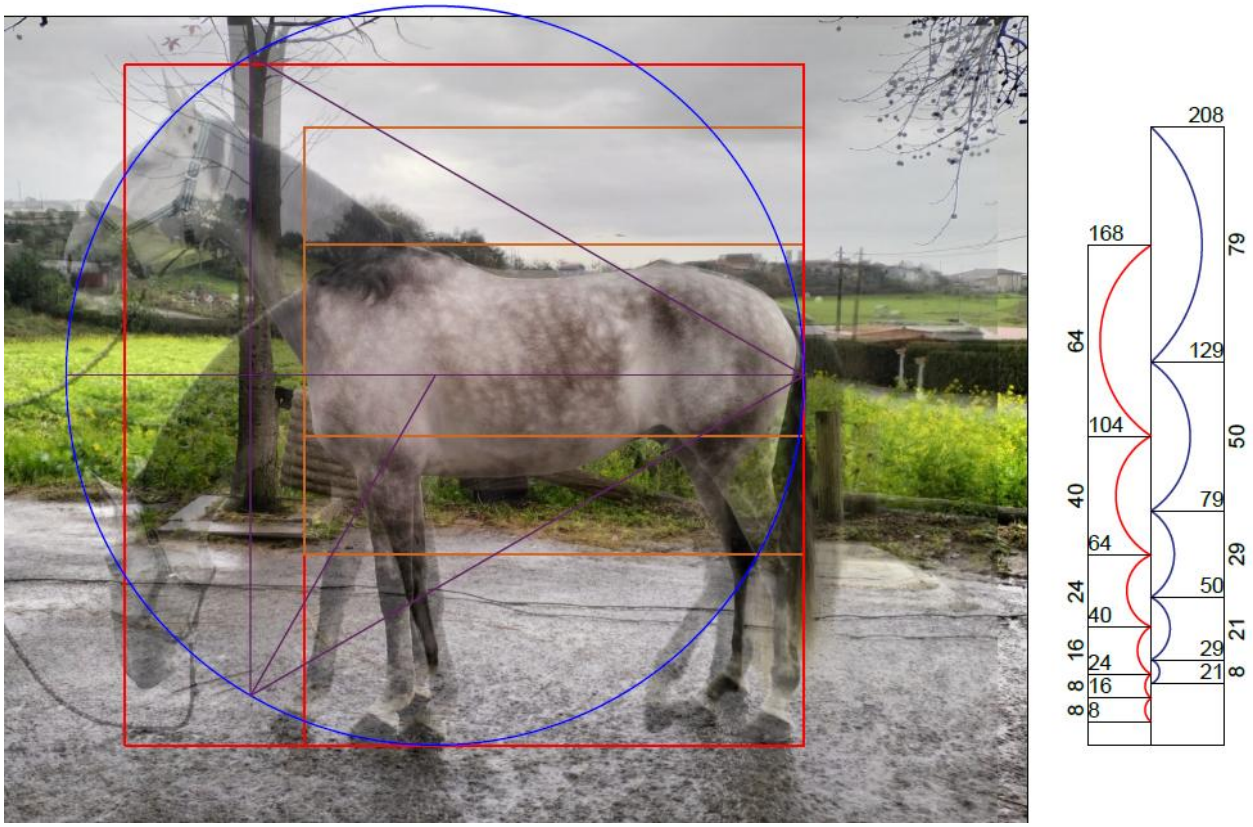

Figure 1: “The horse in a neutral stance and head raised”

24301 | Surrealism and Architecture from A to Z: Collages for a House

Peixoto, Maria Inês; Alegretti, Ana Paula; Hammerschmidt, Tatiane; Monteiro, Beatriz; Vargas, Carmen; Alves, Sandra; Encarnação, Marisa

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Background & Aim: Surrealism, defined by André Breton as pure psychic automatism, emerged at the beginning of the twentieth century with the aim of liberating the unconscious and expressing the true functioning of thought, influenced by Sigmund Freud's psychoanalytic theories. Its relationship with modern architecture is paradoxical, due to the contrast between the rationality of modernism and the imaginative logic of surrealism. Nevertheless, despite this ambiguous relationship, some architects have produced works that employ the tools and modes of thought explored by the surrealists. **Methods:** Inspired by Breton's Dictionnaire abrégé du surréalisme, this study adopts an encyclopaedia/dictionary format to investigate and organise the relationship between architecture and surrealism. The approach aims to engage with and experiment through methods developed by both artists and architects, culminating in an architectural project where the unconscious function as central instrument of the creative process. **Results:** A set of analog collages, demonstrating the different terms and concepts compiled in the encyclopaedia and providing proof of how surrealist methods can be used as tools in architectural design**. Conclusions:** In a world dominated by new technologies, this dissertation seeks to contribute to the exploration of how analog methods can enrich architectural practice. It not only investigates the relationship between surrealism and architecture, but also illustrates potential ways of applying surrealist strategies in architectural design nowadays, particularly through the exploration of the unconscious. This research forms part of the Master's Dissertation for the completion of the Integrated Master's Degree in Architecture, developed under the guidance of Professor Marta Rocha.

Keywords: Surrealism, Collages, Art, Unconscious, Psychic Automatism

24369 | The meaning of inhabiting: architecture as a space for building autonomy for people with autism spectrum disorder

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Background & Aim: The increase in the number of people diagnosed with Autism Spectrum Disorder (ASD) highlights the inability of contemporary housing to respond to their needs. Current solutions split between prolonged permanence in the family home and integration into residential care facilities, that are often misaligned with the specific needs of the diagnosed people. The lack of a housing typology capable of articulating long-term evolution with appropriate sensory and spatial conditions leads to ruptures and compromises life quality. It is relevant to investigate architecture's ability to configure a housing model capable of integrating different life stages, combining domesticity, specialized support, and community integration, within the same place. **Methods:** This housing complex, located in Porto, is the main research instrument. The building splits into three dwelling modes — family nucleus, cohabitation, and independent housing — articulated with support devices and shared-use areas, enable different levels of individual and collective experience. The main point is the possibility of long-term permanence within the same context, through progressive transformation of everyday space. **Results:** The project will reveal that integrating the sensory and spatial specificities of people with ASD, articulated within the same system, allows the development of autonomy without disruptive transitions, consolidating itself within a stable and complete place. **Conclusions:** The main problem of the current housing response resides in the separation of dwelling and care, as well as in the absence of a design approach that considers sensory particularities. By bringing these dimensions together in a single building, the research proposes a revision of the prevailing model, in which autonomy is configured as a progressive process capable of integrating people with ASD, their families, specialized professionals, and wider community. This research is part of the Integrated Master's dissertation in Architecture, supervised by Professor Marta Rocha.

Keywords: Autism**;** Housing; Autonomy

24372 | Healing Enclosure: Architecture between Type and Context

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Background & Aim: Healthcare architecture has often been conceived as a standardized and isolated building model, largely shaped by disease, medical and technological paradigms [1]. This research revisits and challenges the modern concept of the hospital as a universal machine [2], framing it as a situated urban practice embedded within specific geographical, political, cultural, and social contexts. Grounded in psychiatric institutions built in Northern Portugal in the 1970s, the study interprets the hospital as a structure that negotiates care and control, enclosure and exposure. Ultimately, it seeks to articulate a transferable architectural framework that reconciles contextual specificity with adaptability across settings where pre-existing forms enable a cloistered spatial logic. **Methods:** The research adopts a design-based methodology combining theoretical review and architectural practice. Foucault's concept of heterotopia and Saskia de Wit's notion of the enclosed garden structure the analytical framework. The fieldwork and detailed case study of an existing Psychiatric Institution in Bragança, Portugal, informs the development of a cloister-based spatial strategy. **Results:** The study reveals that enclosed gardens and cloistered spatial sequences recalibrate the hospitals' relationship with the urban fabric and nature, introducing alternative temporalities rooted in natural cycles and heightened bodily awareness. The enclosed garden translates heterotopic principles into architectural sequences that support refuge, reflection, and progressive autonomy. Rather than defining a fixed solution, the project establishes a set of adaptable spatial strategies that can be applied when transforming/expanding healthcare environments across varied contexts. **Conclusions:** By reframing healthcare architecture, it proposes a design framework that balances specificity and transferability. Emphasizing spatial principles over fixed formal outcomes, it seeks to inform architectural design at a broader scale.

Keywords: Hospital; Psychiatric Institution; Heterotopia; Enclosed Garden; Bragança

Acknowledgments:

This research constitutes part of a Master's Dissertation within the Integrated Master's Degree in Architecture at Faculty of Architecture of the University of Porto, carried out under the supervision of Professors Carla Garrido de Oliveira (Faculty of Architecture of the University of Porto) and Daniela Arnaut (Instituto Superior Técnico, University of Lisbon).

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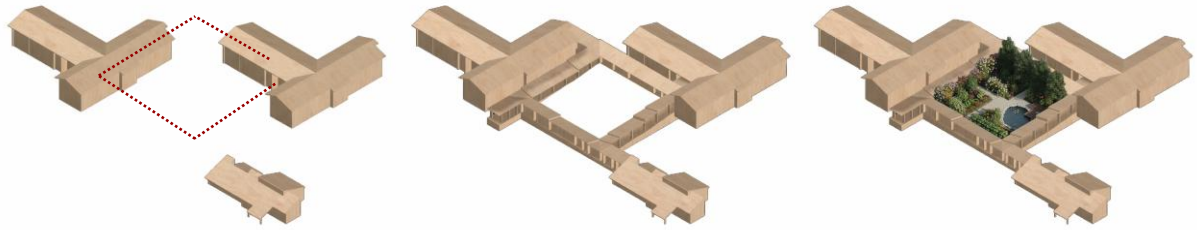

Figure 1: Healing Enclosure: existing buildings; adding the enclosure; development of the enclosed garden.

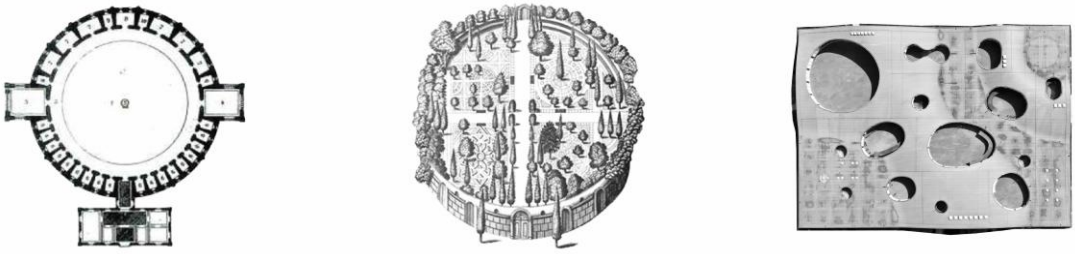

Figure 2: Historical references: *Security Pavilion of Hospital de Rilhafoles*, José Maria Nepomuceno (1896); *Orto Botanico di Padova*, Giacomo Filippo Tomasini (c. 1543); *EPFL's Rolex Learning Center*, Sanaa (2010).

Figure 3: Atmospheres: *The Garden of Eden*, Upper Rhenish Master (c. 1410/1420); *Healing Enclosure view*, author of the abstract (2026); *University Children's Hospital*, Herzog & de Meuron (2024).

24301 | Surrealism and Architecture from A to Z: Collages for a House

Ana Isabel Assunção

Faculdade de Arquitectura

Background & Aim: Surrealism, defined by André Breton as pure psychic automatism, emerged at the beginning of the twentieth century with the aim of liberating the unconscious and expressing the true functioning of thought, influenced by Sigmund Freud's psychoanalytic theories. Its relationship with modern architecture is paradoxical, due to the contrast between the rationality of modernism and the imaginative logic of surrealism. Nevertheless, despite this ambiguous relationship, some architects have produced works that employ the tools and modes of thought explored by the surrealists. **Methods:** Inspired by Breton's Dictionnaire abrégé du surréalisme, this study adopts an encyclopaedia/dictionary format to investigate and organise the relationship between architecture and surrealism. The approach aims to engage with and experiment through methods developed by both artists and architects, culminating in an architectural project where the unconscious function as central instrument of the creative process. **Results:** A set of analog collages, demonstrating the different terms and concepts compiled in the encyclopaedia and providing proof of how surrealist methods can be used as tools in architectural design. **Conclusions:** In a world dominated by new technologies, this dissertation seeks to contribute to the exploration of how analog methods can enrich architectural practice. It not only investigates the relationship between surrealism and architecture, but also illustrates potential ways of applying surrealist strategies in architectural design nowadays, particularly through the exploration of the unconscious. This research forms part of the Master's Dissertation for the completion of the Integrated Master's Degree in Architecture, developed under the guidance of Professor Marta Rocha.

Keywords: Surrealism, Collages, Art, Unconscious, Psychic Automatism

24454| Paths for a Place between Bridges

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Background & Aim: The “Lugar entre Pontes” (Place between Bridges) comprises the riverfront territory of the Douro River in Vila Nova de Gaia, bounded by the Maria Pia Bridge, the São João Bridge, and Avenida Dom João II. Abandoned since 1991, it currently represents an area of segregation and urban rupture. The objective is to consolidate a network of paths that reintegrates this urban void into the urban fabric. **Methods:** The approach was structured into three complementary levels: Maria Pia Bridge Path – The bridge is reactivated as a soft mobility axis. A 3 km connection is established between Campanhã Station, in Porto, and Avenida da República, in Vila Nova de Gaia, branching out into other pedestrian paths; Quebrantões Paths – New connections are proposed, integrated into the existing transport network, articulating the coexistence of road, river, and rail routes; Bridges Museum Path – A gallery is designed, with stairs and a funicular, that runs from the upper to the lower level of the new Bridges Museum, which occupies the pre-existing structures of the former Carbon Sulfide Factory. This system overcomes the elevation gap between the Maria Pia Bridge (level +61.00) and the “Lugar entre Pontes” quay (level +12.00). **Results:** The proposal culminates in an urban and landscape regeneration strategy that transcends mobility infrastructure. The Bridges Museum, the central element of this design exercise, is structured in a succession of volumes, allowing for a stratified reading and use of the program and the hillside. **Conclusions:** The research reiterates the potential of rehabilitating heritage structures as drivers of urban void regeneration. By integrating the topographical difference as a design material, it "sews" the fracture between the city, the “Lugar entre Pontes”, and the southern bank of the Douro River. This research is part of the Dissertation for the Integrated Master's Degree in Architecture, developed under the supervision of Prof. Nuno Brandão Costa and Prof. Marta Rocha.

Keywords: Maria Pia Bridge, Vila Nova de Gaia, Urban Regeneration, Bridges Museum

24560 | Representations of Indo-Portuguese Architecture: Mário T. Chicó, Goa, 1951-1961

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Background & Aim: In 1951, a group of professionals and academics travelled to the Indian subcontinent for a period of just over three months with the aim of documenting, analyzing and researching architecture in *Portuguese India*. The dissertation selects the archive resulting from this trip by the *Study Brigade of the Portuguese India Monuments* as its object of study. **Methods:** Investigation considers the content present in the collection: One must consider the various formats in which the archive is organized, with a focus on photographic records and the associated techniques and methods. To further enhance understanding, the study analysis social and historical context and situation. Chosen practical examples complete the theoretical thinking. The churches of Old Goa that have received the most attention from the *Brigade* are presented and considered on their characteristics of different orders and natures: The Church of the Rosary; the Cathedral; and Convent of Good Jesus. Due to its unique location and context, the Church of Santana, Talaulim, is also mentioned. **Results:** The first goal is to compile an organized catalogue of reference material resulting from the trip. Then, the purpose is to observe the constructed discourse based on *originality*, *monumentality*, and the *fusion of forms*, adopting a *comparative approach* between mainland Portugal and overseas territories. Therefore, the discussion focuses on architecture and architectural photography, considering evident ideas in the visited examples in relation to recording techniques, formats and composition. Comprehending the methodologies used to create imagery, in relation to content and context, should be the subject of research. **Conclusions:** The ultimate objective is to encourage critical discourse on the concept of architecture. The study material also guides the critical observation of architectural photography. The dissertation develops from an exploratory and inquisitive foundation, and the subsequent analysis and interpretation of the reference material should help to enlighten and direct the research thesis.

Keywords: Architecture; Religion; *Portuguese India*; Archive; Photography

Acknowledgments:

To Professor Sidh Losa Mendiratta

To Professor José Miguel Rodrigues

To family and friends

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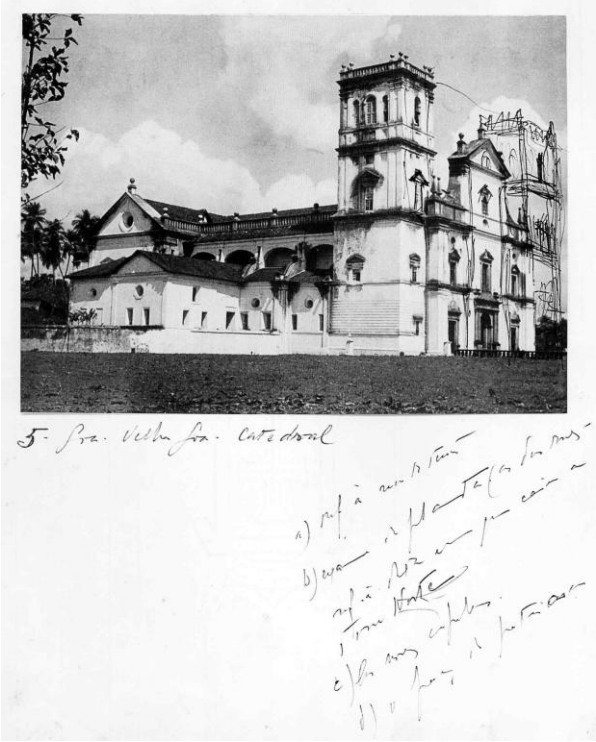

Figure 1: Cathedral of Old Goa, India.

(N/A. Fundação Mário Soares. DTC - Documentos Mário e Alice Chicó. Acessible at HTTP: http://hdl.handle.net/11002/fms_dc_125962. Last accessed in february of 2026.)

27554 | *Where there is smoke, there is fire*. Sucupiras Ecological Park: Living Memorial of the Cerrado

Arthur Machado Dinis[1]; Ana S. B. Alves Costa[1]; Maria Cláudia Candeia de Souza[2];

[1] Faculty of Architecture, University of Porto, Porto, Portugal;

[2] Faculty of Architecture and Urbanism; University of Brasilia, Brasilia, Brasil.

Background & Aim: The work develops an architectural answer to the Management Plan of the Sucupiras Ecological Park in Brasília, Brazil. The Park is an ecological reserve in the central region of the city that protects a piece of the region's native savanna, known as *Cerrado.* Situated adjacent to the Monumental Axis, the Park is the result of a mobilization process by local residents who advocated for the protection of this zone, that has ever since been under constant risk of de-characterization. Encroached by urban area and real-estate pressure, maintaining its status as a ecological reserve requires ongoing mediation between the realities of the city and the forest. To ensure its role of an urban reserve, responsible sectors prepared a Management Plan[1]. This work aims to interpret the Plan through an architectural lens. To do so, it examines contemporary Brasília and its ongoing process of urbanization. **Methods:** The investigation takes place through text and is supported by related graphic material, using architectural tools to interpret and propose within this context, informed by recent investigations that problematize the boundaries between *nature* and *culture.* **Results:** The Park and the *Cerrado* it protects have *vestige* value, serving as a document of the construction of the Brazilian capital's landscape: *natural and built heritage intertwined.* Acknowledging it could allow for additional legal tools to help enforce the proposed Management Plan. **Conclusion:** Although the distinguishment of the Park as a Memorial is not a model solution due to its singular situation, it invites us to consider each point of interest for the different systems that coexist in urban environments as potential forest hotspots. To this end, it is necessary to consider how various environmental *preservation* movements can, in a complementary way, be considered as an expression of a popular demand for environmental *construction*: both a desire and a need to make the city and forest systems coincide.

Keywords: Brasília, Cerrado, Monument, Landscape, Heritage.

References:

[1] This Management Plan establishes guidelines for the park's operation, proposing interventions that better support its use by the public while preserving its ecological reserve status.

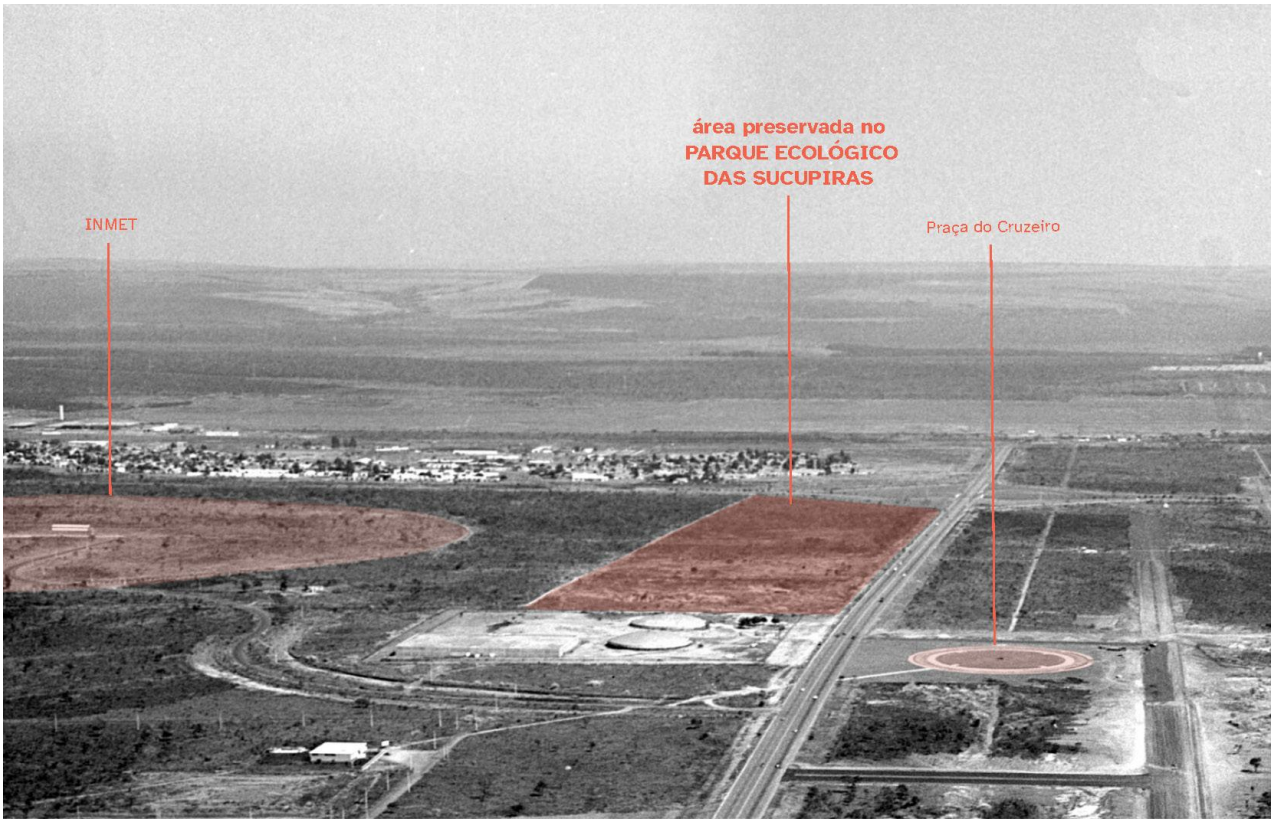

Figure 1: Monumental Axis and *Cerrado* protected by the Sucupiras Ecological Park, 1960. *Arquivo Público do Distrito Federal, edited by the author.*

24740 | *"En lembranza do voso sufrimento"*.

The *Couto Misto* and the *Povos Promíscuos* as border territories of resistance

Patrícia Antunes Reis[1]; Carla Garrido de Oliveira[,2]; Filipa de Castro Guerreiro[3]

[1]Faculty of Architecture - University of Porto, Portugal; [2]Faculty of Architecture - University of Porto, Portugal;

[3]Faculty of Architecture - University of Porto, Portugal

Background & Aim: "*En lembranza do voso sufrimento*"[1] proposes a critical reflection on border territories in the Northwest of the Iberian Peninsula[2]. Until 1864, the *Couto Misto* was a singular jurisdictional enclave, with ambiguous sovereignty and cross-border autonomy. Likewise, the villages historically designated as "*promíscuos*" settlements, were physically crossed by the border. The aim is to analyse how 19th-century border redefinition, confronted with territories and communities of resistance, is a living laboratory to propose contemporary cross-border cooperation. **Methods:** The research combines on-site fieldwork, with archival and bibliographic research, complemented by workshops, interviews and presentations. Drawing is understood not simply as a tool of representation, but as a critical device through which the historical transformations of borders — visible and invisible — are interpreted. **Results:** The study of the Europe oldest political border reveals how administrative decisions and territorial treaties reconfigured settlements, community practices and collective identities. Distinct from formal European cross-border programmes, local cooperation practices emerged from proximity and shared resources, demonstrating adaptive strategies to overcome limits. The *Couto Misto* and the *Povos Promíscuos* embody spatial configurations where resistance materialised not only politically, but also in the morphology of settlements and in collective governance systems. **Conclusions:** The study concludes that the border is more than a line; it is a complex and heterogeneous territory where memory, conflict and solidarity intersect. The *Couto Misto* and the *Povos Promíscuos* are that resistance inscribed in urban form, communal practices and cross-border relations. Their experience offers enduring lessons for contemporary territorial planning, suggesting that sustainable strategies in border regions must emerge from local knowledge, shared governance and the transformation of limits into spaces of encounter.

Keywords: Border; Cross-border; Territory; Couto Misto; Povos Promíscuos

[1] "In memory of your suffering," written in Galician, are the words on the sign at the Cambedo village, expressing the gratitude of Spanish Republicans to the inhabitants of Cambedo during the Franco dictatorship.

[2] This abstract is part of the master thesis "Territórios de Fronteira, Lugares de Encontro" (2025). The dissertation analyses three cases: Couto Misto and the "Povos Promíscuos" —the subjects of this summary—, and Rio de Onor -- presented at the 2024 IJUP.

Acknowledgments: This abstract is part of a dissertation was developed with the support of *Fundación RIA*, under the General Collaboration Protocol established between the Faculty of Architecture of the University of Porto (FAUP) and *Fundación RIA.*

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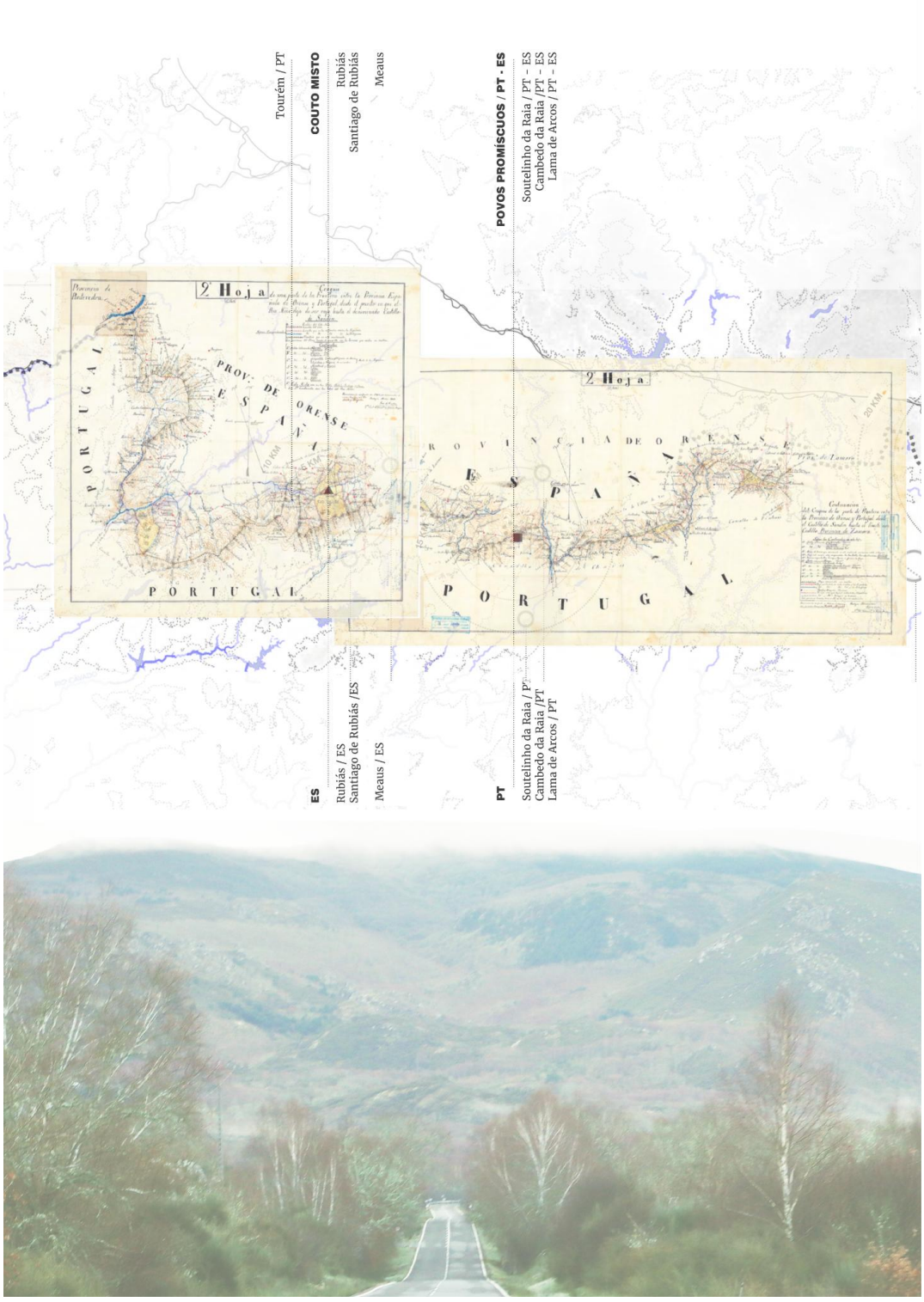
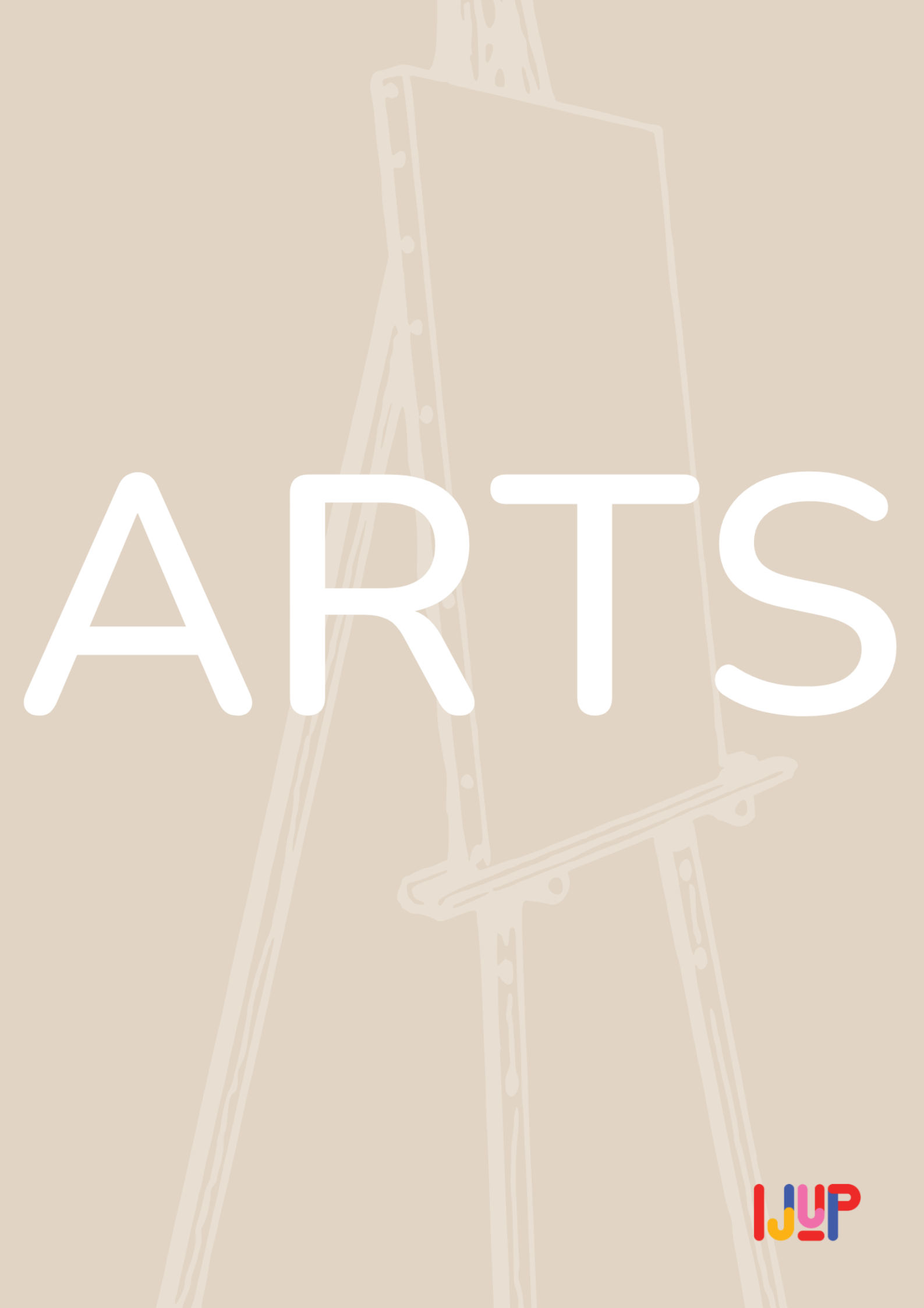


Figure 1: Treaty of Limits. José de Castro López. "Border between Galicia and Portugal. Series of maps "*A Raia*" (1863). Photograph; base map; and collage by the author (2026).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24133 | The Body as Material Trace: Performativity and Memory in Artistic Research

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Background & Aim: This research establishes a practice-led position within contemporary artistic research by reconceptualising absence as a materially produced condition rather than a representational theme. While grounded in discourses on performativity [1], embodied perception [2] and the image as temporal survival [3], the project does not confirm these frameworks but interrogates and displaces them through artistic practice. Drawing on notions of contact [4] and spatial production [5], expanded painting is defined operationally as a field of material procedures—contact, transfer and inscription—through which the pictorial exceeds the image. The research advances the body as material trace: not as representation, but as a generative site where absence is actively produced and sustained. **Methods:** Developed through practice-based research [6][7], the study integrates theory and material operations as structurally interdependent. Methods include frottage, rubbing and gyotaku-inspired transfers [4], moulding, wax casting from votive residues, latex impressions of architectural surfaces, encaustic inscription and performative actions documented photographically. Materials such as graphite powder, wax, latex and translucent supports are selected for their capacity to register pressure, erosion and temporality. Concepts such as spatial production [5], restored behaviour [8] and material memory [3] are not illustrated but reformulated through these operations. **Results:** The research produces a body of work in which absence becomes materially observable. Frottage generates indexical imprints transferring absent bodies and objects onto fragile supports. Wax and latex produce casts and membranes functioning as surrogate skins, creating stratified, translucent structures that articulate loss, preservation and transformation. These processes foreground fragility, layering and partial visibility as material conditions. From these operations, exuvia is formulated as a central concept, defining the body as residual and transitional. Performative actions displace the visible body, producing deferred, indexical traces embedded in material and photographic outcomes. **Conclusions:** The project establishes a non-derivative disciplinary position by demonstrating that absence is a materially producible and verifiable condition. It redefines expanded painting as an operational field grounded in inscription, contact and transformation, rather than theoretical expansion. Its primary contribution lies in formulating trace and exuvia as epistemic categories emerging from practice, and in repositioning performativity as material inscription rather than presence [1][8]. By producing concepts through making, rather than

confirming existing frameworks, the project advances artistic research as an autonomous field capable of generating theory through material engagement.

Keywords: Absence; Body; Artistic Research; Memory; Performativity

Acknowledgments:

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Figure 1: Mariana Maia Rocha, *Exoskeleton and the Corporeality of Touch* (2024). Graphite powder frottages on 90 g tracing paper of family objects and LED lamps, exhibited at the Museum of the Faculty of Fine Arts of Porto. © Nuno Ribeiro.

24194 | Computational Simulation Models in Typeface Legibility Research

Assessing Reliability as an Alternative to Tests with Visually Impaired Participants

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Background & Aim: This study presents the results of a comparative investigation into the effectiveness of typeface legibility testing methodologies for people with visual impairments. The field of accessible type design faces significant challenges, as evaluating the legibility of fonts has traditionally been a complex and time-consuming process that relies on testing with human participants. The rise of technologies such as the "VI-OCR" Optical Character Recognition pipeline and human retina simulation models offers an opportunity to streamline and optimize this process. The primary objective of this research was to determine if simulation technology can serve as a reliable legibility testing method, thereby evaluating if and how a pipeline such as VI-OCR can serve as an efficient substitute for human experimentation. **Methods:** To achieve this goal, the study applied the testing method used by Larson in the development of the Sitka typeface, a validated technique for assessing individual letter recognition, to different fonts. The experimental/quasi-experimental methodology was divided into two parallel approaches. First, legibility was analyzed through a VI-OCR pipeline and a computational retina model, simulating different types and levels of visual impairment. Concurrently, the same tests were conducted with a group of human participants with similar various types of visual impairments. **Results:** The analysis revealed a high degree of correlation between the automated VI-OCR outputs and the letter recognition patterns observed in human participants. Specifically, the simulation models accurately identified the same "problematic" character pairs and legibility bottlenecks as the human participants. While human results exhibited expected variance due to individual fatigue and subjective factors, the VI-OCR pipeline provided a consistent, reproducible baseline for legibility thresholds. These results demonstrate a close link between the data obtained from technological simulations and human participant tests, confirming the hypothesis that the technology could accurately and consistently mimic human visual perception across different levels of acuity and contrast sensitivity. **Conclusions:** The results demonstrated a close link between the data obtained from the technological simulations and the results from the human participant tests, confirming the hypothesis that the technology could accurately and consistently mimic human visual perception.

Keywords: Accessible type design; Visual impairment simulation; Font legibility testing; Optical Character Recognition; Retina Simulation.

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Dr. Qingying Gao, John Hopkins University

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24195 | The Revival of Tia Lira: Reinterpreting cultural legacy in type design through variable font technology

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Technological advances in type design have fostered the growth of type revival—the practice of updating and reinterpreting typefaces from the past [1]. Within this context, the work of Manuel Pereira da Silva [1930–2008]—Portuguese designer, typographer and researcher with significant contributions to the national typographic landscape—remains little recognized in both national and international spheres, despite previous dissemination efforts [2, 3]. This project addresses that gap by revisiting *Tia Lira* (2003), one of his original type designs, through a process of technological reinterpretation that connects cultural heritage with contemporary design tools and practices. The project is based on the reinterpretation of Manuel Pereira da Silva's typeface *Tia Lira* using *OpenType* variable font technologies. It positions type revival as an interpretative and methodological practice, exploring how technological experimentation can coexist with cultural preservation in type design. The methodology is grounded in a Practice-Based Research approach, articulated with a set of complementary methods. The process integrates a thematic literature review, the analysis of *Tia Lira*'s source material, case studies of typographic revivals with a focus on non-historical and experimental approaches, interviews with specialists, the project-based development of a typographic revival validated by type design experts, as well as testing and validation through peer testing and user questionnaires. The project results in a reinterpreted variable type family structured along weight and x-height axes. The resulting work is intended for dissemination through a type distribution platform, contributing to the visibility and critical understanding of Manuel Pereira da Silva's typographic heritage. Ultimately, it proposes a methodological approach to type revival in non-traditional contexts, reinforcing the role of technological reinterpretation as a means of preserving and renewing typographic culture.

Keywords: Technology in design; Type revival; Typographic heritage; Variable fonts

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24200 | Comparing frames: When Orpheus looks back

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Background & Aim: This essay parts from the photographic similarity in-between two film frames, one from Ingmar Bergman's *Persona* (1966) [Figure 1] and another from Céline Sciamma's *Portrait de la jeune fille en feu* (2019) [Figure 2], and by relating them to the myth of Orpheus and Eurydice, it aims to explore how both films portray the ambivalence of desire. **Methods:** At first both frames are analysed in their photographic composition by observing the placement of the actresses and the visible eye on the axis, the techniques to highlight the gaze and the usage of light, shadow and, in the second case, color, for storytelling. This leads to a narrative analysis of the films in relation to Ovidio's version of the myth of Orpheus and Eurydice, followed by a reflection on the portrait of desire based on Anne Carson's *Eros, the bittersweet* (1985) and the myth of the daughter of Bustades by Pascal Quignard (2014). **Results:** As a result, the essay understands that *Eros*, as presented by Anne Carson, is found in the ontological foundation of cinema. It is also in both films, in which desire requires the absence of the loved one to exist. The photographic similarity on the frames leads to understanding that the captured gazes of the actresses are the same of Orpheus, the one that leads him to the immediate loss of Eurydice as a way for *Eros* to keep flowing. **Conclusions:** The essay presents the echoes of the classic myth in cinema and how the ontology of cinema can be understood as a *memoir* of the dead, a looking-back instant right before they're gone. It also finds that the similarity in between the two films goes beyond the photographic composition, as the photography also tells how in both narratives there is an impossibility of a complete conciliation of the lovers and a necessity of letting one part to the "world of the dead" in the name of desire.

Keywords: Cinema; Desire; Memory;

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Figure 1: *Persona*, by Ingmar Bergman, 1966. Ab Svensk Filmindustri.

Figure 2: *Portrait de la jeune fille en feu*, de Céline Sciamma, 2019. Lilies Films.

24214 | Inhabiting Absence: Trust as a Propelling Agent in Sculptural Artistic Research

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Background & Aim: This artistic research examines trust as a foundational condition of human relationships and sculptural practice. Drawing on A. Damásio's understanding of life as biological regulation and D. Winnicott's concept of transitional space, trust is framed as a relational force between interior and exterior, presence and absence, life and death. The study investigates how artistic practice sustains relational bonds and memory in the face of loss, focusing on how these dynamics are materially enacted. **Methods:** The research follows a practice-based methodology combining theoretical inquiry, embodied experience and sculptural production. It includes auto-ethnographic reflection on the anatomical theatre, where contact with cadavers informed an understanding of the body as material and relational presence. These observations translate into sculptural operations through clinical objects, textiles and garments, exploring arrangement, suspension and displacement. The work is further informed by F. González-Torres and Chiharu Shiota, particularly in their use of fragility as a relational strategy. **Results:** The research shows trust as an implicit pact between body, gesture and observer, persisting beyond biological death. Encounters in the anatomical theatre revealed death not as rupture but as a shift in relational condition. This understanding materializes in *In Memoriam*, a sculptural work that reconfigures clinical objects, garments and ritual elements into a spatial composition inviting proximity and slowness. Through minimal gestures, the work establishes tension between vulnerability and support, where absence is activated as a sensitive presence. **Conclusions:** This study positions trust as a core material of artistic practice, enacted through care, proximity and material negotiation. By approaching death as transformative, it contributes to artistic discourse on care, finitude and relational aesthetics, proposing art as a space where absence can be physically and sensorially inhabited.

Keywords: Embodiment; Liminality; Memory; Sculptural Practice; Trust

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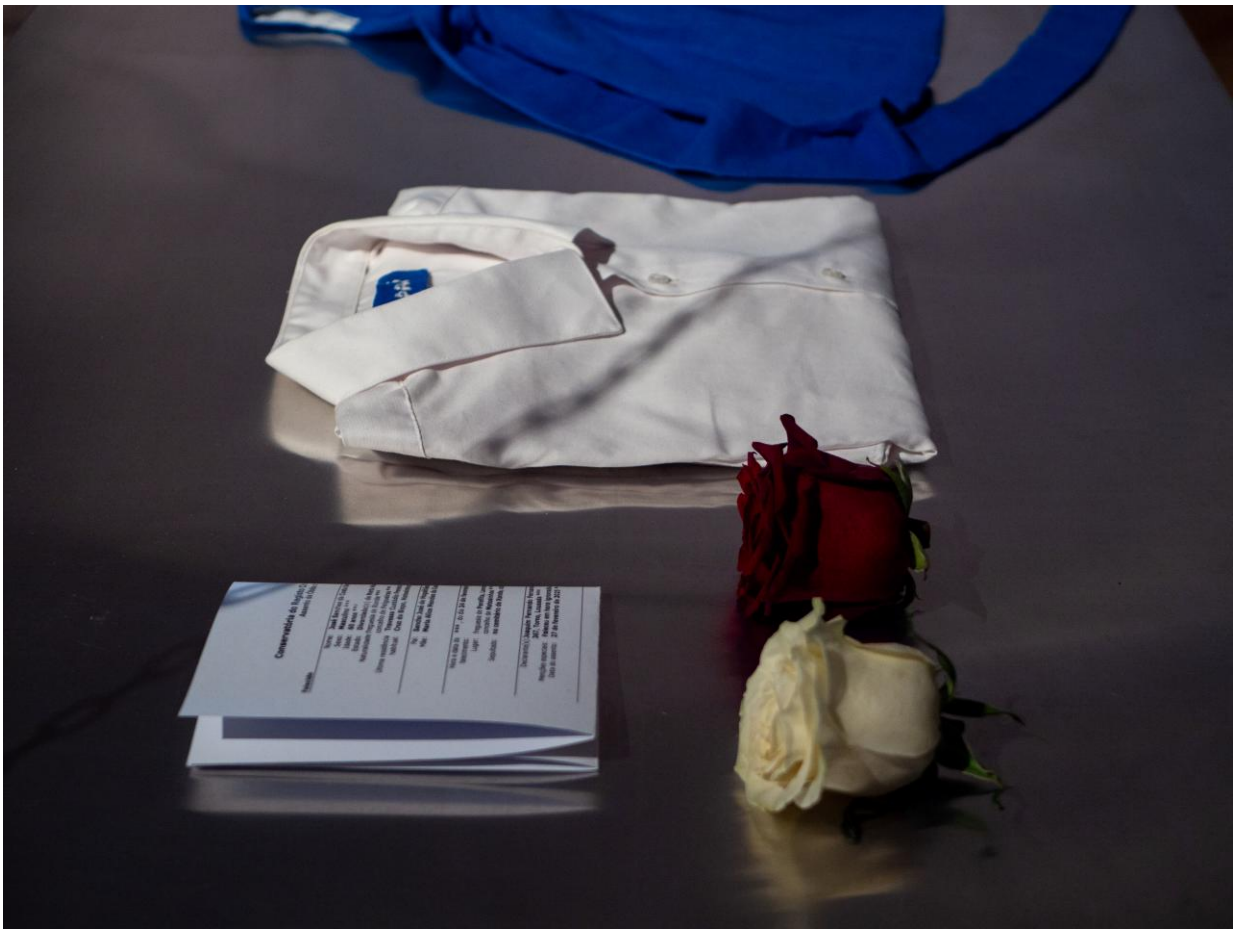

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Figure 1: Detail view of Lino, Rita. *In Memoriam*, 2026.

Figure 2: Lino, Rita. *In Memoriam*, 2026.

Aluminum, bamboo, cotton, linen, paper, roses, steel, wood. 120 × 180 × 80 cm

Figure 3: Detail view of Lino, Rita. *In Memoriam*, 2026.

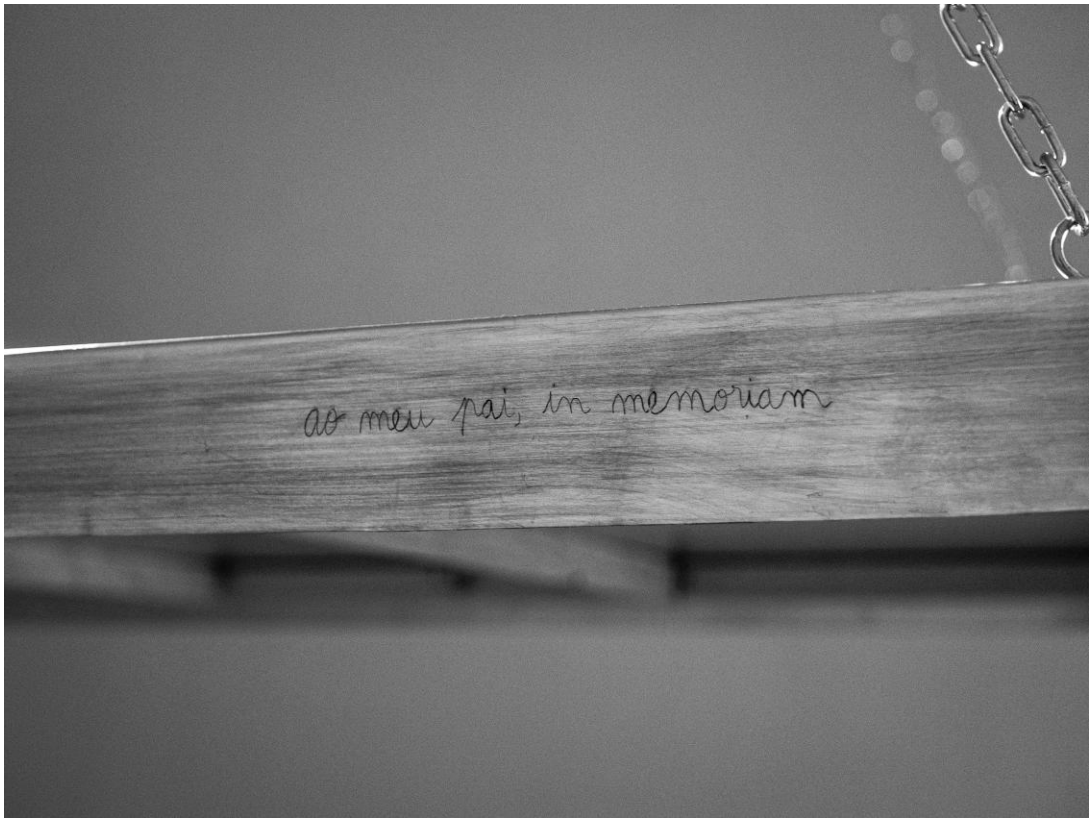

Figure 4: Detail view of Lino, Rita. *In Memoriam*, 2026.

24346 | Alternative Realities: Postmemory and the Reinterpretation of the Family Archive as Artistic Practice

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Background & Aim: The growing attention given to the family archive in contemporary theory and artistic practice reflects the need to revisit personal histories connected to collective memories. Photographs, letters, and inherited objects become mediators between past and present, revealing both silences and absences as well as deep emotional bonds. The aim of this research is to understand how the family archive can function as a living space of identity and emotional reinterpretation, articulating intimacy and history. At the same time, the study analyzes how my artistic practice appropriates my grandfather's military archive—he served in the Portuguese Colonial War between 1968 and 1971—in order to imagine and reconstruct my grandparents' relationship during the period in which they were separated. **Methods:** The article combines a theoretical analysis of authors who study postmemory, such as Marianne Hirsch and Joan Gibbons, in order to understand the influence of the familial past on present generations, as well as the importance of the archive and photography as creative processes based on the manipulation of inherited images, drawing on the ideas of authors such as Hervé Guibert and Roland Barthes. In addition, artists whose work engages with both fields are also referenced, namely Daniel Barroca, Daniel Blaufuks, and Gillian Wearing. **Results:** Through the construction of "alternative realities," the artworks result from interventions on the personal archive, such as the intuitive selection and reprinting of photographs, the association between images according to formal and affective criteria, and digital manipulation through the superimposition of figures.The compositions resulting from this process are subsequently converted into cross-stitch grids, perforated, and manually embroidered onto the printed image. This method reshapes the original narrative, producing hybrid images that bring together individuals who were once separated. Embroidery, by introducing notions of transparency and repetition, generates a ghostly dimension and highlights the overlapping of temporalities, enabling the archive to be reactivated as a sensitive material and opening space for the reinterpretation of histories. **Conclusions:** This reflection demonstrates that the family archive is not a static repository but a dynamic device capable of producing new meanings. In this way, the work contributes to the state of the art by showing how the articulation between postmemory, archive, and artistic practice can generate alternative ways of narrating history, expanding the understanding of intergenerational bonds as a means of creation and identity reconstruction.

Keywords: Family Archive, Postmemory, Collective Memory, Alternative Realities, Embroidery

Acknowledgments: Domingos Loureiro and Joana Rêgo

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Figure 1: Cláudia Pinto, Realidade Alternativa #3, 2024, 89 x 115 cm, embroidery over print on matte paper

24431 | Towards a Forensic Ocean Atlas: Countermapping Angeiras' Underwater Landscapes from the U-1277 Shipwreck

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Background & Aim: Dominant Ocean cartographies reproduce the sea as an abstract, legible, and governable surface aligned with regimes of control, extraction, and tourism. Within this framework, Blue Tourism aestheticizes submerged landscapes, rendering them consumable while erasing their political and historical density. This research challenges such depoliticized representations by positioning mapping as a contested and situated practice. Drawing on post-representational cartography [1], the social construction of ocean space [2], and countercartographic approaches articulated by Nepthys Zwer [3], the project engages forensic methodologies to approach the seabed as an evidentiary field rather than a neutral landscape. **Methods:** Focusing on the U-1277 shipwreck off Angeiras, the research adopts a practice-based methodology combining GIS data, underwater photographic documentation, and archival sources with material experimentation. The atlas is developed through a post-digital approach as a series of haptic maps that translate bathymetry, wreck morphology, and seabed textures into tactile form, destabilizing the authority of screen-based visualizations. Spatio-feminist perspectives inform the process by foregrounding embodied, partial, and situated knowledges. **Results:** The atlas produces tactile cartographic artefacts that reframe the shipwreck as a site of tension between competing regimes of representation. Through layered materializations (relief surfaces) it emphasizes perceptual limits and interpretive uncertainty. By requiring touch and proximity, the atlas repositions the user as an engaged interpreter. **Conclusions:** This research advances a forensic and embodied approach to ocean mapping that operates beyond visual representation. It challenges extractive and aestheticizing logics of Blue Tourism, proposing instead a politically accountable cartography in which submerged landscapes are understood as sites of memory, conflict, and ongoing negotiation. Presented in exhibition contexts, the atlas further activates bodily involvement and tactile exploration, mobilizing imagination as a critical mode of engaging with submerged space.

Keywords: Countercartography; Haptic Cartographies; Post-Representational Cartography; Underwater Cultural Heritage; Critical Ocean Studies

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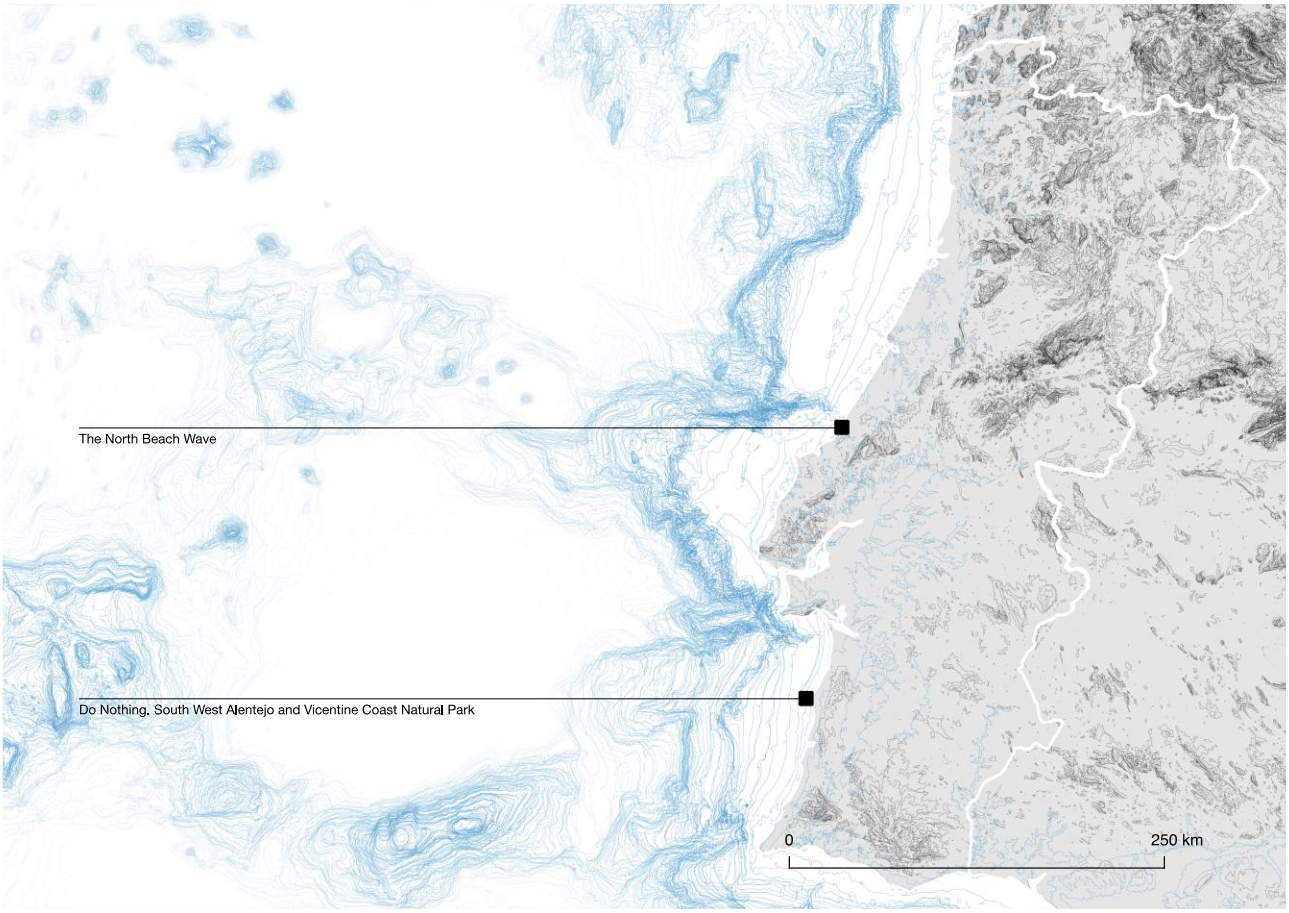

Figure 1: Map developed in QGIS for the book "Atlas of Good Practices: Innovative Blue Tourism, Creative and Cultural Projects" designed for the SEALabHaus project — *Atlantic New Bauhaus Laboratory for Sustainable, Inclusive and Beautiful Blue Tourism, Heritage and Culture of the Sea***.** (Leonor Carvalho, 2025)

Figure 2: “Wreckscapes” — first iteration of the project (Leonor Carvalho, 2025).

Figure 3: “Wreckscapes: An Editorial Apparatus for Submerged Archeologies” — second iteration of the project (Leonor Carvalho, 2025).

24540 | The Support as a Place Where Painting Thinks: What Remains When One Does Not Paint?

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Background & Aim: This research investigates the capacity of the support to operate autonomously as painting, addressing the fundamental question: what remains when one does not paint? Centred on *burel,* a traditional, dense, Portuguese wool fabric, the study posits that pictorial qualities emerge through the material activation of the support prior to any pigment application, thereby precipitating an ontological shift that expands the definition of painting beyond image and surface inscription. **Methods:** The methodology integrates practice-based research with a phenomenological inquiry, drawing on Merleau-Ponty's understanding of the body as the primary condition of perception. The process is structured by three operational phases: the controlled folding of *burel* guided by breath and somatic awareness; the systematic observation of structural responses to pressure, tension, and repetition; and documentation via a visual atlas. The conceptual framework synthesizes the philosophy of the fold (Deleuze), the monochrome (Klein), and the five *Koshas* of yogic philosophy. **Results:** Results, embodied in a body of seven works comprising the *a_dobras* series, demonstrate that these pieces do not merely illustrate a thesis but constitute one. **Conclusions:** The research concludes that painting can be redefined as material activation, where the support's structural memory and rhythmic organization establish the elemental form of the pictorial act.

Keywords: support; fold; materiality; expanded field; somatic practice

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Figure 1: *a_dobras 4* burel, 1.46m x 1.60m, Sónia Jaconiano, 2025

24824 | Photography as illustration in the transition from the 19th to the 20th century. The Portugalia journal

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Background & Aim: Photography is widely used in scientific disciplines as a means of disseminating results. Since the mid-19th century, it has been applied to heritage studies, and archaeological excavations and monuments have been recorded using this method [1]. This work focuses on a set of photographs (and their modification) in the MHNC-UP archive, used to illustrate the journal *Portugalia: materiais para o estudo do povo português*, published between 1899 and 1908. This journal was characterized by the prolific use of imagery (c.1120) to illustrate the results presented. **Methods:** These photographs, not yet inventoried, were stored by blocks in envelopes and scattered throughout the archive. We began with their reorganization according to the articles by comparing with the original journal. Then they were scanned, numbered, and the digital object identified. The physical object was individually packed, with the identification, and placed in appropriate folders. An inventory table was created with the necessary parameters for characterizing the object, and subsequently the data and digital objects were incorporated into the MHNC-UP database for collection management and later public access. **Results:** Of the c.200 images identified so far in the *Portugália* dossier, about 80 are photographs. The authors of the photos were identified, among whom we highlight M. Carneiro, R. Severo and A. Santos Rocha. Sometimes the images were used after manipulation directly on the print. The retouch was made using ink, paint and charcoal pencil. The manipulation was also used to isolate elements within the image. Some prints were also used as the basis for scientific illustration in Indian ink. These situations had already been described in other cases in the archive [2, 3]. **Conclusions:** Photos were used in the articles where architecture, portraits and archaeology were the themes. Retouching is clearly visible and very interesting to study. Photography in Portugalia is used as scientific illustration.

Keywords: Photography; scientific illustration; image editing

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[1] Hissa, Sarah (2015) A fotografia arqueológica: entre a mimese e a criação. *Habitus*, 13:2, pp71-88.

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[3] Gaspar R, Fernandes I M (2024) Aurélia de Souza - Breve incursão pela ilustração científica. As ilustrações para a revista Portugalia. In: Silva, R H et al (coord) *Aurélia de Souza. Catálogo raisonné*, Museu Nacional Soares dos Reis e Instituto de História da Arte da Faculdade de Ciências Sociais e Humanas da Universidade Nova de Lisboa, pp 997-1032.

Figure 1: The photography, original by Manuel Carneiro (1871-1956), corrected with watercolour and displaying the indications for printing in the journal. MHNCUP-FCUP-IA-PORT-5194

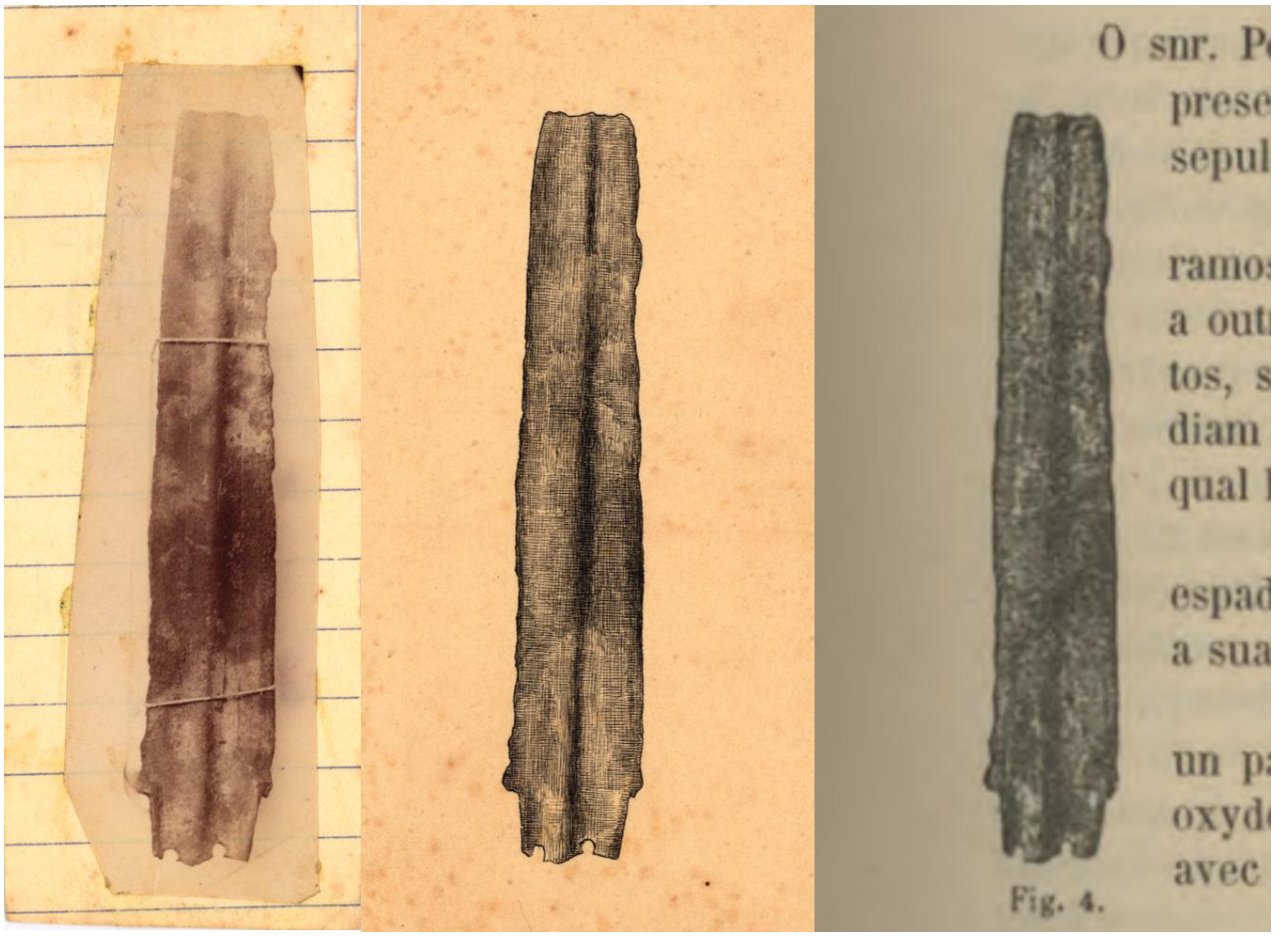

Figure 2: Process of illustrating archaeological materials using photography (left) as base to obtain the scientific illustration (center) and produce the lithography for publication (left). MHNC-UP-IA-PORT-5071 (left) and 5070 (center).

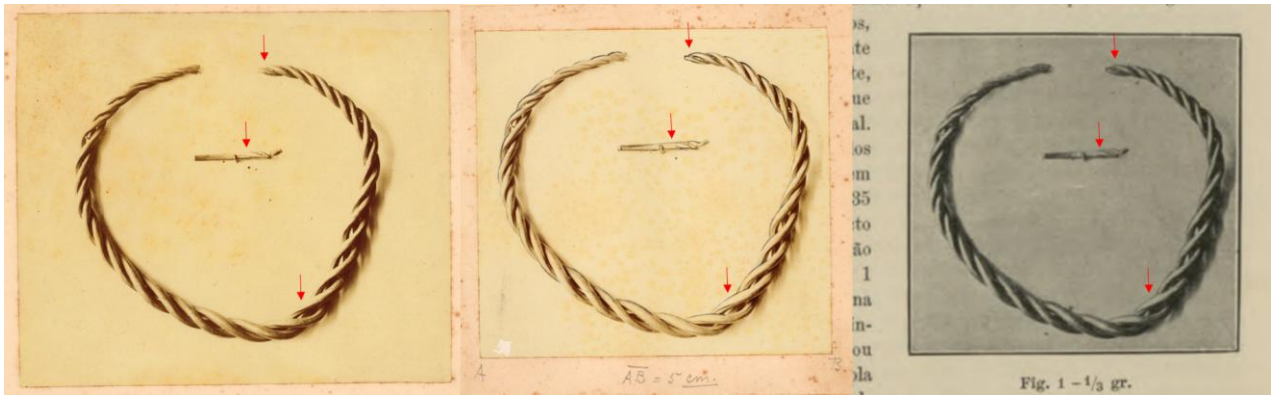

Figure 3: Process of illustrating archaeological materials using photography. The correction of contrast in the paper prof with watercolour in specific places and the lithography in publication (left). MHNC-UP-IA-PORT-5134 (left) and 5135 (center).

24839 | Black and white Artworks for publication. The turn of the century (1899-1908) Portugalia Journal

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Background & Aim: The journal *Portugalia: materiais para o estudo do povo português*, published between 1899 and 1908, was characterized by the profuse illustration of its scientific articles. At the time it was the publication with the greatest care in illustration, constituting an important corpus of artistic material that is, in part, in the archive of the MHNC-UP. For this illustration process, artists from different areas were hired, among them Aurélia and Sofia de Sousa [1]. **Methods:** The drawings and watercolors were not yet fully inventoried, and were scattered throughout the archive. The comparison with the original journal was fundamental for their reorganization, following the scanning, numeration, and identification of the digital object. The physical object was then packed in acid-free paper and identified. The data about the original drawings and watercolors was recorded in the inventory table to subsequently incorporation into the MHNC-UP database for collection management and public access. **Results:** Of the c.210 illustrations in the MHNC-UP archive, about 140 are original drawings and watercolors. The care taken by the journal's editors, namely R. Peixoto, led to the contribution of important artistic figures from Porto. For example, M. Soá, the Italian architect [2] who years later designed the Monumental building on Av. dos Aliados, illustrated several articles, with his amazing pen and ink drawings. Figures from the Porto Academy of Fine Arts and the Porto Polytechnic Academy, as well as from the city's industrial schools, are among the illustrators. C. R. Peixoto, A. Lino and J. Aroso are a few. **Conclusions:** The high graphic quality of the journal *Portugália* is due to the artists invited to participate in its illustrations. Another important factor is the care and high standards of the editors, visible through the editing and printing instructions found in the original works in the archive. This graphic heritage will be available as the result of this study.

Keywords: Watercolors; Indian ink; scientific illustration.

References:

[1] Gaspar R, Fernandes I M (2024) Aurélia de Souza - Breve incursão pela ilustração científica. As ilustrações para a revista Portugalia. In: Silva, R H et al (coord) *Aurélia de Souza. Catálogo raisonné*, Museu Nacional Soares dos Reis e Instituto de História da Arte da Faculdade de Ciências Sociais e Humanas da Universidade Nova de Lisboa, pp 997-1032.

[2] Gaspar R (2023) Michelangelo Soà. In *UIVO 2022*. 12ª Mostra de ilustração da Maia, 19

Figure 1: Pen and ink drawing, original by Michelangelo Soá (1856-1935). MHNCUP-FCUP-IA-PORT-5046

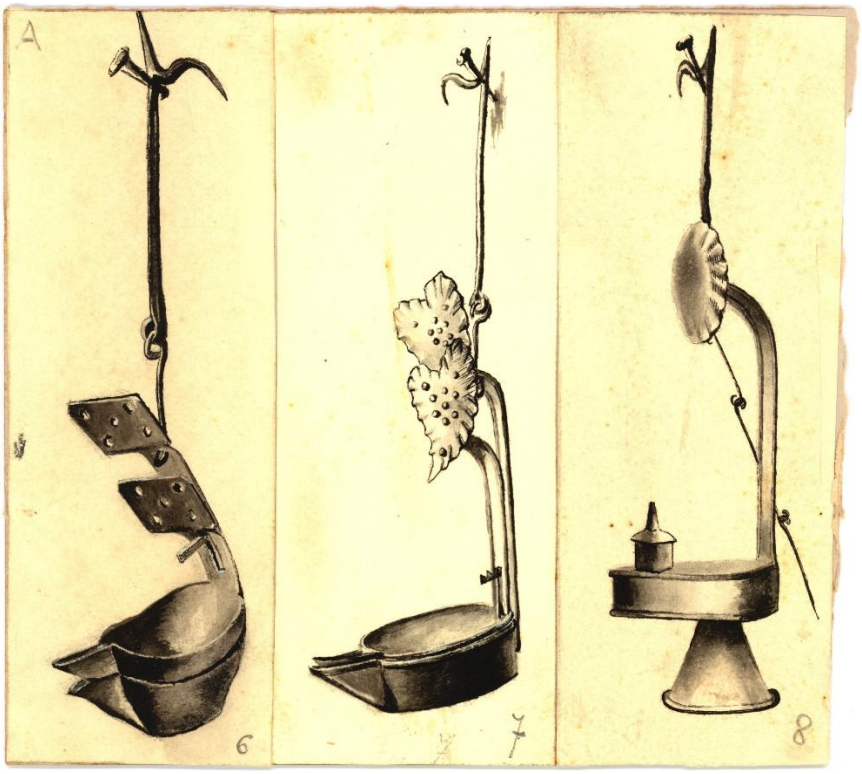

Figure 2: Watercolors by Clotilde Rocha Peixoto. MHNCUP-FCUP-IA-PORT-5159.

Figure 2: Watercolors by José Pinho. MHNCUP-FCUP-IA-PORT-5158.

24908 | Echoes and reflexes of the ovidian myth of Echo and Narcissus in Nuno Júdice and J. W. Waterhouse

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Background & Aim: Through an interart study, this work aims to comparatively analyse the painting *Echo and Narcissus* (1903), by John William Waterhouse, and the poem «Eco e Narciso» (1990), by Nuno Júdice, in the light of the Ovidian version of this myth. Using the idea of symmetry as a departing point, this comparative exercise seeks to understand how this technique can be applied both to a pictorial object and to a literary object, highlighting its main similarities and differences. **Methods:** Through bibliographical research based on theoretical-critical texts, but also through a close reading of the poem and of the painting (although «close-reading» is a concept from literary studies, it can also be applied to visual arts, highlighting the act of reading as a critical exercise), it was possible to establish different connections between the poem and the painting. This paper demanded a close reading of the ovidian text as well, via portuguese translation, which guides the comparative approach in a triangular logic. Among the bibliographical references that sustain this work, authors like Martins (2017), Santos & Atik (2011) e Severino (2018, 2021) were vital to the development of the main arguments. **Results:** Symmetry is essential to comprehend the myth of Echo and Narcissus in Ovid and the same happens with the artistic reiterations developed by J. W. Waterhouse and Nuno Júdice. Not only symmetry, as mainly a visual phenomenon, can also be applied to the internal textual relationships in the poem, but it proved to be a productive concept to think other types of symmetry, namely by analysing how the two objects also mirror the ovidian text. **Conclusions:** Besides a deeper understanding of the poem and of the painting, this paper allows a critical look on the possibilities of painting and poetry as artistic media, on how both can function as imperfect reflexes or echoes of past artworks.

Keywords: J. W. Waterhouse, Nuno Júdice, Myth, Interart, Symmetry.

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[3] SANTOS, Elaine Cristina Prado dos, & ATIK, Maria Luiza Garnieri (2011), «Metamorfose e metaformose: uma leitura mítico-dialógica do mito de Narciso em Ovídio e em Leminski», *in Todas as Musas*, nº 3 (1): 27-36. https://share.google/u4eMZcsi0sP4Y7nNs (20 de novembro de 2025)

[4] SEVERINO, Carlos Mesquita (2018), *Representações das Metamorphoses de Ovídio em J. W. Waterhouse*, Dissertação de mestrado, Universidade de Lisboa. https://share.google/ODujGH2mkr52gQlxB (15 de novembro de 2025)

[5]_____(2021), «Narciso nas Metamorfoses de Ovídio e na pintura de J. W. Waterhouse», *in Forma Breve: olhares de Narciso: egotismo e alienação*, nº17, Aveiro, UA Editora: 119-138, https://share.google/TgXtTLmViOezbPWBu (25 de novembro de 2025)

[6] OVÍDIO (s/d), *Metamorphoses*; ed. ut.: *Metamorfoses*, trad. Paulo Farmhouse Alberto, Lisboa, Penguin Clássicos, 2024.

[7] WATERHOUSE, John William (1903), *Echo and Narcissus*, 109 x 189 cm, disponível https://www.liverpoolmuseums.org.uk/artifact/echo-and-narcissus (5 de novembro de 2025)

Figure 1: *Echo and Narcissus*, by J. W. Waterhouse

24917 | The Body as Emotional Archive or Choreographic Artifact
Exploring the Performative Potential of the Book as a Space of Memory and Design

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Background & Aim: Portugal's dance archives face a critical scarcity, leaving performative experiences underrepresented and at risk of being lost. Despite growing interest in embodied knowledge, there is a lack of editorial tools capable of capturing the ephemeral nature of performance. This project proposes the development of three editorial artefacts — "performance-books", that function simultaneously as archives, choreographic objects, and sensory experiences, preserving and reactivating bodily memory through design. The central question driving the project is not merely how to document a performance, but how the book, as a material and sequential object, can itself become a performative act. **Methods:** The project employs artistic research methodology, combining choreographic experimentation with design processes. It begins with a conceptual and material investigation into the relationship between body, memory, and object. Three performance-books are derived from the performance Ovo Oco - Mirror, Ritual, and Childhood - each conceived as a container for a distinct performative experience and translated into editorial language through specific formal strategies. Mirror uses full-body-scale transparent sheets and photographic layering to invite corporeal identification, making legibility dependent on the reader's own bodily positioning. Ritual takes the form of a scroll containing choreographic scores mapped from above, where the sequential unfolding of the object enacts the tension between repetition and inevitable drift, the beauty of non-replication built into the format itself. Childhood explores instinctive engagement with objects, structuring interaction through tactile cues that operate prior to reading. Across all three, the design process mobilises sequentiality, fragmentation, materiality, and modes of physical interaction as the primary editorial language through which gesture, time, and bodily memory are inscribed into the object. **Results:** The project anticipates an expanded notion of the archive, one that moves beyond the documentary toward an experiential, emotional, and aesthetic dimension. The performance-books are expected to demonstrate that editorial artefacts can embody performative qualities, inviting readers to access sensory and muscular memory through physical interaction. The notion of ruin, as both decay and reconstruction, emerges as a structuring metaphor, highlighting what endures and what is inevitably lost in any act of preservation, and making that loss visible as form. **Conclusions:** "I don't need to be a ruin to be an archive" - a phrase that guided and sustained this project. The study positions graphic and editorial design as an active agent in the preservation and transmission of ephemeral performative knowledge. By proposing the book as a

choreographic, performative, and design-based device, the project aims to foster new ways of experiencing and reflecting on human essence, consolidating design as a socially and artistically engaged practice at the intersection of performing arts and material culture.

Keywords: Archive - Body - Performance - Book - Muscle Memory - Hereditary (or Inherited) - Object - Movement

Acknowledgments: Cristina Ferreira and João dos Santos Martins

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[2] Jacques Rancière, "*The Emancipated Spectator"* (2008)

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[4] Pina Bausch, piece "Palermo Palermo" (1989)

[5] RoseLee Goldberg, "Performance Art: From Futurism to the Present" (1988)

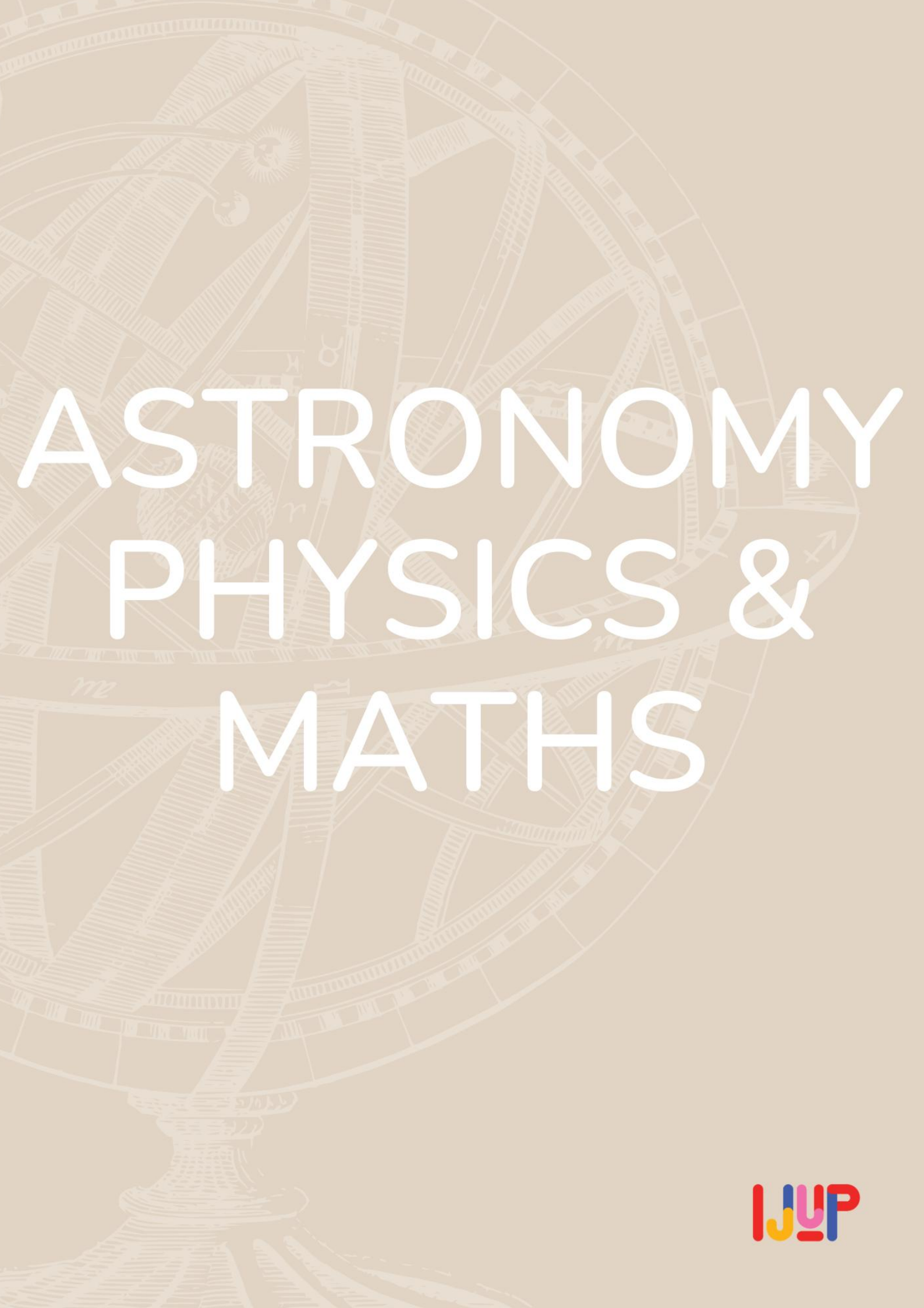
[6] John Berger, "Ways of Seeing" (1972)

[7] Alberto Cortés, piece "Analphabet" (2019)

[8] Marina Otero, piece "Kill Me" (2022)

[9] João dos Santos Martins, "Dança não Dança" (2021)

[10] Lisbeth Gruwez & Maarten Van Cauwenberghe, piece "WASCO!" (2024)



ASTRONOMY PHYSICS & MATHS

24354 | Revisiting the redshift drift forecasts for the SKA

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Background & Aim: The prospect of directly measuring the Universe's acceleration is an exciting topic for future cosmology. The Square Kilometer Array (SKA) will be the largest radio telescope in the world and is expected to finally provide the first ever observation of the Universe expanding in real time, which is known as the redshift drift. In this context, we analyze the SKA capabilities based on its predicted systematics and beyond, varying the channel width, the survey area and the number of antennas, and aiming to determine the ideal SKA configuration for such detection. **Methods:** This experiment can be done by collecting light from HI galaxies at low redshifts in two different epochs and calculating their redshift drift. By using the official SKA sensitivity calculator [1] and a previous simulation of the number of detected sources [2], we estimate the uncertainty on the measured spectroscopic velocity and perform the so-called Fisher analysis with the cosmographic expansion of this velocity. This provides forecasts on the constraints of the Hubble constant H_0, the deceleration parameter q_0 and the jerk parameter j_0, which imply the significance in the acceleration detection. **Results:** It was noted that the channel width is the parameter affecting most the results: the narrower the width, the tighter the constraints, so that the experiment with the SKA standard value of 210 Hz would likely be unfeasible. However, with a channel width of 0.001 Hz, it would be possible to significantly detect the acceleration within 44 years in a realistic experiment with 144 antennas, yielding a 5σ significance in the q_0 value. **Conclusions:** We report that, with channel widths small enough, it is possible to obtain a 5σ detection of the Universe's accelerated expansion, even for the AA* phase of the SKA. Being the channel width an adjustable configuration and considering there are other methods to determine the spectroscopic velocity, we conclude that the SKA is a promising facility for future cosmology.

Keywords: Cosmological parameters; Cosmology: observations; Radio lines: galaxies.

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[3] Kloeckner, H. R., Obreschkow, D., Martins, C., et al. 2015, in Proceedings of Advancing Astrophysics with the Square Kilometre Array — PoS(AASKA14), AASKA14 (Sissa Medialab), 027, doi: 10.22323/1.215.0027
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24342 | Kink-Antikink Collisions and Their Cosmological Implications

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Background & Aim: As the early universe expanded, symmetry-breaking phase transitions may have formed topological defects like domain walls. In multi-degenerate vacuum landscapes, defect dynamics dictate the cosmic network's evolution. This work investigates defect dynamics within generalized scalar field theories featuring tunable, energetically degenerate minima. We focus on how defect fine structure drives populational biases toward lower-mass vacua, and how collisions induce topological sector transitions, spawning topological and non-topological objects. **Methods:** We performed high-resolution lattice simulations of non-linear scalar fields. We introduce two generalized potentials with a tunable shape parameter, interpolating between the phi4/Cosine-Gordon and phi6/Sine-Gordon models. By varying this parameter, we constructed complex landscapes featuring multiple energetically degenerate vacua. We integrated the equations of motion to scan the phase space of defect interactions – varying initial velocities and configurations in 1D, alongside 2D domain wall extensions. **Results:** Simulations reveal diverse interactions: annihilation, oscillon formation, pair creation and sector transitions. Collisions between heavy defects frequently spawn lighter ones, converting excess energy into kinetic energy and radiation. Furthermore, perturbed defects radiate energy asymmetrically into the lower-mass vacuum. To conserve momentum, this emission accelerates the defect oppositely, dynamically expanding lower-mass vacua. Preliminary 2D models confirm thus asymmetric wall acceleration. **Conclusions:** These models elucidate mechanisms behind diverse defect phenomena, including oscillons, annihilation and sector transitions. Crucially, the asymmetric acceleration by radiation emission creates a strong populational bias, dynamically favoring lower-mass vacua expansion.

Keywords: Topological Defects; Domain Walls; Kink Collisions; Scalar Field Theories; Asymmetric Radiation

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24403 | Current-carrying string network evolution in an external magnetic field

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Background & Aim: Cosmic strings are topological defects arising as the Universe undergoes symmetry-breaking phase transitions, whose discovery would offer valuable insight into the high-energy physics that shaped the early Universe. To interpret such a detection, robust theoretical models are essential. The Velocity-dependent One-Scale (VOS) model [1] is particularly prominent: it treats the network as a thermodynamic system, characterizing its key properties, and predicting its large-scale evolution. It was recently extended to include superconducting strings, which carry additional degrees of freedom, giving rise to the Charge Velocity dependent One-Scale (CVOS) model [2, 3]. One limitation is that it only includes loss mechanisms for the charge and current. Here, we extend this model by phenomenologically including a possible energy source mechanism, specifically by allowing current-carrying strings interactions with an external magnetic field. **Methods:** The present work [4] extended the CVOS model by accounting for the possible influence of an external magnetic field and determining the resulting equations of motion. The macroscopic versions of the resulting equations were solved by considering a scaling regime. We obtained several solutions, which were further analyzed through numerical work. **Results:** We found 10 solutions describing the late-time behavior of the string network, which can be packed in three families depending on the values of the velocity and the dominating degree of freedom. One of these solutions allows the network to gain energy. **Conclusions:** The addition of an external magnetic field can be made through a single macroscopic parameter describing its influence. The main impact of the magnetic field on the network is the break on the degeneracy of the charge and current late-time behavior.

Keywords: Cosmology, Cosmic Strings, Magnetic Fields

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24603 | Scaling solutions for varying tension strings

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Background & Aim: Cosmic strings are one-dimensional topological defects resulting from symmetry-breaking phase transitions. Their non-linear dynamics require a combination of analytical modeling and numerical analysis. Our work [1] focuses on the first of these approaches, where the paradigm is to adopt effective descriptions in which the network is treated as a thermodynamical system (i.e. to average the microscopic equations of motion to yield macroscopic counterparts). The canonical model for this purpose is the Velocity-dependent One Scale (VOS) model, which we use to study how two different assumptions for time-varying string tension affect network evolution. **Methods:** Using the VOS model, whose dynamical variables are the root mean square velocity and a characteristic length scale, we explore and classify the possible scaling solutions in cosmological and non-cosmological settings and under two different phenomenological assumptions for the time-varying tension, which rely on different stretching and damping contributions to the string dynamics. We discuss the impact of these assumptions on the standard scaling solutions and on the network density. In addition to power-law cosmological epochs solutions, we discuss the behavior of the network during the radiation-to-matter and matter-to-acceleration transitions. **Results:** Depending on the physical context, the time dependence of the scaling laws can be affected, or the impact of the varying tension might be restricted to changing the quantitative normalization of these laws without affecting their time dependence. As for the network density, though one might expect it to be impacted, we have shown that in several contexts the varying tension effect is canceled out by a corresponding change in the behavior of the characteristic length. **Conclusions:** Overall, our results show that for the same amount of tension variation, a change in the stretching length tends to have a more significant impact on the network than a change in the damping length.

Keywords: cosmology; topological defects; cosmic strings; time-varying string tension

Acknowledgments:

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24333 | Quantum Spin-Orbitronics on Flexible Substrates with AFM/TI Stacks

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Background & Aim: Topological Insulators host surface channels with spin-momentum-locked carriers, which enable efficient spin accumulation through electrical currents (and vice versa) [1,2]. Coupling them to an antiferromagnet (AFM) can enable THz-scale spin dynamics, zero stray fields, and of voltage-tunable magnetic order [3,4], making AFM/TI stacks promising for spintronic devices [5]. The main challenge is engineering clean, well-textured AFM/TI interfaces with controlled stoichiometry, low defects on flexible substrates via scalable processes. Here we fabricate $Mn_{0.5}Fe_{0.5}/Bi_2Te_3$ heterostructures and correlate microstructure with transport to benchmark suitability for flexible spintronics. **Methods:** Thin films are deposited using DC magnetron sputtering on glass and Kapton substrates. Growth optimization focuses on thickness control, interface quality and reproducibility by tuning sputtering parameters, such as current (20-50mA) and deposition time (15-75min). Total thickness is kept below 150nm, with a thicker Bi_2Te_3 layer (100nm) and a thinner $Mn_{0.5}Fe_{0.5}$ layer (5-50nm) to favor topological effects. Structural and morphological characterization is performed using X-ray diffraction, X-ray reflectometry, scanning electron microscopy and energy-dispersive spectroscopy. Electronic and magnetotransport properties are investigated through resistivity, magnetoconductance and weak anti-localization measurements. **Results:** Reproducible growth conditions for $Mn_{0.5}Fe_{0.5}$ and Bi_2Te_3 films are established [6] and fabrication of heterostructures on rigid and flexible substrates is underway. Ongoing structural analysis is expected to confirm good crystallinity and interfaces. Planned transport measurements aim to reveal signatures of topological conduction. **Conclusions:** This work establishes a scalable route to AFM/TI heterostructures compatible with rigid and flexible substrates and provides a platform for future studies of spin-orbit torques, interfacial spin transport and flexible spin-orbitronic devices.

Keywords: Topological insulators; Antiferromagnets; Heterostructures; Spintronics; Spin-Orbitronics

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24366 | Exploring Phase-Change Materials (PCMs) in Topological Insulator Thin Films Deposited by Co-Sputtering on Flexible Substrates for Advanced Photospintronics Applications

Henrique Santos[1], Mafalda Moreira[1], João P. Maganinho[1], Ana L. Pires[1], and André M. Pereira[1]

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Background & Aim: Topological insulators (TI) are quantum materials characterized by an insulating bulk and conducting surface states. Typical TI materials include chalcogenides such as Bi_2Te_3, Bi_2Se_3 and Sb_2Te_3, the latter being a phase-change material (PCM). A PCM is a material that stores large amounts of energy during phase transition. In this research work, we aimed to study the behaviour of thin films of alloys composed of Bi_2Te_3 and Sb_2Te_3 (which are n type and p type semiconductors), and to determine whether the alloys exhibit the properties of a TI. **Methods:** For the creation of the alloys, DC Magnetron Co-Sputtering was used. Co-Sputtering is the simultaneous Sputtering of two different materials (in this case Bi_2Te_3 and Sb_2Te_3). Alloys of deposition times 5min, 15min and 20min were created. The alloys were deposited in clean glass and Kapton substrates. Deposition of material was started with a pressure less than 1mPa, and current 20mA on both sputtering baffles. To study the thermoelectric properties of the alloys, the Seebeck coefficient of each was measured. The resistance of each alloy was measured recurring to a four-wire resistance measurement method. A control film of Bi_2Te_3 was used. **Results:** The obtained Seebeck coefficient showed that the alloys behaved as n type semiconductors. The unexpected oxidation of the sample with deposition time 5min compromised the analysis of seebeck and resistance dependence on deposition time, as the resulting alloy became an insulator and not a semiconductor. It was not possible to measure the resistivity, as time restraints made it impossible to measure the thickness of the samples. **Conclusions:** The research showed that the resulting alloys from of Bi_2Te_3 and Sb_2Te_3 behaved as a n type semiconductor. Due to time restraints, it was not possible to calculate the electric conductivity and to evaluate in detail the dependence of the seebeck coefficient on deposition time. Further research must be done to clarify results.

Keywords: Topological insulators; Spintronics

Acknowledgments

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24720 | From 2D Materials to Machine Learning: Experimental Insights and Neural Network Simulation

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Background & Aim: Modern artificial intelligence algorithms expose the latency and energy inefficiencies of the conventional von Neumann architecture, where the physical separation between memory and processing creates a significant throughput bottleneck. Neuromorphic in-memory computing [1] emerges as a promising solution, offering a highly energy-efficient alternative by fusing these functions within the same physical location. At the core of this shift is the memristor- the fourth fundamental circuit element **[Figure 1]**—which enables high-density crossbar arrays to efficiently execute analog Vector-Matrix Multiplications (VMM). However, the transition from ideal algorithms to real hardware faces challenges associated with the physical non-idealities of the devices, such as stochastic device-to-device (D2D) and cycle-to-cycle (C2C) variability, non-linear weight updates, and parasitic effects like sneak-path leakage currents. To mitigate these physical constraints, atomically thin two-dimensional (2D) materials—such as MXenes and transition metal dichalcogenides (M_oS_2) have emerged as superior alternatives due to their precise ion migration control and ultra-low switching voltages. Consequently, the primary aim of this project is to develop a comprehensive "hardware-aware" simulation pipeline to evaluate the feasibility and robustness of next-generation 2D memristive technologies. This work establishes a bridge between the physical behavior of these devices and the simulation of Artificial Neural Networks (ANNs) by extracting parameters from laboratory-measured hysteresis loops through physical SPICE characterizations [2]. The study quantifies the impact of stochasticity and parasitic resistances on neuromorphic algorithms and image processing tasks under realistic conditions. Ultimately, by implementing adaptive in-situ training algorithms, this research demonstrates the network's capacity to dynamically self-compensate for hardware defects, paving the way for the development of more resilient, autonomous, and low-power edge-computing systems. **Methods:** The study includes both Physical Characterization and Computational Simulation. The device-level evaluation (using the Yakopcic model in SPICE) [2], with a special focus on the measurement and analysis of hysteresis curves, allows to validate non-linear behavior, switching capability, and the extraction of stochastic variability metrics. The development of a *Hardware-Aware* simulation pipeline (using CrossSim [4]) allows to model memristive crossbar arrays and perform proofs of concept for AI models [3], neuromorphic algorithms [5], and image processing tasks (e.g., MNIST dataset classification). The model incorporates strict physical

hardware constraints and critical non-idealities, including input binarization (1-bit DAC), realistic output quantization (calibrated 8-bit ADC), stochastic device variability, thermal noise, parasitic resistances in the crossbar interconnects, and leakage currents. **Results:** The physical characterization results successfully validated the multi-level analog storage capacity of the devices. At the system level, simulations revealed a critical discrepancy between the ideal algorithmic model and realistic hardware. Although the architecture demonstrated high tolerance to stochastic noise (read and write errors), the combined introduction of parasitic resistances and realistic output converters (8-bit ADC) caused a drastic drop in the model's accuracy (from ~70.5% to ~38.3%). This severe degradation occurs because the physical resistance of the wires significantly weakens the output signals, making the different classes indistinguishable for the ADC. Ultimately, this proves that models trained strictly in software (*ex-situ*) cannot be simply transferred to large-scale physical hardware. **Conclusions:** This work establishes a bridge between materials physics and neuromorphic engineering. The main conclusion is that the direct mapping of weights trained on a computer (*ex-situ*) to the hardware is unfeasible in large-scale passive arrays due to severe signal degradation caused by parasitic resistances. It shows that the viability of neuromorphic networks requires the implementation of *Online Learning* (*in-situ* training) models. The transition to algorithms capable of training the network directly on the chip will allow the system to act as a self-compensation mechanism, learning to tolerate its own physical defects.

Keywords: Neuromorphic computing; Memristors; 2D materials; In-memory computing; Hardware non-idealities

Acknowledgments:

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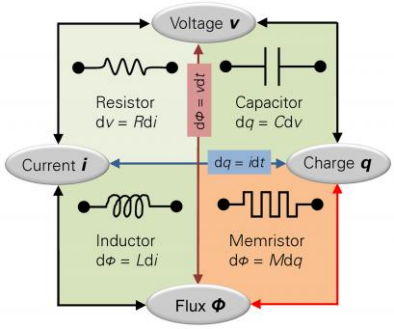
Figure 1:



24497 | Optimization of Water Evaporation–Induced Generators (WEIGs) for simultaneous energy production and desalination

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Background & Aim: Growing demands for sustainable energy and freshwater drive interest in technologies coupling renewable energy harvesting with desalination. Water Evaporation–Induced Generators (WEIGs) produce electricity from capillary-driven flow through charged porous materials. Recent studies highlight their potential as solar evaporators enabling simultaneous energy generation and enhanced evaporation. However, WEIG performance under high-salinity conditions (e.g., seawater, SW) and the role of functional materials within hybrid architectures require further evaluation. This work assesses the impact of SW on the electrical output of a WEIG, explores its application in solar–driven desalination with water recovery by condensation, and optimizes device architecture to maximize power generation and evaporation. **Methods:** The WEIG was tested with deionized water (DI) and artificial SW. Electrical output, and evaporation rate were monitored under controlled temperature, humidity, and simulated solar irradiation. A setup quantified recovered water. Material contributions were analyzed by FTIR, UV-VIS-NIR, SEM, and infrared thermal imaging, followed by selective layer modification. **Results:** The WEIG showed enhanced electrical output with SW compared to DI, reaching 1 V, 0.35 A, and 0.30 mW, indicating ion-mediated effects on surface charge interactions. An evaporation rate of 0.0217 g h^{-1} was achieved. Visible condensation occurred within 2 h, and 7.67 g freshwater was collected after 1 day while maintaining energy generation. PEDOT:PSS ink improved electrical conductivity, while the carbon-based ink promoted photothermal conversion and water-surface interactions. **Conclusions:** These findings show that SW enhances WEIG performance and highlight ionic effects in hydrovoltaic systems. They establish a material optimization strategy for efficient solar-driven desalination with simultaneous power generation, reinforcing the potential of WEIGs as integrated, low-energy water–energy technologies.

Keywords: Water Evaporation-Induced Generators; Hydrovoltaic effect; Energy harvesting; Solar-driven Evaporation; Freshwater recovery

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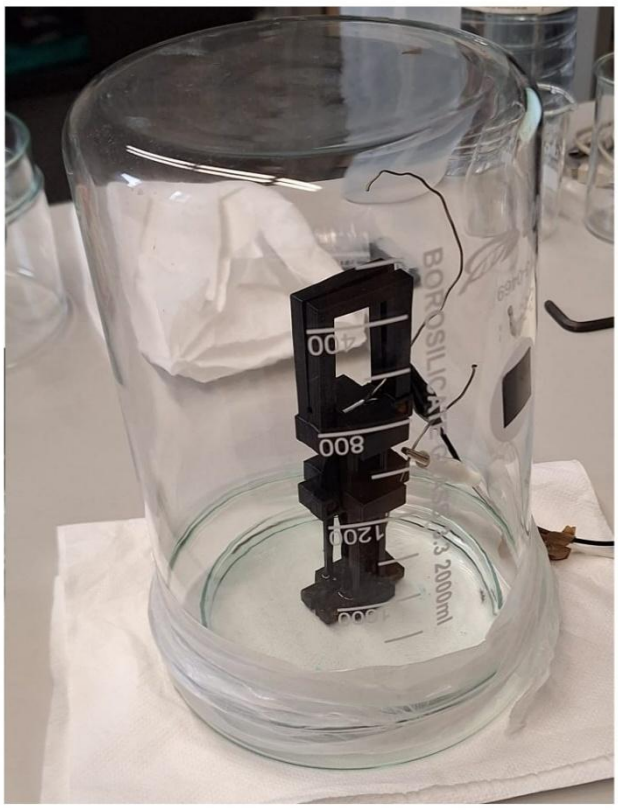
Figure 1. a) Photograph of the mounted experimental setup; b) Condensation observed 2 h after mounting the setup.



a)

b)

24529 | Synthesis and Characterization of Gadolinium Nanowires for Biomedical Applications

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Background & Aim: Nanostructures with high magnetic moment and anisotropy are promising candidates for medical applications, such as magnetic hyperthermia for tumour treatment and advanced imaging for medical diagnosis [1,5]. Gadolinium (Gd) is particularly attractive due to its high saturation magnetization and ferromagnetic behaviour near room temperature. Template-assisted DC electrodeposition in nanoporous anodic alumina (AAO) as well as hydrothermal synthesis allows for the fabrication of ordered nanowires with controlled diameter and aspect ratio [2,3,6]. This work aims to establish an experimental protocol for the synthesis and characterization of Gd nanowires for the purpose of theranostics. **Methods:** Following the approaches of Khan *et al*. [4] and Sharma et al. [6], we perform DC electrodeposition of Gd thin films and the hydrothermal synthesis of Gd oxide nanorods, respectively. They are characterized through X-ray diffraction, Raman spectroscopy, scanning electron microscopy (SEM), and SQUID magnetometry, to assess crystalline phase, morphology, and magnetic dynamics. **Results:** Raman spectroscopy and SEM results show that our films are made of Gd oxide, as expected because Gd oxidizes quickly, but also Gd nitrate (electrolyte), meaning the process must be optimized. SEM results show that the hydrothermal method prior to annealing yields micrometric wires of Gd hydroxide, as expected. The process must be tuned to control rod size. **Conclusions:** Current efforts have allowed us to successfully synthesize Gd oxide thin films and Gd hydroxide microwires. The next step will be the tuning of both processes, production of AAO templates for electrodeposition, and anneal the Gd hydroxide rods, to fabricate Gd oxide nanowires with tailored magnetic and magnetocaloric properties for future theranostic applications.

Keywords: Gadolinium; Nanowires; Electrodeposition; Hydrothermal Synthesis; Theranostics

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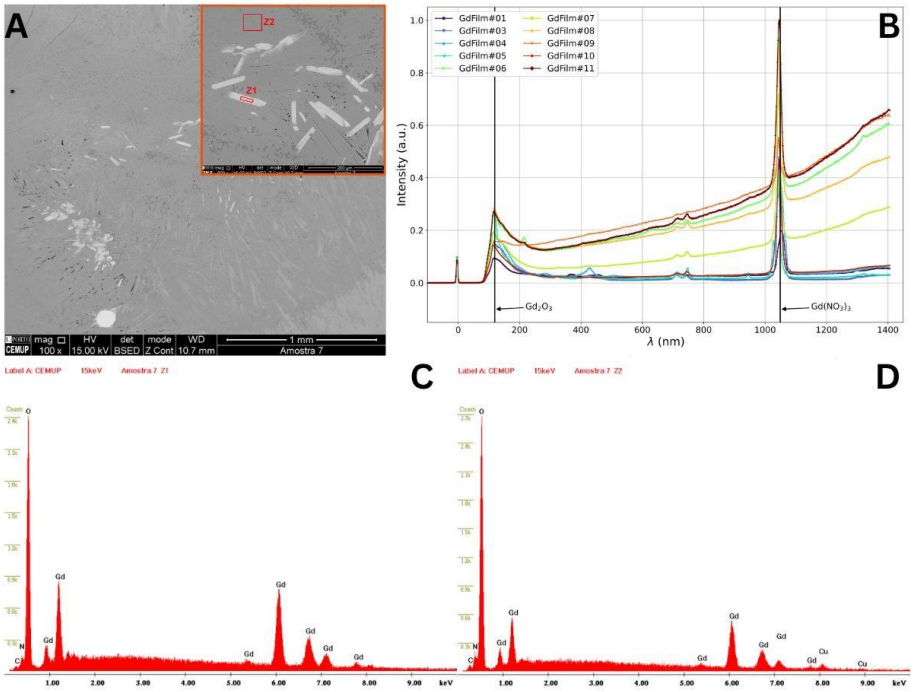

Figure 1: Scanning Electron Microscopy (SEM) image (A) and Energy Dispersive X-ray Spectroscopy (EDS) spectrum (C and D) of two zones of one of the electrodeposited films. Raman Spectroscopy spectrum (B) of all produced films showing the presence of both Gd oxide and nitrate.

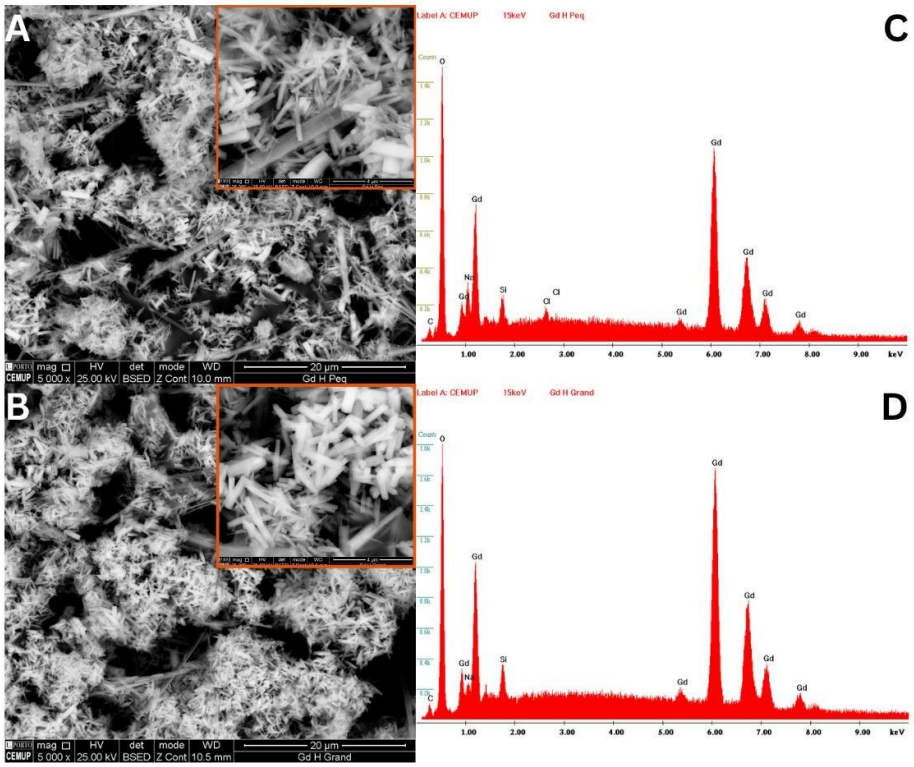

Figure 2: Scanning Electron Microscopy (SEM) images (A and B) and Energy Dispersive X-ray Spectroscopy (EDS) spectrum (C and D) of Gd hydroxide micrometric rods, hydrothermally synthesized in differently sized autoclaves, with similar compositions and sizes, demonstrating method scalability.

24566 | Hardware Neural Networks with Neuromorphic Memristive Device-in-Loop Training

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Background & Aim: Neuromorphic hardware based on memristors with liquid active layer can be used to implement neural network with low power and limited area alongside harnessing the advantages of liquid devices such as low cost, simplicity, and large-scale fabrication. Here, we report the implementation of an artificial neural network (ANN) based on the hardware-in-loop training framework and demonstrate effective learning for benchmark classification tasks such as IRIS and MNIST datasets. **Methods:** A hardware-in-loop training was implemented using memristors where the connected sourcemeters measure the non-linear I-V characteristics as activation function and feed it to the custom-made instrument driver running in PC. Beforehand, the gradient required for the backpropagation algorithm was measured using analog finite difference method using two identical memristors and the results stored in the instrument driver. During training, these stored results are used to compute the weight updates and thus translate suitable programming pulses sent to the memristors through sourcemeters. Additionally, similar training on software environment was performed to draw a comparison with the hardware-based ANN. **Results:** One obtained a stable training loop in a hardware setup able to demonstrate a consistent validation loss reduction and improve accuracy over training epochs. Also, the analog finite difference method was validated for gradient measurement and effective weight updates during the training process. **Conclusions:** This work exhibits that memristors can be used as trainable neuromorphic hardware alongside a static synaptic element. Overall, this work advances neuromorphic hardware by utilizing memristors' properties of non-linearity and power efficiency for efficient on-device learning.

Keywords: Neuromorphic Computing; Low-power ANN; Memristor

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[1] Reaching Bio-Voltages and Controlling Synaptic Dynamics in Liquid-Based Neuromorphic Devices

Andreia V. Silva, Ana T. S. C. Brandão, Carlos M. Pereira, João Ventura, and Catarina Dias

Nano Letters 2025 25 (25), 9944-9951 DOI: 10.1021/acs.nanolett.5c01203

24592 | Self-thermometry measurements of the adiabatic temperature change in first-order phase transition magnetocaloric materials

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Background & Aim: Accurately measuring the magnetocaloric effect is necessary to foster the development of magnetic refrigeration devices. However, current methods are inconvenient, requiring different instruments to measure each individual property or a custom-made setup. We developed a technique to determine the adiabatic temperature change (ΔT_{ad}), a key parameter of magnetocalorics, in materials exhibiting a first-order phase transition (FOPT), using easy-to-obtain magnetometry measurements. Built upon a technique previously proposed for second-order materials [1], this method enables full characterization (magnetic entropy change, adiabatic temperature change, and heat capacity [2]) of any magnetocaloric material, with a single instrument. **Methods:** One can infer the relaxation in temperature, consequent of a magnetic field application, by measuring the correspondent relaxation in magnetization, if one has a proper magnetization-to-temperature conversion. By using equilibrium magnetizations, resulting from each magnetic field application at different temperatures, we were able to construct a magnetization-to-temperature conversion curve, extending the validity of the abovementioned technique to materials exhibiting hysteresis. The FOPT material $Gd_5Si_2Ge_2$ was used as a case study. **Results:** We determined the adiabatic temperature change of $Gd_5Si_2Ge_2$, for a 1 T magnetic field application, under high vacuum (< 0.1 mTorr), using only a commercially available VersaLab® PPMS® from Quantum Design. Compared to a directly measured ΔT_{ad}, our method resulted in a peak ΔT_{ad} of 4.47 K, within 1% of the directly measured value for a sample

of the same composition. **Conclusions:** This work is of interest to the magnetocaloric investigation and refrigeration device engineering communities, as it demonstrates a simpler way to quantify the magnetocaloric effect in FOPT materials, using a single instrument.

Keywords: Magnetocaloric effect; First-order phase transitions; Magnetometry measurements

Acknowledgments:

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24606 | Development of an Integrated SPR-Based Optical and Electrochemical Sensing System

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Background & Aim: The integration of Surface Plasmon Resonance (SPR) with electrochemical methods, also known as electrochemical SPR (eSPR), constitutes a dual-mode analytical platform capable of label-free [1], real-time and high-sensitivity detection of molecular interactions [2,3]. SPR measures variations in the refractive index (RI) near a metal–dielectric interface, such as gold-water. Furthermore, the electrochemical measurements are usually performed in three-electrode configuration, in which the gold chip has the hybrid functions of working electrode (WE) and SPR substrate. Therefore, given the critical role of the gold chip in both sensing modes, it is essential to optimize its optical and electrochemical performance by investigating different adhesion layer materials (Cr, Ti), as well as the effects of annealing and electrochemical activation. **Methods:** RF magnetron sputtering was used for the deposition of gold thin films with annealing treatment at different temperatures. Then, the SPR sensitivity was determined by exposing the substrates to solutions with increasing RI. The electrochemical performance was investigated using cyclic voltammetry (CV) and square-wave voltammetry (SWV) after electrochemical activation in pure electrolyte. **Results:** The current focus of this work is the optimization of gold thin films obtained by RF sputtering. The results obtained were compared with commercially available chips. The study focusing on the adhesion layer materials, Cr and Ti (~3 nm), revealed no significant differences in SPR sensitivity (typical values from 1600 nm/RIU to 2100 nm/RIU) or electrochemical response. Electrochemical activation in phosphate buffer (PB) and sulfuric acid (H_2SO_4) resulted in significant improvements in chip conductivity and redox probe process reversibility ($I_{p,ox}/I_{p,red}$ around 1.0). The annealing study was conducted by subjecting substrates to temperatures of 200 °C and 300 °C, revealing an increased gold adhesion. This adhesion was further evaluated by exposing the chips to repetitive gold oxidation and reduction potentials during CV cycles. Qualitative Scotch tape tests were also performed.

Roughness was measured via ellipsometry. **Conclusions:** The adhesion layer material study demonstrates that a 3 nm adhesion layer does not significantly affect the SPR band. Consequently, chromium was selected as the adhesion material due to its superior stability during the annealing process. The annealing study revealed that at 300 °C, a slight chromium diffusion through the gold film improves adhesion and results in more stable and durable sensor platforms.

Keywords: SPR; Electrochemistry; Electrochemical SPR; Gold films.

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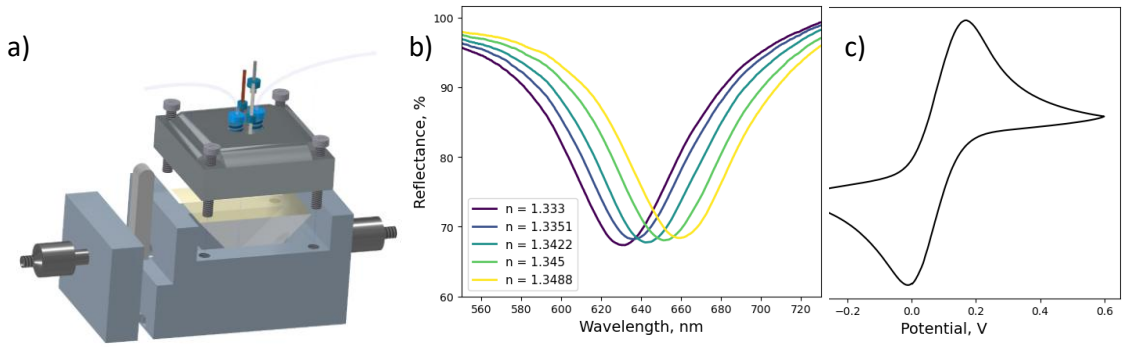

Figure 1: a) Setup for electrochemical and SPR measurements; b) Optical output; c) Electrochemical output (CV).

24680 | Microfluidic structures for artificial synapses

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Background & Aim: As conventional transistor-based architectures reach physical and energy-efficiency limits, memristors are emerging as a promising alternative [1]. These devices can be used for neuromorphic computing, as they mimic the synaptic behavior of the human brain, inspiring new approaches such as liquid-based memristive devices [2, 3]. One of the main challenges that arises when working with liquids is evaporation. In this work, we explored the use of PDMS (polydimethylsiloxane) reservoirs to mitigate this effect and studied possible configurations for a 2×2 memristor array. **Methods:** Multiple PDMS channels were fabricated to serve as reservoirs [2, 4] for aqueous copper sulfate ($CuSO_4$) solutions [3] with different concentrations. For testing with each channel, two electrodes were placed on both ends at varying distances and I-V curves were measured. Two different electrode pairs were used, Cu-Cu and Cu-Pt. For the 2×2 arrays, transparent silicone or PDMS were used to define the reservoirs, attached by a thin layer of PDMS cured at room temperature. The bottom electrodes (Cu, Pt) were defined by photolithography and deposited by sputtering on silicon substrates. **Results:** The characteristic memristive I-V behavior, with transitions between high and low resistance states, was obtained when using Cu-Pt electrodes in a PDMS channel at a distance of ~ 1 mm and a solution of 0.5 mol/L. Preliminary tests on the 2×2 array fabricated with silicone show similar results. **Conclusions:** Stable memristive behavior was achieved by optimizing electrode materials, distance, and electrolyte concentration. The successful operation of a 2×2 array highlights the potential scalability of liquid-based memristive platforms for neuromorphic computing.

Keywords: neuromorphic computing, memristors, liquid medium, PDMS reservoir

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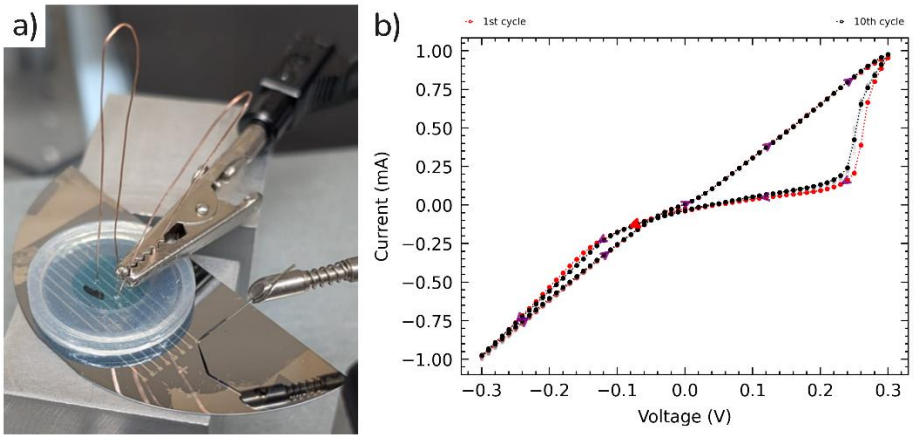

Figure 1: (a) Optical image of the 2×2 memristor array, with Cu and Pt electrodes and a silicone reservoir, and (b) respective I-V characteristic for one cell of the array.

24691 | 5G Energy Harvesting with Screen-Printed Flexible Antennas:

Numerical and Experimental Analysis

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Background & Aim: The recent fifth generation (5G) technology implementation emerged to fulfil the need for faster and seamless communications, allowing the proliferation of the Internet of Things (IoT). Wireless energy harvesting (WEH) addresses the growing need to power the expanding infrastructure of IoT by capturing the excessive ambient radiofrequency (RF) energy and converting it into usable electrical power. An ideal power device for this purpose is a flexible low-loss antenna, which increases adaptability to unconventional environments and applications, such as wearable devices and environmental monitoring systems [1]. The antenna was tailored for a frequency of 3.6 GHz, a widely used 5G NR band (n78) [2]. **Methods:** Using COMSOL Multiphysics simulations (Fig. 1a), several parametric studies were conducted to refine geometry, substrate properties, and matching layers. The optimized antenna was then screen-printed onto a flexible PET dielectric with silver ink (electrical conductivity of $6.3x10^7$ S/m). A vector network analyzer (VNA) was used to characterize reflection coefficient (S_{11}) and gain, while a setup with a 5G transmitter and a rectifying circuit measured time-dependent output voltage. **Results:** The proposed microstrip patch antenna has a metallic patch mounted on a dielectric substrate, with a ground plane on the opposite side. It provides omnidirectional front radiation, achieving S_{11} less than -15 dB, indicating excellent impedance matching and minimal reflection for efficient performance. The real-world WEH performance of the printed antenna was successfully demonstrated (~150 mV). **Conclusions:** The flexible system showed potential to harvest ambient EM radiation from 5G sources. Advanced simulation software played a critical role in optimizing performance and accelerating development. Moreover, the integration of screen-printed antennas enhances scalability and cost-effectiveness. The system's inherent flexibility opens the door to a wide range of applications in low-power, energy-efficient technologies such as IoT sensors.

Keywords: 5G; Rectenna; Energy harvesting; Flexible; IoT

Acknowledgments:

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UIDB/04968/2025 (https://doi.org/10.54499/UIDB/04968/2025), UIDP/04968/2025

(https://doi.org/10.54499/UIDP/04968/2025), 10.54499/2022.05030.PTDC

(https://doi.org/10.54499/2022.05030.PTDC) and NORTE-01-0145-FEDER022096 from NECL.

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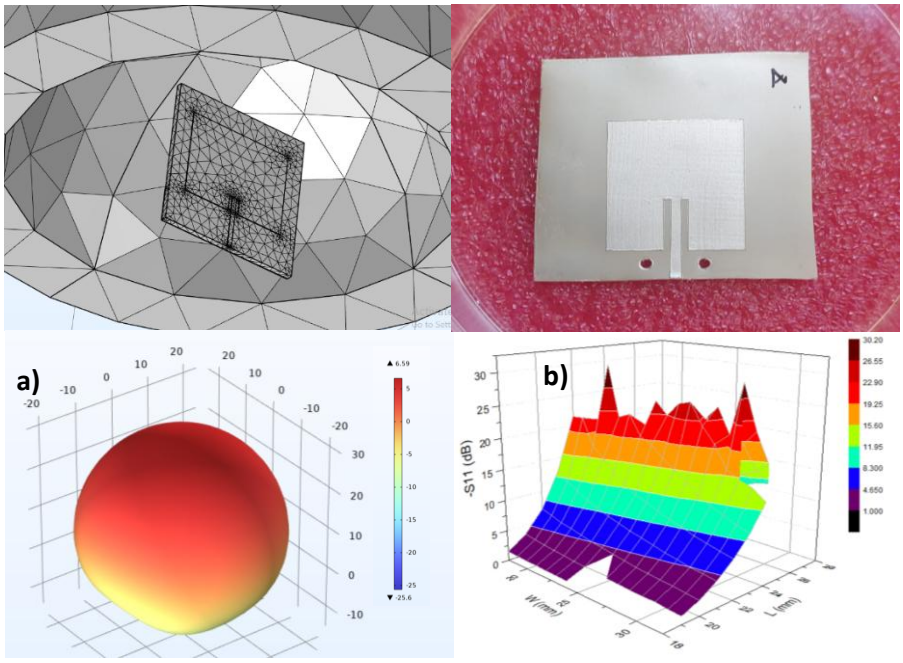

Figure 1: a) COMSOL Multiphysics numerical simulation of antenna with far-field; b) Printed flexible antenna; c) 3D radiation pattern; d) 3D graphic of reflection coefficients (S_{11}).

24606 | Development of an Integrated SPR-Based Optical and Electrochemical Sensing System

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Background & Aim: The integration of Surface Plasmon Resonance (SPR) with electrochemical methods, also known as electrochemical SPR (eSPR), constitutes a dual-mode analytical platform capable of label-free [1], real-time and high-sensitivity detection of molecular interactions [2,3]. SPR measures variations in the refractive index (RI) near a metal–dielectric interface, such as gold-water. Furthermore, the electrochemical measurements are usually performed in three-electrode configuration, in which the gold chip has the hybrid functions of working electrode (WE) and SPR substrate. Therefore, given the critical role of the gold chip in both sensing modes, it is essential to optimize its optical and electrochemical performance by investigating different adhesion layer materials (Cr, Ti), as well as the effects of annealing and electrochemical activation. **Methods:** RF magnetron sputtering was used for the deposition of gold thin films with annealing treatment at different temperatures. Then, the SPR sensitivity was determined by exposing the substrates to solutions with increasing RI. The electrochemical performance was investigated using cyclic voltammetry (CV) and square-wave voltammetry (SWV) after electrochemical activation in pure electrolyte. **Results:** The current focus of this work is the optimization of gold thin films obtained by RF sputtering. The results obtained were compared with commercially available chips. The study focusing on the adhesion layer materials, Cr and Ti (~3 nm), revealed no significant differences in SPR sensitivity (typical values from 1600 nm/RIU to 2100 nm/RIU) or electrochemical response. Electrochemical activation in phosphate buffer (PB) and sulfuric acid (H_2SO_4) resulted in significant improvements in chip conductivity and redox probe process reversibility ($I_{p,ox}/I_{p,red}$ around 1.0). The annealing study was conducted by subjecting substrates to temperatures of 200 °C and 300 °C, revealing an increased gold adhesion. This adhesion was further evaluated by exposing the chips to repetitive gold oxidation and reduction potentials during CV cycles. Qualitative Scotch tape tests were also performed. Roughness was measured via ellipsometry. **Conclusions:** The adhesion layer material study

demonstrates that a 3 nm adhesion layer does not significantly affect the SPR band. Consequently, chromium was selected as the adhesion material due to its superior stability during the annealing process. The annealing study revealed that at 300 °C, a slight chromium diffusion through the gold film improves adhesion and results in more stable and durable sensor platforms.

Keywords: SPR; Electrochemistry; Electrochemical SPR; Gold films.

Acknowledgments:

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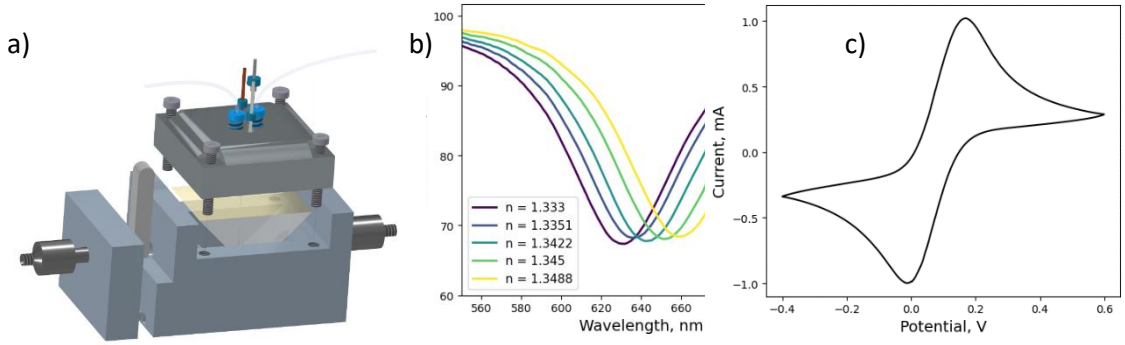

Figure 1: a) Setup for electrochemical and SPR measurements; b) Optical output; c) Electrochemical output (CV).

24715 | Optical properties of metal alloys deposited by RF magnetron sputtering

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Background & Aim: As the global energy paradigm transitions towards sustainable solutions, the development of safe, rapid hydrogen sensors is critical to detect leaks and monitor atmospheric concentrations, given hydrogen's wide flammability range (4-75% by volume in air) and its extremely low ignition energy (0.02 mJ). Palladium (Pd) is widely used for hydrogen sensing due to its low activation energy for the dissociative adsorption of molecular hydrogen (H_2 -> 2H), forming palladium hydride. However, pure palladium suffers from pronounced hysteresis and high degradation caused by metal-hydride phase transitions (α -> β) [1]. Careful controlled ratio of palladium-gold alloys overcomes these problems, suppressing the phase transition, thereby improving its durability and response time [2]. The utilization of a Pd-Au alloy to excite an optical Tamm plasmon at the interface between the metallic layer and a distributed Bragg reflector (DBR) provides a highly sensitive platform for detecting minute changes in the metal's dielectric properties upon hydrogen exposure [3]. **Methods:** Deposition of a Pd-Au alloy thin film on top of a DBR. The whole sensor is deposited using RF magnetron sputtering in a multilayer approach. To achieve a homogeneous alloy, alternating layers of Pd and Au are deposited and subjected to a thermal annealing treatment to promote atomic diffusion between layers. The samples are characterized by Spectroscopic Ellipsometry to determine complex permittivity and Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDS) to determine the composition and morphology of the film. Sensing performance is evaluated using optical reflectance spectroscopy under controlled atmospheric flows. **Results:** During hydrogenation cycles, Pd-Au hydride formation alters the metallic layer's refractive index. This induces a distinct shift in the Tamm plasmon resonance, manifesting as variations in reflected signal intensity, which correlates directly with atmospheric hydrogen concentration. **Conclusions:** The proposed structure effectively senses hydrogen concentrations in controlled

atmospheres. Preliminary results confirm its potential as a quicker and more responsive hydrogen sensor compared to using only palladium for the sensitive medium.

Keywords: Spectroscopic ellipsometry; Hydrogen sensor; Sputtering; Palladium alloy; Tamm plasmon

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24788 | Interaction-sensitive Strain-tunability in ABCA Graphene

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Background & Aim: Rhombohedral graphene (RG) has been a widely popular platform for the study of correlated and topological physics in recent years [1-4]. An insulating layer-antiferromagnetic (L-AFM) phase driven by local Coulomb repulsion has been theoretically predicted [5-7] and seems to fit experimental observations [8-10] at charge neutrality. However, a robust assessment of an effective on-site Hubbard coupling for these systems remains elusive. **Methods:** Using a tight-binding (TB) model and a mean-field approximation that incorporates on-site Coulomb repulsion, we study the strain-tunability of the magnetic ordering of tetralayer rhombohedral graphene (ABCA) at half-filling. **Results:** Our model predicts two regimes (surface and bulk) for a symmetric L-AFM phase, separated by a critical coupling, which has not been explicitly reported in the literature; and a direct impact of longitudinal strain on the magnitude of the L-AFM phase and on the value of the critical coupling, an effect which remains largely unexplored. Our results directly show how the band gap strain-tunability of ABCA graphene is highly sensitive to the value of U0, which suggests a novel way to experimentally assess the effective Hubbard coupling. Notably, our results can be fully explained by direct analogy of RG to a series of open Su-Schrieffer-Heeger (SSH) chains. **Conclusions:** These findings not only deepen our understanding of correlation effects in rhombohedral graphene multilayers, but can also find practical use for controlling the system's insulating states and empirically fine-tuning its tight-binding models.

Keywords: Rhombohedral Graphene; Electronic Topology; Strain; Correlated Phases

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24258 | On the Plurality of Initial States: Operational State Complexity of Multi-Entry Finite Automata

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Background & Aim Multi-Entry Finite Automata (MFAs) [1,2,3] are a generalization of DFAs allowing multiple initial states. This limited form of nondeterminism makes MFAs exceptionally well-suited for high-performance implementation and parallelization, with applications to genomics, linguistics, and network security via speculative evaluation. Despite their recognized utility, the state complexity of language-theoretic operations and the computational complexity of decision problems for MFAs remain largely unexplored. This work aims to bridge this gap by studying the operational and computational complexity of MFAs. **Methods** We define the notion of multi-entry state complexity and adapt automata-theoretic tools, such as fooling sets [4,5] and universal witnesses [6], to provide tight lower bounds for MFAs. These tools allow us to evaluate how MFAs behave under standard operations compared to DFAs and NFAs. **Results** Our findings show that MFAs strike a balance between being expressive and easy to implement. We prove that MFAs efficiently recognize the intersection, union, and the suffix, prefix, and factor closures of languages. In these cases, MFAs achieve the complexity bounds of NFAs while maintaining a structure far more amenable to parallel execution and often remaining exponentially smaller than their equivalent DFAs. Conversely, we prove that even MFA's restricted nondeterminism suffices to render the state complexity of the complement exponential and make the decision problems of equality, inclusion, and universality PSPACE-complete. **Conclusions** This work contributes to a deeper understanding of the structural and complexity-theoretic properties of MFAs, as well as providing fundamental insights into the nature of nondeterminism and its impact on the hardness of computational problems. Additionally, our work provides insight into which applications could benefit from MFAs as well as serving as a basis for future research into the multi-entry state complexity of unary and sub-regular languages.

Keywords: Automata Theory; Operational State Complexity; Regular Languages; Computational Complexity; Limited Nondeterminism.

Acknowledgments: This work was partially supported by FCT through the AVATAR project 2023.12169.PEX

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24361 | Uniformization of Riemann Surfaces and Circle Packings

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Background & Aim: The main purpose of this work is to dive deep in the theory of Riemann surfaces, through its properties, theorems and generalizations of known results, so that, in the end of the first part, we have a total categorization of all simply connected Riemann surfaces in a simple yet beautiful statement present in the Riemann Uniformization theorem. Moreover, we shall also aboard new techniques of approximating the bi-holomorphic mappings from simply connected, bounded and open sets of C given by the Uniformization theorem and investigate new results with these techniques and applications. **Methods:** To establish the Uniformization Theorem, the material on [3] was closely followed, combining fields such as Harmonic and Sub-harmonic Theory, Algebraic Topology and Differential Geometry. Besides, for the approximation methods involving circle packings, the main object of study was the construction of Rodin and Sullivan, [2], where, combining the main result from [1], an improvement on the original article was given. For more information and details, please, consult the full work on the dissertation [4]. **Results:** Both theorems mentioned above have huge implications on the general theory of Complex Analysis as well as promising future applications for the investigation of boundary behaviour of Riemann mappings. Moreover, refining the result from [2], this research provides a precise bound on one parameter of the simplicial maps associated with the circle packings. **Conclusions**: Thus, this project establishes important developments on both subjects of Riemann surfaces and mappings, matters that certainly shall bring forth new corollaries on the study of boundary behaviour of bi-holomorphic functions and, perhaps most interestingly, complex dynamical systems.

Keywords: Riemann surfaces, Uniformization theorem, Riemann Uniformization theorem, Riemann mapping theorem, Circle packings

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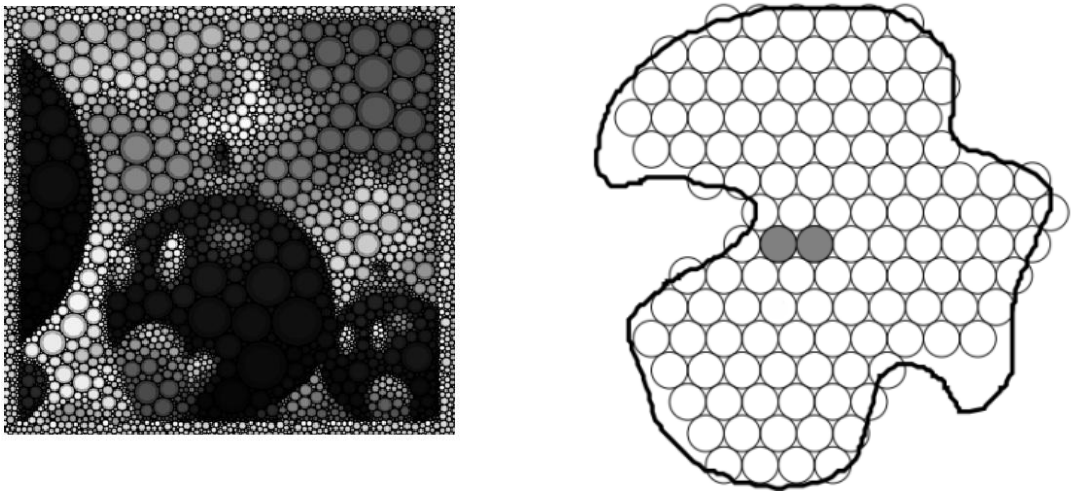

Figure 1: Illustrative examples of circle packings and their inherent approximation characteristics.

24814 | Comparing Classical Parametric Likelihood and Empirical Likelihood Tests for a Changepoint Linear Regression

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Background & Aim: Changepoint regressions assess whether the relationship between a response and a predictor changes over a predictor's domain. The break location is often unknown and requires estimation. This work compares two procedures for testing for a breakpoint and estimating its location: the classical parametric likelihood approach (Quandt, 1958) and the empirical likelihood (EL) method (Liu-Qian, 2009). **Methods:** The classical procedure assumes a fully specified error distribution. Over an admissible set of candidate break locations, it computes a likelihood-ratio statistic comparing the null model (no break) to the alternative model (with a break at that candidate location). In contrast, EL avoids specifying a distribution. For each candidate breakpoint, null-consistent residuals are constructed and assigned probability weights. The EL is maximized subject to a mean-zero moment constraint implied by the null-consistent errors. For both methods, the overall statistic is the supremum over the candidate locations, and the breakpoint estimate is the candidate that yields the maximum. A naïve bootstrap was implemented, since both have nonstandard limiting distributions. **Results:** We simulated three scenarios: (i) strong/(ii) moderate/(iii) weak evidence for the existence of a breakpoint. Results were: (i) rejection by both, (ii) rejection only by the classical test, (iii) neither test rejects. EL rejected less frequently than the classical procedure, indicating that it typically requires stronger evidence to infer a breakpoint, consistent with a more conservative behavior. Breakpoint and segmented coefficient estimates were close to true values when there was evidence of change, and more variable when evidence against the null was weak. **Conclusions:** The parametric approach can be highly powerful when its distributional assumption holds, but it may be sensitive to misspecification. EL, while more conservative, provides an important robustness advantage when the error distribution is uncertain.

Keywords: Changepoint regression; Supremum likelihood-ratio test; Empirical likelihood; Naïve bootstrap.

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24820 | Ensemble clustering of residential characteristics influencing indoor air quality in Europe

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Background & Aim: Air pollution represents a major global health challenge. In Europe, people spend a significant amount of time at home, where housing features may vary across countries and influence indoor air quality (IAQ). This study analyzes housing characteristics in a European IAQ study sample using ensemble clustering. **Methods:** Questionnaire data were collected from 205 households: 25 each in the Czech Republic, Estonia, Italy, the Netherlands, Portugal, Sweden and the United Kingdom and 30 in Slovenia. Clustering was applied to the obtained mixed-type data using the partitioning around medoids (PAM) and the hierarchical models, both with DAFI-Gower dissimilarity, and a finite mixture model (FMM) [1-4]. Internal validation was performed using the silhouette, Dunn and Davies-Bouldin indices, while clustering stability was evaluated by bootstrap and subsetting, from the Jaccard similarity [5]. To obtain a robust partition, ensemble clustering was applied using the Euclidean and the Manhattan methods [6] and compared to isolated results using the Adjusted Rand Index (ARI). Analyses were performed in R. **Results:** All methods suggested two clusters, with high stability. The PAM and the hierarchical model had silhouettes of 0.26 and 0.28, respectively, with the hierarchical exhibiting the best separation and PAM producing more compact clusters. ARI showed highest concordance between PAM and FMM (0.31), while hierarchical and FMM had minimal agreement (0.04). The ensemble was most similar to PAM (0.86) and revealed statistically significant differences between clusters in housing structure, ventilation and exposure factors, identifying more significant variables than hierarchical or FMM. Italy and the United Kingdom clustered together across methods. **Conclusions:** The collected features of European households, affecting IAQ, clustered consistently. Despite some country effects, common patterns emerge, providing a framework for future research, predictive modeling and interventions to improve IAQ.

Keywords: Indoor air quality; Housing characteristics; Mixed-type data; Clustering; Ensemble

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Figure 1: Contingency table of ensemble clusters by country

	Czech Republic	Estonia	Italy	Netherlands	Portugal	Sweden	Slovenia	United Kingdom
Cluster 1	13	2	22	6	9	0	11	24
Cluster 2	11	23	0	13	16	25	18	1

BIOLOGICAL SCIENCES

24126 | Exon Duplication and Loss in Cldn18 Punctuates the Evolution of the Gnathostome Stomach and Lung

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Background & Aim: Understanding the origin of anatomical innovations is key to revealing the evolution of phenotypic diversity. The stomach and its associated digestive functions first evolved in parallel with the appearance of jaws in gnathostomes. A key feature of gastric physiology is the maintenance of epithelial integrity to prevent H^+ leakage, a process where the transmembrane protein Claudin 18 (*Cldn18*) plays a crucial role. This study investigates the evolutionary emergence of *Cldn18*, the timing of its alternative splicing, and its status in gastric and agastric gnathostome species. **Methods:** We used human and elephant shark *Cldn18* amino acid sequences as bait for BLASTP searches. A total of 67 sequences representing major vertebrate groups were aligned using MAFFT. Phylogenetic trees were constructed with PhyML 3.0, with branch support assessed using the aBayes algorithm. We also examined synteny of the gene locus in cartilaginous fish and other lineages. Expression analyses were performed using mandarin fish (*Siniperca chuatsi*) as a model. **Results:** We show that the *Cldn18* orthologue emerged in the ancestor of gnathostomes, with the gene being independently lost in agastric chimaeras and lungfishes. We demonstrate that alternative splicing of exon one in *Cldn18* emerged in the Euteleostomi ancestor. While gastric and lung-specific splicing forms were established in the ancestor of Tetrapoda, the preservation of the *Cldn18* gene in agastric monotremes (platypus and echidna) is explained by the targeted loss of the gastric-specific exon 1. **Conclusions:** Our findings illuminate a complex evolutionary history combining gene duplication, alternative splicing, and secondary exon loss. This research elucidates the paradox

of *Cldn18* retention in agastric species and underscores how these evolutionary forces underpin the intricate phenotypes of both stomach and lung evolution in jawed vertebrates.

Keywords: gene duplication, alternative splicing, gastric evolution, exon loss, pleiotropy

24829 | OF LOSS AND DUPLICATION: THE GENETIC EVOLUTION OF GASTRIC FUNCTION IN CARTILAGINOUS FISH

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Background & Aim: Evolutionary novelty and the mechanisms by which new traits arise remain central, unresolved questions in evolutionary biology[1,2]. The stomach, widely retained in jawed vertebrates, provides a compelling example of independent loss in several lineages, for instance, monotremes and teleost groups (e.g: Cyprinidae, Syngnathidae)[3,4]. In Chondrichthyes, a similar loss of is found in the Holocephali lineage, whereas Elasmobranchs retain the stomach phenotype. This loss is often attributed to the disappearance, alteration, or inactivation of key genes involved in acid secretion, such as *Atp4A* and *Atp4B*, which encode core subunits of the gastric proton pump[3]. This work focuses on investigating the mechanisms of gastric physiology in cartilaginous fish and determine how these adaptations evolved, specifically regarding the role of gene loss the Holocephali. **Methods:** To investigate the genomic basics of stomach loss in chondrichthyans, we'll employ a comparative genomic and transcriptomic framework. First, by performing phylogenetic and synteny analyses of gastric-related genes to determine whether gene loss events occurred independently in Holocephali, and to assess the conservation of the surrounding genomic regions. Second, transcriptomic data from multiple tissues and species will be examined to determine whether candidate gastric genes remain active in the agastric Holocephali lineage. **Results:** As expected, pH sensitive enzymes such as the aspartic proteases (e.g: pepsin & gastricsin) and specific to the stomach in Elasmobranchs, appear to be absent in the five chimaera species analysed. Trehalase, an enzyme responsible for hydrolysing trehalose into two glucose molecules, display the same pattern of loss. Regarding other genes that are almost or totally unique to the stomach of elasmobranchs, exhibit a similar tendency though analysis is ongoing. **Conclusions:** To conclude, this research fills a void in the understanding of how organ decommission is reflected on a genomic level.

Keywords: Stomach, Chondrichthyes, gene loss, agastric, comparative genomics

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24838 | Sweet Loss: Trehalase Inactivation and the Rewiring of Digestive Physiology in Syngnathid Fishes

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Background & Aim: Teleosts represent the most diverse radiation of bony fishes, occupying virtually every aquatic habitat and displaying extraordinary morphological and ecological variation. Their adaptive radiation was accompanied by extensive genomic remodeling, including both gene duplication and gene loss. While duplication has long been viewed as a primary engine of evolutionary novelty, gene inactivation and loss are increasingly recognized as mechanisms of adaptive physiological rewiring, particularly during major ecological transitions. Here, we examine the genomic basis of digestive physiology in the family Syngnathidae, a lineage that includes seahorses, pipefishes, pipehorses, and seadragons. Syngnathids are characterized by highly specialized feeding strategies—rapid suction feeding through elongated snouts, absence of oral processing, and in several species, loss of a morphologically distinct stomach. These anatomical innovations raise fundamental questions about how digestive function has been molecularly reconfigured to accommodate a diet composed largely of small crustaceans and other chitin-rich arthropods. Given this trophic specialization, we focused on the genomic architecture of carbohydrate digestion, with particular emphasis on trehalase—an enzyme responsible for hydrolyzing trehalose, the predominant circulating sugar in arthropods. **Methods:** By integrating comparative genomics and transcriptomic analyses across syngnathid species and teleost relatives, we can detect the presence of the trehalase gene. **Results:** We show that this gene is lost in the Syngnathidae lineage, a puzzling observation given the apparent trehalose-rich prey specialization. However, its presence in members of the families Aulostomidae and Centriscidae suggests that the gene was present in the common ancestor of the suborder Syngnathoidei, followed by multiple independent losses across different families. **Conclusions:** This approach allows us to evaluate how targeted remodeling of a single metabolic node may contribute to the evolution of extreme trophic strategies in teleost fishes.

Keywords: Syngnathidae; Trehalase; Genes

24138 | Probiotic and paraprobiotic effects on gilthead seabream gut health: histomorphology, digestive enzymes, immune and oxidative status

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Background & Aim: With the expansion of aquaculture and the need to reduce fish meal use, plant feedstuffs (PF) have been increasingly incorporated into aquafeeds. However, PF may negatively affect gut health and physiological performance due to the presence of non-starch polysaccharides and other antinutritional factors that impair digestion and induce oxidative stress or inflammation. Functional feed additives, such as probiotics and paraprobiotics, have emerged as sustainable strategies to improve intestinal function, immune competence, and fish welfare. This study aims to evaluate the effects of dietary supplementation with probiotics and paraprobiotics on the intestinal health of gilthead seabream (*Sparus aurata*) fed PF-based diets. **Methods:** Four isoproteic and isolipidic PF-based diets were tested: a control diet and three experimental diets supplemented with probiotics (*Bacillus* sp. or *Saccharomyces* sp.) or a paraprobiotic yeast (*Saccharomyces* sp.). Each diet was fed to triplicate groups of fish (173 g) twice daily to visual satiation for 34 days. At the end of the trial, intestines of 3 fish per tank were collected to measure digestive (amylase, lipase, and alkaline proteases) and antioxidant (catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), and superoxide dismutase (SOD)) enzyme activities, lipid peroxidation (LPO), and the expression of oxidative stress-related genes (*cat, gpx, gr, sod*, and heat shock protein 70 (*hsp-70*)). Histomorphological and immune-related gene expression analyses are ongoing. **Results:** No significant differences were observed among dietary treatments for digestive or antioxidant enzyme activities, LPO levels, or oxidative stress-related gene expression. **Conclusions:** Feed additive supplementation had no major effects on the measured digestive and antioxidant enzyme activities. Likewise, oxidative stress-related gene expression was unaffected. Intestinal morphology and immune response are currently under evaluation.

Keywords: Aquaculture; *Bacillus* sp.; Plant feedstuffs; *Saccharomyces* sp.; *Sparus aurata*.

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24242 | A Dual Identity: How the Spectraplakin Shot Drives or Restrains Tumorigenesis

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Background & Aim: Spectraplakins are a family of large cytoskeletal crosslinkers that uniquely bind to all three major cytoskeletal components: actin, intermediate filaments, and microtubules. Members of the spectraplakin family, such as DST and MACF1, are involved in several human diseases, including cancer (1). However, DST has a context-dependent role in tumor development: it promotes invasion in oral squamous cell carcinoma (2), but acts as a tumor suppressor in breast cancer (3). This biological complexity, along with the genetic redundancy among spectraplakin family members, has made it difficult to fully understand their functional roles *in vivo*. In contrast, *Drosophila melanogaster* has a single spectraplakin gene, *Short stop* (*Shot*), which shows strong evolutionary conservation with human *DST* (73% similarity). Our goal is to determine which isoforms or functional domains of Shot are responsible for its tumor-promoting or tumor-suppressing functions. **Methods:** The *Drosophila* wing imaginal disc is a well-established model for studying epithelial growth and tumor formation. Using this tissue, we seek to uncover the fundamental principles by which cytoskeletal regulation influences the duality of gene function. **Results:** Our preliminary data indicate that overexpressing full-length Shot increases hinge length while decreasing pouch growth. Notably, growth in the hinge results from increased cell proliferation. Additionally, overexpression of Shot lacking the actin-binding domain specifically reduces the pouch area without affecting the hinge, emphasizing domain-specific and region-specific roles. **Conclusions:** Overall, these initial findings suggest that growth effects in the wing disc are region-specific. Insights gained from *Drosophila* could have important implications for human cancer biology and for developing strategies that target pro-oncogenic mechanisms without disrupting essential cellular functions.

Keywords: Cytoskeleton, Gene duality, Spectraplakins, Tumorigenesis, Shot

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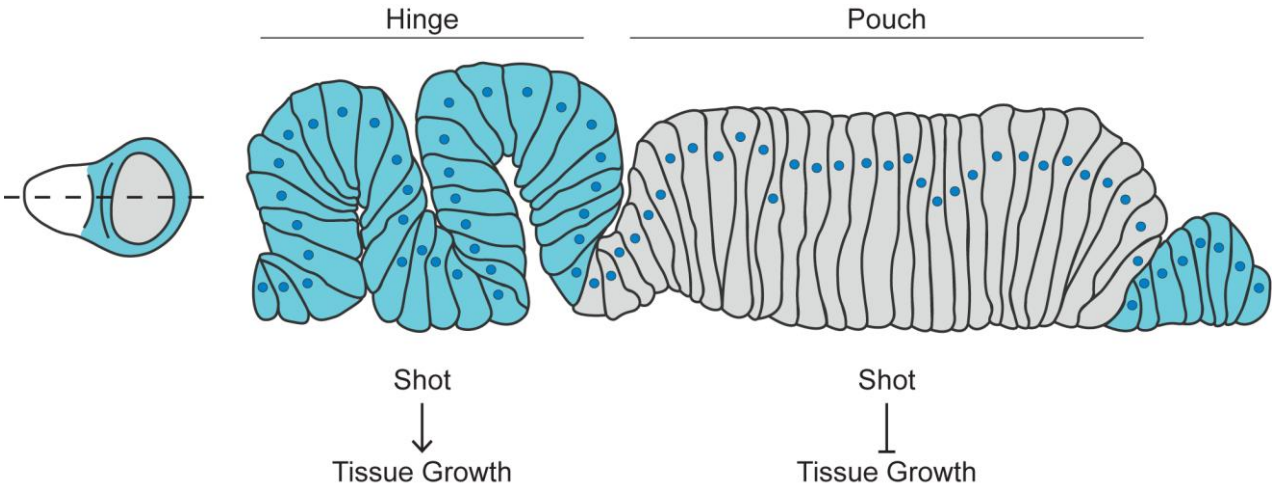

Figure 1: Shot-mediated region-specific growth regulation of the wing imaginal disc. The pouch (grey) and hinge (blue) compartments exhibit distinct regulatory mechanisms.

24293 | Exploring the role of calcium signaling and endoplasmic reticulum (ER) stress in the repair of plasma membrane damage

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Background & Aim: The plasma membrane (PM) preserves cellular integrity and homeostasis. PM disruption during bacterial infection leads to stress signaling and ionic imbalance, particularly calcium (Ca^{2+}) influx[1]. Among the most potent PM-damaging agents are pore-forming toxins such as pneumolysin (PLY), produced by *Streptococcus pneumoniae*[2]. Previous work from our group showed that both PLY intoxication and *S. pneumoniae* infection induce remodeling of the endoplasmic reticulum (ER), namely ER enlargement. Since these changes follow pore formation and Ca^{2+} influx, we hypothesized that Ca^{2+} drives ER remodeling and aimed to determine whether ER enlargement is a consequence of extracellular Ca^{2+} influx. ER expansion has been associated with activation of the unfolded protein response (UPR), an adaptive stress pathway mediated by PERK, IRE1 and ATF6 that restores ER homeostasis[3,4]. We therefore aimed to assess if PLY activates UPR. Understanding these mechanisms can clarify how cells adapt and repair PM injury and may contribute to the development of complementary therapeutic strategies. **Methods:** To understand the role of Ca^{2+}, HeLa and A549 (cervical and lung epithelial, respectively) cells were intoxicated with PLY (0.25–0.5 nM, 10 min) in the presence or absence of extracellular Ca^{2+}, and ER morphology was assessed by immunofluorescence. To evaluate UPR activation, A549 cells were infected with wild-type and deficient-PLY *S. pneumoniae* strains (MOI 50, 1-6h), and the expression of key proteins involved in the UPR branches was analyzed by western blot. **Results:** ER enlargement induced by PLY was dependent on extracellular Ca^{2+}, supporting a direct link between Ca^{2+} influx and ER remodeling. Ongoing analyses are evaluating if PLY activates UPR signaling during infection. **Conclusions:** These findings show that Ca^{2+} influx drives ER enlargement following pneumococcal pore formation and provide insight into early host responses to PM damage. Whether PLY activates UPR pathways requires further study.

Keywords: *Streptococcus pneumoniae*; Pneumolysin; Endoplasmic Reticulum; Calcium, Unfold Protein Response.

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24294 | Structural and molecular dissection of the role of Copine-1 and Copine-3 in the repair of plasma membrane damage

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Background & Aim: Plasma membrane (PM) integrity is critical for cellular homeostasis. *Streptococcus pneumoniae* (*Spn*) is the leading cause of community-acquired pneumonia and a major driver of lung injury[1]. Its pore-forming toxin pneumolysin (PLY) disrupts PM integrity and triggers calcium (Ca^{2+}) influx that coordinates pro-survival cell responses[2-5]. Copine-1 (CPNE1) and Copine-3 (CPNE3) are described as Ca^{2+}-binding proteins[6]. They are enriched in extracellular vesicles released by PLY-intoxicated cells, are recruited to cortical repair sites upon PLY challenge and are required for efficient PM repair[7]. We aim to define the structural basis of Ca^{2+} binding in CPNE1/3 and determine their contribution to PM repair during *Spn* infection. **Methods:** AlphaFold-based structural modeling with Ca^{2+}-docking was used to predict domain organization and ion-coordination sites. Structural conservation was assessed by FoldSeek and sequence alignment. Predicted Ca^{2+}-binding residues were targeted by site-directed mutagenesis to generate Ca^{2+}-deficient variants and their recruitment to cortical repair sites is currently being evaluated by confocal and live-cell imaging to determine the requirement of Ca^{2+} binding. CRISPR/Cas9-edited lung epithelial cells lacking CPNE1 or CPNE3 were generated and are being challenged with PLY or infected with wild-type and Δply *Spn*. **Results:** Both paralogs exhibited a conserved C2–C2–VWA architecture with high-confidence Ca^{2+}-binding clusters predominantly within C2 domains. Comparative modeling supported strong homology with canonical Ca^{2+} sensors. Gene-specific A549 knockout clones were successfully validated, establishing robust models to investigate CPNE function during toxin-induced injury. **Conclusions:** CPNE1 and CPNE3 emerge as Ca^{2+}-responsive scaffolds linking toxin-induced Ca^{2+} influx to PM repair. These findings provide a structural-genetic framework to dissect epithelial resilience to infection. Functional analyses are ongoing to define their mechanistic contribution.

Keywords: Copine proteins; Calcium signaling; Plasma membrane repair; *Streptococcus pneumoniae*; Pneumolysin

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24300 | Comparative Evaluation of HRP, APEX2, and TurboID for Selective Surface Labeling at Astrocyte–Neuron Interfaces

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Background & Aim: Despite decades of research, the molecular interactions underlying astrocyte–neuron interactions and their influence on neural circuit function remain poorly understood. Defining the organization of these contact sites requires tools that label proteins with high spatial precision while preserving native cellular architecture [1]. Proximity-dependent biotinylation has become a central strategy for spatial proteomics [2]. However, enzymes used for this purpose differ in their characteristics [3]. Here, we directly compare three such enzymes, HRP, APEX2, and TurboID, to identify the best tool for cell-surface labeling in astrocytes and neurons. **Methods:** Membrane-targeted constructs of HRP, APEX2, and TurboID were first validated in HEK293 cells to evaluate expression and labeling efficiency under controlled conditions. To test performance in the mouse brain, constructs were delivered to the developing cortex via *in utero* electroporation, enabling cell-type-specific expression in either neurons or astrocytes. For TurboID-mediated labeling, biotin was administered systemically for one week prior to tissue collection to allow continuous biotinylation *in vivo*. In contrast, peroxidase-based labeling with HRP or APEX2 was performed *ex vivo*: acute brain slices were prepared and exposed to biotin-phenol and hydrogen peroxide to initiate rapid surface-restricted labeling. **Results:** It was observed that peroxidase-based systems demonstrated superior performance for cell-surface labeling in both neurons and astrocytes, with HRP consistently performing better than APEX2, which performed better than TurboID. **Conclusions:** By defining the strengths and limitations of each system, this work provides a methodological framework for spatially resolved proteomic analysis of neuron–glia interactions, with HRP-based proximity labeling emerging as

the most promising approach for selective surface proteomics at astrocyte–neuron contact sites.

Keywords: Astrocyte–neuron interactions; Proximity-dependent labeling; Cell-surface proteomics; Peroxidases; Biotin ligase

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24328 | Improving the Physiological Relevance of In Vitro Intervertebral Disc Models through Mechanical Loading Optimization

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Background & Aim: The intervertebral disc (IVD) is a cartilaginous structure and part of the spinal articulation. [1] In degeneration, there are profound changes to the composition and biochemistry of the IVD, with substantial evidence supporting a role for mechanical stimulation in its pathway, although the role of mechanical loading on specific IVD cell populations remains largely unexplored. [2,3] As such, it is vital to study the way different loading conditions of mechanical stimulation impact the IVD, and in particular IVD cells fate. **Methods:** In this work, an in vitro model for IVD tissue mechanical loading was established. For that, bovine nucleus pulposus (NP) 9mm-punches were prepared as described [4]. NP punches were then placed in a 6-well plate under cell culture inserts and subjected to mechanical loading in a TA.XTplus Texture Analyzer during 4-5 days, with 2 forces: 27N to simulate degeneration and 2N for a more physiological condition [5, 6], in PBS pH 7.4. Measurements of the NP's physical properties such as height and compressive Young's Modulus, and evaluation of changes to its composition through protein loss are taken into consideration to evaluate the effectiveness of the model. **Results:** After mechanical stimulation of the NP during 4-5 days, a maximum decrease in disc height of 2mm was noted, while diameter increased around 4mm. Total protein released into the surrounding buffer was quantified, showing an increase during time and with stronger compressive force. The compressive Young's Modulus depends on load intensity, but seems to decrease throughout the experiment, as much as 16kPa loss for 27N, and 2,2kPa for 2N. **Conclusions:** Overall, these results point towards the fact that the texture analyzer can be used to induce mechanical loading in bovine IVD, and that the loading influences the biomechanics and biochemical composition of the disc. In the future this model can be used to address the impact of mechanical loading on IVD degeneration, namely in specific IVD cells.

Keywords: Intervertebral Disc, Mechanical Stimulation, Biomechanics, In vitro models, Bioreactor

Acknowledgments: Alexander Humboldt Foundation, Research Group Linkage Program for the project “IVDD- RESIDE: Intervertebral disc degeneration resolution through resident immune cells targeting by extracellular vesicles from Mesenchymal Stromal Cells”.

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24329 | Decellularized Aligned Matrices for Studying Intervertebral Disc Biology

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Background & Aim: Intervertebral disc (IVD) degeneration is a multifactorial condition, often leading to structural and biochemical alterations in the extracellular matrix (ECM) [1]. This process contributes to low back pain (LBP) and IVD herniation, which affect up to 3% of the population annually and carry significant social and economic burdens [2]. The annulus fibrosus (AF), a structural component of the IVD, plays a critical role in maintaining IVD integrity [3]. Nonetheless, the underlying mechanisms of AF failure remain largely unclear, partly due to the limited availability of appropriate experimental models [4]. This study aimed to create a decellularized (d)AF scaffold from bovine origin with native ECM composition and structure to support human AF cell culture, to advance the study of IVD biology. **Methods:** A decellularization protocol using sodium dodecyl sulfate (SDS), DNAse and sonication was optimized for bovine AF tissue. The main components of the tissue were quantified (collagen, glycosaminoglycans (GAGs) and elastin). The resulting dAF scaffolds underwent characterization, including histological analysis, hydration and degradation tests. Finally, the biological performance of the scaffolds was assessed through repopulation studies using human AF cells, with metabolic activity and cytotoxicity evaluations. **Results:** Cell removal was confirmed with an efficiency about of 77% DNA decrease, with preservation of the main ECM components. Histological analysis of decellularized AF tissue confirmed maintenance of the characteristic lamellar architecture. Swelling assays demonstrated the scaffold´s capacity to retain medium over 28 days, while exhibiting about 35% degradation in protein content. When seeded on top of dAF scaffolds, human AF cells were viable, metabolic active and interacting with the collagen lamellae. **Conclusions:** These results demonstrate that bovine dAF scaffold is a promising 3D model for in vitro studies of AF biology, particularly to understand AF failure mechanisms.

Keywords: Intervertebral disc, Annulus fibrosus, Decellularization, Tissue engineering, Annulus fibrosus Study Model.

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24332 | Effects of Methylphenidate oral treatment on the levels of MAP2 and PSD-95 in the brain of male and female spontaneously hypertensive rats

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Background & Aim: Attention-deficit/hyperactivity disorder (ADHD) is associated with functional and structural alterations in fronto-striatal and hippocampal circuits. Methylphenidate (MPH) is the most widely prescribed pharmacological treatment for ADHD [1]. Many of its effects remain misunderstood, therefore we aimed to study key influences of MPH in specific brain regions using the spontaneously hypertensive rat (SHR), a well-established animal model of ADHD. **Methods:** 17 male SHR and 6 female SHR were assigned to two groups, one treated with MPH, via gavage, while the control group received an equivalent volume of 5% sucrose solution [2]. Administration started on postnatal day 15 (human infancy equivalent) for 15 days, and on the 30th, the animals were sacrificed, and their brains dissected. Immunofluorescence was performed targeting microtubule-associated protein 2 (MAP2) and postsynaptic density protein 95 (PSD-95), focusing on the prefrontal cortex (PFC), nucleus *Accumbens* (NAc), hippocampal subfields (CA1, CA3, dentate gyrus and hilo), and striatum. Optical density was quantified using a custom-developed image analysis pipeline in FIJI. Weight variations during the protocol and sex-based comparisons were also performed. Statistical analysis was conducted in GraphPad Prism 8. **Results:** MPH treatment did not alter the total body weight growth curve. PSD-95 immunoreactivity levels did not change in SHR rats following treatment. Meanwhile, MPH significantly reduced MAP2 optical density in the hilo region of females. Furthermore, female controls had significantly higher MAP2 levels in the hilo subregion

than male controls. **Conclusions:** By characterizing basal synaptic markers in ADHD-relevant SHR brain regions, this study highlights region- and sex-dependent differences in therapeutic responses to MPH.

Keywords: Attention Deficit Hyperactivity Disorder (ADHD), Methylphenidate (MPH), Spontaneous hypertensive rat (SHR).

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24387 | The endoplasmic reticulum undergoes ultrastructural modifications in response to plasma membrane damage induced by *Streptococcus pneumoniae* infection

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Background & Aim: *Streptococcus pneumoniae* (*Sp*) is a major cause of pneumonia and a global health threat[1]. Although vaccines and antibiotics exist, rising antimicrobial resistance limits treatment[2,3], highlighting the need for new therapeutic strategies. For that, we need to better understand host-pathogen interactions. *Sp* produces pneumolysin (PLY), a pore-forming toxin (PFT) that acts as a key virulence factor by disrupting the host plasma membrane (PM). At lytic concentrations, PLY causes cell death; at sub-lytic doses, it activates Ca^{2+}-dependent PM repair responses[4,5]. Our unpublished data show that PLY induces remodeling of the actomyosin cytoskeleton concomitantly with PM blebbing. Similar effects caused by other PFTs have been linked to endoplasmic reticulum (ER) chaperone Gp96 and ER vacuolation[6,7]. Whether ER morphology is altered by PLY and contributes to protection against damage remains unclear. We aim to characterize ER ultrastructure during PM damage and repair. **Methods:** A549 and HeLa (lung and cervical epithelia, respectively) cells were infected with *Sp* WT or Δ*ply* (MOI 50, 1 h), intoxicated with PLY (0.25-0.5 nM, 10 min), or left untreated. ER organization was analyzed by confocal microscopy, correlative light & electron microscopy, and electron tomography. **Results:** Infection with *Sp* WT strain, but not with Δ*ply* mutant, and PLY intoxication altered ER structure in both cell lines. 3D reconstruction revealed marked ER enlargement (200-2000 nm) compared to untreated cells (<50 nm), which was reversed in recovering cells after PLY removal. Recovering cells also displayed increased number and size of multivesicular bodies (MVBs), endocytic organelles proposed to degrade other PFTs and implicated in PM repair[8,9]. **Conclusions:** *Sp* infection induces reversible ER enlargement mediated by PLY, and increased formation of enlarged MVBs during recovery. Whether ER remodeling and MVBs emergence are connected events and are part of an active cellular repair mechanism requires further study.

Keywords: *Streptococcus pneumoniae*; Pneumolysin; Plasma Membrane Repair; Endoplasmic Reticulum; Multivesicular bodies

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24808 | FREEZE OR FILTER? The Evolutionary Puzzle of Antifreeze Proteins and Aglomerular Kidneys in Notothenioids

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Background & Aim: The ecological success of notothenioid fishes in the Southern Ocean is linked to extreme physiological adaptations, most notably the evolution of antifreeze glycoproteins (AFGPs). It has been proposed that to prevent the urinary loss of these costly molecules these fishes evolved aglomerular kidneys. However, the genetic mechanisms underlying this phenotypic loss remain unclear. Glomerulus-associated genes such as nephrin (NPHS1) and podocin (NPHS2) appear to be retained rather than lost, unlike what has been reported in other aglomerular teleost lineages (e.g. syngnathids). Furthermore, the link between AFGPs and aglomerulism is not universal: in gadids, which independently evolved AFGPs, both glomerular and aglomerular species coexist. This project investigates whether aglomerulism in notothenioids represents a fixed trait or a developmentally regulated physiological state. **Methods**: Gene expression patterns will be analysed using RNA-seq across developmental stages, focusing primarily on juvenile and subadult individuals, additionally we also manage to obtain larval samples. In parallel, juveniles (n = 92) will be exposed to environmental conditions predicted to favour glomerular filtration, including elevated temperature and reduced salinity. Differential gene expression analyses will assess the regulation of genes associated with glomerular structure and renal function. **Results:** Preliminary transcriptomic analyses indicate that these genes show expression on early development stages. **Conclusions:** This work revisits the assumption that notothenioid kidneys represent a complete loss of glomerular function linked to AFGPs. Instead, it explores whether aglomerulism in this lineage reflects regulatory changes in renal physiology while retaining the genetic machinery for glomerular filtration. Clarifying these mechanisms refines current models of kidney evolution in Antarctic fishes and may help assess how highly specialized notothenioids respond to rapid environmental change.

Keywords: EcoDevo; Fish renal function; Notothenioid; Glomerulus; Antifreeze glycoprotein.

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24844 | Seaweeds as Sustainable Protein Sources: *In Silico* Identification of Bioactive Peptides Targeting sACE for Hypertension and Fibrosis Management

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Background & Aim: Hypertension affects nearly 30% of the global population. Although synthetic angiotensin-converting enzyme (ACE) inhibitors are effective, their lack of domain selectivity often leads to adverse side effects. Somatic ACE (sACE) consists of two homologous domains: the C-domain, which regulates blood pressure, and the N-domain, involved in fibrotic pathways. This study aimed to identify, for the first time, seaweed derived peptides through an *in silico* workflow that selectively targets these domains to provide dual antihypertensive and antifibrotic benefits. **Methods:** A library of 300 seaweeds protein sequences from Swiss-Prot (UniProtKB) was subjected to simulated gastrointestinal digestion using pepsin, trypsin, and chymotrypsin (BIOPEP-UWM server). From that, a pool of 8037 new and potentially bioactive peptides was obtained and screened using molecular docking (GOLD software, PLP scoring) against the C-domain (PDB: 6F9T) and the N-domain (PDB: 6EN5). Three sequential selection criteria were applied to ensure high affinity and domain specificity. **Results:** The *in silico* screening identified eight novel bioactive peptides originating from five seaweed species. Peptides CGVEVTES, DAASVEY, GDGVAEAW, and QVQVSF exhibited preferential binding to the N-domain of sACE, suggesting potential antifibrotic activity. In contrast, ATQAR, PGPIGDVY, TDPSAEDF, and TTDGEEQTL displayed high affinity for the C-domain, indicating significant antihypertensive potential. Furthermore, allergenicity predictions classified DAASVEY, PGPIGDVY, and TDPSAEDF as non-allergens, supporting their potential safety for integration into sustainable food systems. **Conclusions:** This research highlights the potential of seaweeds as a sustainable source of bioactive compounds for the development of functional foods capable of simultaneously mitigating hypertension and fibrosis. Additionally, it provides molecular

scaffolds that can be used by the pharmaceutical industry for the development of selective inhibitors targeting the C- and N-domains of sACE.

Keywords: Seaweed; Bioactive Peptides; Angiotensin Converting Enzyme; Molecular Docking; Hypertension.

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24390 | Functional analysis of knock-out mutants for the typical aspartic proteinases from *Arabidopsis thaliana.*

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Background & Aim: Aspartic proteinases (APs; EC 3.4.23) are a class of endopeptidases widely distributed among various taxa. Plant APs are localized along several tissues and are linked with diverse processes such as protein processing and degradation, the activation of zymogens during flower development and seed germination, among others. Some plant APs have a one hundred amino acidic sequence called PSI (Plant Specific Insert), which is associated with distinct functions, such as the subcellular protein trafficking, APs structural stability and substrate specificity. In *Arabidopsis thaliana,* 70 APs are found but only AP1, AP2 and AP3 have the PSI domain. The main goal of this work is to focus on these three APs, resorting to T-DNA insertion mutants, transcriptional reporters and overexpression lines to understand the role of each during plant development, particularly in reproductive tissues. **Methods:** To better perceive the role of APs in plant reproduction, T-DNA insertion lines were used, and several phenotypical assays were performed to characterize the mutant reproductive performance. Also, gene expression analysis was evaluated to better understand the APs behaviour in reproductive tissues. **Results:** Two knockdown homozygous lines were obtained (*ap1, ap2*), that showed slower development of the vegetative organs when compared with wild-type (WT). Regarding the reproductive tissues, *ap2* had a slower pollen tube growth rate, and both T-DNA lines demonstrated an increased percentage of seed abortion and unfertilised ovules, and consequently a decrease in the production of viable seeds, when compared to WT. Gene expression analysis demonstrated that the genes coding for the three APs of Arabidopsis are differentially expressed in flowers. **Conclusions:** These APs seem to play different roles during

plant reproduction. Altogether, the results here obtained paved the path to better understand the roles of *AP1*, *AP2* and *AP3* in Arabidopsis reproductive processes.

Keywords: Aspartic Proteinases; Plant Specific Insert; Plant reproduction; Pollen tube; *Arabidopsis thaliana*.

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24417 | Engineering Magnetic-Induced Tenogenic Cell Sheets for the Development of a Cell-Free Injectable Hydrogel for Tendon Regeneration

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Background & Aim: The use of decellularized extracellular matrix (dECM) derived from *in vitro* cultures has recently emerged as an alternative approach to traditional tissue-derived dECM, with the potential to provide better control over the final composition, while improving practicality and reproducibility, holding promise for applications in regenerative medicine, namely for tendinopathies, where specific biochemical signals are essential to guide cellular behavior.[1] Previous studies have shown that magnetic stimulation increases the production of a robust ECM by human adipose stem cells (hASCs) in a cell-sheet, and so, the main objective of this work will be to optimize the culture conditions leading to a more exuberant production of tenogenic ECM.[2] Subsequently, that matrix will be decellularized with the aim of producing an injectable cell-free hydrogel to use in tendon regeneration.[3] **Methods:** The work will be developed as represented in Figure 1. hASCs are used to produce tenogenic cell-sheets. Medium supplementation and magnetic stimulation are optimized throughout various timepoints in order to facilitate that production in terms of quantity and quality. The optimized sheet will then be decellularized under optimized protocols and then characterized for cell removal efficiency and ECM preservation. Afterwards, the best decellularized matrix will be lyophilized, milled and digested in pepsin, to produce an injectable hydrogel, which will then be evaluated in terms of injectability, biocompatibility, mechanical properties and regenerative potential. **Results:** Preliminary results show that magnetic-induced hASCs cell-sheets were successfully produced, showing a time dependent development, with the presence of tenogenic proteins, with and without TGF-β3 supplementation. Dynamic magnetic stimulation led to a higher sheet robustness. **Conclusions:** Magnetically stimulated cell-sheets demonstrate the potential to mimic the tenogenic ECM. Ongoing studies include the optimization of decellularization protocols for further development of the envisioned injectable hydrogel.

Keywords: Tendinopathy, Cell-Derived Matrix, Decellularization, Tissue Engineering, Magnetic Stimulation

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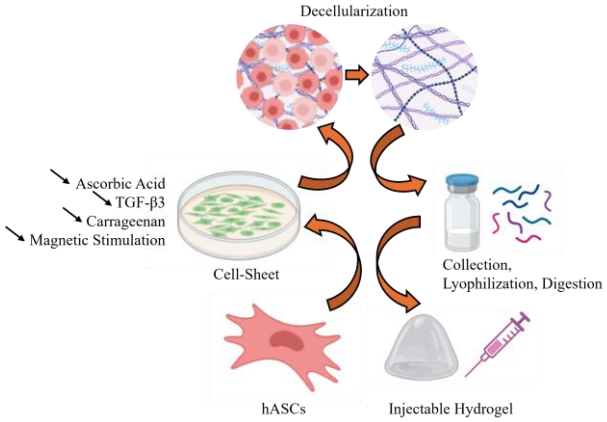

Figure 1: Workflow of the present work: production of hASCs cell-sheets, optimization of their production; optimization of their decellularization; collection, lyophilization and digestion; production and characterization of the injectable hydrogel. Figure created in BioRender.

24418 | Novel Peptidomimetic-Ionic Liquid Conjugates to tackle Diabetic Foot Ulcers

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Background & Aim: Antimicrobial resistance is reducing the effectiveness of current treatments for different types of infections, such as diabetic foot ulcers (DFU), creating an urgent need for new therapeutic modalities [1]. Gomes *et al.* previously demonstrated that peptide-ionic liquid conjugates are promising candidates for topical treatment of DFU, as they simultaneously promote wound-healing and antimicrobial action owing to their cosmeceutical peptide and ionic liquid building blocks, respectively [2]. Yet, despite showing numerous advantages, peptides have also a few drawbacks, which can be overcome by using peptidomimetics [3]. This project aims to produce peptidomimetic-ionic liquid conjugates and to evaluate how their antimicrobial activity and cytotoxicity compare to those of their parent peptide-ionic liquid conjugates. **Methods:** The peptides/mimetics were synthesised *via* Solid Phase Peptide Synthesis, and next linked to conveniently modified ionic liquid, using the copper(I)-catalysed alkyne-azide cycloaddition "click" reaction. The conjugates' toxicity to human keratinocytes (HaCat cells) and dermal fibroblasts (HDF cells) were determined by the resazurin method. In parallel, their antimicrobial activity was evaluated against susceptible bacterial strains, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. **Results:** Short wound-healing peptides (3 and 5 amino acids), their peptidomimetics, and a longer 12-mer peptide, were successfully synthesised. Shorter peptides showed antimicrobial activity only after covalent conjugation to the ionic liquid, while the longer one showed no antimicrobial activity. Cytotoxic effects were observed only for the covalent conjugates, and no significant differences were found between the parent peptides and their peptidomimetics. **Conclusions:** Based on these results, we can conclude that peptide/peptidomimetic conjugation to the ionic liquid, despite increasing cytotoxicity, is of chief importance for the display of antimicrobial activity.

Keywords: Antimicrobial resistance; Diabetic Foot Ulcer; Ionic Liquids; Peptides; Peptidomimetics.

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24952 | Development and Validation of Genetic Tools to Monitor Endosomal Recycling Dynamics in Invasive Triple-Negative Breast Cancer

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Background & Aim: Triple-Negative Breast Cancer (TNBC) is the most aggressive BC subtype with high metastatic potential and limited therapeutic options. FER has been established as a driver of TNBC progression by regulating endosomal recycling (ESR), a key process for cell invasion [1]. Having previously identified SEC16A as a downstream FER effector, we performed *in silico* analysis and identified Tyrosine 1320 (Y1320) on SEC16A as a FER phosphorylation site [2]. This study aims to functionally characterize the FER-SEC16A interaction and develop fluorescent genetic tools to monitor ESR dynamics during TNBC invasion. **Methods:** Site-directed mutagenesis was used to generate shRNA-resistant SEC16A constructs: phospho-null (Y1320A) and phosphomimetic (Y1320D) mutants. These were transfected into SEC16A-depleted MDA-MB-231 (MM231) cells to evaluate Rab4 vesicles and Paxillin-rich focal adhesions (FA) via immunofluorescence. To visualize ESR dynamics, lentiviral constructs encoding Rab4-mCherry and Rab11-GFP were generated for introduction into MM231 cells and Patient-Derived Xenograft Organoids. These tools were validated by FACS, Western blot, and high-resolution live-cell imaging. **Results:** Quantitative imaging revealed that SEC16A depletion significantly reduces both Rab4 vesicle numbers and Paxillin-rich FA. While the Y1320A mutant failed to rescue these phenotypes, the Y1320D mutant successfully restored Rab4 vesicles to levels statistically comparable to wild-type rescue. For FAs, Y1320D provided a partial rescue, showing improvement over the Y1320A mutant. To study whether microenvironmental cues reshape ESR via FER and SEC16A interactions to promote invasion, cells constitutively expressing Rab4-mCherry and Rab11-GFP were successfully generated. **Conclusions:** Altogether**,** these findings suggest that Y1320 phosphorylation is critical for SEC16A to regulate oncogenic vesicular trafficking, identifying the FER-SEC16A axis as a potential therapeutic target in TNBC.

Keywords: Triple-Negative Breast Cancer; FER kinase; SEC16A; Endosomal Sorting and Recycling

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24943 | Computational and in vivo dissection of pancreatic cis-regulatory elements driving T2D phenotypes

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Background & Aim: Type 2 Diabetes (T2D) is a common pancreatic disease with a genetic component linked to the disruption of non-coding cis-regulatory elements (CREs)[1]. CREs are open chromatin regions that serve as docking sites for transcription factors (TFs) and are essential for controlling gene expression[2]. Although Genome-Wide Association Studies suggest that CREs disruption contribute to T2D, the underlying mechanisms remain unclear[3]. This project aims to investigate whether CRE function is governed by sequence-based rules across multiple CREs and, using zebrafish as an *in vivo* model, whether CRE perturbations can lead to T2D-like phenotypes. **Methods:** Focusing on the *GCK* gene, a key diabetes locus, we identified a CRE that in humans is associated to T2D risk. Using CRISPR/Cas9, we aimed to delete a putative orthologous CRE in zebrafish and screened for the resulting mutation via genotyping. In parallel we used chromatin-accessibility datasets from human β-cells and our own generated data from zebrafish β-cells to computationally predict TF binding sites. Unsupervised clustering methods were then applied to group CREs sharing TFs binding profiles. **Results:** We were able to identify a *gck* regulatory mutation, which we are currently phenotyping for T2D related traits. Regarding the clustering of CREs, when applied to a subsample of the total β-cell dataset, this approach was able to identify groups of putative functionally equivalent CREs with potentially shared regulatory logic between humans and zebrafish, despite sequence divergence. **Conclusions:** By integrating computational predictions, cross-species mapping, and *in vivo* validation, this project will elucidate how CRE sequences govern pancreatic gene regulation and how their disruption contributes to T2D-associated phenotypes.

Keywords: Binding profiles, Unsupervised clustering, Zebrafish model, GCK gene, Type 2 Diabetes

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24421 | Neuro-immune axis in post-traumatic osteoarthritis

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Background & Aim: Post-traumatic osteoarthritis (PTOA) accounts for 12% of symptomatic osteoarthritis (OA), a leading cause of pain, disability and socio-economic burden, affecting 500 million people [1, 2]. Up to 50% of individuals who suffer injury develop PTOA, underscoring the need to elucidate mechanisms of its initiation and progression to develop effective therapies [3]. Although early damage-associated molecular patterns (DAMPs) released from injured or dying cells have been identified, their role in PTOA initiation remain unclear [4, 5]. We hypothesize early DAMPs drive dysregulation of the neuro-immune signaling in the joint microenvironment. Chondrocytes, immune cells, and sensory neurons express DAMPs receptors, promoting immune cell recruitment and aberrant nerve sprouting, hallmarks of advanced OA transition [6, 7]. This work seeks to develop a system to mimic PTOA and uncover potential disease-modifying therapeutic targets. **Methods:** To model PTOA, 3D cartilage microtissues derived from OA patients chondrocytes were exposed to controlled physical or biochemical stimuli. They were characterized for cell viability, extracellular matrix (ECM) content and gene expression. Its secretome was analyzed for protein content and applied to macrophages and embryonic dorsal root ganglia (eDRGs) to assess cell recruitment and neuronal response, respectively. Compartmentalized microfluidic devices selectively exposed axonal terminals to secretome, mimicking the *in vivo* spatial organization. **Results:** Gene expression data indicate early overexpression of neurotrophic factors and ECM components, with downregulation at later timepoints. The secretome contained established DAMPs like alarmins and ECM fragments, differing between early and later timepoints. Functional neuronal response assays remain under analysis. **Conclusions:** Cartilage damage correlates with DAMPs presence and neurotrophic/catabolic signaling. The model shows great potential as a predictive degenerative cartilage-on-a-chip.

Keywords: Osteoarthritis, Post-traumatic Osteoarthritis, Organ-on-chip, Neuro-immune axis, Dorsal root ganglia

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24222 | Characterization of a putative beta-oxidation pathway in Cyanobacteria

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Background & Aim: Little is known about how cyanobacteria, photosynthetic microorganisms with a great impact on the global carbon cycle, degrade fatty acids and other lipids [1]. Beta-oxidation, the primary pathway for fatty acid degradation in living systems, was believed to be absent in cyanobacteria [2]. However, we identified a putative beta-oxidation gene cluster in cyanobacterial genomes. Therefore, we aim to investigate the functionality of the beta-oxidation pathway using in vivo, in vitro and in silico approaches to characterize the predicted beta-oxidation enzymes, as well as an associated putative lipase. **Methods:** Beta-oxidation genes were analyzed using bioinformatic tools, followed by cloning and heterologous expression in *E. coli.* The recombinant proteins were isolated and purified for biochemical characterization. To evaluate their catalytic activity, in vitro enzymatic assays were performed. Additionally, in vivo supplementation assays with isotopically labeled fatty acids were performed to assess fatty acid degradation. Metabolite analysis was carried out using LC-MS and complementary spectrometric methods. **Results:** Predicted structural analyses of the enzymes support the hypothesis of a beta-oxidation pathway in cyanobacteria. LC-MS data provide evidence for predicted enzymatic activity in at least two proteins encoded by the gene cluster. Furthermore, in vivo supplementation experiments have shown fatty acid degradation patterns consistent with beta-oxidation. **Conclusions:** Functional beta-oxidation genes are present in cyanobacteria, challenging the assumption that this metabolic pathway is absent in this phylum.

Keywords: Cyanobacteria, Beta-Oxidation, Free Fatty Acids, Enzyme Characterization

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24667 | Aspergillus niger growth on cyanobacterial biomass and regolith simulants for bioregenerative life support systems

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Background & Aim: Current human presence aboard the International Space Station depends on continuous resupply missions from Earth. However, with the upcoming Artemis project aiming to establish a sustained human presence on the Moon [1], advanced resource recycling and production technologies are needed. In this sense, two relevant complementary approaches are bioregenerative life support systems (BLSS) and *in situ* resource utilization (ISRU) [2,3]. While plants and cyanobacteria have been extensively studied within these frameworks [4,5], fungi remain comparatively underexplored [6]. The aim of this study is to provide a proof-of-concept for the integration of filamentous fungi into future BLSS for lunar and Martian exploration, evaluating whether fungal growth can be supported by ISRU-derived resources and byproducts of pre-established BLSS. **Methods:** *Aspergillus niger* N402 was grown on minimal media (MM) and in different space-resource media formulations, which included water-released minerals from lunar and Martian regolith simulants (LHS-1 and MGS-1, respectively), and lysed biomass from cyanobacteria *Anabaena* sp. PCC 7120 and PCC 7938 –strains which could be used as oxygen producers in space habitats [7]. *A. niger* was grown under standard Earth gravity and under simulated microgravity using a clinostat with FEP bag bioreactors. Citric acid production, relevant for food preservation in future space habitats, was assessed through LC-MS/MS. **Results:** Lysed *Anabaena* sp. PCC 7938 supported fungal growth at 30% of the positive control (MM), when diluted in either lunar or Martian minerals solutions, under both standard gravity and simulated microgravity. Citric acid was present in FEP bags from cultures grown with

Martian minerals under both gravitational conditions. **Conclusions:** This work showcased that *A. niger* can recycle cyanobacterial biomass and use lunar and Mars minerals as space resources to grow, further emphasizing the potential of integrating filamentous fungi in sustainable BLSS.

Keywords: life support systems, space microbiology, *Aspergillus niger*, *in situ* resource utilization, space exploration

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24424 | Functional biomembranes based on polysaccharides and polyphenols to improve oral mucositis

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Background & Aim: Oral mucositis is a common and debilitating complication of radiotherapy and chemotherapy. This inflammatory condition is characterized by severe pain, erythema, and ulceration, leading to reduced oral intake, significant weight-loss, and deterioration in patients' quality of life [1]. As current therapeutic approaches rely mainly on basic oral care, anti-inflammatory agents, and pain management, more effective and accessible treatments are needed [2]. This study aims to develop functional biomembranes based on natural polysaccharides and plant-derived polyphenols with wound healing properties as a novel strategy for oral mucositis management. **Methods:** The biocompatibility of various polysaccharides was evaluated using oral cell lines (HSC-3 and TR146) through MTT and Neutral Red assays. The selected polysaccharides were used to develop biomembranes by solvent casting method. Different families of plant-derived polyphenols (tannic acid, punicalagin, anthocyanins and procyanidins) were selected to enrich the biomembranes and several concentrations were assessed in cell viability assays. **Results:** Pullulan, sodium alginate, carboxymethylcellulose, pectin, banana and orange peel extracts showed moderate to high biocompatibility (60-120%) and were selected for further studies. Biopolymer solutions were optimized to determine the lowest concentration capable of forming the biomembranes (1% (w/v), 0.03g of polymer per 21.2 cm^2 Petri dish). Further cell viability assays demonstrated that tannic acid maintained biocompatibility at lower concentrations (0.25, 0.5 and 1%), while higher concentrations resulted in a dose-dependent reduction in viability. **Conclusions:** The selected polysaccharides demonstrated promising biocompatibility, indicating their potential as innovative alternatives for oral mucositis management. Ongoing studies are focused on further evaluating the biocompatibility of the polyphenols, as well as assessing the wound-healing performance of the functional biomembranes.

Keywords: Oral mucositis, polysaccharides, polyphenols, biomembranes, cell viability

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24464 | Isolation and characterization of an environmental caffeine-resistant bacteria strain

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Background & Aim: Caffeine (1,3,7-trimethylxanthine) is a plant-based compound with a negative environmental impact. Human production and consumption of coffee are the main responsible, due to the high amounts of caffeine-rich residues continuously discarded [1]. Bioremediation can be a relevant tool to surpass this problem. For example, *Pseudomonas putida*, a Gram-negative, rod-shaped environmental bacterium, presents potential applications in this area, since it is caffeine-resistant, promoting its biodegradation [2,3]. The aim of this work was to isolate from the environment and to characterise a bacteria strain able to grow in a caffeine-fortified culture media. **Methods:** Bacteria were isolated from potting soil with a coffee plant and coffee leaves. Strains of *Escherichia coli* ATCC (25922) and *Pseudomonas* spp. were used as negative controls. The bacteria strains obtained from the samples were subjected to different concentrations of caffeine (0.0, 2.5, 5.0 and 15 g/L) and distinct media. A single strain of interest was selected for biochemical and genetic characterisation. Moreover, timed aliquots of its spent broth were analysed through HPLC-DAD for caffeine quantification. **Results:** A Gram-negative, rod-shaped bacterium was selected from the soil sample, tolerating up to 15 g/L of caffeine in its culture medium, at 30 ºC. Biochemical testing identified this strain as *P. putida* or *P. fluorescens*, while 16S rRNA gene sequencing recognized it as *P. putida* or *P. alkylphenolica*. Whole-genome sequencing validated the strain as the later, also showing potential xenobiotic-resistance genes. No caffeine degradation or metabolites were observed in the spent broth media. **Conclusions:** A bacteria strain capable of growing in culture media fortified with caffeine was isolated and characterized as *P. alkylphenolica*. While not showing caffeine-degradation, it demonstrated alternative mechanisms of xenobiotic-resistance.

Keywords: *Pseudomonas putida*; coffee plant; *P. alkylphenolica*; xenobiotic resistance.

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24469 | Prokaryotic Fingerprints of a Changing Ocean: Deciphering Water Mass Structuring in the Pacific Arctic Region

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Background & Aim: Prokaryotes are key to Arctic marine food webs and nutrient cycling. The Arctic Ocean is warming, causing sea ice loss[1] and increased inflow of Pacific waters, which alter stratification and nutrient availability. These changes affect prokaryotic productivity, community structure, and biogeochemical cycling[2], yet their distribution across environmental gradients in the Pacific Arctic remains poorly understood[3]. The aim is to describe the spatial variation in prokaryotic community composition and diversity across different depths and water masses and to identify the main environmental factors influencing its structure. **Methods:** This project studies prokaryotic communities using seawater samples collected during the 2023 campaign aboard the *Research Vessel Mirai*. Microbial DNA was extracted from Sterivex filters (0.22 µm) and analysed via 16S rRNA gene sequencing (V4–V5). Bioinformatic analyses characterized taxonomic composition, diversity, and ecological structure by integrating sequencing data with environmental parameters (CTD and nutrient profiles), examining variation along depth gradients and across water masses. **Results:** Preliminary results reveal strong vertical and water mass structuring. Surface assemblages were associated with temperature and chlorophyll-a (Cyanobacteria), while deeper communities were linked to salinity and nitrate (Nitrospinota). Only two ASVs were shared among all water masses, and null intersections suggest ecological barriers shaping microbial distribution rather than random assembly. **Conclusions:** Arctic Pacific prokaryotic communities are strongly structured by physicochemical gradients. This study provides a baseline for understanding microbial distribution across vertical gradients and contrasting water masses and identifies key environmental drivers influencing ecosystem functioning. Integration of 2024 data will reveal responses to ongoing Arctic change.

Keywords: Arctic, Pacification, Prokaryotic Communities, Diversity, Water Masses

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24608 | Hybridization extent between European and Mountain hares across fragmented contact zones

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Background & Aim: Human actions significantly impact ecosystems. Land-use changes, human-mediated introduction of non-native species and accelerated climate change have been reshaping species distributions [1]. Such human-induced range shifts may disturb contact zones between closely related species, altering hybridization dynamics [1]. Understanding how hybridization affects genetic variation is vital for conservation, as gene flow between species can compromise their genetic integrity by introducing deleterious or maladaptive traits [2]. Yet, hybridization may also promote the transfer of adaptive genetic variants, potentially enhancing species resilience [3]. In this work, we explored admixture patterns between Mountain hares (*Lepus timidus*) and European hares (*L. europaeus*) across several contact zones under contrasting ecological and anthropogenic-impacted scenarios where interbreeding may threaten Mountain hares' persistence [4]. **Methods:** Using genomic data, we investigated patterns of hybridization between Mountain and European hares in a natural contact zone, the French Alps, and an anthropogenic contact zone, Peak District**. Results:** Our analyses show that human-mediated contact produces a higher frequency of interspecific crosses than natural contact. Moreover, gene flow direction is predominantly from European to Mountain hares, evidencing spatial invasion followed by backcrosses. Finally, ancient introgression in the French Alps contrasts with recent hybridization in the Peak District, indicating that historical contact zones are more stable than recently established anthropogenic contact zones. **Conclusions:**

Human-mediated contact between hares intensifies hybridization, promoting asymmetric introgression from the invading into native species and threatening genetic integrity. In contrast, stable natural contact zones show older and more limited gene flow, reinforcing the need to manage introductions to preserve species resilience.

Keywords: Genomics; hares; introgression; biodiversity conservation

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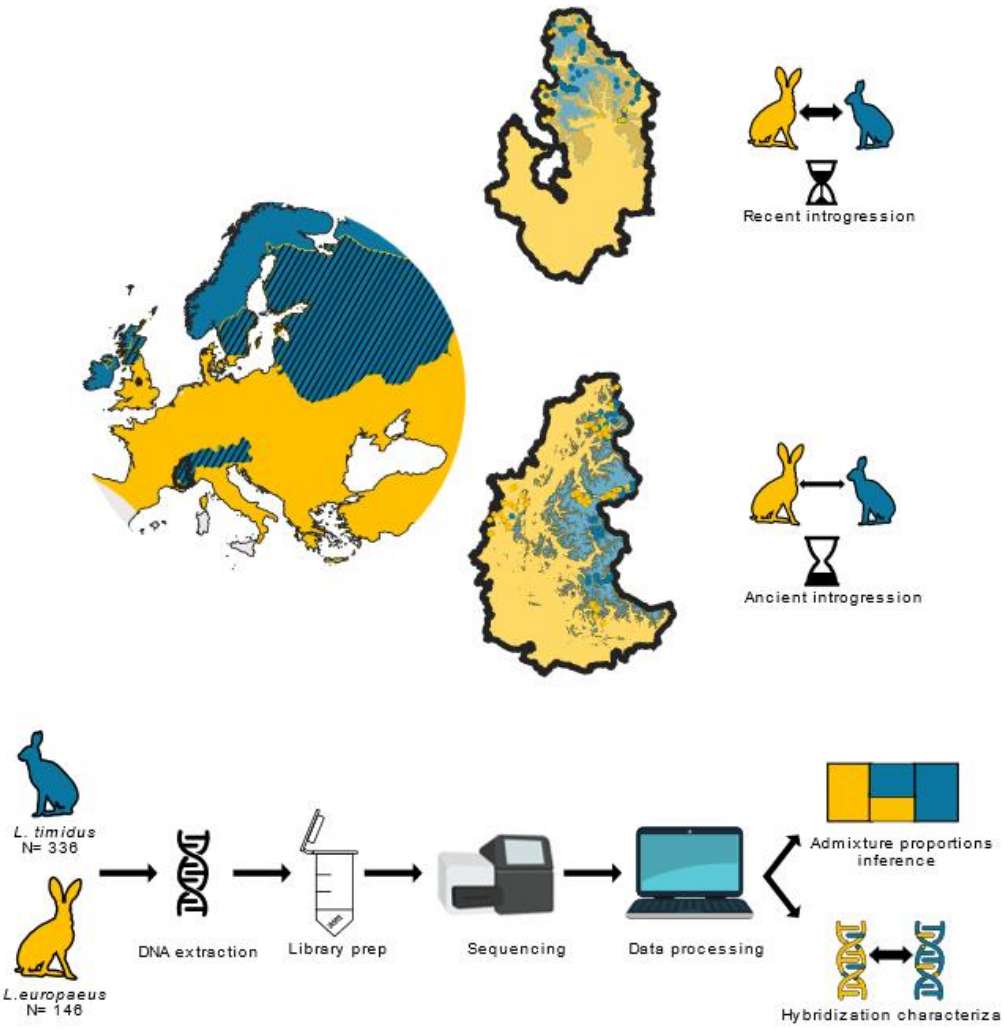

Figure 1: Graphical abstract.

24644 | Who thrives and who lingers? A camera-trapping baseline of mammals in Peneda-Gerês National Park

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Background & Aim: As Portugal's only National Park, Peneda-Gerês (PGNP) is a biodiversity stronghold, yet it lacks a systematic monitoring network for medium and large-sized mammals. To bridge this gap, ICNF and BIOPOLIS-CIBIO are implementing a long-term, park-wide camera-trapping monitoring program. The present work is a precursor to this initiative, leveraging camera-trap data collected by park managers to establish the first baseline of mammal species occupancy and assemblage structure. Additionally, it explores how these patterns relate to conservation zoning - the main tool for managing human disturbance - as a pilot assessment of management efficacy. **Methods:** We analyzed camera-trap data (2021-2025) from 45 stations distributed opportunistically across the park. Using a Bayesian community occupancy model, we estimated species-specific occupancy and evaluated species responses to conservation zonation. **Results:** We recorded 13 of 14 mammal species that were detectable via camera-trapping and had a confirmed occurrence in PGNP. Three species of low conservation concern (red fox, wild boar, and roe deer) were widespread (Ψ>0.65). Surprisingly, the endangered Iberian wolf exhibited intermediate occupancy values ($\Psi\approx0.40$), reflecting its extensive spatial use. The remaining species were rather rare (Ψ <0.30), including conservation-priority species (e.g., European wildcat, pine marten, and Iberian ibex) and species of least concern, such as the red deer. Overall, the assemblage structure revealed a weak association with the conservation

zoning, with generalist species maintaining similar occupancy across zoning classes. **Conclusions:** The non-detection of one species, combined with low overall mammal occupancy and apparent disconnection from the zoning scheme, raises concerns about the current state of this assemblage. Given that this refers to a protected area, these results emphasize the need for a long-term park-wide monitoring framework to detect future shifts and enhance management actions.

Keywords: Mammal assemblage; Protected areas; Ecological monitoring; Camera-trapping; Community occupancy models

Acknowledgments: We thank Instituto da Conservação da Natureza e das Florestas, I.P. for providing the data and for their overall support in the development of this work.

24831 | Dual-Faced Behavior of Natural Chromophores in Skin Health

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Background & Aim: In the context of skin health, natural chromophores often exhibit a "dual-faced" behavior, depending on the biological and photochemical context. In this study 4 food-derived compounds (riboflavin, chlorophyllin, quercetin and curcumin) were investigated to understand their bioactivity either as photoprotectors against UV radiation and/or as photosensitizers for treatment of skin abnormalities [1, 2]. **Methods:** Compounds were characterized regarding solubility, absorption spectra, photostability, and antioxidant capacity (DPPH). Cyto-and phototoxicity were assessed in human keratinocytes and human melanoma cells. PDT experiments were performed with a violet-blue LED irradiation device and UV-induced damage was evaluated using a UVB lamp. Cell viability was determined by resazurin-based fluorescence assays. **Results:** Quercetin and curcumin showed the highest antioxidant activity (EC_{50} = 2.3 and 4.9 µg/mL, respectively) followed by chlorophyllin (766.8 µg/mL), while riboflavin showed negligible response. Pre-treatment of skin cells with the compounds before UVB exposure revealed a pro-oxidant synergism in the case of chlorophyllin and curcumin, which increased UVB-induced cell death. In PDT assays quercetin showed no photodynamic response up to 25 µM, whereas riboflavin, curcumin, and chlorophyllin displayed clear photosensitizing activity against melanoma cells. Chlorophyllin stood out for its superior photostability compared with riboflavin and curcumin. **Conclusions:** Quercetin showed strong antioxidant activity with negligible photodynamic response, supporting its role primarily as a photoprotective compound. Riboflavin, curcumin, and chlorophyllin demonstrated clear photosensitizing activity, highlighting their potential for PDT applications, with the latter showing superior photostability. Curcumin displayed the most marked dual behavior, acting as an antioxidant in the dark but shifting toward pro-oxidant activity and exacerbating UV-induced damage at higher concentrations.

Keywords: Natural chromophores; skin photobiology; photoprotection; phototoxicity; oxidative stress

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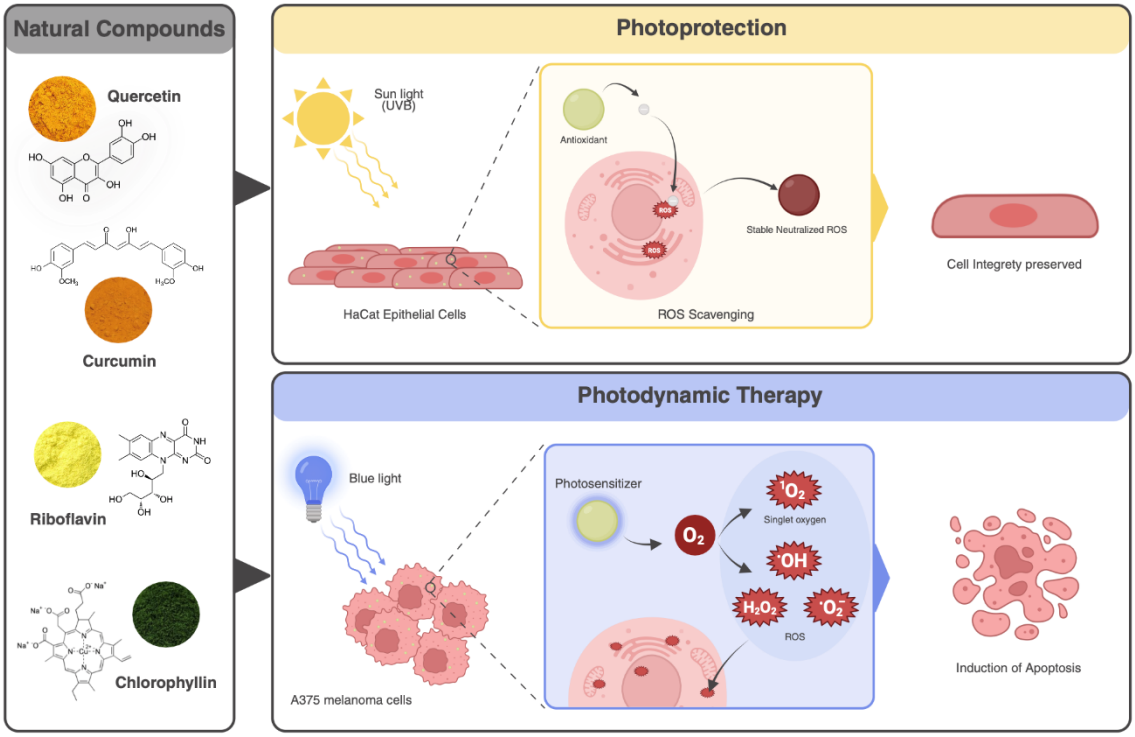

Figure 1. Graphical abstract representing the context-dependent dual photoactivity of food-derived compounds. *Created with BioRender.com.*

24523 | Urinary Metabolomic Signatures in Users of Electronic Cigarettes, Heated Tobacco Products, and Conventional Tobacco

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Background & Aim: Tobacco smoking remains the leading cause of preventable death and disease worldwide. The rising use of alternative products such as electronic cigarettes (ECs) and heated tobacco products (HTPs) raises concern about their systemic metabolic effects. This study aimed to compare urinary metabolic profiles of EC and HTP users, conventional tobacco smokers (CT), and non-smokers to evaluate the biochemical impact of different tobacco products and identify metabolites associated with product-specific exposure. **Methods:** Urine samples from CT, EC, HTP users, and non-smokers were analyzed by gas chromatography mass spectrometry (GC-MS). Samples underwent enzymatic urea removal, protein precipitation, and chemical derivatization by methoximation followed by silylation[1]. Metabolites were putatively identified using spectral library matching, and semi-quantitative data were evaluated by multivariate and univariate statistical analyses. **Results:** A total of 75 metabolites were identified, including amino acids, organic acids, sugars, and other small polar compounds. Although no distinct separation was evident among the four groups in multivariate analysis, pairwise comparisons revealed significant metabolic differences. CT smokers showed higher levels of quinate, furoylglycine, guaiacol, and 2-hydroxyhippurate, and lower sorbitol compared to non-smokers. EC users showed higher levels of beta-alanine and 3-methylhistidine compared to non-smokers and CT smokers, while citrate levels were lower only relative to CT smokers. HTP users showed elevated 2-hydroxyhippurate compared to CT smokers and decreased 3-methylhistidine, scyllo-inositol, 3-aminoisobutyrate, uracil, and one unknown compound compared to EC users. **Conclusions:** Urinary metabolomic profiling revealed distinct metabolic alterations associated with different tobacco products. These findings support the potential of metabolomics as a sensitive tool for the toxicological assessment of emerging tobacco and nicotine products.

Keywords: Electronic cigarettes; heated tobacco products; conventional tobacco; metabolomics; gas chromatography mass spectrometry (GC-MS)

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24761 | A synthetic community approach to control the pear pathogen *Stemphylium vesicarium* and grapevine pathogen *Phyllosticta ampelicida*

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Background & Aim: Brown spot of pear, caused by *Stemphylium vesicarium*, and grape black rot, caused by *Phyllosticta ampelicida*, are among the most threatening diseases in pear orchards and vineyards, respectively [1,2,3,4]. The use of fungicides has increased antimicrobial resistance and raised environmental concerns. Biological strategies, such as the use of Biological control agents (BCAs) and synthetic microbial communities (SynComs) has been increasing for disease management [5,6]. This project aims to identify and characterize pear and grapevine-associated bacterial endophytes with antagonistic activity against *S. vesicarium* and *P. ampelicida* as a first step toward SynCom design. **Methods:** A collection of 161 endophytic bacteria from pear trees and grapevines was identified through 16S rRNA sequencing. In vitro screening of the antagonistic activity against both pathogens was performed through a dual culture method. The most promising candidates to control both diseases are being characterized phenotypically and their tolerance under environmental stressors. From these experiments, a selection of at least 3 top BCAs will be used to assess the compatibility matrix over multiple generations, guiding the SynCom assembly. **Results:** All bacterial endophytes were taxonomically identified and comprised, among others, several species of Bacillus, *Pseudomonas graminis, Paraburkholderia gramminis.* Antagonism assays against the target pathogens were performed, and six potential BCAs showed more than 60% growth inhibition. These strains were selected for further characterization through a comprehensive set of phenotypic assays. **Conclusions:** Pear- and grapevine-associated microbes with robust antagonistic activity against *S. vesicarium* and *P. ampelicida* have been identified, and compatible strains have been selected as building blocks for tailored SynComs. These results will support the development of synthetic microbial communities as environmentally friendly tools for integrated control of fungal diseases.

Keywords: Biological control agents; Syncom; Phytopathogenic fungi; Brown spot of pear; Black rot disease

Acknowledgments:

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24476 | Unravelling the Evolutionary History of Least Weasels (*Mustela Nivalis*): Insights from Whole Genome Data

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Background & Aim: Understanding the evolutionary processes generating and maintaining diversity is a key aim of biological research. High-throughput sequencing technologies now allow using genome-wide data to more effectively study the evolutionary history of species. The least weasel (*Mustela nivalis*) is a widespread carnivore that displays substantial morphological diversity, which has been used as evidence for its subdivision into various recognised subspecies. However, previous genetic studies, based on mitochondrial (mtDNA) markers, have shown a discordance between mtDNA-based population structure and morphology-based classifications. Here, we use whole-genome sequencing (WGS) data to probe the evolutionary history and extant population structure of the least weasel. **Methods:** Our dataset includes WGS data of 11 least weasels, covering most of Eurasia and spanning most of the previously identified genetic groups. Using genome-wide data, we characterised population structure and genetic diversity and inferred phylogenetic relationships among individuals. We will further use coalescent-based demographic reconstruction to explore recent demographic history. **Results:** Our data supports the identification two major clades further subdivided into four distinct genetic groups along a geographical continuum. We find evidence of admixed individuals, suggesting recent interbreeding between genetic groups. **Conclusions:** The results of this study expand our understanding of population structure in Eurasian least weasels, showing more detailed genetic sub-structuring, compared to mtDNA inferences. Our data highlights the value of using genomic approaches to clarify the evolutionary history of natural populations.

Keywords: population genetics; phylogenetics; demography; whole-genome resequencing;

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24422 | Characterization of a putative beta-oxidation pathway in Cyanobacteria

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Background & Aim: Little is known about how cyanobacteria, photosynthetic microorganisms with a great impact on the global carbon cycle, degrade fatty acids and other lipids [1]. Beta-oxidation, the primary pathway for fatty acid degradation in living systems, was believed to be absent in cyanobacteria [2]. However, we identified a putative beta-oxidation gene cluster in cyanobacterial genomes. Therefore, we aim to investigate the functionality of the beta-oxidation pathway using in vivo, in vitro and in silico approaches to characterize the predicted beta-oxidation enzymes, as well as an associated putative lipase. **Methods:** Beta-oxidation genes were analyzed using bioinformatic tools, followed by cloning and heterologous expression in *E. coli.* The recombinant proteins were isolated and purified for biochemical characterization. To evaluate their catalytic activity, in vitro enzymatic assays were performed. Additionally, in vivo supplementation assays with isotopically labeled fatty acids were performed to assess fatty acid degradation. Metabolite analysis was carried out using LC-MS and complementary spectrometric methods. **Results:** Predicted structural analyses of the enzymes support the hypothesis of a beta-oxidation pathway in cyanobacteria. LC-MS data provide evidence for predicted enzymatic activity in at least two proteins encoded by the gene cluster. Furthermore, in vivo supplementation experiments have shown fatty acid degradation patterns consistent with beta-oxidation. **Conclusions:** Functional beta-oxidation genes are present in cyanobacteria, challenging the assumption that this metabolic pathway is absent in this phylum.

Keywords: Cyanobacteria, Beta-Oxidation, Free Fatty Acids, Enzyme Characterization

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24581 | Polyphasic characterization of LEGE 10371 (Pseudanabaenales), a novel marine cyanobacterium with anti-inflammatory potential

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Background & Aim: Thin filamentous cyanobacteria within Pseudanabaenales are taxonomically challenging due to morphological simplicity and frequent homoplasy [1]. Species delimitation requires integrative approaches combining morphological and molecular evidence within a polyphasic framework [2]. Marine cyanobacteria are also recognized as sources of bioactive metabolites with anti-inflammatory properties [4]. This study aimed to describe a novel marine cyanobacterial strain isolated from a sponge on the Portuguese coast (LEGE 10371) and to assess its preliminary anti-inflammatory potential through *in vitro* bioassays. **Methods:** Morphological characterization was performed using light microscopy and TEM/SEM. Phylogenetic analyses were based on 16S rRNA gene sequences following current taxonomic recommendations [2]. Pigment composition was analyzed by HPLC-PDA and protein profiling by MALDI-TOF MS. Bioactivity was assessed through *in vitro* antioxidant assays (superoxide anion and nitric oxide radical scavenging) and cytotoxicity testing in HaCaT keratinocytes. The inhibition of enzymes involved in inflammation and production of inflammatory mediators in a mammalian cell model (RAW 264.7) will be evaluated. **Results:** Phylogenetic inference placed strain LEGE 10371 in a well-supported and distinct clade within the genus *Thalassoporum* (Pseudanabaenales). Genetic divergence, together with stable morphological traits, including elongated trichomes and a marine epizoic lifestyle, supported its recognition as a new species [3]. Biomass fractions of different polarities showed radical scavenging activity against reactive oxygen and nitrogen species, without detectable cytotoxic effects in HaCaT keratinocytes. **Conclusions:** Morphological and molecular evidence supports the establishment of the new species. Its antioxidant activity suggests a potential anti-inflammatory role mediated by reactive species modulation, highlighting sponge-associated cyanobacteria as promising sources of bioactive compounds.

Keywords: *Thalassoporum;* polyphasic taxonomy; marine cyanobacteria; antioxidant activity; anti-inflammatory potential

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24613 | Discovery of cyanobacterial natural products through heterologous expression

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Background & Aim: Cyanobacterial natural products (CNPs) comprise diverse chemical structures and bioactivities highly relevant for research and industry[1]. They are synthesized by adjacent and co-expressed genes called biosynthetic gene clusters (BGCs). The majority of BGCs are considered orphan since they lack a corresponding metabolite, meaning that most of CNPs are still to be discovered[2]. Genetic engineering can be used to deorphanize various bacterial BGCs, yet most cyanobacteria are not genetically tractable; therefore, heterologous expression is a good alternative to reach this goal[3-5]. At CIIMAR, LEGEcc is a culture collection of over 1,100 cyanobacterial strains, representing a notable biological source of novel chemistry. Through heterologous expression, this work aims to deorphanize BGCs from LEGEcc strains and elucidate new CNPs. **Methods:** AntiSMASH analysis was performed to predict BGCs from LEGEcc strains. Target cyanobacteria were cultivated, and their gDNA extracted. BGCs were amplified and cloned in replicative vectors. These were then transformed into *E. coli* and into the model cyanobacterium *Anabaena* sp. PCC 7120 for heterologous expression. Organic extracts from the expressing cells were analyzed by LC-MS. **Results:** Three BGCs from LEGEcc strains were predicted to encode a polybrominated diphenyl ether, a ribosomally-synthesized and posttranslationally modified peptide, and an indole. Their correct assembly into vectors was verified by sequencing and their transformation into the hosts was confirmed by PCR. The expression of BGCs and GFP (a reporter) was induced and GFP signal was detected. Comparative metabolomic analysis of the organic extracts is ongoing to identify relevant masses. **Conclusions:** Biotechnological tools were applied to heterologously express BGCs with putative chemical novelty, showing a suitable and promising approach to deorphanize BGCs from LEGEcc strains or from other culture collections to expand knowledge in cyanobacterial chemistry.

Keywords: cyanobacterial natural products; biosynthetic gene clusters; heterologous expression

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24683 | Seasonal variation outweighs spatial variation in Mediterranean lotic water mite communities (Acari, Hydrachnidia)

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Background & Aim: Water mites (Hydrachnidia) are among the most abundant freshwater arthropods, inhabiting most types of water bodies[1]. Water mite communities in Mediterranean rivers are poorly studied, despite their high diversity and importance as bioindicators [2]. This study aims to assess how water mite specimen abundance, genus richness, diversity and community composition vary across seasonal and spatial factors (season, river and elevation), while testing if the observed patterns reflect community-wide or taxon-specific variation. **Methods:** Water mite communities were sampled in spring, summer, and fall across three rivers (Alva, Mondego and Zêzere) in the Serra da Estrela region, in Portugal. Four sites were sampled per river across an elevational gradient from 100 to 1,100 m a.s.l. Adults were morphologically identified to genus level. Abundance and genus richness were modelled using generalised linear models, which included rivers, seasons and altitude as explanatory variables. Diversity (Hill numbers) per river and season was estimated using coverage-based standardisation. Community composition was analysed using multivariate generalised linear models with the same variables used in the GLM. **Results:** In total, 7,309 adult water mites were collected. While some specimens are still pending final identification, 30 genera were already found. Abundance and richness varied seasonally, peaking in summer/fall. While abundance did not vary significantly between rivers or altitude, genus richness was higher in Mondego than in Zêzere, with Alva having intermediate richness. Community composition varied with season, river and elevation, with these variations being driven by only a few genera. **Conclusions:** These results show that, in the Mediterranean rivers studied, water mite community composition varies mostly seasonally, but also spatially. They also indicate that water mite genera within communities do not vary consistently; instead, a few genera drive the observed variation.

Keywords: Freshwater ecology, Mountain rivers, Portugal, Species Abundance, Genus Richness.

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24665 | Tailoring *Ulva* spp. composition through nutrient stressing

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Background & Aim: *Ulva* spp. are a valuable source of bioactive compounds with biostimulant properties that can be used in environmentally friendly products to improve agricultural production[1]. Nutrient availability affects *Ulva* sp. biochemical composition[2,3]; by manipulating it, polysaccharide, phenolic compound and pigment contents can increase, enhancing antioxidant capacity. This work aims to optimise *Ulva* spp. cultivation to increase polysaccharides and phenolic compounds, tailoring the biomass for agricultural applications. **Methods:** *Ulva* sp., collected on a rocky shore in northern Portugal (Amorosa, 41.641470N, -8.823320W), was cultivated using Provasoli-enriched seawater at 18°C, light intensity of 80 µmol photons m^{-2} s^{-1}, 16:8 (L:D) photoperiod and 3 gL^{-1} stocking density. After 1 week of optimal conditions, nutrient stress was applied by renewing the seawater without nutrient addition. Biomass growth and photosynthetic efficiency[4] was measured at the start of the stress, and after 12, 24, 48 and 96 h. Samples from each timepoint were dried and analysed for polysaccharides[5,7], proteins[6], pigments and phenolic compounds[7]. **Results:** Growth rate was 8.5±0.4 $\%d^{-1}$ during optimal conditions. At the end of the stress period, growth rate was 8.4±0.4 $\%d^{-1}$, while control cultures grew 9.9±1.1 $\%d^{-1}$. Maximum Electron Transport Rate decreased during stressing, averaging 37.5±8.9 µmol e^{-1} m^{-2} s^{-1}, whereas controls averaged 67.3±10.3 µmol e^{-1} m^{-2} s^{-1}. During this period, the biomass became lighter in colour, but showed no deterioration. No clear polysaccharides variation was observed, averaging 27.3±1.4%. Variation in protein, pigments and phenolic compounds content, as well as the further increase with prolonged stress periods, will be presented and discussed. **Conclusions:** This work evaluated *Ulva* spp. potential as a source of compounds with biostimulant properties. Further work will build on these results to optimize cultivation conditions for tailored biomass for agricultural uses.

Keywords: *Ulva* spp., Biostimulant, PAM, Bioactive compounds, Polysaccharides

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24477 | Cyanobacteria as a new eco-friendly platform for oral antigen delivery and fish vaccination

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Background & Aim: With the growth of aquaculture, the importance of promoting safe and effective strategies for the development of aquatic animals has also increased. To warrant fish wellbeing, it is essential that we find sustainable solutions to protect them from disease outbursts. One of the most efficient solutions is vaccination, either by injection, immersion or oral route, with the latter being the most preferred by producers. Advances in the understanding of cyanobacterial molecular and cell biology have placed them at the forefront of biotechnology[1]. The continuous development of bioengineering makes it possible to create cyanobacteria that are capable of producing heterologous proteins that may function as immune system modulators[2]. With this project, our objective is to test cyanobacterial cells as a biotechnological tool in fish, with applications in the delivery of proteins/antigens, to stimulate specific adaptive immune responses. **Methods:** To this end, we are using standard molecular and synthetic biology techniques, including PCR amplification, cloning, DNA sequencing, bacterial transformation, Western blotting, biomass cultivation and oral immunization of fish with cyanobacterial cells. **Results:** Genes from the pathogenic bacterium *Vibrio* spp., encoding proteins known as potent antigens, were amplified from genomic DNA and cloned into suitable plasmids. Constructs have been transferred to *Synechocystis* sp. PCC 6803, and heterologous protein expression is presently being carried out. Next, genetically modified cyanobacterial cells will be used to feed zebrafish as a model, and to assess the effectiveness of said cells we will test their ability to confer protection against infection through bacterial challenge assays[3]. **Conclusions:** This work tries to expand the toolbox for antigen delivery to aquatic animals, making use of microbial photosynthetic systems, particularly cyanobacteria, as feed or dietary supplements, while serving as an oral antigen delivery platform.

Keywords: Microbial Biotechnology; Cyanobacteria; Vaccine; Aquaculture

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24486 Environmental gradients determining the distribution of intertidal organisms at small scales

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Background & Aim: Rocky intertidal ecosystems are exposed to strong thermal and hydrodynamic variability driven by tidal cycles. Small-scale differences in surface orientation and inclination create pronounced microclimatic heterogeneity, potentially shaping the distribution of sessile invertebrates. During emersion, organisms experience rapid fluctuations in temperature and wave action, often operating near their physiological limits. This study aims to determine how centimetre-scale environmental gradients influence the spatial distribution of sessile species, and whether surface orientation (north- vs south-facing) and inclination drive differences in presence and abundance. **Methods:** Sixty 25×25 cm quadrats were established on three rocky shores along the northwestern Portuguese coast, equally distributed between north- and south-facing surfaces with a 45 ± 10° inclination. Quadrat vertices were permanently marked with screws and a numbered washer, and equipped with temperature and pressure loggers recording hourly data. High-resolution images were collected monthly and processed using photogrammetry tools for organism identification. Downloaded thermal profiles were analysed in R to determine the precise conditions experienced by organisms. **Results:** Temporal analyses revealed clear variability in biological cover at the quadrat scale across all shores. Monthly changes were heterogeneous, with some quadrats showing marked shifts while others remaining relatively stable. Analysis of thermal profiles revealed pronounced thermal heterogeneity between orientations, with south-facing surfaces displaying higher thermal stress potential. **Conclusions:** Our preliminary results show that topographic complexity drives biodiversity patterns even at the centimetre scale, showing biological cover changes over small temporal scales across many quadrats and shores. These findings highlight the importance of considering small-scale dynamics when studying the effects of thermal stress in intertidal communities.

Keywords: Intertidal; Sessile species; Logger; Photogrammetry; Spatial heterogeneity

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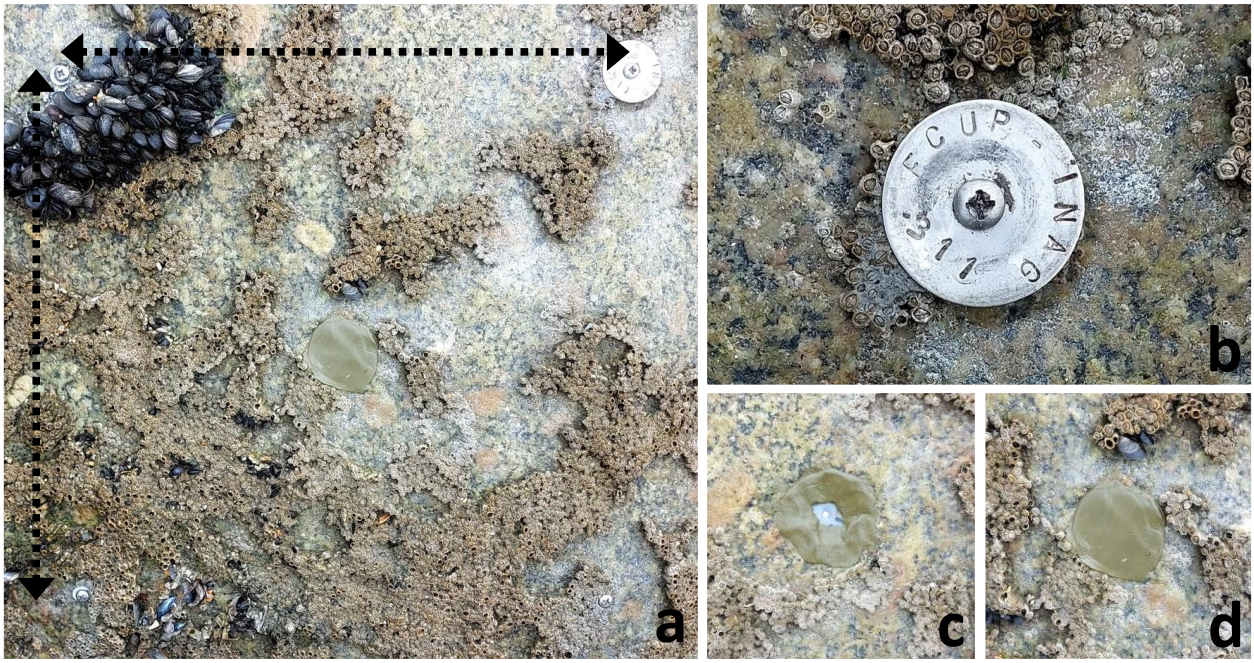

Interplay Between Geographic Variation, Tidal Cycles and Microhabitat Temperatures. *Frontiers in Marine Science*, *7*.

Figure 1: Installation and setup of a sampling area: (a) 25×25cm square (black arrows) permanently marked with stainless steel screws at each corner and a numbered washer affixed on the top right screw; (b) close-up of the identification washer on the top-right corner; (c) close-up of a temperature and pressure logger (EnvLogger TP1.1), secured with marine-grade epoxy resin inside of a shallow hole drilled into the rock substrate; and (d) close-up of a temperature-only logger (EnvLogger T7.3), secured following the same method.

24506 | Does testosterone drive impulsivity? A case study on the common waxbill, *Estrilda astrild*

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Background & Aim: Impulsivity, defined as the tendency to act rapidly, disregarding potential risks or reduced rewards [1], is a key component of decision-making and can have important consequences for survival and fitness. While studies in mammals suggest that testosterone can modulate impulsive behaviour (e.g. [2-4]), this relation remains poorly understood in birds. To address this gap, we experimentally manipulated testosterone levels in a bird model to test the hypothesis that elevated testosterone increases impulsive behavior. **Methods:** We used 24 common waxbills (*Estrilda astrild*; 12 females and 12 males; figure 1), a highly social species [5] that presents hormone-dependent traits [6]. Impulsivity was assessed using a detour-reaching task, in which individuals must inhibit a direct approach to visible food behind a transparent barrier and instead detour around it. More impulsive individuals attempt to reach the food directly through the barrier, while those that successfully detour demonstrate greater inhibitory control. Before the trials, each bird received either a control (saline) or testosterone treatment injection (150µg), with birds tested under both treatments in a balanced order. **Results:** Testosterone treatment significantly reduced detour success compared to control, indicating decreased inhibitory control under elevated testosterone. Sex differences were also observed, with females outperforming males. In addition, performance improved in the second test and was higher earlier in the day. **Conclusions:** Our findings show that experimentally elevated testosterone levels decrease inhibitory control and increase impulsivity in the *Estrilda astrild.* These results extend evidence from mammals to birds and suggest that testosterone may play a role in modulating decision-making processes across vertebrates. Moreover, the observed effects of sex, test order, and time of day highlight the importance of considering both biological and contextual factors when assessing cognitive performance.

Keywords: Cognition; Decision-making; Testosterone; Birds; Detour-reaching test

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24539 | *Uncovering the role of CFAP-36 in cilia and sensory neurons using C. elegans*

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Background & Aim: Cilia are highly specialized organelles that play critical roles in neuronal development, signaling pathways and cognitive functions. Their dysfunction is implicated in a wide range of neurological disorders associated with human ciliopathies. A recent study has shown that CFAP-36 is described as a conserved, cilia-specific adaptor of the intraflagellar transport (IFT), a trafficking system that enables the bidirectional movement of signaling and other molecules along the ciliary axoneme. **Methods:** The aim of this project is to assess the importance of CFAP-36 in the transport of different cargoes and cilia-mediated behaviors. To do so, we used CRISPR-Cas9 to generate a loss-of-function *cfap-36* mutant in *Caenorhabditis elegans*, a genetically tractable model with conserved ciliary architecture and a well-characterized nervous system. Ciliary function was assessed using osmotic avoidance and dye-filling assays, as well as live fluorescence imaging of IFT markers. Additionally, non-ciliary phenotypes were evaluated through liquid locomotion and reproductive output assays. **Results:** Loss of *cfap-36* did not result in prominent alterations in the ciliary parameters analyzed. Mutant animals also exhibited apparently normal cilia-dependent behaviors, comparable to wild-type controls, suggesting that *cfap-36* is not critically required for the functions evaluated. **Conclusions:** Our findings indicate that *cfap-36* is not essential for the maintenance of core ciliary functions in *C. elegans*. These results open the possibility that additional ciliary cargo adaptors may exist and have redundant roles with CFAP-36. Alternatively, CFAP-36 may have very specific roles/cargoes, through interactions with other IFT components or within specific neuronal subtypes. Accordingly, further studies will be required to elucidate the precise molecular function of CFAP-36 within cilia and its contribution to sensory neuron biology.

Keywords: Neuronal cilia, Intraflagellar transport, CFAP-36, *Caenorhabditis elegans*

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24545 | Not all seals detox alike: Species-specific Variability of PXR Activity in Seals

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Background & Aim: Pinnipeds are marine mammals that depend heavily on water and soil for survival, currently exposed to multiple environmental contaminants. Multiple harmful effects of exposure to xenobiotics include endocrine disruption, changes in the immune system, development, and reproduction. Biotransformation acts as a barrier to these substances, promoting their metabolism into less reactive molecules and facilitating excretion from the organism. The Pregnane X receptor (PXR) known as the "master xenobiotic receptor", functions as a sensor that activates the transcription of biotransformation enzymes after binding to ligands. Studies report differences in PXR sensitivity between humans and polar bears, with the latter having a narrower ligand specificity; while in cetaceans, the PXR gene is pseudogenized. The present study aims to evaluate the conservation and functionality of PXR in pinnipeds when exposed to endogenous ligands and environmental contaminates. **Methods:** First, *silico* screening of potential ligands was conducted using comparative homology modelling. We predicted three-dimensional models of the Weddell seal (WD) and the Hawaiian monk seal (HMS), using them as input for and molecular docking assays. Secondly, experimental assays were performed to characterize the ligand binding in target PXR transactivation assays using a Dual-Luciferase Reporter assay system. **Results:** Results show a novel frameshift mutation in the HMS PXR gene affecting the receptor's C-terminal region, which may influence ligand binding. In contrast, the WD PXR does not carry this frameshift and shows a high degree of conservation relative to humans. Transactivation assays reveal differences in activation potential among WD, HMS, and human PXR. **Conclusions:** This study contributes with new findings regarding the susceptibility of pinnipeds to xenobiotics and offers insights into the endogenous detoxification capacity of these species.

Keywords: PXR, pinnipeds, molecular docking, toxicogenomic

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24786 | Development of a noninvasive video-recording system to study the role of microglia in early-life sleep structure

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Background & Aim: Sleep is a conserved physiological state essential to life. While mechanisms controlling adult sleep are well described, sleep is most prevalent in prenatal and postnatal life [1], a critical period of brain development in which sleep is believed to play a key role [2, 3]. Despite this, postnatal sleep still has a lot of secrets that remain unknown. Microglia, the brain immune cells, sense wake/sleep states and regulate adult sleep through sleep-promoting factors [4, 5]. Given their critical role in brain development [6], could they also contribute to the regulation of early-life sleep? This project aims to develop a non-invasive methodology to identify sleep and wake in mouse pups and define the role of microglia in postnatal sleep structure. **Methods:** A noninvasive video-recording system was implemented to register the behavior of an isolated pup and the deep learning tool DeepLabCut tracked the position of 9 body parts over time. Movement amplitude of selected body parts was used to classify behavioral states: atonia defined quiet sleep; atonia with myoclonic twitches defined active sleep; and high-amplitude movements defined wake. Microglia were depleted using PLX5622, a CSF1 receptor inhibitor essential for microglia survival. **Results:** Our video-based tracking system reliably identified periods of wake, quiet and active sleep-in representative videos of each state. In videos of undisturbed and sleep-deprived pups, the system accurately detected that most time was spent in sleep and wake, respectively. Microglia depletion reached 91% at P5 and 89% at P8, demonstrating high depletion efficiency. **Conclusions:** We established a reliable video-based tracking system to detect sleep/wake-like states in mouse pups. Combined with postnatal microglia depletion, it will reveal the role of microglia in early-life sleep. In addition, our system can be applied to study molecular, cellular, and circuit mechanisms regulating early-life sleep, as well as sleep alterations in pathology.

Keywords: Early-life Sleep; Microglia; Brain development.

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24609 | Actinobacteria-Mediated Solid-State Fermentation of Macroalgae for Biotechnological Applications

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Background & Aim: Macroalgae are a promising resource for aquaculture, although their direct incorporation into feeds is limited by suboptimal nutritional profiles and digestibility. Solid-state fermentation (SSF) offers a sustainable strategy to enhance macroalgal biomass through microbial biotransformation, improving its nutritional value while generating added-value metabolites [1]. In this context, marine-derived actinobacteria, particularly *Streptomyces* spp., represent an attractive but still underexplored group of microorganisms for SSF systems, given their well-known capacity to produce diverse bioactive compounds [2]. The present study explores the use of macroalgae-associated actinobacteria in SSF as a biotechnological approach for macroalgal biomass valorization within a circular economy framework. **Methods:** Ten *Streptomyces* strains previously isolated from *Chondrus crispus* and *Codium tomentosum* were individually subjected to SSF of macroalgal substrates for 3 weeks [3]. Based on sporulation intensity and substrate degradation, two strains were selected for 6-week SSF. Fermented biomass was lyophilized and extracted with ethanol or methanol:acetone (1:1). Crude extracts (1 mg/mL) were screened for antimicrobial activity against representative human and fish pathogens using the disc diffusion assay. Antioxidant activity (DPPH assay, phenolic content) and nutritional profiling (fiber, ash, protein content) assessments are ongoing. **Results:** Two strains exhibited enhanced sporulation and substrate degradation during preliminary SSF. Extraction yields varied according to solvent and substrate, with methanol:acetone and *C. tomentosum* providing higher yields. To the moment, no antimicrobial activity was detected under the tested conditions. **Conclusions:** These preliminary findings demonstrate that *Streptomyces* spp. isolated from macroalgae are efficient fermenters of solid macroalgae substrates. Solvent and

substrate type influenced crude extracts recovery efficiency. Ongoing bioactivity assays and compositional analyses will further clarify the potential of SSF as a sustainable strategy for marine biomass valorization in aquaculture applications.

Keywords: Solid-State Fermentation; Marine *Streptomyces*; Macroalgae; Bioactives; Aquafeed Valorization

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24489 | Novel Spastic Paraplegia–Associated *TUBB2A* Variant Affects Egg-laying Behavior in *Caenorhabditis elegans*

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Background & Aim: Hereditary spastic paraplegias (HSPs) are a heterogeneous group of rare neurodegenerative disorders characterized by progressive lower limb spasticity and weakness. Although over 80 genes have been implicated, many HSP cases remain genetically unsolved. Altered microtubule dynamics link HSPs to tubulinopathies, typically associated with neurodevelopmental brain malformations; however, recent reports with spastic phenotypes suggest an expanded clinical spectrum. In our HSP cohort [1], we identified a novel heterozygous *TUBB2A* variant (c.1241A>G; p.Asn414Ser) in a Portuguese family with progressive spastic paraplegia and intellectual disability. Therefore, we aimed to functionally assess its pathogenicity *in vivo*. **Methods:** We generated a *Caenorhabditis elegans* model carrying the orthologous N414S substitution in *tbb-4* via CRISPR/Cas9 microinjection. Mutant animals were functionally characterized using reproductive fitness, development, and lifespan.

Neuronal function was assessed via behavioral assays and fluorescence microscopy. **Results:** Neither the *tbb-4* N414S nor the knock-out strains exhibit locomotion or sensory defects. However, reproductive behavior was impaired, particularly regarding total progeny and egg-laying pattern. These findings align with previous reports showing that *tbb-4* is expressed in the hermaphrodite-specific neuron (HSN) and vulval muscle cells [2], both essential for egg-laying regulation. Notably, heterozygous *tbb-4 N414S* worms produced more offspring than homozygous mutants and wild-type animals, suggesting a possible dosage-dependent or dominant-negative effect, a mechanism implicated in tubulinopathies. Ongoing fluorescence microscopy studies aim to assess HSN motor neuron integrity and exclude gonad/sperm defects contributing to this phenotype. **Conclusions:** These findings provide supportive evidence for the pathogenicity of our heterozygous *TUBB2A* variant associated with HSP, further expanding the phenotypic spectrum of *TUBB2A*-related disorders.

Keywords: *Caenorhabditis elegans*; *TUBB2A*; Hereditary Spastic Paraplegia (HSP); Tubulinopathies; Dominant-negative effect

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24492 | Biomaterials for Modulating Nerve–Bone Crosstalk in Bone Regeneration

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Background & Aim: Although bone has intrinsic regenerative capacity, large defects remain an orthopedic challenge, leading to repeated surgeries and disability [1]. Bone tissue engineering (TE) has advanced with osteoimmunomodulatory biomaterials [2]. However, bone-guided biomaterials frequently overlook innervation in TE. Understanding these interactions requires models integrating biomaterial-driven osteogenesis and neuronal input [3]. Thus, next-generation biomaterials must regulate multicellular crosstalk within the regenerative microenvironment [4]. In this context, kefiran, an exopolysaccharide, has emerged as a candidate for TE due to its physicochemical and biological properties [5]. This study aims to leverage a kefiran-based biomaterial to promote neural integration and modulate nerve-bone crosstalk, enhancing bone regeneration. **Methods:** Kefiran hydrogels at 5 %, 2 % and 1 % (w/v) were assessed for biocompatibility using human osteoblasts (HOBs) and sensory neurons derived from dorsal root ganglia (DRGs). Compartmentalized microfluidic DRG explant cultures exposed axonal terminals to kefiran hydrogels, mimicking bone spatial organization. Neurite outgrowth was quantified using the Axofluidic software [6]. Alkaline phosphatase (ALP) activity in HOB was evaluated at days 7, 14 and 21. Engineered 3D bone microtissues (EBM) were created by encapsulating HOB in kefiran hydrogels and characterized. **Results:** Preliminary results indicate that kefiran-based biomaterials sustain neuronal viability and neurite extension. HOBs cultured within these matrices showed increased metabolic activity and elevated ALP levels at day 7. EBMs displayed enhanced structural integrity and more organized cellular morphology compared to controls (Figure 1). **Conclusions:** These findings highlight kefiran-based biomaterials as a promising platform for promoting neural integration and shaping nerve-bone interactions during bone regeneration. Ongoing studies are examining how EBM influences sensory neuron activity.

Keywords: Bone regeneration; Nerve-bone crosstalk; Microfluidics; Kefiran; Engineered bone microtissues

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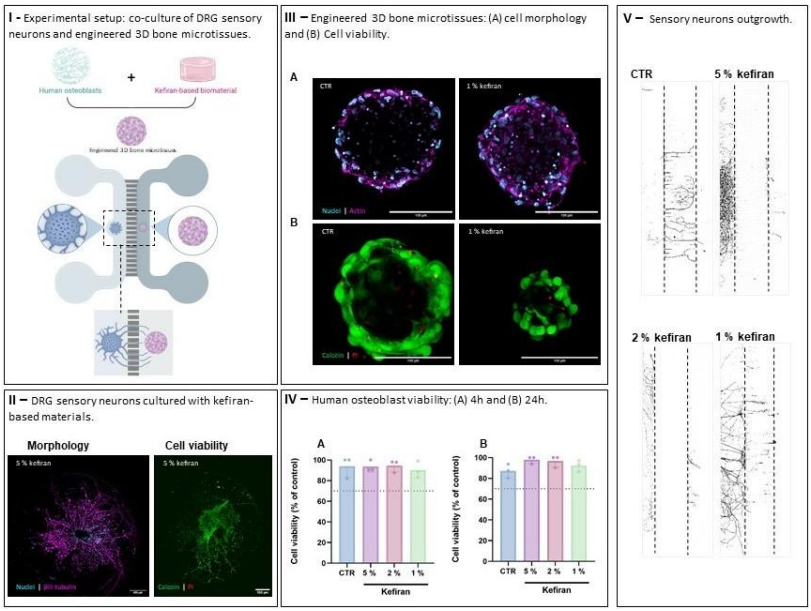

Figure 1: Experimental assessment of kefiran-based biomaterials in neuronal and osteogenic *in vitro* models.

CHEMISTRY

24208 | Influence of Phenyl Position on Electronic Correlation in Phenanthrenes: Experimental and Computational Approach

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Background & Aim: Based on a molecular thermodynamics approach, the primary objective of this work is to experimentally quantify π-electron correlation in phenylphenanthrene derivatives, evaluate its optical and structural manifestations, and investigate its origin[1,2] .These compounds are particularly relevant in the field of organic electronics, where they are widely used as building blocks of organic semiconductors[3], underscoring the importance of a detailed understanding of their electronic properties. **Methods:** The studied compounds were synthesized via the Suzuki–Miyaura cross-coupling reaction. Their thermodynamic properties were investigated using Knudsen effusion combined with a quartz crystal microbalance (KNQ)[4] and differential scanning calorimetry (DSC). In addition, UV-Vis spectroscopy was used to characterize their optical properties. Geometry optimizations and vibrational frequency calculations were carried out using density functional theory (DFT) to complement and support the experimental data. **Results:** DSC successfully characterized the melting and crystallization behavior of all compounds. Knudsen effusion measurements provided vapor pressure data, enabling the derivation of sublimation thermodynamic properties. UV-Vis spectra of the phenanthrene derivatives revealed characteristic electronic absorption bands. Computational results initially provided an evaluation of the molecular energetic at different levels of theory. **Conclusions:** The higher melting temperature and lower volatility of 2-phenylphenanthrene, compared to its isomer 9-phenylphenanthrene, arise from entropic effects associated with positional disorder in the crystal lattice. Two polymorphs were identified for 9-phenylphenanthrene, 2,7-diphenylphenanthrene exhibited crystal-liquid behavior. The UV-Vis absorption bands showed bathochromic shift in the order 9-phenylphenanthrene -> 2-phenylphenanthrene -> 2,7-diphenylphenanthrene, consistent with increasing π conjugation.

Keywords: Phenanthrene derivatives; Thermodynamic properties; Theoretical studies; Electronic correlation.

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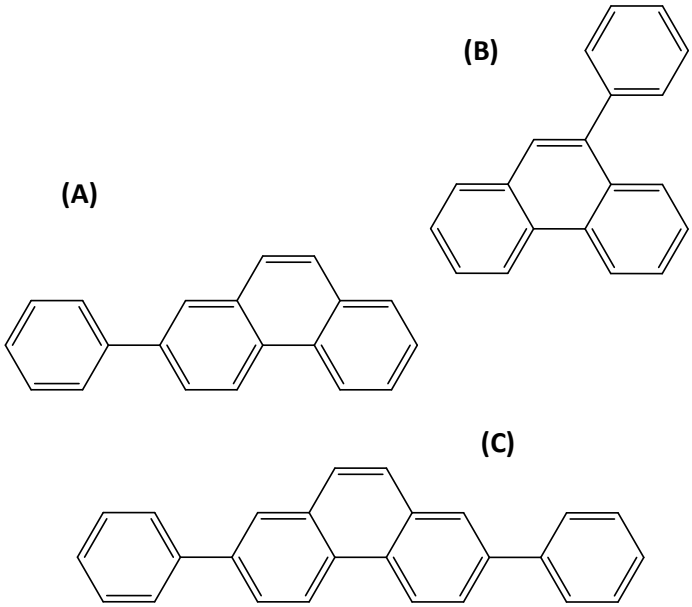

Figure 1: Structure of the phenanthrenes studied: **(A)** 2-Phenylphenanthrene; **(B)** 9-Phenylphenanthrene; **(C)** 2,7-Diphenylphenanthrene.

24260 | Synthetic antifouling agents and somatosensory transient receptor potential (TRP) ion channels

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Background & Aim: One of the biggest problems facing by the maritime sector is marine biofouling, defined as the adhesion of organisms to submerged surfaces. An alternative to traditional coatings made from hazardous metals and biocides is bioinspired antifouling agents. Understanding the mechanism of action (MoA) of new candidates is essential, since eco-friendly antifoulants require robust MoA evidence to progress toward commercialization.[1] Transient Receptor Potential (TRP) channels have been implicated in larval settlement of marine macrofoulers, namely mussels, including surface detection and adhesive release.[2,3] Xanthones patented by our group interfere with settlement behavior in *Mytilus galloprovincialis* mussels, and interestingly, marine 4-substituted xanthones (chrexanthomycins) have recently been described as human TRPV1 inhibitors. [4-6] These observations led us to hypothesize that our synthetic xanthones could act by modulating TRP channels. The aim of this work was to synthesize 4-hydroxyxanthone and 4-substituted derivatives as simplified analogues and evaluate their ability to modulate TRPV1 channels, as long as a small in-house library of antifouling xanthones, exploring structure-activity relationships. **Methods:** 4-Hydroxyxanthone was prepared through an optimized diaryl-ether route and derivatized at C-4 position to afford nine analogues, characterized by IR and NMR techniques. A Ca^{2+}-fluorescence assay was established in HEK293T cells expressing human TRPV1 to investigate how synthetic compounds modulate TRPV1 channel activity: agonism and antagonism assays, using capsaicin and capsazepine as positive controls. **Results:** The assays showed that four derivatives exhibited antagonistic behavior, reducing the TRPV1 Ca^{2+} signal evoked by capsaicin, similarly to capsazepine. None of the tested compounds exhibited agonistic behavior like the control capsaicin. **Conclusions:** 4-substituted xanthones can antagonize TRPV1, supporting TRP-channel modulation as a plausible antifouling MoA. In the future, this approach will be extended to mussel TRP channels and used to correlate TRP antagonism with settlement inhibition.

Keywords: TRP, Xanthones, Antifouling

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UID/04423/2025, UID/PRR/04423/2025 and LA/P/0101/2020, and under the scope of the project PTDC/CTA-AMB/0853/2021. Bruno da Silva Martins Loureiro received financial support from Fundação Amadeu Dias or Soja de Portugal under the BYTplus program of CIIMAR – 6th edition of BYTplus / 2024-2025.

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24302 | Synthesis of Peptides by Solid-Phase Approach and Solution Macrocyclization

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Background & Aim: Linear tetrapeptides, owing to their structural flexibility, can adopt multiple conformations and interact with diverse biological targets. These compounds are not only relevant in the pharmaceutical field; several other industries already use peptides in their daily operations. In addition, they are important precursors for the synthesis of cyclic derivatives [1]. Due to the potential and high demand of this class of compounds, peptide chemistry has made remarkable strides in recent decades. Solid-phase peptide synthesis (SPPS) is currently the most widely used method for peptide synthesis and offers several advantages [2]. For macrocyclization, several synthetic approaches have also been explored in recent years [1,2]. This work describes the synthetic route of a series of linear tetrapeptides. The cyclization of one tetrapeptide was also performed. **Methods:** Tetrapeptides were synthesized by SPPS through repeated cycles of coupling, deprotection, and washing until the peptide sequence was fully assembled. The structures of the synthesized tetrapeptides were established by IR, ^{1}H and ^{13}C NMR, and HRMS. The strategy for achieving peptide cyclization was the conventional method using a coupling agent. **Results:** SPPS afforded the desired peptides with yields of 56.70% - 86.86% and simplified purification. The spectroscopic data corresponded to the proposed structures. The cyclization of one synthesized peptide was performed, and the cyclic peptide was characterized using the LC-HRMS technique. **Conclusions:** The synthesis and structure elucidation of tetrapeptides were successfully achieved. One cyclic peptide was also synthesized by conventional cyclisation, but due to difficulties in purification and the low quantity obtained, other cyclisation methodologies must be explored in future work. Moreover, the biological activity of the synthesized peptides will be evaluated, and they will be used as building blocks to obtain other derivatives.

Keywords: cyclic peptide; macrocyclization; solid-phase peptide synthesis; tetrapeptide

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24307 | Synthesis of Chitosan Derivatives via Schiff base reaction and click chemistry

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Background & Aim: Chitosan, a linear polysaccharide obtained from the deacetylation of chitin, possesses reactive amino and hydroxyl groups that allow chemical modifications, serving as a platform for the development of advanced materials. Among the available approaches, regioselective and quantitative functionalization stands out for enabling the production of well-characterized derivatives [1]. Schiff base reactions, which rely on the condensation of aldehydes or ketones with primary amines to yield imines, represent a straightforward and efficient synthetic approach due to their versatility and mild conditions [2]. Moreover, click chemistry is an efficient, selective, and high-yielding method for the modular synthesis of complex polymers under mild conditions, paving the way for new compounds with promising applications [3]. The aim of this work is to obtain promising chitosan derivatives using both synthetic strategies. **Methods:** A xanthone derivative was obtained by the synthesis of a benzophenone intermediate followed by acylation via microwave-assisted reaction. Chitosan–xanthone derivatives were synthesized via Schiff base reactions or click chemistry, two synthetic strategies that offer distinct structural and functional possibilities. Four reaction steps were performed to obtain the click-derivative: protection of chitosan amino groups, substitution of OH by an electron-withdrawing group and then by an azide group, and finally the coupling reaction with a suitable functionalized xanthone. The structures of the xanthone and chitosan derivatives were established by spectroscopic methods. **Results:** Two chitosan–xanthone derivatives were obtained via multistep synthetic procedures involving either a Schiff base reaction or click chemistry. All structures were confirmed by spectroscopic methods. **Conclusions:** The synthesis, purification, and structural analysis were successfully achieved. Both strategies yielded promising results in the synthesis of the target compounds. Future studies will focus on evaluating their biological activity

Keywords: chitosan; xanthone derivative; Schiff base; click chemistry

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24337 | Innovative Strategies Against Antimicrobial Resistance: RNA Riboswitches Binders as Novel Drug Discovery Platforms

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Background & Aim: With the rise of antimicrobial resistance (AMR), antibiotics are increasingly losing effectiveness against several bacterial infections. There is an urgent need for innovative antibacterial agents with new mechanisms of action (MoAs) 1,2. Nevertheless, one of the major hurdles in the discovery and development of new antibacterials is identifying first-in-class molecules that operate through unique MoAs. Therefore, this work has its main goal: the discovery and development of new small molecules to explore new antibacterial therapeutic targets, namely non-coding RNA structures called riboswitches[3,4]. **Methods:** This work involves tailoring known antibacterial scaffolds with fragments that may bind to non-coding RNAs, potentially yielding dual-target antibacterial agents. In this context, sulfur- and nitrogen-containing heterocycles were introduced onto the ciprofloxacin scaffold via esterification, amidation, and Suzuki reactions to attain new ciprofloxacin derivatives, as exemplified in Figure 1. **Results:** To date, a small set of these compounds, namely those with modifications at position 3 of the fluoroquinolone core, has been successfully synthesized and is currently undergoing phenotypic evaluation of antibacterial activity against Gram-positive and Gram-negative bacteria using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. **Conclusions:** The designed and synthesized compounds exhibit potential antibacterial activity, representing a promising advance in the fight against AMR.

Keywords: Antibacterial resistance (AMR); RNA Riboswitch; Ciprofloxacin; Heterocycle

Acknowledgments:

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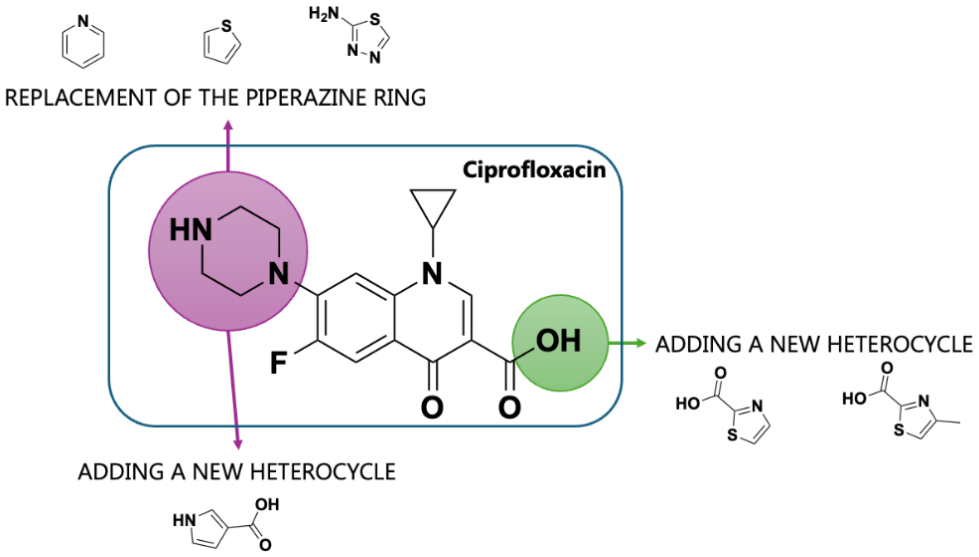

Figure 1: Rational Design of new antibacterials based on ciprofloxacin scaffold

24350 | Physicochemical characterization of new CBD-based terpenyl-*N*-acyl-aryl-hydrazones

Catarina Ribeiro[1]; Graziella do Reis Rosa Franco[2]; Isabella Marie Fernandes[2]; Vanessa Silva Gontijo[2]; Núbia Tuão Korch[2]; Laiane Santos Mota Tavares[2]; Sara Cravo[1,3]; Cláudio Viegas Jr.[2]; Maria Emília Sousa[1,3]

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Understanding the physicochemical properties of new chemical entities is a critical step in early drug development, because it influences formulation design, bioavailability, and therapeutic efficacy. Solubility and stability are, among others, key parameters that determine whether a candidate compound can progress or not. In this context, the present work is focused on the preformulation characterization studies of six lead compounds, new cannabidiol (CBD)-based terpenyl-*N*-acyl-aryl-hydrazones with anti-nociceptive and anti-inflammatory effects (**Figure 1**), aiming to evaluate their solubility and stability profiles. Analyses were conducted by HPLC (High Performance Liquid Chromatography). The kinetic solubility of the compounds was studied using the shake-flask method. Stability was evaluated by monitoring the compounds at two different temperatures over a period of 45 days. Results showed that solubility varied among the compounds, but none of these showed high solubility in water or phosphate-buffered saline (PBS). The most soluble compounds exhibited moderate solubility. Regarding stability, all the investigated compounds underwent degradation over time in both conditions, and further studies are required to clarify this aspect. Nonetheless, the majority were found to be stable for the period of 8 h at 23°C. This study provided valuable insights into the physicochemical behavior of new CBD-based terpenyl-*N*-acyl-aryl-hydrazones leads [1], and small structural variations were found to affect solubility and stability in this series of compounds. It also calls for future optimization, contributing to the selection and development of promising drug candidates with improved pharmacokinetic profiles.

Keywords: lead compounds; solubility; stability; HPLC; cannabidiol derivatives.

Acknowledgments: This research was supported by national funds through FCT within the scope of Base Funding UIDB/04423/2020 and UIDP/04423/2020 (Group of Natural Products and Medicinal Chemistry - CIIMAR).

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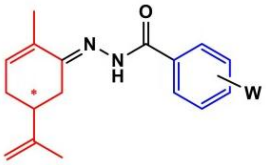

Figure 1: General structure of the compounds investigated.

24411 | Routine Quality Control and NIR Method Development for Isocyanate Determination in Paints and Coatings Industry

Caetano, Bárbara[1]; Machado, João[1;] Silva, Elisabete[1]; Lopes, Vasco[1]

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Background & Aim: Ensuring excellence in industrial products quality control is essential, and the coatings and paints industry is no exception. For both raw materials and finished products, this control can be ensured through a diversity of routine tests and analytical tecnhiques, including Near-Infrared Spectroscopy, High-Performance Liquid Chromatography, Fourier Transform Infrared Spectroscopy and X-ray Fluorescence [1-3]. Specifically, NIR stands out due to its rapid response, low sample preparation, and suitable for routine industrial analyses. In this context, this work also focuses on developing a quantative NIR method for the determination of isocyanate content in raw materials [3]. A reference method based on ISO 11909:2025 was used to obtain isocyanate contents, which were subsequently applied in the development and validation of a NIR calibration method [4]. This approach aims to provide a rapid reliable alternative for isocyanate content determination, reducing analysis time and reagents in quality control and recontrol processes. **Methods:** Polisocyanate samples react with an excess of dybutilamine, and the unreacted amine is subsequently titrated with hydrochloridric acid, using bromophenol blue as indicator. In parallel, NIR spectra are aquired for each sample. The collected spectra are processed using chemometric software to develop calibration models for the quantitative determination of isocyanate content. **Results:**A PLS regression model that best fits the purpose employed, as pre-treatment process, baseline correction and derivate. The selected spectral regions were 6172-7413 cm^{-1}, 4629-377 cm^{-1} and 8062-8872cm^{-1}. Using eight latent factors, the model achieved an R^2 of 0,9987 and an RMSEP of 0,1623. **Conclusions:** The developed PLS model demonstrated promising predictive performance for isocyanate content determination. However, the limited number of samples used for calibration highlights the need for continued spectral acquisition to improve model robustness and reliability.

Keywords: Coatings and Paints, Quality control, Near-Infrared Spectroscopy, Isocyanate

Acknowledgments: The authors acknowledge CIN – Corporação Industrial do Norte, S.A for providing the facilities and technical support that enabled this work.

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24351 | Innovative 3D-printed colorimetric sensors for rapid evaluation of bioactive compounds

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Background & Aim: Antioxidants are an important class of bioactive compounds due to their ability to quench free radicals and prevent oxidative damage. Several methods are available to determine antioxidant activity, and the ABTS radical assay is one of the most widely used. However, this assay presents some limitations, including time-dependent results, susceptibility to spectral interference, and a lack of standardization [1]. This work explores an innovative strategy to address these issues by using Polymerizable Deep Eutectic Solvents (PDES) to produce low-cost 3D-printed structures that can be applied in the development of a colorimetric sensor for evaluating antioxidant activity [2,3]. **Methods:** PDES resin was prepared by mixing under heating an hydrogen bond acceptor and a photocurable hydrogen bond donor in a fixed molar ratio, then combined with a photoinitiator and a cross-linker agent. Then, the fibers were washed in 2-propanol and subjected to post-printing curing by UV light. Next, they were impregnated with ABTS radical solution and tested at different Trolox concentrations in 1% ethanol and compared with a blank solution (1% ethanol). Detection was performed by capturing images with a smartphone over time while the fiber was immersed in the solution, until the colour disappeared, followed by analysis of RGB variations. **Results:** This work, which is still under development, has demonstrated promising results, as the 3D-printed sensors were observed to react with solutions containing antioxidants, producing a visible color change within a few minutes. **Conclusions:** This work shows potential for developing colorimetric sensors using 3D printing, offering reduced spectral interference and improved standardization. At the same time, PDES represents an environmentally sustainable source for polymer production.

Keywords: 3D printing; Antioxidant Activity; Polymerizable Deep Eutectic Solvents; Colorimetric sensors; Functional Materials.

Acknowledgments: This work received support from Portugal national funds (FCT/MECI, Fundação para a Ciência e a Tecnologia and Ministério da Educação, Ciência e Inovação) through

the project UID/50006/2025 DOI 10.54499/UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

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Routine Quality Control and NIR Method Development for Isocyanate Determination in Paints and Coatings Industry

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Background & Aim: Ensuring excellence in industrial products quality control is essential, and the coatings and paints industry is no exception. For both raw materials and finished products, this control can be ensured through a diversity of routine tests and analytical tecnhiques, including Near-Infrared Spectroscopy, High-Performance Liquid Chromatography, Fourier Transform Infrared Spectroscopy and X-ray Fluorescence [1-3]. Specifically, NIR stands out due to its rapid response, low sample preparation, and suitable for routine industrial analyses. In this context, this work also focuses on developing a quantative NIR method for the determination of isocyanate content in raw materials [3]. A reference method based on ISO 11909:2025 was used to obtain isocyanate contents, which were subsequently applied in the development and validation of a NIR calibration method [4]. This approach aims to provide a rapid reliable alternative for isocyanate content determination, reducing analysis time and reagents in quality control and recontrol processes. **Methods:**Polisocyanate samples react with an excess of dybutilamine, and the unreacted amine is subsequently titrated with hydrochloridric acid, using bromophenol blue as indicator. In parallel, NIR spectra are aquired for each sample. The collected spectra are processed using chemometric software to develop calibration models for the quantitative determination of isocyanate content. **Results:**A PLS regression model that best fits the purpose employed, as pre-treatment process, baseline correction and derivate. The selected spectral regions were 6172-7413 cm^{-1}, 4629-377 cm^{-1} and 8062-8872cm^{-1}. Using eight latent factors, the model achieved an R^2 of 0,9987 and an RMSEP of 0,1623. **Conclusions:**The developed PLS model demonstrated promising predictive performance for isocyanate content determination. However, the limited number of samples used for calibration highlights the need for continued spectral acquisition to improve model robustness and reliability.

Keywords: Coatings and Paints, Quality control, Near-Infrared Spectroscopy, Isocyanate

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24445 | Vitamin B12-Enriched Plant Sprouts as Novel Functional Food products to Reach Nutrient Needs Vitamin B12 encapsulation and evaluate their potential for the biofortification of alfalfa sprouts

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Background & Aim: Vitamin B12 deficiency is a major concern in plant-based diets due to its low natural availability in vegetal foods and limited absorption from conventional supplements [1]. Biofortification of edible sprouts using nanotechnology represents a sustainable and innovative strategy to improve micronutrient intake [2, 3]. This work aimed to develop lipid nanoparticles for vitamin B12 encapsulation and evaluate their potential for the biofortification of alfalfa sprouts. **Methods:** Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), with and without vitamin B12, were produced and characterized regarding particle size, polydispersity index, zeta potential, encapsulation efficiency and loading capacity. Stability was assessed over 8 weeks at room temperature, and release studies were performed at different pH. Alfalfa seeds were incubated with B12-loaded nanoparticles, followed by a 7-day germination period and evaluation of growth parameters and vitamin extraction. **Results:** Both nanoparticle systems showed nanoscale size, low polydispersity and high encapsulation efficiency, with good stability over time, particularly for SLN-B12. Release assays showed a controlled and progressive release of vitamin B12 under simulated digestive conditions. Germination assays revealed no significant differences in sprout growth, fresh weight, dry weight or length between treatments and control, indicating good compatibility with plant development. The analysis of extracts showed B12 retention by the sprouts. **Conclusions:** Lipid nanoparticles, especially SLN, proved to be stable and effective carriers for vitamin B12 delivery and suitable for the biofortification of alfalfa sprouts without affecting plant development. This strategy may contribute to the development of functional plant-based foods to help address vitamin B12 deficiency.

Keywords: Vitamin B12; Nanoparticles; Biofortification; Alfalfa sprouts; Functional foods

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Figure 1: Alfalfa sprouts grown with the vitamin B12-loaded nanoparticles.

24460 | Structure-Guided Evaluation of Lithocholic Acid-Based Compounds for Breast Cancer Therapy

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Background & Aim: Breast cancer remains one of the leading causes of cancer-related mortality worldwide.[1] Despite significant therapeutic advances, effective treatment options remain limited, particularly for aggressive subtypes such as triple-negative breast cancer (TNBC). The vitamin D receptor (VDR) has emerged as a promising molecular target in hyperproliferative diseases.[2,3] Although the clinical application of its primary endogenous ligand, calcitriol, is restricted due to hypercalcaemic side effects.[2,3] Lithocholic acid (LCA), identified as an alternative VDR modulator with antiproliferative properties and a more favourable calcaemic profile, represents a promising scaffold for the development of safer VDR-targeted therapies.[4] **Methods**: A focused library of rationally designed LCA-derived compounds was generated using a structure-guided approach supported by molecular modelling studies to optimise VDR binding interactions. Selected candidates were synthesised through strategic chemical modifications of the parent scaffold. Their antiproliferative potential was evaluated in hormone-responsive and TNBC cell lines. **Results**: *In silico* studies supported the selection of compounds with improved predicted VDR binding scores (139.5-161.2%) compared with LCA (100%). The synthesised derivatives were obtained with high chemical purity (> 95%) and subjected to biological evaluation. Several compounds demonstrated relevant antiproliferative effects in breast cancer cell models, including aggressive triple-negative cells, showing encouraging activity when compared with reference therapeutic agents. **Conclusions**: Structure-guided optimisation of the LCA scaffold enabled the identification of novel derivatives with improved predicted interaction profiles toward VDR and promising antiproliferative activity in breast cancer models. These findings support further investigation of LCA-based compounds as potential VDR-targeted therapeutic candidates.

Keywords: Breast Cancer; Calcitriol; Lithocholic Acid; Vitamin D Receptor.

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24483 | From waste PET to efficient functional MOFs for the capture and conversion of CO_2

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Background & Aim: The rapid accumulation of plastic waste has become a critical environmental issue, resulting in persistent plastic residues across ecosystems. Polyethylene terephthalate (PET) bottles residues pose a critical ecological concern due to limited recycling feasibility once dispersed in environment. Chemical hydrolysis of PET waste offers a viable strategy for material recovery, enabling the extraction of terephthalic acid (TPA)[1], which can subsequently serve as a ligand in the construction of porous metal-organic frameworks (MOFs)[2], [3]. On the other hand, CO_2 is the most important greenhouse gas subject to over-emission due to human activity, having significant impact on climate changes. The main objective of this research is to study the hydrolytic depolymerization of PET residues under environmentally benign and sustainable conditions, emphasizing the use of green solvents and energy-efficient methodologies. The recovered TPA is subsequently used in the development of MOFs later employed as a recyclable catalyst for the direct valorization of CO_2 through its capture and conversion into value-added products (Figure 1.). **Methods & Results:** The basic hydrolysis of PET bottles was studied under a 3-hour ohmic heating procedure, using a NaOH solution (1:1 water: ethanol), subject to a 2nd hydrolysis, with an overall conversion of 64% of PET residue into TPA. The synthesis of MOF-5 is studied using a solvothermal method employing TPA extracted from PET waste and commercial. Both porous structures were characterized, via PXRD, FTIR, and TGA, confirming the preparation of the desired MOF structure. The CO_2 adsorption capability of the MOFs is under evaluation, and their catalytic performance in the conversion of CO_2 in added value products has been investigated. **Conclusions:** The present study addresses both the global issue of plastic waste and greenhouse gas as means to extract TPA for MOF synthesis, and subsequent valorization of CO_2 and its value-added products.

Keywords: PET Hydrolysis; TPA recovery; Porous MOFs; Eco-MOF; CO_2 mitigation; CO_2 valorization.

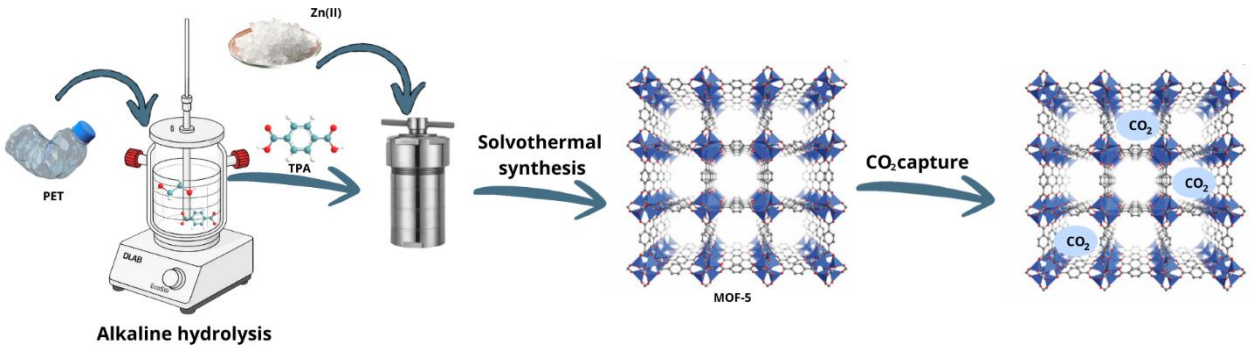

Figure 1: Schematic of the approach for upcycling PET waste into porous MOF structures and its high potential of capture for effective catalytic application.

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24488 | Synthesis and structure elucidation of nor-methylone for further cytotoxicity assessment

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Background & Aim: Methylone is a synthetic cathinone, part of one of the largest groups of new psychoactive substances (NPS). Being β-keto analogs of amphetamines, synthetic cathinones are widely abused due to their psychostimulant properties [1]. The biotransformation of these NPS yields metabolites that may contribute to their overall biological effects or toxicity [2]. The metabolism of methylone has been reported in literature involving pathways such as *O*-demethylenation, *N*-demethylation, β-keto reduction [3]. However, studies on the effects of these metabolites are still limited. The aims of this work were the synthesis and structure elucidation of one of the major metabolites of methylone, which results from the *N*-demethylation of the parent compound. **Methods:** The synthetic plan to obtain nor-methylone involved five steps starting with a Grignard reaction from piperonal followed by an oxidation to the corresponding ketone. The third step involved bromination of the α-carbon followed by a substitution with an azide group. At the end, hydrogenation was performed to achieve nor-methylone. The structures of the intermediates and the final product were elucidated by spectroscopic methods. **Results:** The synthesis of nor-methylone was successfully achieved in five synthetic steps, using commercially available piperonal as the starting material. The synthetic steps were performed with yields of 48-92%. ^{1}H-NMR, ^{13}C-NMR and IR were used to confirm the structure of all intermediates and the final product. **Conclusions:** Nor-methylone and intermediates were successfully synthetized with high yields and high purity. As future work, the metabolite will be evaluated for its cytotoxicity to compare with the parent compound and understand its contribution to methylone's toxicity.

Keywords: Metabolism; Metabolites; Methylone; Nor-methylone; Synthetic cathinones

Acknowledgments:

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24525 | Amino Acid Signatures as Origin Tracers in *Trachurus trachurus*: A Machine Learning Approach

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Background & Aim: Seafood fraud threatens consumer trust and sustainable fisheries [1]. A survey (n=102) revealed high fraud awareness (68%) but low detection confidence (56%), underscoring the need for robust verification. While spectroscopic/elemental methods are established [2-4], amino acid (AA) profiling remains underexplored for origin authentication. This study investigates whether AA profiles can reliably discriminate Atlantic horse mackerel (*Trachurus trachurus*) harvested along the Portuguese coast using interpretable machine learning. **Methods:** Muscle from 150 specimens (n=30/location) was collected from five ports (Matosinhos, Peniche, Sesimbra, Sines, Olhão). After n-hexane defatting, samples were hydrolyzed with 4 M methanesulfonic acid/tryptamine under N_2 purge. Seventeen AAs were quantified by HPLC following OPA/FMOC derivatization. Fat content (FC) was determined gravimetrically and crude protein by Kjeldahl. Discriminant models (LDA, QDA, RDA, SDA, PCA-LDA) were assessed using repeated 5-fold cross-validation (100 blind train/test partitions). **Results:** A significant FC gradient was observed (13.5% Peniche–18.2% Sines dry weight; ANOVA: $F(4,145)=24.26$, $p<0.001$; ANCOVA: $F(4,143)=11.06$, $p<0.001$). However, FC reflects triacylglycerols and seasonal variations, limiting its suitability as an origin marker. Crude protein ranged 76.2–80.2%. Linear Discriminant Analysis (LDA) achieved the best performance: 84% classification efficiency across 100 blind test simulations. The most informative AAs were tyrosine, valine, glutamic acid, isoleucine, and alanine. **Conclusions:** AA profiles provide a robust biochemical fingerprint for geographical authentication. Achieving 84% blind test accuracy across five sites, remarkably high given *T. trachurus*' mobility, demonstrates the power of simple, interpretable LDA modeling over complex black-box approaches [5], offering a highly effective tool for seafood traceability.

Keywords: seafood authentication; amino acid profiling; discriminant analysis; food fraud; geographical origin

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24571 | Grape Pomace: A source of bioactive compounds for valorization

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Background & Aim: Wine production is one of the most important agricultural activities worldwide, and in addition to large annual wine production values, it also generates waste, notably grape pomace (GP). Traditionally, it was seen as waste, but this residue has been reanalyzed from the perspective of the principles of bioeconomy and circular economy, proving to be a rich source of bioactive compounds with great potential for valorization [1]. Its heterogeneous composition accumulates a variety of polyphenols, dietary fibers, flavonoids and other secondary metabolites that give to GP biological properties such as antioxidant, anti-inflammatory, and antimicrobial activities. Aimimg GP valorization it is of utmost importance to optimize extraction methodologies to obtain the bioactives responsible for such properties. **Methods:** A conventional solid–liquid extraction was performed with different variables (time, temperature, volume, pH e water/alcohol ratio) to determine total phenolic and flavonoids contents, anthocyanins, and antioxidant activity (DPPH and FRAP assays), through spectrophotometric methods. **Results:** In optimizing this method, it was possible to conclude that, for temperature, the best results appeared when extraction was performed at 40°C during 60 min. The volume variation showed that the highest values were achieved at 25 ml, and 50:50 of ethanol mixture stood out as the best achieved. Finally, pH variations in relation to the original sample showed worse results when compared to the initial pH. **Conclusions:** The analysis carried out leads to the conclusion that GP, often treated as waste without economic value, is indeed a strategic resource in line with contemporary requirements of industrial sustainability. For this, the methodologies studied must be optimized, allowing their reuse not as a mere solution for waste management, but as an opportunity to create new products with high added value.

Keywords: Circular economy; environmental responsibility; bioactive compounds; conventional extraction.

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24582 | Preparation of Molecularly Imprinted Nanogels for Sensing: from Environmental Monitoring to Disease Diagnosis

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Background & Aim: Molecularly imprinted polymers (MIPs) have been developed by design as synthetic materials with specific binding sites for the "recognition" of target analytes. MIPs combine high selectivity with chemical stability and scalable, cost-effective production, making them reliable alternatives to biological receptors in sensing applications. [1] This work aims to develop and optimize molecularly imprinted nanogels (nanoMIPs) as biomimetic recognition elements targeting chemically and biologically relevant compounds, such as contaminants and biomarkers. **Methods:** NanoMIPs were synthesized via precipitation polymerization, in which acrylate-based functional monomers interact with the template molecule and crosslinkers stabilize the polymeric network to preserve complementary binding sites after template removal (Fig. 1). The resulting imprinted nanoparticles (NPs) were then characterized by dynamic light scattering (DLS) to determine their size distribution. Finally, affinity studies were performed to assess the molecular recognition performance of the imprinted nanogels toward contaminants and/or protein markers. **Results:** NanoMIPs were successfully prepared for the selective recognition of target analytes. DLS analysis confirmed the formation of spherical NPs within the nanometric size range. Preliminary affinity studies revealed that the imprinted materials exhibit enhanced binding toward target molecules when compared to corresponding non-imprinted NPs (NIP NPs), highlighting the effectiveness of the imprinting strategy for the generation of recognition sites. **Conclusions:** Precipitation polymerization approach proved to be a simple and efficient strategy for the preparation of nanoMIPs, particularly when compared to alternative established methodologies (e.g., solid-phase synthesis), which typically involve more laborious and time-consuming procedures. Furthermore, nanoMIPs demonstrate strong potential as selective synthetic alternatives to biological receptors in analytical assays.

Keywords: Molecularly imprinted nanoparticles (MIP NPs or nanoMIPs); Imprinted nanogels; Biomimetic recognition

Acknowledgments:

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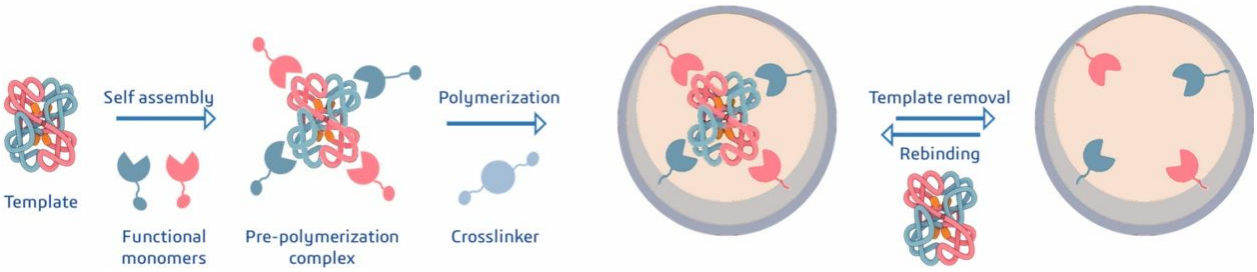

Figure 1 : Schematic illustration of MIPs synthesis. Figure reproduced from reference [2].

24584 | Functional nanomaterials development for application in anti-counterfeiting sensors

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Background & Aim: The textile industry faces the ever-growing issues of counterfeits and theft [1], thus resorting to anticounterfeiting sensors that trace products throughout the entire value chain [2], such as digital passports and smart security labels (e. g. QR codes, RFID tags) [3]. This work aims at developing multifunctional inks with electrochemical, magnetic and optical properties for application in anticounterfeiting textiles, and evaluate the smart labels encoding and security capabilities. **Methods:** Functional nanomaterials with electrochemical (carbon-based materials), magnetic (metal-based oxides) and optical (carbon quantum dots, CDs) features were synthesized via liquid-phase approaches. An optimization study was conducted by varying experimental conditions and precursors in the synthesis of CDs to determine the optimal optical response. The materials were characterized through spectroscopic and microscopy techniques, X-ray diffraction, magnetometry and electrochemical measurements. The magnetic and electrochemical-active materials were incorporated in functional inks and coated onto textile substrates using well-implemented industrial techniques. **Results:** Magnetic nanoparticles (NPs) and functionalized carbon materials were successfully prepared and incorporated into stable inks based on polymer-based formulations. Both formulations when printed kept their respective properties (Fig. 1)**.** The modified textiles with carbon-based materials exhibited electrochemical response, which was dependent of the material loading. The prepared CDs absorbed radiation in the range of 325-340 nm and emitted around 410-445 nm (blue color). **Conclusions:** The properties for magnetic NPs and carbon-based materials were active and characterization results were as expected, which validates the synthesized materials. The results' variability between nanomaterials also show that they can provide different responses when printed onto the textile, increasing the range of security codes that can be generated.

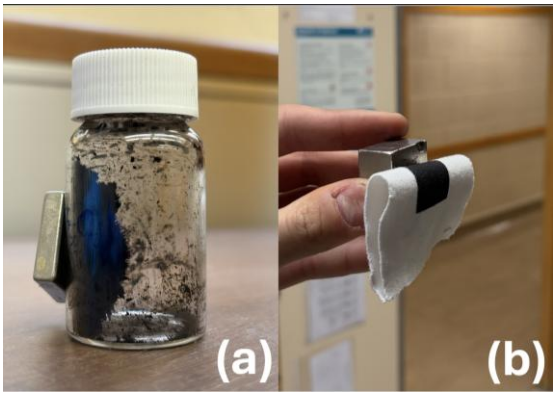

Figure 1: Metal-based material (a) and textile printed with formulation containing magnetic materials (b) being attracted by a magnet.

Keywords: Nanomaterials, textile, anti-counterfeiting, ink, security.

Funding: This work was supported by the Exploratory Project SafeTex (Ref. 2023.13995.PEX), funded by the Fundação para a Ciência e a Tecnologia and Ministério da Educação, Ciência e Inovação (FCT/MECI).

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Hydroxyapatite-Biochar Renewable as Promising Catalysts for (Electro)chemical Processes

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Background & Aim: This research focuses on Goal 9 & Goal 13 of the UN Sustainable Development Goals[1], promoting a circular economy. Using renewable biomass materials, fishbones (FB) and shrimp shells (SS) cutoffs, by-products from sea food distributors, were employed to create a combined material. This is being explored in electrocatalytic carbon dioxide reduction, to remove CO_2 from the atmosphere, and catalytic hydrogen transfer of levulinic acid (LA) and furfural (FF) to biofuel additives. Hydroxyapatite (HA) was prepared from FB utilized for its Ca and K rich composition, ion-exchange capacity & tunable acid-base ability. It was combined with shrimp shell biochar (BioC), improving electron transfer capabilities. **Methods:** HA was prepared by alkaline hydrolysis[2] and calcination[3]. Shrimp shells were converted using pyrolysis at 900°C. The BioC & HA were combined at various ratios: 1:0.5, 1:1 & 0.5:1[4,5]. Material was characterised using FTIR, SEM, elemental analysis, ICP, BET. Initial electrochemical tests have followed the oxygen reduction reaction (ORR), to understand the electrochemical potential of the raw materials. Shown Fig 1. **Results:** BET showed BioC to have the highest surface area (~275 m^2/g) and with a slight decrease for materials with more HA. Electrocatalytic ORR with BioC showed the most promising results with jL of -4.07 j/mA cm^{-2} and nO_2 of 3.35 electron on average Fig 2. Catalytic hydrogen transfer has been briefly evaluated for LA and FF conversion with conversions up to 97% and a selectivity of 98% towards Isopropyl 4-oxovalerate Fig.3. **Conclusions:** The preliminary results for BioC:HA catalysts showed they are promising materials compared with state of the art. Replacing catalysts in industry with more sustainable ones brings us one step closer to creating a zero-waste circular economy.

Keywords: Catalysts; Biochar; Sustainability; Shrimp shells; Renewable.

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This work received financial support through PT national funds COMPETE2030-FEDER-01199900, and COMPETE2030-FEDER-00814400 co-financed by COMPETE 2030, through Portugal 2030 and FEDER in the scope of the project "Shrimp4NanoCosmetics" and "MareCircularis".

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Figures:

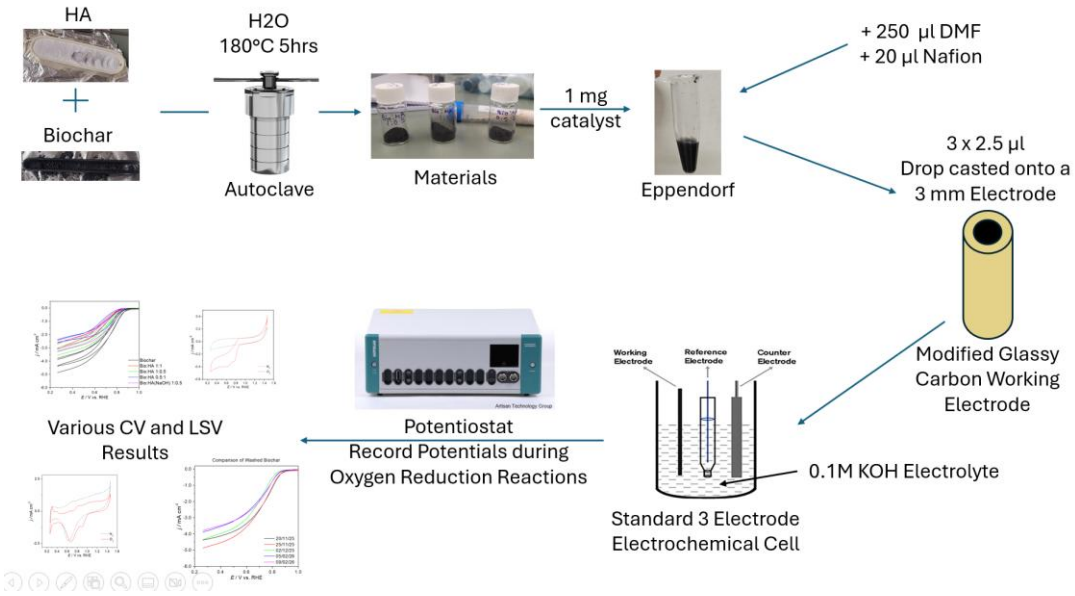

Figure 1: basic diagram of the method and procedure.

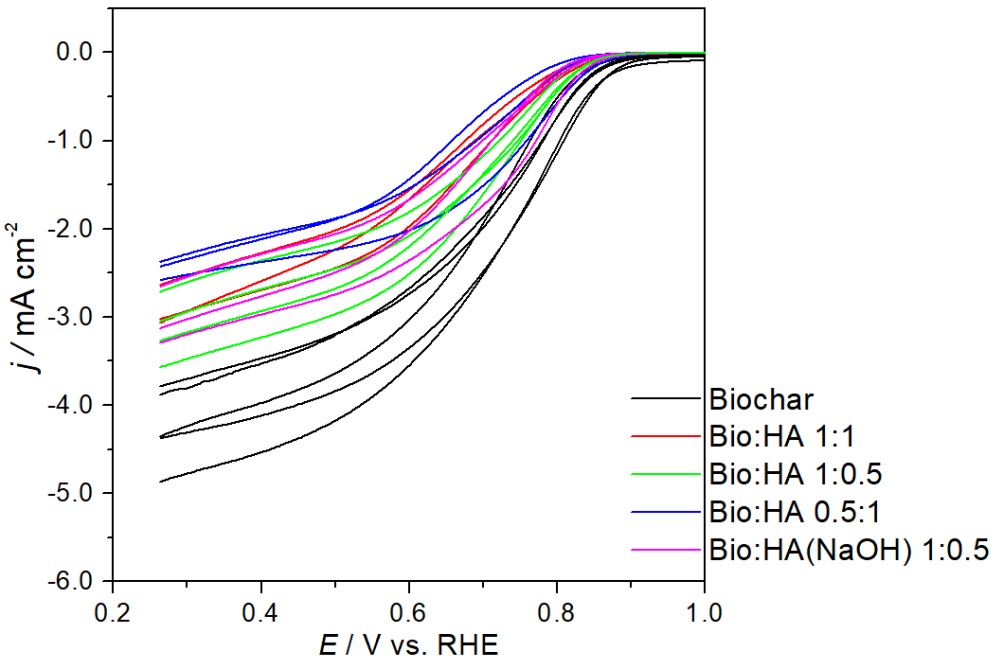

Figure 2: Electrochemical ORR following all materials prepared. BioC shown in black.

Catalyst Name	Conversion %	Selectivity for (Isopropyl 4-oxovalerate) %	Selectivity for (Y-Valerolactone) %
HA	69.9	100	0
Bio:HA 0.5:1	97.9	98	2

Figure 3: Table showing the conversion and selectivity of HA and BioC:HA 0.5:1 towards different biofuel additives. Conditions: Isopropanol, Levulinic acid, tetradecane in a high-pressure reactor, 10 hrs at 160°C.

24622 | Optimising Broad-Polarity Bioactive Compounds Recovery: A Novel Ultrasound- and Enzyme-Assisted Cascade Extraction for *Magnolia grandiflora* Seeds

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Background & Aim: Conventional extraction methods targeting lipophilic and polar metabolites (e.g., Bligh and Dyer) [1] rely on hazardous solvents like chloroform and methanol, raising sustainability and health concerns [2]. This study designs an innovative, zero-waste cascade extraction using greener solvents to sequentially fractionate steroids, phenolic compounds, and other metabolites from *Magnolia grandiflora* L. seeds. **Methods:** Dried and ground seeds are initially defatted by ultrasound-assisted extraction (UAE) with n-hexane. The spent biomass (Residue 1) undergoes UAE with ethanol:water (95:5). Residue 2 undergoes enzymatic digestion and repeated hydroethanolic UAE. Finally, Residue 3 is solubilised in water, leaving insoluble matter as the final residue [3]. The hydroethanolic extracts are later fractionated by sequential solid-liquid extractions with cold water-ethanol mixtures of increasing ethanol content. **Results:** This sequential green solvent strategy enables exhaustive phytochemical fractionation with minimal waste. UAE facilitates oil recovery with minimal n-hexane volumes; hydroethanolic UAE extracts most remaining secondary metabolites, whilst enzymatic digestion ensures full valorisation of cellulosic material. Fractioning both hydroethanolic extracts separates metabolites by solubility, from glycosides to neolignans (magnolol and honokiol), other phenolics, and bound or free steroids [4]. The remaining water-soluble and insoluble materials can be valorised for full circularity. **Conclusions:** This cascade methodology provides a greener, scalable, and circular alternative to conventional extractions for isolating valuable phenolics and steroids from *M. grandiflora* seeds, applicable to other lipid and polar metabolite-rich biomasses. Simultaneous extraction of steroids and polyphenols holds great value in natural products research, targeting pharmaceutical, food, or cosmetic industries.

Keywords: *Magnolia grandiflora*; Green extraction; Cascade fractionation; Phenolics; Steroids

Acknowledgments: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

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24669 | Enantioseparation and Structural Modification of Nadifloxacin for Enantioselective Ecotoxicological Studies

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Background & Aim: Fluoroquinolones (FQs) are widely used antibiotics and emerging environmental contaminants due to their persistence and incomplete removal in wastewater treatment plants [1]. Nadifloxacin (NAD) is a chiral FQ marketed as a racemate, although its *S*-enantiomer is significantly more potent. Despite its relevance, due to their role in the spread of antibiotic resistance [2], enantioselective environmental data for NAD are scarce. Thus, this work aimed to achieve the enantioseparation of NAD, synthesize its stereoisomeric prodrugs, and evaluate stereochemical effects on enzymatic hydrolysis relevant to environmental fate. **Methods:** NAD was enantioseparated by semi-preparative normal-phase liquid chromatography using a CHIRALPAK® AD-H column (10 mm I.D. x 250 mm L, 5 µm). Stereoisomeric prodrugs were synthesized by coupling reaction with a racemate NAD with enantiomerically pure alanine derivatives, followed by deprotection [3]. All compounds were elucidated by IR and NMR. Preliminary enzymatic hydrolysis assay of NAD prodrugs was evaluated using porcine liver esterase (PLE) and monitored by TLC. **Results:** NAD enantiomers were obtained with enantiomeric purities above 98%, and the corresponding diastereomeric prodrugs were successfully synthesized and separated. Enzymatic studies revealed pronounced stereoselectivity: the D- prodrug with D- alanine was fully hydrolyzed within 20 min, whereas the L-alanine required approximately 60 min, indicating a higher susceptibility of the D-form to esterase-mediated cleavage[4]. **Conclusions:** The combined enantioseparation and synthesis strategy provided enantiomerically pure NAD and prodrug standards forenantioselective ecotoxicological studies

. Stereochemical differences strongly influenced enzymatic hydrolysis, impacting both pharmacological behavior and environmental fate. These findings highlight the importance of chirality in assessing the ecotoxicological risk and transformation pathways of fluoroquinolone antibiotics.

Keywords: Fluoroquinolones; Chirality; Enantioseparation; Environmental fate; Enzymatic Hydrolysis.

Acknowledgments:

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24681 | Development of Nanoformulations for Oral Administration and Treatment of Crohn's Disease

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Background & Aim: Methotrexate (MTX) exhibits poor oral bioavailability due to its low intestinal permeability and aqueous solubility, limiting its efficacy in Crohn's disease therapy.[1] Recent nanomedicine-based approaches have proven effective in enhancing drug's oral bioavailability and therapeutic outcomes. Thus, this work aims to develop lipidic MTX-loaded nanoparticles (NPs) for oral administration. **Methods:** Lipid-based NPs, nanostructured lipid carriers (NLCs) and solid lipid nanoparticles (SLNs) were prepared by solvent emulsion-evaporation and double emulsion methods, respectively.[2-4] The sizes and polydispersity index (PDI) of the NPs were determined by Dynamic Light Scattering and their superficial charge (ζ-potential) by Electrophoretic Light Scattering. Plus, MTX encapsulation efficiency (EE%) and Drug Loading Content (DLC%) were determined by Ultra High-Performance Liquid Chromatography. NPs shelf-stability was monitored for 31 days at 4°C and room temperature. Plus, NPs were incubated with mucins to assess mucoadhesive properties. **Results:** The prepared NPs demonstrated sizes<200 nm, PDI<0.3, and ζ-potential values between -25 to −40 mV. The determined EE% and DLC% were found to be 77% and 1.38% for SLNs and 41% and 0.74%, respectively. Incubation in a range of different pH, resulted in an increase of ζ-potential, although the NPs remained stable. Mucin incubation also increased ζ-potential, indicating mucin-NPs interaction. Evaluation of shelf-stability demonstrated that SLNs exhibited greater physicochemical stability over time compared to NLCs. **Conclusions:** The prepared NPs demonstrated physicochemical properties suitable for oral deliver.[5, 6] Plus, NPs demonstrated to be stable in a range of different pH and to have mucoadhesive properties, both important features for the administration of NPs via oral route.[3] Due to the improved EE%, DLC% and

shelf-stability, SLNs proved to improve MTX bioavailability and efficacy, and requiring further study.

Keywords: Crohn's Disease, Methotrexate, Lipid Nanoparticles, Oral Delivery, Mucoadhesive Properties.

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24738 | Influence of Temperature and Storage Duration on the Phenolic Stability of Commercial Fruit Smoothies

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Background & Aim: Fruit smoothies are popular for convenience and perceived health benefits. Rich in fruits and vegetables, they provide phenolic compounds linked to antioxidant and protective effects against chronic diseases[1,2]. Phenolics contribute to antioxidant capacity[1,2] and sensory properties but are sensitive to storage time and temperature, which may affect stability and bioavailability. Monitoring their evolution is essential to ensure quality during shelf life[2]. This project, developed with Sumol+Compal S.A., aimed to characterize the phenolic profile of selected smoothies and evaluate the effect of storage time and temperature. Samples were analyzed at 0, 1, 2 and 4 months under different storage temperatures. Three varieties were studied: green ("Saúde Digestiva"), yellow ("Imunidade") and pink ("Energizante"). **Methods:** Smoothies were freeze-dried; 1 g of dried sample was extracted with methanol/water (50:50), centrifuged (13,000 rpm, 10 min, 4 °C) and filtered (0.45 µm). Individual phenolics were identified by HPLC. Total phenolic content (TPC) was determined by Folin–Ciocalteu. Antioxidant capacity was assessed by FRAP and DPPH assays. **Results:** The green smoothie contained chlorogenic acid, epicatechin, quinic, cinnamic and caffeic acids, coumaroylquinic acid, quercetin and phloridzin derivatives. The yellow contained chlorogenic acid, rutin, phloridzin, gallic and quinic acids, galloyl glucose, caffeic acid, procyanidins, coumaroylquinic acid and naringin. The pink contained chlorogenic acid, caffeoylmalic acid, punicalin, malic and quinic acids, pedunculagin, ellagic acid, epicatechin and phloridzin derivatives. Total polyphenols decreased from 0 to 4 months. Refrigerated samples showed higher TPC than those at room temperature, and antioxidant activity followed the same trend. **Conclusions:** Longer storage

and higher temperature reduced phenolic stability and antioxidant potential, highlighting the importance of adequate storage conditions to preserve smoothie quality.

Keywords: Smoothies; Polyphenols; Antioxidant Activity; Functional Food; Shelf-life

Acknowledgments:

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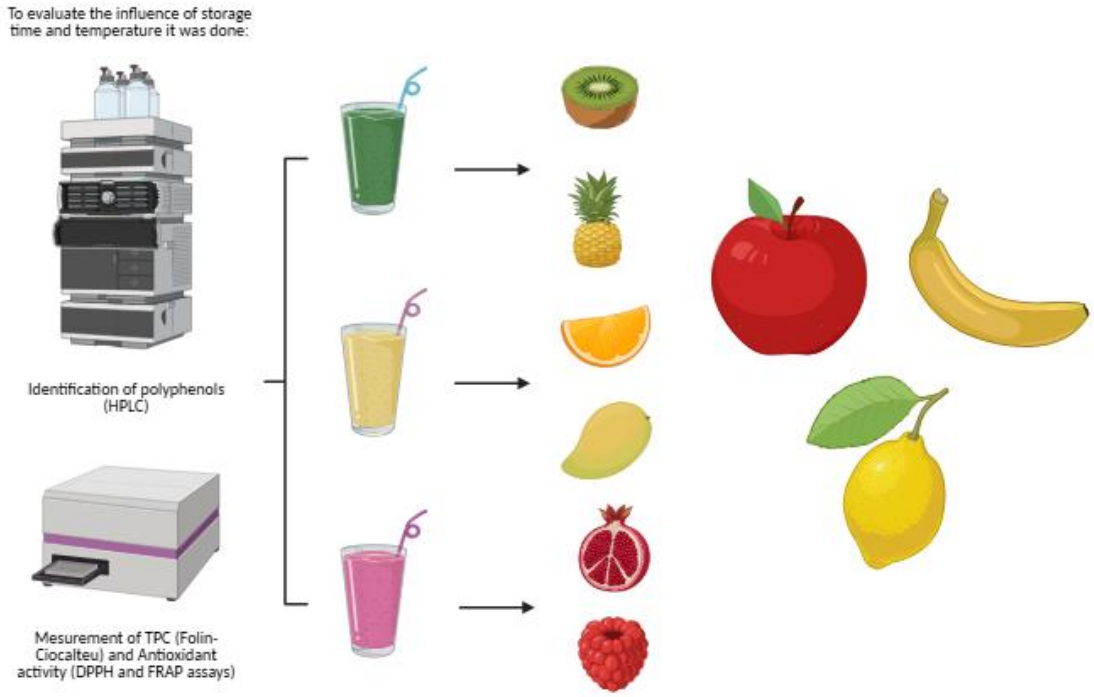

Figure 1: Graphical abstract of the analyses performed and the composition of the smoothies.

24750 | Synthesis of a flavone scaffold for coupling to a chiral moiety to increase the therapeutic performance

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Background & Aim: Flavonoids are natural products associated with a wide range of therapeutic activities [1-2]. Among them, flavones are valuable in Medicinal Chemistry due to their diverse biological activities, particularly their antitumor properties [2]. However, their use is limited owing to the poor selectivity and bioavailability [2]. Association with a chiral moiety proved to increase the therapeutic performance of many flavonoids with antitumor activity [2]. This work focuses on the first step of the protocol to improve the methodology to synthesise a flavone for subsequent coupling with enantiomerically pure amino esters. **Methods:** The synthetic route of 4'-methylchrysoeriol (LuMe) involving Mentzer's cyclocondensation reaction between phloroglucinol and ethyl 3,4-dimethoxybenzoylacetate with subsequent esterification with methyl bromoacetate, under alkaline conditions, led to the intermediate which was hydrolysed to the respective carboxylic acid, 2-((2-(3,4-dimethoxyphenyl)-5-hydroxy-4-oxo-4H-chromen-7-yl)oxy) acetic acid. This intermediate served as the building block for the coupling reaction with enantiomerically pure amino ester and amino acids. **Results:** The most efficient methodology in terms of yield, purity, and reaction time to scale up the reaction to obtain LuMe was the microwave-assisted synthesis, providing the highest yield (84 %), allowing further coupling with the chiral moiety. Structural elucidation was performed by Infrared and nuclear magnetic resonance spectroscopy. Enantiomeric purity was assessed by liquid chromatography using a commercial chiral column with carbamate of cellulose as the stationary phase. **Conclusions:** This work enabled a four-step synthetic protocol aimed at obtaining new chiral flavone derivatives with promising antitumor activity. The overall enantiomeric ratios were above 98 % for all derivatives. These results confirm the success of the synthetic strategy to achieve chiral derivatives of flavone for future biological activity evaluation.

Keywords: flavones; synthesis; antitumor activity

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24860 | Extraction, quantification and characterization of polyphenols from multifruit pulpy juices as stability evaluation through different storage conditions

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Background & Aim: During storage, variations in phenolic profile of juices may occur, affecting their antioxidant capacity and shelf-life [1,2]. This study aims to evaluate stability through the polyphenolic profile of three multifruit pulpy juices under different storage conditions. **Methods:** Three juices were analyzed: A (apple, mango, banana and baobab), B (apple, mango, guava, grape and pineapple) and C (apple and mango). Samples were stored for 7 days/40 °C, 14 days/40 °C and 3 months/20 °C and 5 mL of each sample was diluted 1:1 with ultrapure water and centrifuged at 13000 rpm for 1 hour at 4 °C. The supernatant was collected and filtered using 0.22 µm syringe filters, yielding 8 mL. A C18 solid-phase extraction column was used for purification, with elution using MeOH, MeOH + acetone (1:1) and MeOH + acetone (1:1) acidified with HCl, 5 mL each. The eluate was evaporated to ~1 mL in a rotary evaporator and adjusted to 2 mL with ultrapure water. All samples were injected into HPLC and LC-MS to obtain chromatographic profiles for polyphenol characterization, using 0.5% formic acid in water (A) and acetonitrile (B) as the mobile phase and a C18 reversed-phase column (Agilent 4.6 × 250 mm; 2.7 µm) as stationary phase. Data were processed in Excel and GraphPad and expressed as equivalents of chlorogenic acid (hydroxycinnamic acid), gallic acid (hydroxybenzoic acid), mangiferin (xanthone), quercetin-3-O-Glc (flavonol), phloridzin (dihydrochalcone) and catechin (catechin). **Results:** The main polyphenols originated from the apple base in each sample, with chlorogenic acid and phloridzin as markers. Considering the concentration of each sample, all juices showed stability under different conditions. **Conclusions:** The C18 solid-phase extraction method was efficient and reproducible for recovering a broad range of polyphenols from multifruit pulpy juices. All samples maintained a stable polyphenolic profile under all storage conditions.

Keywords: Polyphenols; HPLC; LC-MS; multifruit juices.

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This work was performed under VIIAFOOD Project with multifruit juices kindly provided by SUMOL+COMPAL, S.A.

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24790 | Development of Bio-based Adsorbents from Grapevine Pruning Residues and Fishbones for Phosphate Removal from Wastewater

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Background & Aim: Industrial and agricultural effluents are major sources of nutrient pollution, accelerating eutrophication and posing risks to aquatic ecosystems and public health [1]. At the same time, phosphorus, is a critical and non-renewable resource, reinforcing the need for efficient and sustainable recovery strategies [2]. Biochar-based adsorption offers a promising approach, particularly when produced from waste biomass and reused as slow-release fertilizers. In this study, magnesium-loaded biochars from codfish bones (FB) and grapevine pruning canes (GPC) were prepared and evaluated for phosphate removal from synthetic and real wastewater. **Methods:** The preparation process involved biomass pre-treatment, magnesium impregnation and controlled pyrolysis, with operating conditions optimized through a Face-Centered Composite Design. The materials were extensively characterized to determine their physicochemical properties, and their adsorption performance was assessed under batch and continuous-flow conditions. Phosphate concentrations were measured using the Vanadate-Molybdate method, while adsorption behavior was described using kinetic (pseudo-first and pseudo-second order) and equilibrium (Langmuir and Freundlich) models. **Results:** Optimization results indicated that the magnesium loading ratio was the key factor governing adsorption performance, whereas pyrolysis temperature showed no significant influence on either yield or phosphate uptake. Maximum adsorption capacities reached 91.92 mg P/g for FB and 373.33 mg P/g for GPC at the highest magnesium content. Column experiments further demonstrated the suitability of both materials for continuous wastewater treatment. **Conclusions:** Overall, magnesium-modified biochars, especially those obtained from GPC residues, exhibit strong potential as low-cost and sustainable adsorbents for phosphorus removal and recovery from water streams.

Keywords: Adsorption; Phosphate; Biomass; Biochar; Wastewater.

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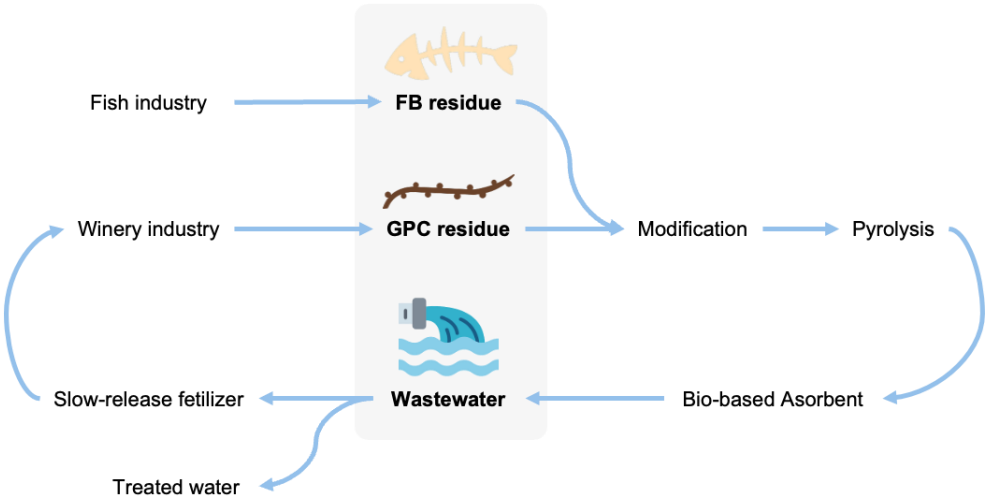
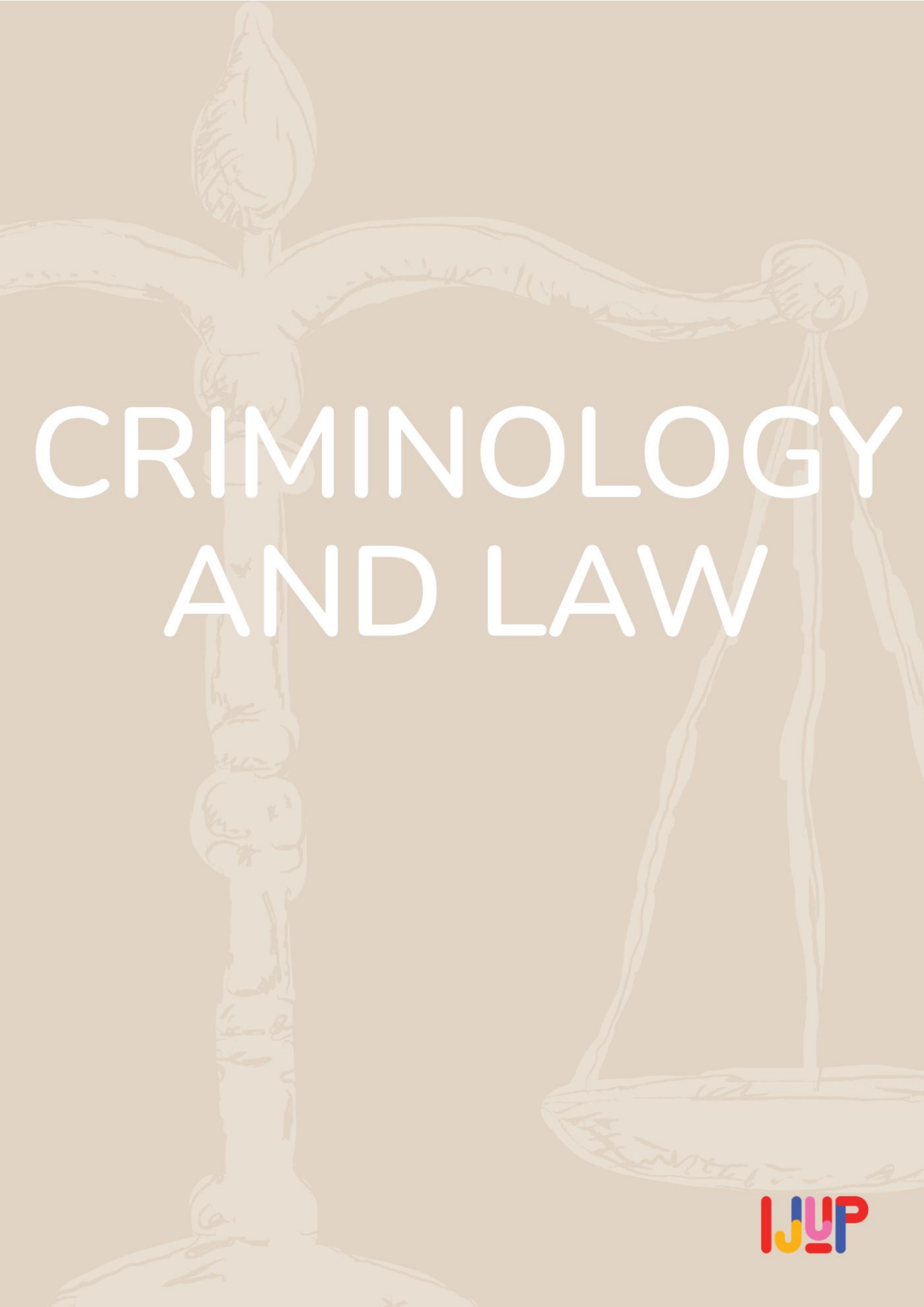


Figure 1: Conceptual flow diagram of the valorization pathway: GPC and FB are converted into a Mg-modified biochar adsorbent for phosphate removal and reused as a slow-release fertilizer.



CRIMINOLOGY AND LAW

24434 | Reentry experiences of former inmates: A gendered perspective

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Background & Aim: After serving a prison sentence, the former inmate begin community reintegration, a process that requires readjusting to life outside prison while managing barriers that may hinder successful reentry.[1][2] Prior research highlights both shared and gender-specific experiences, underscoring the importance of examining differences in this process.[3][4][5]Thus, following this rationale and adopting a gendered perspective, this study seeks to explore former inmates' views on prison-based support and key reintegration dimensions, including stigmatization, employment, housing, family relationships, parenting, and formal support. **Methods:** A qualitative design was used, based on semi-structured interviews and a sociodemographic questionnaire. Of the 22 potential participants, 2 declined, 5 were excluded, and 1 withdrew, resulting in a final sample of 14 Portuguese participants (37–55 years), evenly distributed by gender. **Results:** Prison-based support was generally perceived as insufficient and poorly tailored to individual needs. Institutional selectivity in leisure and occupational activities appeared to reproduce traditional gender stereotypes. Reentry was largely described as challenging, particularly due to stigmatization, financial hardship, and limited access to employment. Housing did not emerge as a major barrier, as most relied on family support. Gendered patterns were evident, with women reporting have experienced double stigmatization for both offending and transgressing traditional norms of femininity. Single mothers faced additional difficulties balancing employment and caregiving, sometimes relying on social benefits due to limited labor market flexibility. **Conclusions:** Gender shapes both structural reintegration challenges and the meanings attributed to imprisonment and release. The findings underscore the need for gender-responsive reintegration policies, attentive to multiple vulnerabilities and capable of turning imprisonment into a constructive life transition.

Keywords: Prison sentence; Reintegration process; Barriers to reentry; Gender; Social support

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24273 | Fear of crime and police perception in rural context in Portugal

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Background and Aim: Research on fear of crime has predominantly cantered on urban contexts, leaving rural environments comparatively underexplored, a pattern also evident in Portugal. Addressing this gap, the present study examines fear of crime within rural Portuguese communities. **Methodology:** Using a quantitative, cross-sectional design, a paper-and-pencil questionnaire was administered to a sample of 500 participants aged between 18 and 99 years residing in rural areas. The instrument collected data on sociodemographic characteristics, fear of crime and victimization, neighborhood factors (perceptions of disorder and crime, collective efficacy, and place attachment), and attitudes toward the police (satisfaction, perceived effectiveness, and trust). The findings indicate that predictors of fear of crime in rural areas resemble those documented in urban contexts. **Results:** Specifically, women, older individuals, and those with prior victimization experiences reported higher levels of fear of crime, greater perceived risk of victimization, and a stronger tendency to adopt protective behaviors compared to men, younger individuals and non-victims. In contrast, greater social cohesion, stronger community values, and higher levels of social trust relates to lower levels of fear and risk perception. However, these factors did not translate into an increased adoption of protective behaviors. Additionally, heightened perceptions of neighborhood disorder were positively associated with fear of crime, elevated perceived risk, and more frequent engagement in security-related behaviors. Similarly, perceptions of police performance were related to fear of crime, such that higher perceived police efficacy and procedural justice were associated with lower levels of fear and risk perception, whereas lower perceived police visibility corresponded to slightly higher fear levels. **Conclusions:** Finally, although some patterns of fear of crime in rural contexts mirror those in urban environments, the rural setting merits recognition as a distinct area of inquiry requiring dedicated research into its unique characteristics. This study further addresses its theoretical and methodological implications, alongside its limitations, with the objective of informing and guiding future research in this context.

Keywords: Fear of crime; Victimization; Rural areas; Portugal; Policing

24855 | The Legal Personality of Commercial Corporations, its Disregard and the Material Undercapitalization as a Ground

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Background & Aim: The legal personality of commercial corporations represents the backbone of contemporary legal-economic structures, institutionalizing commercial risk through limited liability to stimulate investment. Using the corporate form for alien objectives raises significant questions as to whether legal personhood is restricted by its socio-economic functions. While Common Law jurisdictions have pierced the corporate veil through judicial precedent, the Portuguese Civil Law tradition, abiding primarily by codified legal norms, lacks express statutory provisions. This study examines material undercapitalization as means of transferring business risk to creditors. **Methods:** This study employs historical and dogmatic legal analysis of the evolution of legal personhood. The normative legal framework is analyzed via the Commercial Companies Code and the Civil Code. General Private Law principles, specifically the abuse of right and tort law, are used to bridge the gap in the (insufficient) legal text. **Results:** Analysis reveals corporate personality can be accurately framed as a legal right, functionalized to intervene in commerce for profits. While undercapitalization materially constitutes an "institutional abuse of right" by disproportionally transferring the business risk from investors to creditors, shareholder responsibility cannot be directly extracted from this subsidiary institute alone, as it only restrains the exercise of legal entitlements. Instead, the abuse of right establishes "wrongfulness" – a core requirement for Aquilian liability, the Portuguese legal system's standard mediator for imputing damages **Conclusions:** The disregard of legal personhood is a subsidiary mechanism ensuring coherence between for limited liability's material grounds and Private Law principles. This non-statutory doctrine grounds the recognition of limited liability in its material purposes, ensuring legal personhood exceeds formal status.

Keywords: Legal Personality; Commercial Corporations; Piercing the Corporate Veil; Material Undercapitalization; Abuse of right.

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24467 | Money Laundering as a Transnational Organized Crime Threat: Perceptions of Portuguese Experts and Judicial Decisions Analysis

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Background & Aim: Money laundering (ML) enables transnational organized crime to sustain [1], expand [2], and infiltrate legitimate economies [3], causing an estimated annual loss of 2–5% of global gross domestic product [4]. Its consequences span economic destabilization, political corruption, and erosion of public trust [5], [6]. Portugal's financial vulnerabilities, high circulation of people and capital through its tourism-driven economy, and geopolitical proximity to high-risk jurisdictions amplify its attractiveness to criminal networks and international ML flows. [7]. This study seeks to understand through criminological frameworks how transnational ML and its associated criminality operate within and through Portugal, and analyze how public and private actors cooperate to defend the country's economic integrity. **Methods:** A qualitative exploratory design was adopted, comprising two sub-studies. The first sub-study analyzed six Portuguese Court of Appeal rulings retrieved from the DGSI database, selected through intentional sampling requiring ML convictions. The second involved semi-structured interviews with anti-money laundering professionals and experts. Data were analyzed using Mayring's Qualitative Content Analysis through combined deductive and inductive coding. **Results:** The analyses of judicial decisions identified diverse actor typologies — white-collar criminals, professional enablers, and social recruits — operating through hierarchical, transnational networks using offshore layering, real estate, and virtual markets. Findings from interviews revealed dedicated ML organizations functioning as “crime as a service” and a multi-layered defense architecture spanning private compliance, financial intelligence units, and judicial cooperation. **Conclusions:** These findings contribute to the identification of systemic vulnerabilities within the Portuguese economic system, suggesting improvements for security policies, and informing the ongoing debate surrounding the growing threat of ML.

Keywords: Money Laundering; Transnational Organized Crime; Anti-Money Laundering; Predicate Offences; Compliance

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24499 | The role of digital technologies on intimate partner violence among young people

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Background & Aim: Intimate partner violence (IPV) among young people is a growing social and public health concern. It encompasses physical, psychological, and emotional abuse that is often minimized or unrecognized. Digital technologies have extended violence into online environments, where violent behavior normalization may hinder identification and reporting, while also creating opportunities to support victims by facilitating access to information, specialized services, and support networks. This study examines IPV among young people in digital contexts, focusing on the role of emerging technologies in its perpetuation and response. **Methods:** Semi-structured interviews were conducted with victim support professionals to explore their perceptions of the impacts of digital technologies on IPV among young people, and on intervention practices. Interviews were conducted via videoconference, audio-recorded, transcribed, and analyzed using thematic analysis. **Results:** Ten victim support professionals participated, all women aged between 20 and 45 years. Preliminary results point to professionals' perception that IPV in digital contexts among young people has a high prevalence, with dynamics that are specific to this sub-population, as well as multiple associated risks factors that serve to sustain and intensify digital IPV among youths. Digital technologies may also be used to prevent IPV and provide support to young people. **Conclusions:** IPV among young people in digital contexts is perceived as a complex and growing phenomenon. The ambivalent role of digital technologies underscores the need to strengthen awareness and digital literacy adapting intervention practices to online environments. The study highlights the importance of developing institutional, social, and policy strategies that integrate the digital context into the prevention and protection of young victims, promoting responses that are better aligned with contemporary realities.

Keywords: Intimate Partner Violence; Young People; Digital Technologies; Victim Support; Prevention.

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24851 | "Insecurity in urban contexts and gender identity: an exploratory study based on the experiences of transgender individuals"

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Background & Aim: Insecurity is a multidimensional phenomenon that encompasses both an objective dimension (actual crime) and a subjective dimension (individual perception). Historically, criminological research has focused on binary and cisnormative analyses, neglecting the specificities of trans populations. This study is based on the hypothesis that gender identity and conformity to gender norms directly influence subjective perceptions of insecurtiy. The aim is to understand how experiences of insecurity, fear of crime, victimization and self-protection strategies are shaped by the gender identity of transgender men and women in urban settings. **Methods:** A qualitative and exploratory study was conducted. The sample consisted of 9 transgender individuals (5 trans men and 4 trans women) residing in Portugal. Data were collected through semi-structured interviews, which allowed for an exploration of daily routines, experiences of victimization (both direct and indirect), and the adoption of protective behaviors. The data analysis followed a thematic approach, cross-referencing the narratives with the theoretical concepts of passability and structural vulnerability. **Results:** The experiences of insecurity are influenced by multiple factors, among which the social reading of the body and passability, the trans condition as a vulnerable existential position, and exposure to risks associated with the female condition stand out. Trans women described greater exposure to threats associated with their gender expression, and reported feeling particularly vulnerable in certain physical and social contexts. Trans men, on the other hand, shared concerns related to pressure to conform to standards of masculinity and the possibility of their identity being questioned. Protection, avoidance, and self-defense strategies were strongly influenced by previous experiences of victimization and the perception of threat in certain spaces and at certain times. Lack of trust in institutions contributes to a heightened sense of insecurity in the face of what they perceive to be recurrent exposure to transphobia. **Conclusions:** The study concludes that gender identity is a critical determinant in urban safety management. Passability acts as a protective mechanism, while the visibility of trans identity increases vulnerability to crime and stigma. The study contributes to the state of the art by challenging the classic gender

dichotomy in criminology and demonstrating that trans insecurity is inseparable from structural transphobia.

Keywords: Feeling of insecurity; risk perception; adoption of behaviors; gender identity; passability

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ECONOMICS AND MANAGEMENT

24207 | Who Sits at the Top Matters: TMT Human Capital, Fit, and Firm Performance

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Background & Aim: Who sits at the top matters, but how Top Management Teams' education and professional experience translates into firm performance remains insufficiently understood. Drawing on Upper Echelons (Hambrick & Mason, 1984) and Human Capital (Becker, 1993; Davenport, 1999/2007) theories, this study examines how the composition and alignment of TMTs' formal education and professional experience affect firm productivity and financial outcomes. **Methods:** Our analysis uses a uniquely and economy-wide panel of over 360,000 Portuguese firms, across industries, between 2010 and 2023, covering 2.18 million firm-year observations, based on the *Quadros de Pessoal* and *Sistema de Contas Integradas das Empresas* databases. We apply fixed-effects panel econometric techniques, having the logarithm of real gross value added per worker, at market prices, as our main dependent variable. **Results:** Both the level and fit of TMT human capital matter with higher average education and industry-specific experience significantly enhancing productivity. Additionally, curated heterogeneity in education levels have a positive impact, while excessive dispersion or misalignment in experience undermines performance. These findings help to reconcile mixed evidence on diversity by distinguishing between breadth and dispersion, showing that managerial fit amplifies firm-specific value creation. **Conclusions:** Firms perform better when TMTs combine strong educational backgrounds and industry-relevant experience. Diversity in knowledge and experience only helps when it complements firms' strategic and operational needs. Excessive variation or poor match between managerial backgrounds and firm activities can harm outcomes. This study advances the Upper Echelons and Human Capital literatures by considering a large sample of Portuguese firms linking human capital to productivity and by distinguishing curated breath and dispersion, with implications for TMT composition design and for productivity and innovation strategies.

Keywords: Top Management Teams; Managerial Human Capital; Firm Performance; Education and Experience Alignment; Team Diversity

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24730 | A Reappraisal of the Delayed Overshooting Puzzle: A Critical Assessment and a Dynamic Modification of Dornbusch (1976)

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The behavior of exchange rates following monetary disturbances remains a key issue in international macroeconomics, one that many scholars have sought to understand. Dornbusch (1976) presents a theoretical framework that attributes exchange rate volatility in response to nominal disturbances to the immediate overshooting driven by differences in the adjustment process of goods and money markets. Following the author, after a monetary shock, the exchange rate must immediately exceed its long-run equilibrium value in order to guarantee asset market equilibrium and, consequently, uphold Uncovered Interest Rate Parity. Nonetheless, empirical evidence suggests a delayed response of exchange rates following monetary shocks. This finding has given rise to what is widely known as "delayed exchange rate overshooting" puzzle, sparkling a broad and outgoing discussion about the mechanisms underlying this exchange rate adjustment process. The present research aims to take stock of the current debate on this puzzle, highlighting different perspectives found in economic literature to explain this anomaly. Concurrently, a dynamic modification of Dornbusch (1976)'s exchange rate dynamics is conducted, in order to assess whether it can account for this puzzle. The extensive review of the economic literature suggests that delayed overshooting may be explained in light of the rejection of the Efficient Market Hypothesis, frictions and rigidities in asset market or firms' environment or in light of the interplay between foreign exchange rate and monetary policies. Notwithstanding, some scholars contend that the apparent delayed response of exchange rates is merely the result of misidentified monetary policy shocks. The dynamic approach undertaken in this research leads to the conclusion that a modest modification to the exchange rate dynamics of Dornbusch (1976)'s — and, consequently, to the exchange rate adjustment mechanism — is sufficient to replicate the "delayed overshooting puzzle".

Keywords: Delayed Overshooting; Exchange Rate Dynamics; Uncovered Interest Rate Parity (UIP)

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24963 | Powering Independence: A Quantitative Analysis of the EU's Energy Transition and Fossil Fuel Dependence (2010-2024)

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Background & Aim: The EU's commitment to achieving the net-zero emissions goal by 2050 has been established by Member States through the European Green Deal. The continent's reliance on imports of fossil fuels has become more evident at the start of the Russian-Ukrainian war, highlighting the need to obtain energy independence and more ambitious climate objectives in the face of environmental and geopolitical challenges. However, significant differences remain within the bloc as the transition progresses unevenly across the countries. The aim of this study is to conduct a comparative analysis of the impact of renewable energy capacity expansion and investments in R&D for green technologies on the EU's dependence on fossil fuels, by dividing the Member States into two clusters of countries. **Methods:** To assess the effect of energy transition metrics on the dependent variable, namely the national energy import dependency, the study employs a linear regression model and Ordinary Least Squares (OLS) relying on historical data from a 14-year period (2010-2024) for the 27 EU's Member States, sourced from Eurostat and the International Energy Agency (IEA). **Results**: We anticipate that an increase in renewable energy capacities and investments in R&D for green technologies will negatively impact fossil fuel dependence, strengthening EU's energy security and self-sufficiency. The adoption of renewable energy is expected to vary across Member States, shaped by different national sectoral and policy priorities. **Conclusions:** The disparities observed within the Union indicate that uneven deployment and investment in renewable energy could undermine continent-wide environmental targets. Therefore, the necessity to establish coordinated energy policies among European countries, allowing for greater flexibility in the measures to be adopted by each Member State is essential to ensure both energy security and the attainment of climate objectives.

Keywords: Green transition; European Union; Renewable energy; Fossil fuel imports; Energy security

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24145 | Technology Readiness and Generative AI acceptance factors among B2B sales professionals in Portugal: A TAM-based study

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Background & Aim: Generative AI is changing how B2B sales work, but adoption varies widely. Most research treats technology readiness as an antecedent, missing how it might change the strength of other relationships. Blut and Wang (2020) found that positive readiness factors (optimism, innovation) and negative ones (insecurity, discomfort) work in opposite ways and need separate analysis. We still know little about how AI awareness and trust affect salespeople's decisions. This study examines what makes Portuguese B2B sales professionals accept or reject GenAI, focusing on how their readiness changes the link between perceptions and actual usage intentions. **Methods:** We survey B2B sales professionals in Portugal using a quantitative approach. Our model builds on TAM but adds technology readiness as moderator, splitting it into motivators and inhibitors (bidimensional). We also include AI awarenss and trust as factors shaping how useful and easy people find GenAI. Analysis uses PLS-SEM to test whether different readiness levels make cognitive beliefs more or less likely to become usage intentions. **Results:** Data collection is ongoing. The model suggests readiness works asymmetrically: people high in optimism and innovation should show stronger links between perceived usefulness, ease of use, and adoption intention, while those high in insecurity and discomfort show weaker links. AI awareness and trust should increase both usefulness and ease perceptions. The study will reveal which salespeople accept GenAI most readily and why.

Conclusions: Treating technology readiness as moderator with separate positive and negative dimensions fills a gap in understanding GenAI adoption professionally. Adding AI awareness and trust provides a fuller adoption picture. Findings should help companies introduce GenAI to sales teams in ways that account for individual differences, not just technology features.

Keywords: Generative Artificial Intelligence; B2B Sales; Technological Readiness; Technology Acceptance Model; Trust

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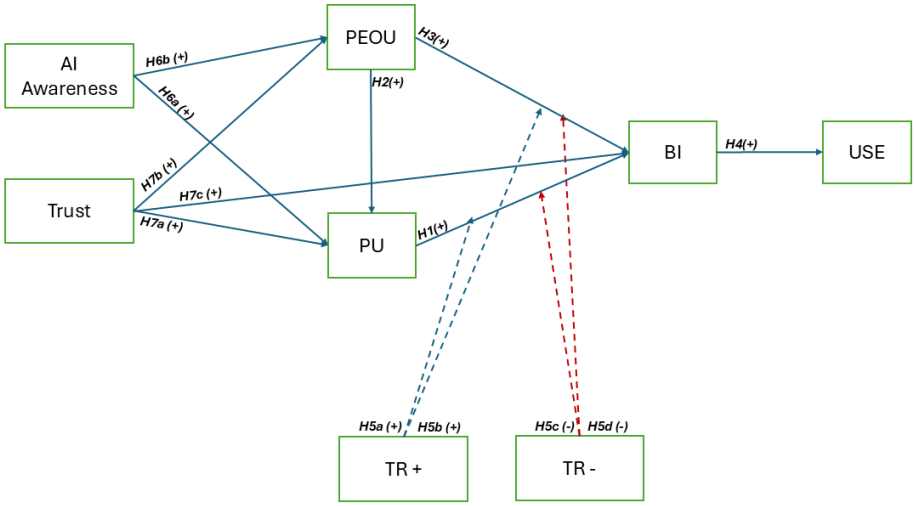
Figure 1: Proposed Conceptual Model | Source: Author's own elaboration



ENGINEERING

24161 | Numerical simulation of textile dyeing with purple onion peel pigments: key phenomena and parametric analysis

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Background & Aim: Environmental concerns surrounding synthetic dyes have intensified the search for sustainable alternatives in the textile industry [1]. In this context, natural dyes from renewable sources stand out as a safer environmental alternative to conventional dyeing processes, which consume large amounts of water and release toxic compounds. Thus, studying natural dyeing is essential, and modeling helps reduce costs and materials while enabling the analysis of extreme cases. However, textile dyeing models are limited and mainly focus on chemical [2] dyes or effluent treatment [3]. This study presents a new mathematical model to simulate wool and cotton dyeing with pigments from purple onion skins (natural dye), considering dye diffusion and sorption/desorption in textile fibers. **Methods:** A one-dimensional numerical approach was adopted, combined with volumetric average calculation techniques, to analyze mass transfer through the textile sample thickness and predict the temporal evolution of dye concentration. **Results:** Strong agreement was obtained between the numerical and analytical results (qualitative tendencies), confirming the correct implementation of the mathematical approach. Parametric studies were conducted to explore the main influences on the dyeing process. The results revealed the following: i) increasing fabric thickness results in surface dye accumulation, hindering penetration; ii) increasing the solid fraction fivefold and fiber density sixfold reduces liquid-phase dye concentration by 1.4 and 1.7 times, respectively; iii) decreasing the diffusion coefficient 100-fold prevented dye penetration after 600 s and iv) increasing the convective coefficient and mass concentration in dyeing solution enhanced dye uptake by the textile. **Conclusions:** Further quantitative validation (e.g., using dye concentration data) is required; however, the developed model provides a basis for predicting and optimizing dye–fiber interactions.

Keywords: Textile dyeing; natural dye; numerical simulation; dye diffusion in water; dye sorption in fibers

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The authors are grateful to Tintex Textiles S.A. for the supply of textile substrates used in this work and for technical assistance.

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24188 | Exploring the influence of low molecular weight organic gelators on liquid crystal gelation: a rheological and electro-optical study

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Background & Aim: Physically gelled soft materials based on the self-assembly of low-molecular weight organic gelators, particularly liquid crystal physical gels (LCPGs), form a versatile platform where supramolecular gel networks coexist with liquid crystal order [1], enabling reversible gelation and tunable electro-responsive properties [2–5]. However, the influence of electric fields on the interplay between these two self-assembly processes remains mostly unexplored. Here, this gap is addressed by probing LCPGs formulated from MBBA (as the liquid crystal) and suitable gelators. **Methods:** Rheological properties were measured using a stress-controlled rotational rheometer, coupled with electrorheological testing to assess the effect of electric fields applied during gelation and/or testing. For conductive formulations, a custom device was used, allowing controlled field application. Transition phenomena were characterized through differential scanning calorimetry and polarized optical microscopy and fiber morphology was examined by transmission and scanning electron microscopy. **Results:** Oscillatory testing showed a clear gel-like signature at low strains, with 9-O-Me forming a stronger network, while both networks remained robust under both short and long-timescale deformations. The addition of gelators lowered the crystallization temperatures, by 8 °C (9-O-Me) and 14 °C (6-O-Me), consistent with cavity formation that stabilizes the mesophase, with the larger delay for 6-O-Me arising from higher chiral aggregate content despite a sparser network. Moreover, electric fields applied during testing weakened the gel via mesogen reorientation, while application during gelation disrupted network formation, leading to anisotropic fiber growth. **Conclusions:** Stable gel formation was present for all cases and delays in crystallization highlighted the potential for low-temperature applications. Electric field application proved to influence the self-assembly process, potentially enhancing its tunability.

Keywords: liquid crystal; gelation; liquid crystal physical gel; rheology; electrorheology

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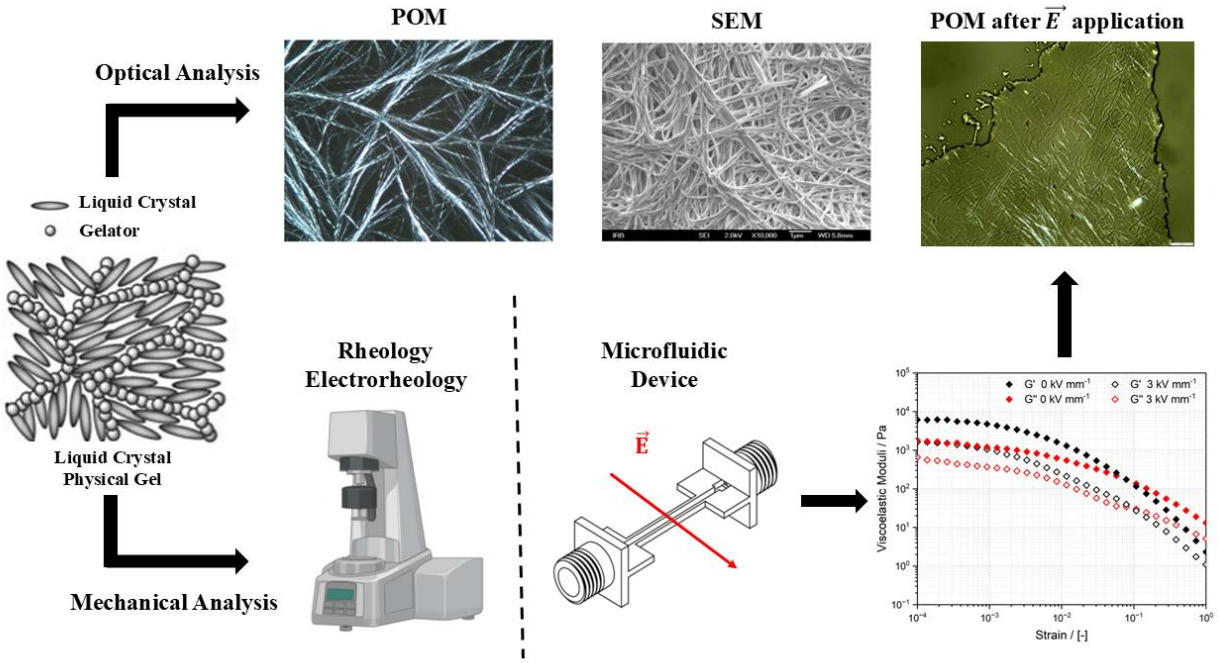

Figure 1: Graphical abstract.

24190 | Development of Sustainable Bio-Based Microcapsules for Textile Functionalization

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Background & Aim: Textile functionalization is a growing industry that converts fabrics beyond their traditional role, designing well-being and health-focused clothes [1]. A major approach is the incorporation of essential oils (EOs) with potential antimicrobial, anti-inflammatory and antioxidant effects. However, EOs are highly sensitive and prone to degradation due to external factors [2]. Microencapsulation offers an effective strategy to protect these actives by enclosing them with a shell that enhances stability and allows controlled release [2–4]. Currently, the commercial shell materials are toxic or plastic-based, raising environmental and health concerns [5,6]. This project studied the sustainable microencapsulation of an EO of interest using biodegradable, bio-based shells – chitosan and a protein –, followed by impregnation to produce health and wellness-oriented fabrics. **Methods:** Microencapsulation was achieved by complex coacervation, a scalable and solvent-free method [2,7]. Formulations were optimized through a cyclic approach of testing and characterization by optical microscopy (OM) and laser diffraction (LD). Chemical structure and encapsulation efficiency (EE) of selected formulations were studied through FTIR and GC-MS analysis. Textiles were impregnated using the pad–dry method, after which they were characterized by scanning electron microscopy (SEM). **Results:** Promising formulations showed spherical microcapsules with low aggregation, achieving an EE of (91.7 ± 0.9) % and a mean particle size of (19.71 ± 0.16) µm. OM and LD analysis confirmed formulation stability after 21 days. SEM showed partial capsule adhesion to textiles. Padding roller pressure led to capsule rupture, highlighting the need to improve shell resistance and fabric adhesion. **Conclusions:** A stable and sustainable microcapsule formulation was developed using an eco-conscious and scalable process. Preliminary textile applications demonstrated promising performance, although further optimization is needed.

Keywords: Functional textiles; Microencapsulation; Biopolymers; Sustainability.

Acknowledgments:

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The authors gratefully acknowledge the R&D team – Susana Dinis, Ivo Aroso and Catarina Moreira – of HeiQ Iberia Unipessoal Lda. for providing the textiles for functionalization.

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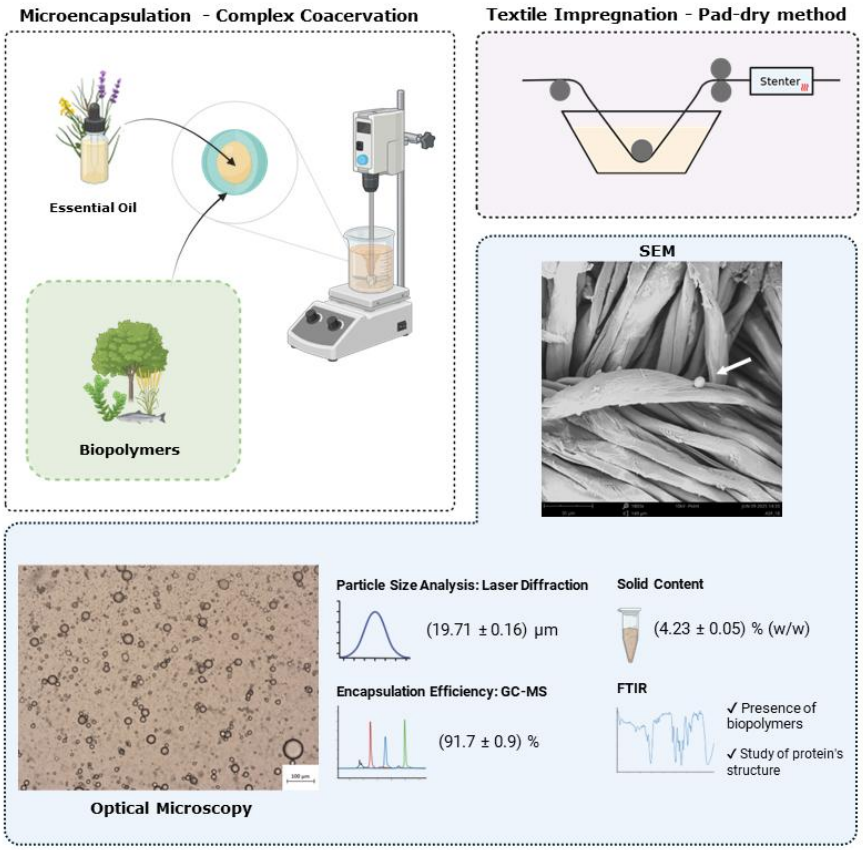

Figure 1: Graphical abstract of the project.

24308 | The potential of CO_2 hydrogenation to produce e-fuels: thermodynamic and techno-economic analysis

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Background & Aim: Petroleum has underpinned societal and industrial progress, as a primary source of heating, power and fuel. However, its environmental effects and in urban areas have highlighted the urgency to transition toward sustainable energy alternatives. CO_2 hydrogenation to electrofuels (i.e., e-fuels) emerges as promising for cleaner energy production. This study focuses on 12 different e-fuels: methane, ethane, propane, butane, pentane, gasoline, kerosene, diesel, methanol, ethanol, butanol and dimethyl ether. **Methods:** For thermodynamic assessment, Aspen Plus® V14 was used to simulate an equilibrium reactor fed with pure CO_2 and H_2 streams under different temperatures, pressures and H_2/CO_2 ratios. Initial economic analysis considered the determination of a production phase rentability parameter; a value above 1 indicates fuel sales surpass the combined costs of green H_2 and operational expenditure. Potentially viable cases advance to a rigorous economic evaluation. **Results:** Thermodynamic assessment results showed an increase in productivity and CO_2 conversion with higher pressure and lower temperatures. Excluding capital expenditure and assuming fuel prices equivalent to those from conventional production, potential rentability was revealed for gasoline under both best- (200 °C, 50 bar) and worst-case (350 °C, 20 bar) conditions, corresponding to the highest and lowest productivities, respectively, whereas diesel only under the best-case scenario. When capital expenditure and parameters such as workforce were considered, the initial investment was estimated at approximately 7-8 million euros, excluding costs associated with gas purification, transport, and storage. Cash flow progressively decreases, suggesting the unviability of the project under current conditions. **Conclusions:** A sensitivity analysis of hydrogen costs indicated potential feasibility for gasoline and diesel production, if H_2 prices drop to half their current levels, achievable by decarbonization investment.

Keywords: CO_2, e-fuels, renewable energy.

Acknowledgments:

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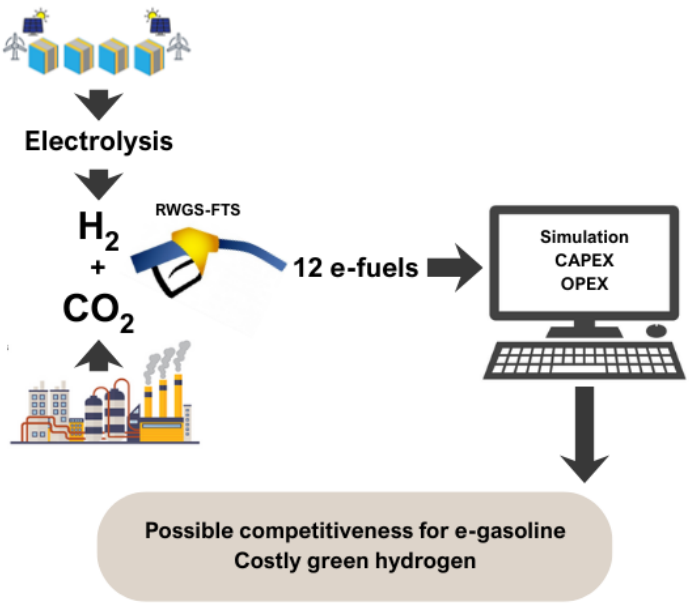

Figure 1: Graphical abstract.

24334 | Study of the geology and geochemistry of rare earth element ores and their effect on beneficiation and extraction processes

Jorge Pinto[1]; Maria de Lurdes Dinis[1]; Maria Cristina Vila[1]

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Background & Aim: This study provides a comprehensive overview of the mineralogical and geochemical characteristics of Rare Earth Element (REE), undertaken as part of the European REESource project. The research focuses on the Fen deposit in Norway, which serves as the primary case study. The FEN deposit consists of several types of carbonatites, among which Fe-dolomite carbonatite is of most significant economic interest due to the presence of bastnäsite, parasite, synchysite, and monazite (Silva et al., 2023). Bastnäsite and monazite deposits, together with xenotime, are considered the most economically viable for exploitation (Gaiendra et al., 2025). This work aims to develop a study of the geology and geochemistry of RRE ores and their effects on beneficiation and extraction processes, to understand how ore characteristics influence processing strategies. **Methods:** The methodology combines a review of existing literature with the generation of new experimental data. Petrographic analyses are carried out, alongside geochemical profiling using X-ray fluorescence and inductively coupled plasma mass spectrometry. Laboratory-scale beneficiation trials are also conducted on Fen deposit samples to assess processing performance. **Results:** The findings of this study provide essential insights into the mineralogical and geochemical behaviour of REE-bearing ores, which are critical for optimising recovery efficiency. The results demonstrate that variations in mineralogy and geochemical signatures have a substantial effect on the selection of processing methods and reagents, as well as on extraction yields. **Conclusions:** While established extraction processes exist for minerals such as bastnäsite, monazite, and xenotime, many REE-rich deposits contain more complex or less conventional minerals. The outcomes highlight the necessity for tailored processing approaches to maximise REE recovery from a diverse range of ore types. This will guide future exploration and processing strategies within the sector.

Keywords: REEs; Geology; Geochemistry; Mineral processing; FEN deposit

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24338 | Engineering Localized Dynamic Magnetic Fields for Targeted MNP Transport in Flexible Substrate

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Background & Aim: Magnetic manipulation of magnetic nanoparticles (MNPs) is increasingly explored for drug delivery and magnetic targeting, using magnetic forces to transport and concentrate therapeutic carriers at specific sites [1,2]. Yet, the design of localized, dynamically varying magnetic fields (rather than static magnets) is still comparatively underdeveloped, even though coil-based actuation can provide spatiotemporal control of particle trajectories and local mixing in biological environments [2,3]. In this work, we implement localized dynamic magnetic-fields using planar coils on flexible substrates and ferrimagnetic $MnFe_2O_4$ MNPs, establishing a controlled platform for targeted, field-driven MNP transport on dressing-like materials. **Methods:** Planar coil geometries and actuation strategies were optimized through COMSOL simulations to maximize magnetic field gradients while keeping a compact size suitable for integration into wound dressings. The magnetic field was generated via controlled current actuation. The coils were fabricated by screen-printing silver conductive ink onto Kapton and PET substrates. $MnFe_2O_4$ MNPs were synthesized via co-precipitation and characterized by XRD, SEM/EDS, and MPMS, then incorporated into a commercial wound dressing. **Results**: Simulation studies enabled identification of coil parameters that maximize near-surface gradients and geometries capable of generating enhanced magnetic field gradients. Magnetic characterization confirmed the formation of ferrimagnetic $MnFe_2O_4$ MNPs with suitable magnetization for magnetophoretic actuation. Preliminary assays in the dressing demonstrated controlled MNP release and field-driven displacement across the substrate surface. **Conclusions:** These initial results demonstrate the feasibility of integrating engineered planar coils with magnetically responsive nanoparticles toward enhanced biofilm disruption in future studies. This approach provides a controlled, fully characterized magnetic actuation strategy, addressing a limitation often reported in magnetophoretic studies.

Keywords: Magnetic nanoparticles; Magnetophoresis; Dynamic magnetic fields; Flexible planar coils; Wound dressings.

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24340 | Development of a Functional Knee Model for Anatomy Education

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Background & Aim: The knee is one of the most complex and essential joints in the human body, critical for mobility, balance, and weight support. Due to its structural complexity and frequent use, it is especially vulnerable to injury and degeneration, requiring a great understanding of its anatomy and biomechanical properties [1]. This project presents the development of an accurate and functional knee model, designed for anatomy education, capable of simulating muscle action. **Methods:** The procedure involved acquiring and using CT images to segment the bones of the knee joint in 3D Slicer, obtaining a 3D model. The soft tissues, including menisci and ligaments, were then sculpted around the model in Blender, respectively labelled and with connection points for their integration. Afterwards, a single-acting pneumatic piston, controlled via an Arduino and pneumatic circuit, was added to simulate the extension of the quadriceps muscle group, using custom-designed fittings. Finally, the model was printed, in PLA for the bone structures and TPU for the soft tissues, and the piston and its functional circuits were integrated. **Results:** The bone model accurately replicated the properties of a real bone and retained the details for connection with the other pieces. The soft tissues were more flexible, as intended, and kept their resolution in the areas of interest. The addition of the pneumatic piston, along with its controlled circuit, allowed for the demonstration of the knee extension, offering an interactive experience for anatomical education. The final model was very similar to the virtually designed model and worked as intended, though it was limited by a lack of passive relaxation and material wear. **Conclusions:** This work provides a simple procedure for creating anatomically accurate models of the human body, serving as a foundation for more comprehensive and functional anatomical models for medical education, thereby making it more engaging and comprehensive.

Keywords: 3D Anatomical Model, Artificial Muscle Actuators, Knee Anatomy, Medical Education

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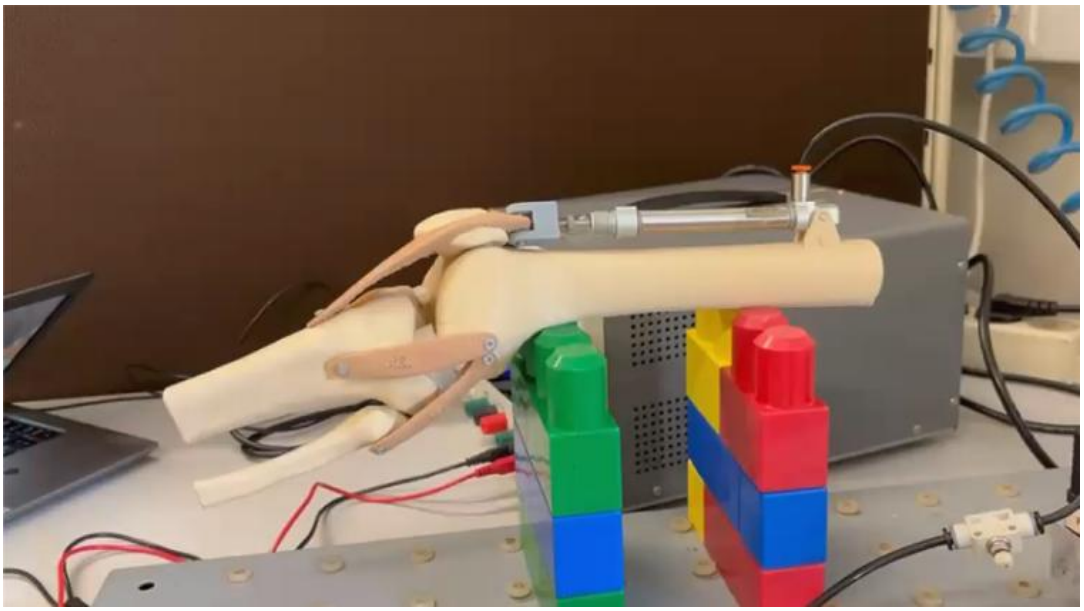
Figure 1: Functional Knee Model



24396 | Transferrin and fluorine-modified PLGA nanoparticles for brain targeting and delivery of donepezil

Rafael Silva[1,2]; Maria J. Ramalho[1,2]; Stéphanie Andrade[1,2]; Maria Carmo Pereira[1,2]; Joana A. Loureiro[1,2,3]

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Background & Aim: Alzheimer's Disease (AD), the most predominant form of dementia, is a progressive neurodegenerative disease, with its prevalence rising with age [1]. Therapeutic approaches almost exclusively focus on symptomatic treatment, with donepezil (DPZ) among the drugs often prescribed [2]. The main obstacle to the development of new treatments, and to the effectiveness of those already approved, is the blood-brain barrier (BBB) [3]. Nanoparticles (NPs) have emerged as drug delivery systems, enabling safe and efficient transport across this barrier [3]. In this work, poly(lactic-*co*-glycolic acid) (PLGA) NPs loaded with DPZ were functionalized with transferrin (Tf) to improve BBB passage by receptor-mediated transcytosis and fluorine to enhance the barrier's permeability and inhibit amyloid-beta (Aβ) aggregation [4,5]. **Methods:** DPZ-loaded PLGA NPs were produced by double emulsion, and chemically conjugated via carbodiimide coupling. Encapsulation efficiency (EE) and loading capacity (LC) were determined by UV-Vis spectroscopy. Size, polydispersity index (PdI), and zeta potential (ZP) were determined by dynamic and electrophoretic light scattering after production and over time to evaluate the nanoformulation stability in storage conditions (C_{PLGA} = 10 mg·mL^{-1} aqueous suspension, 4˚C). Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) was performed to confirm effective conjugation of the ligands, and transmission electron microscopy (TEM) allowed visualization of the NPs' morphology. **Results:** The developed double-decorated DPZ-loaded NPs presented a diameter of 148 ± 6 nm, a PdI of 0.07 ± 0.03, a ZP of -18.3 ± 0.6 mV, an EE of 32 ± 9%, a LC of 3 ± 1%, and were stable for 15 days. ATR-FTIR confirmed NPs' functionalization, and TEM images revealed spherical, homogenized, and individualized NPs. **Conclusions:** A double-functionalized nanocarrier was formulated successfully with promising features to aid the challenges of current therapies.

Keywords: Alzheimer's Disease, Nanotechnology, Surface modification, Drug delivery

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24518 | Robotic-Assisted Reconstruction of the Anterior Cruciate Ligament

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Background & Aim: Anterior cruciate ligament (ACL) reconstruction is one of the most frequently performed orthopaedic procedures worldwide [1], yet consistent and anatomically accurate femoral tunnel placement remains a major clinical challenge [2]. Variability in tunnel positioning can compromise knee biomechanics, leading to instability, graft failure, and increased revision rates [2-4]. Although computer-assisted and robotic-assisted technologies have demonstrated improvements in surgical accuracy, their widespread adoption is limited by workflow complexity, invasiveness, and reliance on bone-mounted tracking markers [5], with limited exploration of markerless and less invasive alternatives [6]. This study aims to develop and experimentally validate a markerless robotic-assisted framework for ACL reconstruction to enable precise, repeatable, and clinically relevant femoral tunnel placement. **Methods:** The proposed framework integrates three-dimensional depth sensing using the Ensenso N36 depth camera for real-time knee localisation and tracking, iterative closest point-based registration for accurate alignment between virtual anatomical models and physical anatomy, and robotic guidance with the Kuka LBR MED to assist controlled femoral tunnel positioning using the Bernard and Hertel grid [7] as a coordinate system. The system will be evaluated using a knee phantom under simulated surgical conditions. **Results:** System performance will be assessed through target registration error and femoral tunnel placement deviation relative to the planned anatomical target. Repeatability and execution time will also be analysed across multiple experimental trials. The proposed approach is expected to achieve errors below 3 mm [6] and improved placement consistency compared with conventional manual techniques. **Conclusions:** The proposed markerless robotic-assisted framework establishes a foundation for reducing invasiveness while maintaining clinically relevant accuracy in ACL reconstruction.

Keywords: Robotic-assisted surgery, Anterior cruciate ligament reconstruction, Computer-assisted orthopaedic surgery, Markerless tracking

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24624 | Knee Arthroplasty Navigation System based on Spatial Augmented Reality

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Background & Aim: Total knee arthroplasty (TKA) is one of the most frequently performed orthopaedic procedures worldwide, and its use continues to increase due to aging populations and rising rates of obesity and osteoarthritis [1]. Although TKA is generally effective in relieving pain and restoring function, approximately 7.3 to 20% of patients remain dissatisfied [2], often due to persistent pain or implant malalignment [3]. Robotic-assisted systems have improved surgical precision and alignment accuracy [4]. However, they are costly and require significant operating room space and infrastructure. This project aims to develop a knee arthroplasty navigation system based on spatial augmented reality (SAR) that provides accuracy comparable to robotic-assisted systems while reducing costs and improving surgical workflow efficiency. **Methods:** An Ensenso N36 stereo camera is used to acquire three-dimensional (3D) coordinates of the knee. By determining the geometric relationship between the camera and a projector, the system calculates which projector pixel corresponds to each 3D point on the knee surface. A patient-specific Computer-Aided Design (CAD) model derived from Computed Tomography (CT) imaging enables anatomical registration and real-time tracking, allowing precise projection of planned bone resection planes directly onto the surgical field. **Results:** The expected outcome is a reliable and accurate navigation system capable of real-time knee surface identification and precise projection of surgical cut planes, achieving clinically acceptable alignment accuracy. **Conclusions:** This SAR based system has the potential to offer a cost effective and space efficient alternative to robotic assisted TKA while maintaining high surgical precision.

Keywords: Total Knee Arthroplasty, Spatial Augmented Reality, Computer-assisted Orthopedic Surgery, Markerless Tracking

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24668 | Development of Immobilized Enzymes to support Biofuel production from residual feedstocks

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Background & Aim: Conventional biodiesel production relies on chemical catalysis, toxic fossil-derived methanol, and virgin oils that compete with the food sector. Aiming for a more bio-based process, this study utilized residual feedstock (Used Cooking Oil, UCO), ethanol, and enzymatic catalysis. To overcome high enzyme costs, physical immobilization was also explored, which might enable biocatalyst reuse. **Methods:** The quality of the biofuel, produced by transesterification, was evaluated based on its FAME (fatty acid methyl esters) content, after product purification. To assess biocatalyst and residual feedstock use viability, the conversions of UCO and virgin sunflower oil, with lipase in its free form as catalyst, were compared. This lipase was then adsorbed onto spent coffee grounds (SCG), carbon nanotubes (CNTs), and alumina. Their catalytic activity and reusability in the reaction between UCO and methanol were compared against those of commercial immobilized and free lipases. The use of ethanol instead of methanol, using both commercial enzymes as catalysts, and UCO as substrate, was also studied. **Results:** Free enzyme catalysis resulted in 100% FAME using virgin oil and 89 ± 5 wt.% with UCO, validating both the biocatalyst and the waste feedstock. SCG demonstrated exceptional potential as enzyme support: its performance matched the commercial immobilized enzyme, under similar conditions and, under optimized conditions, it achieved the free lipase efficacy (both $p > 0.05$). CNT-immobilized enzyme enabled favorable conversions (77 ± 7 wt.%), whilst the one immobilized on alumina failed to catalyze the reaction. Effective reusability was not verified for any biocatalyst. Ethanol substitution proved viable, showing no significant differences compared to methanol ($p > 0.05$). **Conclusions:** Integrating UCO, ethanol, and enzymatic catalysis has the potential to create a stronger bio-based and sustainable framework for biodiesel production. SCG is a remarkably promising residual carrier that might minimize immobilized biocatalyst costs, aligning with Circular Economy principles. Future work must optimize immobilization to achieve successful reusability.

Keywords: Biodiesel; Enzymes; Immobilization; Bio-based; Circular Economy.

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24377 | Co-Designing a Serious Game to Support Emotion Regulation in Adolescents

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Background & Aim: Difficulties in emotion regulation (ER) during adolescence are associated with increased risk of anxiety, depression, and behavioral problems [1]. Preventive digital interventions, particularly serious games, offer promising opportunities to promote emotional skills in engaging and accessible ways. This work aims to design and evaluate an evidence-based serious game that supports the development of ER skills in adolescents through a participatory co-design approach. **Methods:** The project follows a participatory design approach that includes workshops, iterative prototype testing, and evaluation. Adolescents aged 10–14, together with educators and mental health professionals, will contribute to co-design activities to ensure that the content is appropriate for their age and relevant to their everyday lives. The game design is informed by Gross's Process Model of Emotion Regulation [2]. Usability, engagement, and motivation will be assessed using self-report instruments (e.g., SUS [3], IMI [4], GEQ [5]), while emotional outcomes will be explored using measures of affect and ER strategies. **Results:** It's expected to identify adolescents' key emotional challenges, preferred game mechanics, and design requirements for emotional learning and engagement. Prototype testing is expected to show high usability, strong engagement, and increased awareness of emotional responses and coping strategies. **Conclusions:** Co-designing serious games with adolescents and stakeholders appears to be a promising approach for developing theory-driven and contextually relevant digital tools for ER. The proposed game contributes to mental health promotion by providing an interactive environment in which adolescents can practice adaptive ER strategies. The findings are expected to inform the design of scalable educational interventions targeting emotional well-being.

Keywords: Emotion regulation; Serious games; Adolescents; Co-design; Mental health

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24748 | Incentive-based Ensemble Forecasting for Renewable Energy

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Background & Aim: This project is developed at INESC TEC in collaboration with Elia Group, the transmission system operator in Belgium and Germany (50Hertz). The collaboration aims to operationally demonstrate a collaborative forecasting platform developed by INESC TEC, applied to Belgian national offshore wind and solar photovoltaic generation. The platform [1] combines forecasts from multiple providers using ensemble methods to deliver deterministic and probabilistic forecasts (10th/90th percentiles) at a 15-minute resolution for day-ahead horizons. A SoftMax-based weighting method assigns weights based on historical errors using an exponential decay function, linking forecast accuracy to ensemble influence and remuneration per provider. Dynamic Time Warping is used to remove anomalous submissions. The main goal is to evaluate the robustness of the current ensemble approach and develop adaptive mechanisms to improve accuracy and error stability. **Methods:** Several ensemble strategies were benchmarked [2], including linear regression and gradient boosting. Given their heterogeneous performance across operating conditions, adaptive classifiers and regressors were developed. Trained on meta-features of the daily submissions (e.g., maximum, correlation, variance), the classifier dynamically selects the best ensemble strategy, while the regressor estimates weights to combine them. **Results:** So far, the meta-learning approaches outperformed initial ensemble methods, reducing average forecasting errors by up to 6% in a specific month, and significantly decreasing variance of monthly average errors by up to 10%. **Conclusions:** Results indicate that adaptive meta-learning allows robustness more effectively than static ensemble schemes. By dynamically adapting combination logic to daily forecast profiles, the platform improves operational reliability and reduces imbalance costs. This provides an adaptive, risk-aware framework for handling the volatility of renewable generation.

Keywords: Ensemble forecasting; Forecasting markets; Renewable energy.

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24778 | Host Robot Intelligence Layer

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Background & Aim: Host robots guide users and shape social trust, but scripted dialogues lack adaptability. While Large Language Models (LLMs) offer conversational flexibility, they suffer from hallucinations and lack spatial and temporal grounding. This project aims to build an AI Agent acting as a grounded decision agent for decision and navigation. The ultimate goal is to create an Intelligence Layer allowing a ROS 2 device to become a host robot for live campus tours and guidance at FEUP and/or INESC TEC, aware of spaces and temporal issues. **Methods:** The system utilizes a modular architecture featuring a full Speech-to-Text (STT), LLM, and Text-to-Speech (TTS) pipeline. Reasoning is to be based on an agentic approach that includes skills, Vectorial Databases, and Retrieval-Augmented Generation (RAG). Validation is based on a custom 2D Pygame event driven simulator. **Results:** The STT-LLM-TTS pipeline is fully implemented, and preliminary findings show RAG successfully restricts the AI Agent's output to valid environment data. The Intelligence Layer accurately translates user intents into ROS 2 behaviours (navigation goals and visual feedback information). In simulation, the system demonstrates environmental awareness, successfully handling contextual events and producing emotion information to be displayed in a custom-built LED display. **Conclusions:** This Intelligence Layer transforms an adequate ROS2 robot into a host robot. By functioning as a grounded AI Agent, the system can dynamically guide guests through complex campuses while seamlessly handling spatial and time-sensitive constraints.

Keywords: Social Robotics; Large Language Models (LLMs); Dialogue Systems; Human–Robot Interaction (HRI); System Architecture

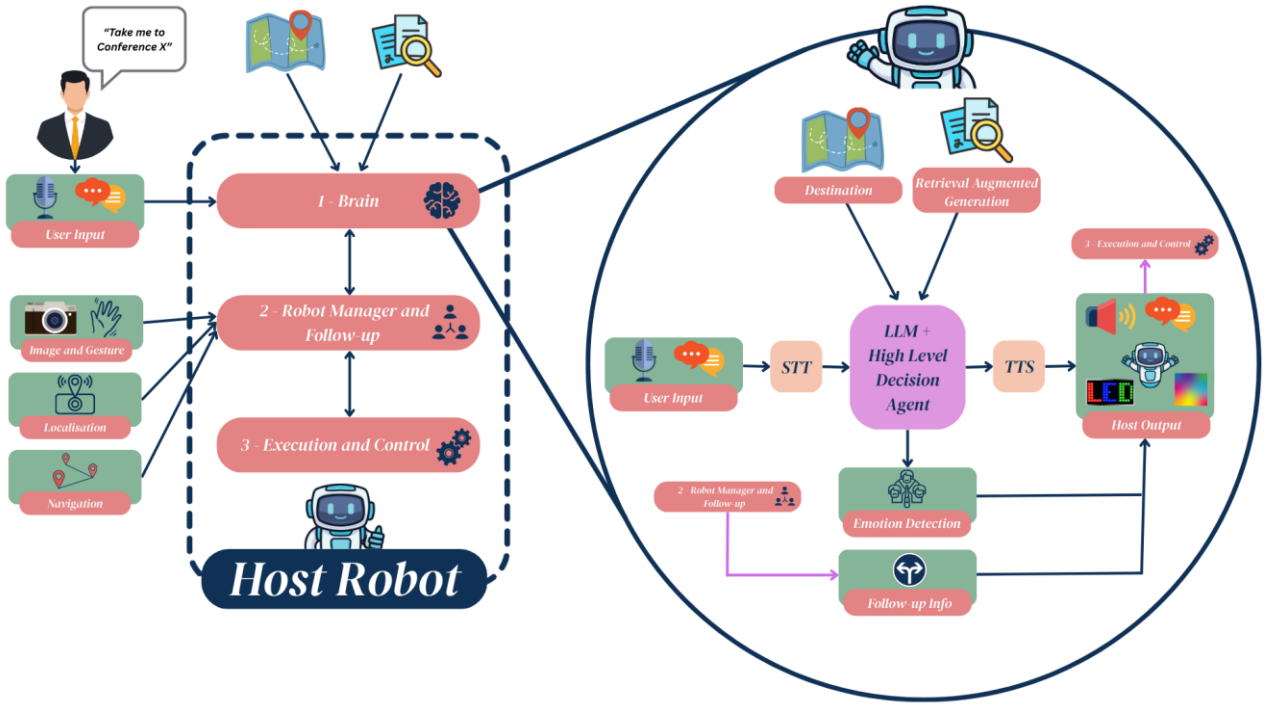

Figure 1: Graphical Abstract

24806 | Protein-Functionalized Carbon Nanotubes for the Selective Recovery of Platinum Group Metals

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Background & Aim: The recovery of Platinum Group Metals (PGMs), classified by the European Commission as critical raw materials [1], is increasingly relevant due to their scarcity and high demand across several industrial sectors like emerging green technologies. Conventional mining processes are energy-intensive and generate toxic byproducts, whereas spent automotive catalytic converters (SACCs), the most predominant secondary source, offer significantly higher PGMs concentrations than natural ores. Developing efficient, environmentally benign recovery strategies from secondary sources is key to improving material circularity and supply resilience [2]. Bio-derived materials emerged as alternatives for metal recovery through adsorption, offering high selectivity, sustainability and environmental benefits [3]. In this work, ovalbumin (OVA) functionalized multiwalled carbon nanotubes (CNTs) were tested for this purpose, defined as OVA-CNTs. **Methods:** OVA immobilization on the CNTs was studied by optimizing various parameters. After, OVA-CNTs were tested for selective PGMs recovery from synthetic HCl-based multi-metallic solutions. Several characterization techniques (FTIR, Raman, TEM, TGA, and XPS) aided in understanding differences between pristine CNTs and OVA-CNTs, while shedding light on the adsorption mechanism. **Results:** OVA immobilization was optimized, achieving Langmuir-type adsorption with OVA loading of ~126 $mg \cdot g^{-1}$ at pH 7 within 30 min, yielding a strong, durable bioconjugate material. In metal adsorption tests, OVA-CNTs consistently proved its potential for PGMs recovery, achieving high Pd and Pt adsorption capacities (6.94 and 4.41 $mg \cdot g^{-1}$). Characterization techniques clarified the advantages OVA brings to the adsorption process when compared to pristine CNTs. **Conclusions:** While CNTs provide durability and loading capacity, OVA functional groups are crucial for achieving selective and efficient metal adsorption. This poses as a novel approach to tackle the global PGMs supply imbalance.

Keywords: Critical metals; multi-walled carbon nanotubes; ovalbumin; platinum group metals; selective adsorption.

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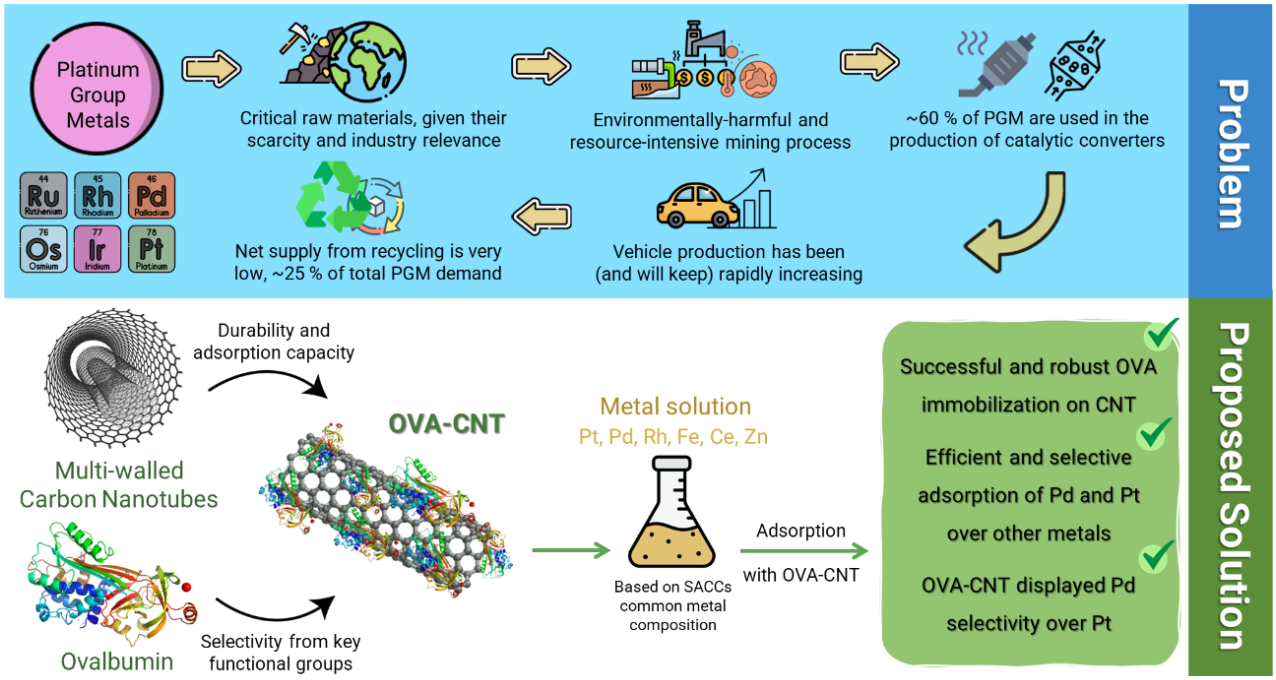
Figure 1: Graphical Abstract



24863 | Study of the Deformation of Metallic Components Produced by 3D Printing

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Background & Aim: Additive Manufacturing (AM) enables the production of complex geometries with high efficiency, but processes like Powder Bed Fusion (PBF) face critical challenges such as part warping due to high thermal stresses and non-uniform cooling. This work aims to identify the most suitable simulation conditions in Abaqus to achieve reliable results with minimal computational cost and to study the optimal interlayer waiting time to reduce deformation in a turbocharger turbine. **Methods:** Finite element analysis was conducted using the Abaqus 2022 AM Modeler plug-in. A custom Python script was developed to convert G-code into toolpath and roller input files. Six mesh sizes (1 mm to 6 mm) were tested to evaluate the balance between stress accuracy and CPU time. Nine interlayer time variations (0s to 45s) were simulated for the turbine geometry. The methodology was validated through a Directed Energy Deposition (DED) simulation and a subsequent tensile test replication. **Results:** Through a convergence study, an ideal mesh of 3 mm was reached for the printing simulations, which ensured the best balance between result accuracy and computational cost. Based on this mesh, it was determined that an interlayer time of 20 seconds is ideal to obtain the turbine geometry while minimizing deformations and maintaining an efficient production time. In contrast, continuous printing proved preferable for minimizing residual stress. The validity of the study was confirmed by replicating a DED case study and a tensile test, where the simulated stress-strain curve showed high agreement with experimental data. **Conclusions:** The study successfully optimized simulation parameters for metallic AM, demonstrating that strategic cooling pauses (20s) are essential for dimensional accuracy in thin-walled components like turbine blades. The high correlation between simulated and experimental DED data confirms the reliability of the proposed numerical framework for industrial optimization.

Keywords: Additive manufacturing; Abaqus; Powder Bed Fusion; Direct Energy Deposition; Finite Element Analysis

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24882 | Auxetic patterns for the development of vascular grafts via melt electrowriting

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Background & Aim: Anastomoses of vascular grafts can be compromised by the axial shortening under pulsatile pressure caused by conventional positive-Poisson materials. Auxetic ([1]) structures can be used to avoid these kinds of complications. The aim was to generate continuous auxetic toolpaths for melt electrowriting (MEW) and assess how geometrical and printing parameters control the scaffolds mechanical response. **Methods:** A MATLAB G-code generator was developed to produce toolpaths for the desired patterns. MEW was used to fabricate planar and tubular samples across printing conditions. Planar samples (n=3 per condition) underwent uniaxial tensile testing to measure unfolding strain; and the printed structures were characterized through optical imaging. **Results:** Tubular and planar auxetic scaffolds were fabricated with MEW. Different printing parameters and toolpaths resulted in differences on the angle of the unit cells, and therefore, affected the unfolding strain: samples with larger-than-angles unfolded less and showed reduced extensibility, indicating sensitivity to toolpath and printing parameters. **Conclusions:** Continuous MEW auxetic patterns are manufacturable and their unfolding mechanics are highly sensitive to small geometric deviations introduced during printing. This work provides an algorithmic and fabrication pathway to design MEW scaffolds with non-typical Poisson ratios, advancing the state of the art in mechanically informed graft design. By enabling tunable longitudinal compliance at the scaffold level, these toolpaths offer a route to reduce anastomotic stress and to improve mechanical matching between graft and native vessel. Next steps include moving towards microporous auxetic scaffolds that can be used as the core of a vascular graft that does not shorten axially under pulsatile pressure.

Keywords: melt electrowriting; vascular graft; longitudinal compliance; auxetics;

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24914 | 3D-printed hybrid scaffolds containing 2D nanomaterials for photothermal therapy of cancer

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Background & Aim: Local cancer treatments remain limited by the difficulty in achieving effective tumor control without significant systemic toxicity [1]. Photothermal therapy (PTT) has emerged as a promising approach, enabling spatially confined heating through near-infrared (NIR) light activation of photothermal agents [2]. 2D nanomaterials, such as copper sulfide (CuS), are particularly attractive candidates given their high surface area and strong NIR absorption [3]. This work aims to develop 3D-printed polymer scaffolds incorporating CuS nanoparticles capable of acting as photothermal agents for cancer therapy. **Methods:** Photothermal heating performance of CuS was evaluated under a custom-developed LED NIR irradiation device (24-LED system to fit a 48-well plate) using thermocouples to record temperature increase. Physicochemical characterization was performed by TEM, Raman spectroscopy, FTIR, and Zetasizer. Formulations are being studied to incorporate CuS into printable polymer matrices (PCL) to be processed into filaments for 3D printing. Scaffolds with defined geometry and porosity were designed (CAD model). In vitro biological assays will assess biocompatibility and anticancer efficacy with and without NIR activation. **Results:** NIR irradiation of CuS (250-500 µg mL^{-1}) for 30 min produced consistent temperature increases relative to controls, reaching values within therapeutic ideal ranges (52-57 °C), demonstrating its suitability for anti-cancer PTT. Additional characterization (Raman, TEM, Zetasizer, FTIR) confirmed that the materials presented ideal physicochemical properties for 3D-printed hybrid scaffolds production. **Conclusions:** Early results validate the use of CuS nanomaterials as photothermal agents, supporting their integration into 3D-printed scaffolds for PTT in cancer treatment. This approach

has the potential to generate a reproducible and geometrically customizable platform for localized therapy, reducing systemic exposure and side effects, and improving therapeutic precision.

Keywords: 3D printing; scaffolds; 2D nanomaterials; photothermal therapy; drug delivery

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24920 | Lipid matrix composition effect on the structural optimization of caffeine-loaded solid lipid nanoparticles for skin applications

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Background & Aim: Caffeine is a bioactive compound widely used in cosmetic and dermatological formulations due to its antioxidant, anti-inflammatory and lipolytic properties [1]. However, achieving optimal skin delivery and sustained activity remains challenging with conventional topical formulations [2]. Solid lipid nanoparticles have emerged as a promising solution capable of enhancing compound stability, skin penetration and controlled release [3]. This study aimed to evaluate the influence of lipid matrix composition on the optimization of caffeine-loaded SLNs for skin delivery applications [4]. **Methods:** Several solid lipids were screened to evaluate their compatibility with caffeine and nanoparticle stability. SLNs were produced by high-shear homogenization followed by probe ultrasonication and characterized in terms of hydrodynamic diameter, polydispersity index and zeta potential, together with encapsulation efficiency and loading capacity. Stability was evaluated by accelerated stability assays, *in vitro* release studies by UV-Vis spectroscopy and morphological analysis by transmission electron microscopy. Thermal behavior was analyzed by differential scanning calorimetry and further characterization included antioxidant activity, spreadability, occlusive properties and pH assays. Cytocompatibility was assessed using human dermal fibroblasts. **Results:** Compritol® 888 ATO produced the most suitable nanoformulation, exhibiting a mean size of 160±27 nm, PdI of 0.30±0.01 and zeta potential of -12±2 mV, achieving an EE of 65±4%. Morphological analysis confirmed spherical particles, while studies at different temperatures showed no physicochemical changes after 1 month. The formulation showed sustained caffeine release and high cell viability. **Conclusions:** These results highlight the critical role of SLN's lipid

matrix composition in their physicochemical properties and performance. The optimized formulation represents a promising lipid-based platform for enhancing topical caffeine delivery.

Keywords: Nanoparticle engineering; lipid matrix design; nanoencapsulation optimization; caffeine delivery systems; topical application

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ENVIRONMENT

24203 | EU-Relevant Pharmaceuticals, Pesticides, and UV-Filters in Drinking Water: A SPE-LC-MS/MS Method for Environmental Monitoring and Risk Assessment

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Background & Aim: Scientific concern regarding the presence of micropollutants (MPs) in aquatic environments has been growing in recent years [1]. Most conventional wastewater and drinking water treatment plants (WWTPs and DWTPs, respectively) are not specifically designed to completely remove many of these MPs, meaning their occurrence in DW remains likely, potentially posing risks to public health [1, 2]. Therefore, this work aims to provide an analytical method for the determination of MPs in DW, providing a preliminary insight into their potential human health risks. **Methods:** An offline solid-phase extraction liquid-chromatography tandem mass-spectrometry (SPE-LC-MS/MS) method was optimised, validated, and applied for the determination of 23 MPs listed in recent European Union frameworks. Validation was performed according to international guidelines. The validated method was applied to 50 DW samples collected across Portugal. A preliminary human health risk assessment was conducted through the estimation of hazard quotients (HQs). **Results:** Method optimisation included the addition of 13.5 mg of sodium thiosulfate to DW samples to improve analyte recovery. The method demonstrated high analytical sensitivity with method detection limits below 1.50 ng L^{-1}. Only 4 MPs - fluconazole, irbesartan, dimoxystrobin, and benzotriazole - were detected in 43 samples, with up to 3 MPs per positive sample. Quantifiable concentrations ranged from 0.28 to 98.8 ng L^{-1}, although benzotriazole and dimoxystrobin exceeded the upper calibration limits in 1 and 3 samples, respectively. HQs indicated no relevant human health risks. However, these HQs were evaluated for each substance individually in each sample and do not reflect realistic exposure scenarios since potential mixture effects were not considered. **Conclusions:** This study provides high-quality data on the occurrence of EU-relevant MPs in Portuguese DW, addressing a significant knowledge gap.

Keywords: micropollutants; tap water; analytical chemistry; European legislation; hazard quotients.

Acknowledgments:

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24206 | Down the drain: Insights on the ecotoxicological effects of stormwater runoff in rivers

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Background & Aim: Stormwater runoff, especially in urban areas, can transport a wide range of contaminants into freshwater ecosystems. Because these discharges often enter waterways untreated or poorly treated, they threaten aquatic life and ecosystem integrity. This study aims to evaluate the ecotoxicological effects of stormwater using 2 model organisms: the bacterium *Escherichia coli* and the fish *Danio rerio*. **Methods:** Water samples were collected from 3 freshwater bodies (Este river, Sousa river and Vale das Flores stream). At each site, sampling was carried out upstream, at stormwater effluents points, and downstream. Bacterial cell viability assays were performed using two *E. coli* strains (ATCC25922 and an environmental isolate) exposed to unfiltered water samples. Fish Embryo Acute Toxicity (FET) tests were performed, evaluating mortality, hatching success, and morphological abnormalities after exposure to filtered (0.45 µm) and unfiltered water samples. **Results:** *E. coli* ATCC25922 was consistently more sensitive than the environmental strain across most exposure times and samples. Sousa river samples caused the highest reduction in bacterial viability whereas Vale das Flores stream samples showed the lowest impact. In the FET assays, no consistent pattern emerged between filtered and unfiltered samples. All sampling sites induced morphological alterations in embryos (e.g., body curvature, hemagglutination, and pericardial edema), with Este river and Vale das Flores stream showing the highest percentages (5 - 25%). Sousa river samples significantly impaired embryo hatching and resulted in the highest mortality rates (reaching up to 95%). **Conclusions:** Overall, the results revealed distinct toxicity profiles among the urban-influenced waterbodies studied, with Sousa River samples inducing the most lethal effects. These findings highlight the relevance of integrating multi-level bioassays to strengthen ecotoxicological assessment and improve monitoring practices in urban freshwater systems.

Keywords: Water quality; Rainwater; Aquatic Ecosystems; Ecotoxicology; Model organisms

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24228 | Beyond Single Stressors: How do metformin and thermal stress interact to modulate *Daphnia magna* feeding behavior?

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Background & Aim: Metformin (MET) is a widely prescribed antidiabetic drug for type II diabetes mellitus, a major public health concern worldwide. Due to its high consumption and limited removal capacity from wastewater treatment plants, MET is released into freshwater ecosystems, endangering aquatic biota. Concurrently, climate-change-driven thermal stress (e.g., global warming and extreme cold events) can alter contaminant toxicity, challenging ecological risk predictions. This study evaluated how temperature shifts modulate the ecotoxicological effects of metformin in *Daphnia magna*, integrating feeding behavior responses and sub-individual biomarkers. **Methods:** *D. magna* feeding inhibition assays (24h; 0 to 80 mg/L; no light) were conducted at: 20°C (standard), 24°C (warming scenario; + 4°C increase projected by the IPCC until 2100), and 15°C (cold-wave scenario; - 5°C decrease defined by cold wave duration index). Biomarkers for oxidative stress, detoxification, and neurotoxicity were quantified. **Results:** A significant interaction was observed between MET and temperature across all endpoints. The impact on feeding rate was less pronounced at 15°C (EC_{50}= 85.23 mg/L) and 24°C (EC_{50}= 66.65 mg/L) compared to 20°C (EC_{50}= 60.35 mg/L), indicating potential short-term adaptive capacity. At 15°C and 20°C, increased antioxidant/detoxification defenses (CAT and GSTs activities) were insufficient to prevent oxidative damage, as shown by raised lipid peroxidation. At 24°C, these defenses were more effective, reducing oxidative damage and neurotoxic effects. Acetylcholinesterase (AChE) inhibition occurred mainly at 20°C, whereas AChE activity increased at 15°C, suggesting compensatory mechanisms (e.g., pseudocholinesterases activation). **Conclusions:** Temperature shifts significantly modulate MET toxicity and the related biochemical disruptions observed in *D. magna*. These findings reinforce

the need for multi-stressor and multi-level approaches in ecological risk assessment under climate change scenarios.

Keywords: Bioassays; Pharmaceuticals; Climate Change; Biomarkers; Ecotoxicity

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24325 | Assessment of Total Volatile Organic Compounds in Primary School Indoor Environment

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Background & Aim: Indoor air quality (IAQ) has a significant influence on human health and well-being, as people spend about 90% of their time indoors, where pollutant concentrations are typically higher [1]. Children spend approximately 7-8 h per day in school; additionally, they are more susceptible to the adverse effects of poor IAQ [2]. Volatile organic compounds (VOCs) are among the most common indoor chemical pollutants, with adverse effects on children's health and well-being. Given the possible health risks, the World Health Organization (WHO) identified 30 chemicals relevant to indoor air of child settings that require further attention, out of which 17 are VOCs [3]. **Methods:** This study aimed to investigate levels of total VOCs (TVOCs) in twenty primary schools (S1–S20) situated in Porto, Portugal. Real-time TVOCs were continuously monitored by VelociCalc® Multi-Function Ventilation – model 9565, TSI Inc., Shoreview, USA. In each school, sampling was conducted in four classrooms (n=80) over five weekdays, February 2024–June 2025; a 1-min logging interval was used to obtain a broad temporal distribution of the data. **Results:** TVOC means ranged from 60 µg/m³ (at S3) to 856 µg/m³ (S19), with an overall mean of 373 µg/m³. The existing guideline of 600 µg /m³ was exceeded in 15% of schools. During the occupied period, the mean TVOCs was 6 times higher than during the unoccupied period, with the largest difference between the two periods being 1025 µg/m³ (at S8) and the smallest being 72 µg/m³ (at S3). **Conclusions:** The results show a significant impact of human occupancy on indoor TVOC levels. It also highlights that some schools did not meet legislative requirements, thus posing a potential health risk to students and teachers.

Keywords: IAQ; TVOCs; children; classrooms;

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24357 | Behavioural changes in juvenile isopods after exposure to triclosan: open-field and colour enriched arena test approach

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Background & Aim: Irrigation with treated wastewater and the use of biosolids can result in the spread of the antimicrobial triclosan in agricultural soils, which can reach up to 10 mg kg $^{-1}$ [1]. This emerging contaminant may affect isopods, and essential soil maintenance organisms [2]. The objective of this study was to evaluate the behaviour of juvenile terrestrial isopods *Porcellionides pruinosus* after consumption of triclosan, through colour-enriched arenas and open field arenas never used before for these organisms. **Methods:** *Porcellionides pruinosus* from laboratory cultures (23 ± 1 °C; 16:8 L:D) were exposed to triclosan-spiked food discs (1 mg/g) in plastic boxes with moist filter paper (1 organism/box). After 48 h, open-field tests were performed in colourless and colour-enriched arenas, videos were recorded for 15 min, and analysed in AnimalTA® for exploration patterns. Statistical analyses to determine behavioural patterns used Generalised Linear Mixed Models (GLMMs), Self-Organising Maps (SOMs) and Hidden Markov Models (HMMs). **Results:** Behavioural parameters such as velocity and thigmotaxis demonstrated an impact of triclosan in *P. pruinosus*. In addition, it was observed preference for the colours black, green, and orange. **Conclusions:** For the first time, colour preference is reported by *P. pruinosus* and triclosan-induced thigmotaxis changes are reported for open-field behaviour tests, highlighting ecotoxicological risks of this pharmaceutical.

Keywords: Isopods; Open-field test; Behaviour; Triclosan.

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24371 | Effects of urban stormwater runoff on the growth of the *Lemna minor*

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Background & Aim: Urbanization alters freshwater ecosystems through intensified anthropogenic pressures, particularly the expansion of impervious surfaces that enhance urban stormwater runoff [1,2]. This runoff transports a complex mixture of contaminants, including metals, hydrocarbons, nutrients, microplastics, and pesticide residues, which can impair water quality and disrupt ecological processes [3]. This study investigated the effects of urban stormwater runoff collected from Portuguese water bodies, representing different urban runoff sources on the standard macrophyte *Lemna minor*. **Methods:** Water samples were collected from three urban-influenced Portuguese waters: Este river, Sousa river, and Vale das Flores stream. In each ecosystem, samples were collected from three sites: an upstream site, the stormwater discharge outlet, and a downstream site influenced by urban runoff. Growth inhibition assays with *L. minor* were performed following the OECD guideline 221 [4] using both filtered (0.45 µm) and unfiltered water samples. After 7 days of exposure period, frond number, fresh biomass, and total frond area were measured as endpoints of growth and physiological performance [5,6]. **Results:** Overall, exposure to unfiltered water samples resulted in more pronounced inhibitory effects on plant performance. This suggests that particulate-bound contaminants or suspended solids played an important role in driving the observed toxicity. Among the studied rivers, Vale das Flores stream showed the highest growth inhibition, evidenced by a reduced number of fronds, lower biomass and smaller total frond area. In addition, a clear spatial pattern was observed, with the highest levels of inhibition occurring at discharge sites. **Conclusions:** Urban stormwater runoff reduced *L. minor* growth, showing that contaminant mixtures carried by runoff can harm primary producers. These results highlight the

ecological risk of urban runoff and the need to include plant growth endpoints in water quality assessments.

Keywords: Water quality; Ecotoxicological assays; Aquatic ecosystems; Macrophyte

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24379 | Doors to the sea: ecological assessment of two estuaries in the North of Portugal

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Background & Aim: Estuaries are important transitional water bodies, especially located near urban areas, where they strongly influence water quality and ecosystem health. Monitoring these habitats is essential to protect environmental and human well-being. This study aims to evaluate the ecological status of two estuaries exposed to different levels of human pressure: the Douro estuary, a highly urbanized system, and the Lima estuary, a comparatively more natural ecosystem, based on the assessment of benthic invertebrate communities' composition. **Methods:** In each estuary, nine sediment samples were collected— three from the lower (4.1), middle (4.2) and upper (4.3) estuarine zones—during winter and summer of 2025. The macroinvertebrate community was evaluated using ecological metrics (Margalef Index, Shannon-Wiener diversity, AMBI index) and the biotic index Benthic Assessment Tool (BAT) was applied to determine the ecological status of each site. Sediment samples were analyzed for organic matter content and granulometric composition. **Results:** BAT results showed that the Lima estuary generally had a better ecological status than the Douro estuary. Lima estuary was mostly classified as good, with seasonal variation at 4.1 (high) and 4.3 (moderate), while Douro ranged from moderate in winter to good in summer. The Douro estuary showed higher species richness and diversity at 4.1 during summer and at 4.3 during winter, whereas 4.2 presented a more unbalanced community. Similarly, in the Lima estuary, higher richness and diversity were recorded at 4.1 (summer) and 4.3 (winter). However, a more unbalanced community was observed at site 4.3 in summer and at site 4.2 in winter. Both estuaries were dominated by gravel sediments. **Conclusions:** Despite differences between estuaries, these transitional waters were classified as having a moderate ecological status. However, the Douro estuary is under strong urban pressure, resulting in generally lower ecological status at its sampling sites.

Keywords: Transitional Waters, Water Framework Directive, Ecological Monitorization; Macroinvertebrates

24392 | Scaling Up Venlafaxine Electrocatalytic Removal: A Factorial Design Approach

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Background & Aim: Emerging pollutants present in surface waters are a high concern and, to a certain extent, are a consequence of proper quaternary treatment in wastewater treatment plants [1]. In this sense, advanced oxidation processes (AOPs) aim to remove these substances, allowing better water quality for both the environment and human health. **Methods:** The work started with direct electrolysis by assessing the influence of different electrode materials and electrolytes in the removal of venlafaxine (VFX). A full factorial design of three levels and three factors was made to scale-up the process. Then, grape must-derived biochars, synthesised at different temperatures, were used as a suspension electrocatalyst in order to develop an efficient three-electrode system capable of increasing electro-active surface area and originate novel oxidative pathways. **Results:** The electrode pair was optimised in electrolytic conditions, with bored-doped diamond as anode and stainless steel as cathode achieving VXF removals of ca. 70% and total organic carbon removals of ca. 50% in 120 min using sodium sulphate electrolyte. The best electrolyte proved to be sodium chloride, achieving a considerable time reduction of the process and improving VFX removal up to 98% in 15 min. The influence of electrode size, reactor volume and electrolyte concentration was investigated with the full factorial design and the biochar synthesis (temperature and yield) was optimised for the electrocatalysis process. **Conclusions:** Direct electrolysis allowed for a nearly complete removal of VFX in a synthetic water, while the addition of a biochar electrode further improved the system. In this way, the implementation of electrochemical AOPs can be promising solution for the removal of emergent pollutants present in wastewaters.

Keywords: Biochar; Advanced oxidation process; Electrocatalysis; Particle electrode.

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24443 | Characterization of Indoor Air Quality in Portuguese Primary School Classrooms: PM_{10} and $PM_{2.5}$

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Background & Aim: School classrooms constitute a critical indoor microenvironment for children's exposure to air pollutants, as pupils spend a substantial proportion of their time there [1]. Moreover, children are more susceptible to the adverse effects of poor indoor air quality [2]. Of particular concern is particulate matter (PM), namely PM_{10} (aerodynamic diameter ≤ 10 µm) and especially $PM_{2.5}$ (≤ 2.5 µm) due to their ability to penetrate into the lower respiratory tract. The present study aimed to assess PM_{10} and $PM_{2.5}$ levels in Portuguese primary schools and to evaluate the potential implications for children's health. **Methods:** PM_{10} and $PM_{2.5}$ were monitored in 80 classrooms across 20 primary schools in Porto, Portugal (February 2024–June 2025). In each school, 4 classrooms were continuously assessed by Lighthouse Handheld Particle Counter (model 3016 IAQ), which allowed simultaneous monitoring of 6 size fractions (300 nm – 10 µm), with a time resolution of 1 min, resulting in a large dataset (n = 291,600). **Results:** Across the 20 schools, the median PM_{10} was 46.9 µg/m^3 (range of medians: 23.5-112 µg/m^3), and 11.7 µg/m^3 (4.60-32.4 µg/m^3) for $PM_{2.5}$. During occupied periods, median concentration was 121 µg/m³ (62.3–256 µg/m³) for PM_{10} and 13.4 µg/m³ (4.80–33.3 µg/m³) for $PM_{2.5}$, representing 4.3-fold and 1.2-fold increases, respectively, compared with non-occupied periods (PM_{10}: 28.2 µg/m³, range 13.2–74.5 µg/m³; $PM_{2.5}$: 10.8 µg/m³, range 4.50–32.0 µg/m³). When occupied, the recommended guideline of 50 µg/m³ for PM_{10} [3] was exceeded in all 80 classrooms, whereas $PM_{2.5}$ exceeded its respective guideline of 25 µg/m^3 in 18% of classrooms. Consistently, the median $PM_{2.5}/PM_{10}$ was 0.103 (range: 0.064-0.205) during occupied periods, indicating a

predominance of the coarse fraction, being significantly higher than in non-occupied periods (0.383; 0.285-0.644).

Conclusions: The obtained findings highlight the need for improved air-quality control in respective schools.

Keywords: Indoor air quality; Particulate matter; Primary schools; School children.

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24618 | Lithium influence on nitrogen metabolism in *Arabidopsis thaliana*

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Background & Aim: Lithium (Li) is an alkali metal, raising concerns due to its increasing environmental concentration resulting from anthropogenic activities, which may make it toxic to the environment and living beings, including plants. Nitrogen metabolism is influenced by high metal doses; therefore, this study aimed to investigate how Li influences nitrogen metabolism in the shoots (S) and roots (R) of stressed *Arabidopsis thaliana* plants. **Methods:** Wild-type *A. thaliana* were grown for 21 days in MS medium supplemented with 7.5 and 15 mM Li. Nitrogen metabolism parameters, such as glutamine synthetase (GS), nitrate reductase (NR), NO_3^- and NH_4^+ levels, and GS gene family expression, were studied. To complement these results, some biometric and biochemical parameters of oxidative stress were evaluated. The ultrastructure of the leaf and R cells was also evaluated by transmission electron microscopy. **Results:** Only the 15 mM Li concentration significantly affected plants' biometry, in which the R length and the total S area decreased. The NO_3^- and NH_4^+ levels did not change in either organ with any treatment. Despite this, GS and NR activities increased, along with the expression of several GS-encoding genes. Significant changes in stress indicators, such as increased hydrogen peroxide in both organs, were observed, especially with 15 mM Li. Considering the cellular ultrastructure, R and S cells presented differences compared to the control. **Conclusions:** This study showed that Li influenced the nitrogen metabolism in *A. thaliana*. All GS-encoding genes and GS and NR activities were significantly upregulated in R at 15 mM Li, suggesting that these enzymes are involved in plant adaptation to Li-induced stress. Because the NO_3^- and NH_4^+ levels did not change up to 15 mM Li, it suggests that Li does not interfere with the balance of these nitrogen forms. Because many of the stress indicators evaluated changed, this indicates that Li induced oxidative stress and altered the cell ultrastructure.

Keywords: alkali metal, oxidative stress, cellular ultrastructure, glutamine synthetase, nitrate.

24709 | Assessment of stress-related biochemical parameters in *Solanum lycopersicum*'s leaves in response to silver

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Background & Aim: Silver (Ag) has been accumulating in the biosphere due to anthropogenic activities. Ag is a non-essential heavy metal, and exposure to Ag may cause stress, negatively influencing plant growth. This work aimed to study the effects of high Ag concentrations on *Solanum lycopersicum* leaves by quantifying several stress indicators: H_2O_2, malondialdehyde (MDA), photosynthesis-related pigments, and reduced glutathione (GSH). The plant's biometry was also assessed. **Methods:** *S. lycopersicum*'s wild-type MicroTom seeds were cultivated in MS medium and left to grow for 18 days after germination. After this, the plants were transplanted into a mixture of vermiculite and perlite and exposed to 3 silver concentrations (0, 25, and 50 mg/L) for 21 days by supplying them in Hoagland's solution. The plants' 4th and 5th leaves were collected to quantify the abovementioned stress factors. The obtained results were analysed using a one-way ANOVA followed by a Dunnett multiple-comparison test. Results with p <0.05 were considered statistically significant. **Results:** Increasing Ag concentrations reduced total plant mass (33 & 42%), root length (37 & 26%), and shoot length (24 & 27%). In the leaves of the plants exposed to 50 mg/L Ag, H_2O_2 levels increased (60%) while MDA levels decreased (11%). Chlorophyll a content rose (109 & 150%) with increasing Ag concentration, whereas chlorophyll b increased (66%) only with 50 mg/L Ag. β-carotene levels remained unchanged, while lutein increased (49%) with 50 mg/L Ag. GSH levels increased (48 & 52%) in both concentrations. **Conclusions:** Ag exposure negatively affected plant growth and elevated H_2O_2 levels. However, this rise did not result in severe oxidative damage, as indicated by reduced MDA levels and increased chlorophyll content. Together with the rise in GSH, these findings suggest that, in addition to GSH, other antioxidant mechanisms may contribute to the plant's defence against Ag-induced oxidative stress.

Keywords: oxidative stress, antioxidant metabolism, heavy metals

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24883 | Failures in Implementing National Adaptation Plans and Local Adaptation in Portugal

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Background & Aim: Climate adaptation faces a contradiction: local communities develop effective territory-based solutions, while national policies remain centralized. This study analyzes the disarticulation between Local-Led Adaptation (LLA) and National Adaptation Plans (NAPs). The aim is to identify gaps between international commitments and practical implementation in Portugal. **Methods:** Critical document analysis of legal instruments, UNFCCC NAP Technical Guidelines[4], UNEP Adaptation Gap Report 2025, and national strategies (Climate Base Law [2], RNC2050 [5], PNEC 2030 [6], P-3AC [7] and ENAAC [8]). Assessed integration of LLA principles into public policies, focusing on governance and financing. **Results:** Results show Portugal maintains centralized, hierarchical approaches despite Paris Agreement alignment. There is an absence of direct financing mechanisms for communities, public participation limited to non-deliberative consultations, and significant delays in Municipal and Regional Climate Action Plans (PMACs/PRACs). Over half of municipalities lack published plans. Communities emerge as passive beneficiaries, perpetuating climate injustices and weakening territorial resilience against extreme events. National instruments (PNEC 2030 [6], P-3AC [7], RNC2050 [5]) prioritize mitigation over adaptation, lacking social sustainability indicators and local capacity-building mechanisms. The Climate Base Law [2] establishes rights but fails operational decentralization. Vertical integration remains fragmented, reproducing administrative centralization rather than empowering local actors [3,4]. It is urgent that Municipal and Regional Climate Action Plans gain institutional strength, financial autonomy, and decision-making power to operationalize adaptation at the territorial level, ensuring timely, context-specific responses to climate risks. **Conclusions:** Climate adaptation in Portugal functions as normative compliance rather than territorial transformation. Transition to LLA models with predictable financing and participatory governance is urgent. Without power and resource redistribution, climate action risks becoming an institutional project instead of real resilience.

Keywords: Climate adaptation; Local-Led Adaptation; Climate justice; Territorial governance; National Adaptation Plans

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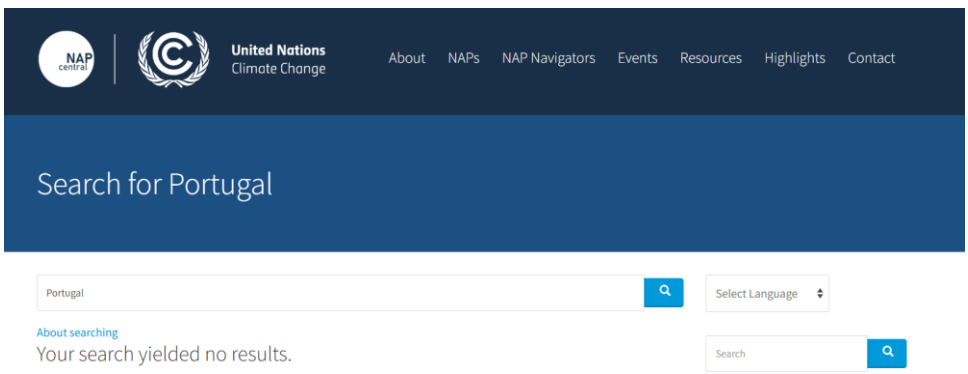
Figure 1: NapCentral. Submitted NAPs from developed country Parties



HEALTH SCIENCES

24234 | Stability Study of a New Compounded Medicine for the Treatment of Oral Mucositis

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Background & Aim: Oral mucositis (OM) is an inflammatory and ulcerative disease that affects patients undergoing cancer treatment, namely chemotherapy and radiotherapy, significantly compromising their quality of life. Despite its high incidence and clinical impact, current therapeutic options remain limited, reinforcing the need for new treatment approaches. In this context, the Instituto Português de Oncologia do Porto FG, EPE (IPO-Porto) developed an innovative mouthwash containing dexamethasone sodium phosphate (DSP), ascorbic acid, and sodium hyaluronate for the treatment of OM. In this work the physicochemical stability of this formulation was evaluated to ensure its quality and safety as compounded medicine. **Methods:** Exploratory studies were performed under accelerated aging conditions (40 ± 2 °C), followed by stability studies at real time (20 ± 2 °C) and refrigeration conditions (4 ± 2 °C), as well as in-use stability studies simulating daily patient handling. At each sampling time, physicochemical characterization was carried out (pH, color, odor, and precipitate formation) as well as the DSP quantification using high-performance liquid chromatography. The acceptable range of 90.0–110.0% of DSP strength, was used as the primary stability criterion. **Results:** Results showed that the formulation remained stable at high temperatures (40 °C) on short exposure times (≤6 h), and for 30 days at 4 °C in closed amber glass bottles. In-use stability studies performed during the 7 days after opening (considering three daily applications) showed that the DSP concentration remained above 90%. **Conclusions:** The results obtained enabled the definition of storage conditions and beyond-use date, since they showed that the prepared formulation is stable when stored under refrigeration. Further studies are currently being developed with a view to its clinical use as a compounded mouthwash in a hospital setting for the treatment of oral mucositis.

Keywords: Cancer therapy; oral mucositis; dexamethasone; mouthwash; stability studies, compounded medicines

24590 | Humanization in the Emergency Department: Anxiety Reduction and Environmental Perception as Determinants of Global Satisfaction

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Background & Aim: Humanization is recognized as a core dimension of healthcare quality, integrating relational, ethical, and environmental components [1] within established quality-of-care frameworks [2,3]. This study aimed to determine which factors contribute to global satisfaction of the patient within the emergency departments (ED). **Methods:** Prospective cohort study including 107 adult patients randomly selected from those attending the ED. Satisfaction was assessed via structured telephone questionnaire adapted from SERVPERF. Anxiety was measured at triage and discharge, and the variation calculated. **Results:** Anxiety reduction was moderately associated with global satisfaction ($\rho = 0.514$; $p < 0.001$). A positive correlation was observed between overall satisfaction with medical care and the perceived time spent in the consultation ($\rho = 0.379$, $p < 0.001$). Pearson correlation coefficients for satisfaction in the six stages of the ED journey (admission, triage, medical care, nursing, auxiliary and diagnostic technicians) revealed that all variables show positive and statistically significant associations with each other ($r = 0.283$ to $r = 0.682$, with p-values <0.05). Environmental perception correlated strongly with anxiety variation ($\rho = 0.596$; $p < 0.001$). In the multivariable analysis, better acclimatization ($\beta = 0.364$; $p = 0.005$) and cleanliness ($\beta = 0.329$; $p = 0.015$) were independent predictors of anxiety reduction. Also, the presence of an accompanying person correlated significantly with anxiety reduction ($d = 0.81$; $p < 0.001$). **Conclusions:** Patients perceived quality of care throughout all phases of the emergency process as a coherent and integrated phenomenon. Professional involvement from admission onwards has not only clinical and organizational relevance but also emotional value, contributing to reduced anxiety and a safer hospital experience.

Keywords: Health literacy; Anxiety; Emergency department; Humanized care; Patient-centered care

Acknowledgments:

The authors are sincerely grateful to all the patients who generously agreed to participate in this study. Their trust and contribution made this research possible and help demonstrate that there is still meaningful progress to be achieved in the humanization of healthcare.

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24216 | Impact of front-of-package labels on caregivers' and children's decision-making- A systematic review

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Background & Aim: Front-of-package labelling (FOPL) is widely promoted to support healthier choices, but its role in early childhood – where caregivers decide – remains unclear. This systematic review synthesizes quantitative and qualitative evidence on how FOPL influences food-related decisions for children aged 0–12 years. **Methods**: Five bibliographic databases and one grey literature source were searched through July 2025. Studies assessing interpretive and non-interpretive FOPL on packaged foods were included. Behavioral, nutritional, and cognitive-affective outcomes were synthesized. PRISMA 2020 was followed; risk of bias was assessed with JBI tools, and certainty with GRADE and GRADE-CERQual. **Results:** Thirty-nine studies were included. Moderate-certainty evidence indicates that nutrient-specific warning labels reduce caregivers' purchase intentions for less healthy products, while comparative RCT evidence suggests that warning labels outperform calorie labels or text-only warnings for this outcome (high certainty). Evidence for purchasing/selection, perceived healthfulness, comprehension, and interpretability was mixed and mostly low certainty; nutritional outcomes were largely very low certainty. Educational add-ons showed no consistent added benefit (very low certainty). **Conclusions:** Qualitative evidence suggests caregivers often use FOPL as a heuristic, shaped by comprehension and everyday constraints. Overall, FOPL most consistently affects early decision processes, while nutritional change in early childhood remains uncertain.

Keywords: Front-of-package labelling; Food choice; Food Policy; Nutrition; PRISMA

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24430 | Circulating MicroRNAs as Diagnostic and Prognostic Biomarkers in Pulmonary Hypertension: A Systematic Review

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Background & Aim: Pulmonary hypertension (PH) is a complex and incurable cardiovascular disorder, often diagnosed late due to nonspecific symptoms and the lack of reliable biomarkers[1]. Circulating microRNAs (miRNAs) are small, stable non-coding RNAs that regulate fundamental cellular processes and are readily detectable in blood-derived samples[2-4]. In PH, miRNAs are involved in key pathogenic mechanisms, including pulmonary vascular remodeling, maladaptive cell proliferation and inflammatory signaling, highlighting their potential as non-invasive biomarkers[4, 5]. However, current evidence remains fragmented regarding their diagnostic and prognostic utility. Moreover, published evidence often focuses on specific mechanisms or PH subtypes, providing limited comparative assessment of their performance as biomarkers [4, 5]. To address these gaps, this systematic review aims to synthesize both clinical and experimental evidence on circulating miRNAs, evaluating their potential as diagnostic and prognostic biomarkers in PH. **Methods:** PubMed, Web of Science, Scopus and ClinicalTrials.gov databases were searched, following PRISMA 2020 guidelines[6]. Eligible studies included human PH patients or mammalian *in vivo* models reporting circulating miRNA levels in plasma/serum with diagnostic and/or prognostic outcomes. Data were extracted on study design, population, PH subtype, sample type, assays, normalization, differential expression, and performance metrics. Risk of bias and reporting quality were assessed using QUADAS-2[7], QUIPS[8], SYRCLE[9] and ARRIVE[10] tools. **Results:** Studies reported a set of circulating miRNAs dysregulated in PH associated with disease severity and prognosis. Although miRNA panels outperformed single biomarkers, substantial methodological heterogeneity was observed across studies. **Conclusions:** Circulating miRNAs demonstrated potential as diagnostic and prognostic biomarkers in PH, but further standardized and prospective studies are needed to support clinical translation.

Keywords: Pulmonary Hypertension (PH); Circulating microRNAs (miRNAs); Diagnostic Biomarkers; Prognostic Biomarkers; Systematic Review

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24501 | Assessment of Food Insecurity in a Population of Adults with Type 2 Diabetes

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Background & Aim Food insecurity is a critical social determinant of health, disproportionately affecting individuals with type 2 diabetes mellitus. Inadequate access to sufficient and nutritious food compromises dietary quality, treatment adherence and glycemic control, contributing to poorer clinical outcomes. Despite increasing global evidence, data from Portuguese primary care remain limited. This study aims to evaluate the association between food insecurity and metabolic control in adults with type 2 diabetes. **Methods:** A cross-sectional study is conducted in a Primary Healthcare Unit in northern Portugal. Adults with type 2 diabetes are recruited between October 2025 and March 2026. Data collection includes sociodemographic and clinical variables, alongside food insecurity assessment using a validated scale. Metabolic control is assessed through glycated hemoglobin (HbA1c), lipid profile and body mass index. Statistical analysis includes descriptive statistics and multivariate regression models to determine the independent association between food insecurity and metabolic outcomes. **Results:** Food insecurity is associated with significantly poorer metabolic control, particularly higher HbA1c levels. Affected individuals also demonstrate less adequate dietary patterns and greater challenges in diabetes self-management, reinforcing the link between socioeconomic vulnerability and adverse clinical outcomes. **Conclusions:** Food insecurity emerges as a significant determinant of suboptimal metabolic control in type 2 diabetes. These findings strengthen current evidence by highlighting the clinical relevance of social determinants and support the integration of food insecurity screening and targeted interventions into diabetes care and public health strategies.

Keywords: food insecurity¸type 2 diabetes mellitus¸metabolic control; glycemic control; Social determinants of health

24181 | Nursing interventions for the self-concept of individuals requiring mental health care: A scoping review

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Background & Aim: Self-concept is a fundamental component of mental health, encompassing self-esteem, personal identity, and body image. Nursing plays a vital role in this domain, yet there is a lack of studies on interventions specifically addressing self-concept. This gap underscores the need for a comprehensive synthesis of existing evidence. This scoping review aimed to map nursing interventions targeting self-concept in individuals requiring mental health care, and explore the associated nursing diagnoses, the key attributes of interventions, and relevant assessment tools and outcomes. **Methods:** The review was conducted in accordance with the Joanna Briggs Institute (JBI) methodology and reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) checklist. The databases MEDLINE and CINAHL (via EBSCOhost) were searched, incorporating quantitative and qualitative studies, with no restrictions on language or publication date. Title and abstract screening, full-text evaluation, and data extraction using a tool developed by the authors, based on JBI recommendations, were conducted. The extracted data included details about participants, concept, context, study methods and main conclusions relevant to the review questions. **Results:** A total of 811 articles were identified, of which 36 met the inclusion criteria. The most frequently reported interventions encompassed cognitive-behavioral therapies, creative interventions, and psychoeducation, mainly delivered in inpatient units and community-based settings. The most predominant outcome was self-esteem and the most utilized assessment tool was the Rosenberg Self-Esteem Scale. Only one study explicitly mentioned nursing diagnoses related to self-concept. **Conclusions:** Findings underscore the necessity of developing more interventions that address the multidimensional nature of self-concept, while also systematising nursing diagnoses and a broader range of assessment instruments.

Keywords: Nursing interventions, Self-Concept, Mental Health

24193 | Exploring fermented-derived bioactive compounds from red grape juice to mitigate Metabolic Dysfunction Associated Steatotic Liver Disease

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Background & Aim: Metabolic dysfunction–associated steatotic liver disease (MASLD) is a prevalent chronic disorder linked to obesity, diabetes, and metabolic syndrome. Since dietary intervention is a cornerstone of MASLD management, there is significant interest in bioactive compounds like polyphenols, known for their antioxidant and metabolic-regulating properties. This study evaluated fermented red grape juice as a complementary nutritional strategy, focusing on how fermentation enhances the chemical stability and systemic bioavailability of these health-promoting metabolites. **Methods:** The study compared three matrices: non-fermented red grape juice (control), fermented juice with yeast cells, and fermented juice without yeast cells. Comprehensive chemical profiling was conducted via HPLC and LC–MS to determine concentrations of phenolic compounds, vitamins, and sugars, including 3-NPH derivatization for organic acid analysis. To evaluate the digestive fate of these compounds, samples were processed through the SIMGI dynamic in vitro gastrointestinal model. Gastric and intestinal digests were analyzed to measure the bioaccessibility and molecular biotransformation of the bioactive components. **Results:** Fermentation successfully transformed the juice's profile, significantly reducing sugar content while increasing key organic acids, such as pyruvic and succinic acids. The process enriched the phenolic profile, specifically increasing stable anthocyanin derivatives and bioavailable phenolic acids like gallic, caffeic, and p-coumaric acids. Additionally, B-complex vitamins, particularly B2 and B5, were notably elevated. During simulated digestion, pyranoanthocyanins exhibited superior stability, and stilbenes—including polydatin—underwent biotransformation into forms with higher potential for absorption and biological activity. **Conclusions:** Fermentation optimized the nutritional and

bioactive signature of red grape juice, markedly improving the stability and bioavailability of its functional compounds. These results support the use of fermented red grape juice as a potent, complementary dietary tool for the prevention and management of MASLD.

Keywords: MASLD; Fermented red grape juice; (Poly)phenols; Anthocyanins; Bioavailability

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24246 | Nutritional Guidelines and Practices in Daycare Centres: A Scoping Review of International Recommendations

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Background & Aim: The first three years of life are critical for establishing lifelong dietary and health patterns, with daycare centres providing key environments for implementing early nutritional interventions. This scoping review aimed to analyse existing national and regional guidelines for food provision in daycare settings, identifying common principles, inconsistencies and gaps. **Methods:** Searches were performed on the official websites of national and regional health ministries from December 2024 to January 2025, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) Checklist. This scoping review is registered in the Open Science Framework (DOI: 10.17605/OSF.IO/CWG8E). **Results:** Twenty-nine official documents from seventeen countries were included. Findings revealed wide heterogeneity in age ranges, portion sizes and recommended food frequencies. Most guidelines recommend the daily inclusion of breastmilk, meat, fish, eggs, vegetables, fruits and dairy products, alongside a broad consensus on limiting added sugars and salt. However, several gaps were identified, including frequent inclusion of red and processed meat, insufficient emphasis on fish and plant-based protein sources, limited promotion of whole grains, and permissiveness toward fruit juices and ultra-processed foods. Quantitative recommendations were often incomplete, and few guidelines addressed vegetarian diets or texture progression. **Conclusions:** This review highlights the urgent need for harmonised, evidence-based, and context-specific nutritional guidelines tailored to daycare centres. Standardised frameworks could improve the quality of food provision, foster healthier eating habits in early childhood, and support long-term public health benefits.

Keywords: food provision, daycare centres, guidelines, early childhood

24938 | IL-10 Regulation Controls P2X7 receptor-mediated protection in neonatal Group B Streptococcus infection

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Background & Aim: Group B Streptococcus (GBS) is a major cause of neonatal sepsis, with an estimated mortality of 17.6% and significant impact on neurodevelopment sequelae. Neonatal immunity is characterized by a bias toward anti-inflammatory responses that pathogens can exploit to evade host defense. GBS infection induces production of the anti-inflammatory cytokine interleukin-10 (IL-10), contributing to disease progression. The purinergic receptor P2X7 (P2X7R) is a key regulator of inflammatory/neuroinflammatory signaling. Previous work from our group demonstrated that activation of P2X7R improves the outcome in neonatal GBS infection by shifting the immune response from an IL-10 anti-inflammatory state to a pro-inflammatory profile. Considering the role of IL-10 in neonatal immune regulation, this study aimed to determine whether the protective effect of P2X7R activation depends on IL-10 signaling during neonatal GBS infection. **Methods:** Pups born from BALB/c dams intravaginally inoculated with GBS were divided into treatment groups and subcutaneously administrated: anti-IL-10 mAbs (50 µg) at postnatal day (PND) 1 and 4, either alone or prior Bz-ATP (selective P2X7R agonist, 2.5 mg/kg/day, from PND1 to PND4) administration. Controls received isotype mAbs or saline. Survival rate, clinical score and neurodevelopmental milestones were assessed until weaning. **Results:** P2X7R activation and IL-10 neutralization individually improved survival and clinical outcome in infected pups, indicating that partial relief of the immune suppression enhances host defense ($p<0.01$). In contrast, the combined administration of anti-IL-10 antibodies and Bz-ATP abolished the protective effect observed with either treatment alone($p<0.05$), with increased mortality, disease severity and impaired performance in motor neurodevelopmental. **Conclusions:** These findings demonstrate that the protective effect of P2X7R activation relies on a tightly controlled balance between IL-10–mediated anti-inflammatory signaling and pro-inflammatory responses during neonatal GBS infection.

Keywords: Neonatal Sepsis, P2X7 Receptor, Group B *Streptococcus*, IL-10

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24456 | Identification of Immune-Mediated Mechanisms Induced by Vaccination Operating at the Maternal-Fetal Interface During Neosporosis

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Background & Aim: *Neospora caninum* is a major cause of bovine abortion, leading to severe economic losses. Since no commercial vaccine is available, understanding protective immunity during pregnancy is crucial. This study aimed to evaluate the immune response and protection elicited by an experimental vaccine (three doses of parasite membrane proteins encapsulated in DODAB:MO liposomes with CpG ODN adjuvant) in a bovine pregnancy model. **Methods:** The study included ten 2-year-old Holstein-Friesian heifers (4 immunized, 6 controls) that were artificially inseminated and intravenously infected with 5×10^7 *N. caninum* tachyzoites at 16 weeks of gestation. Blood samples were collected at the day of infection and at several time points after infection to quantify serum parasite-specific IgG subclasses and cytokines (IFN- γ, IL-2, IL-4, IL-8, IL-10, IL-17A, TNF- α, TGF-β) by ELISA and to assess T-cell proliferation (CD4, CD8, TCRγδ) by flow cytometry. Nineteen days post-infection, maternal and fetal tissues were collected and DNA was extracted to quantify parasite burden by qPCR. **Results:** Immunized heifers showed a predominant Th1/Th17 response, with an early serum IFN-γ peak and sustained IL-17A levels, whereas controls were more Th2-biased. Fetuses from vaccinated dams presented marked elevated serum IFN-γ levels. Vaccination induced robust antigen-specific IgG1 and IgG2 production and enhanced CD8+ T-cell proliferation. However, preliminary qPCR data showed parasitic DNA in all analyzed fetal and placental tissues, indicating that the vaccine, despite its high immunogenicity, did not fully prevent vertical transmission under these experimental conditions. **Conclusions:** The liposomal vaccine successfully triggered a strong cellular and humoral response associated with protection. Although vertical transmission was not blocked in this particular trial, the induction of Th1/Th17 immunity and T cell memory

represents a promising step forward. These results, together with the study of placental and fetus' tissues, will contribute to the understanding of host-pathogen interactions at the maternal-fetal interface and guide the refinement of future immunization strategies.

Keywords: *Neospora caninum,* Maternal–fetal interface, Liposomal vaccine, Th1/Th17 immune response, Vertical transmission.

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24640 | Malignancies reported in patients with Clostridium septicum infection: a systematic review of clinical reports

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Background & Aim: Clostridium septicum is a Gram-positive anaerobe frequently found in tandem with digestive malignancies, where mucosal compromise facilitates bacterial dissemination [1-3]. This systematic review summarises case reports and case series to characterise clinical presentation, diagnostic timing, and outcomes. **Methods:** Following PRISMA 2020 guidelines, we searched PubMed, Scopus, and Web of Science databases from inception to September 2025 [4]. Study selection and data extraction were carried out independently by two reviewers, and 125 articles were included. Methodological quality was independently assessed by two authors using the Murad et al. (2018) tool for case reports and series [5]. **Results:** Among 139 cases (mean age 68.6; 59.7% male), the cecum and right colon were the most frequent primary tumour sites (35.3% and 34.5%, respectively), and adenocarcinoma was the predominant histology (87.8%). Metastatic cancer was reported in 36.7% of patients. In most reports, C. septicum infection preceded cancer recognition and served as the presenting event of an occult malignancy (82.0%), with 61.2% of cancers diagnosed during the same admission. Clinically, gas gangrene was a common presentation (36.7%), and management often required invasive source control, with 73.4% undergoing surgery or drainage. Diabetes was the most common comorbidity (36.0%). Overall mortality reached 41.0%, primarily due to infection (82.5%). Methodological quality was judged as good and fair in 40.3% of reports each. **Conclusions:** Although causality cannot be established, a marked clinical pattern involving elderly, diabetic patients with right-sided adenocarcinomas is evident. As infection frequently represents the first manifestation of occult malignancy and carries a poor prognosis, immediate oncological investigation is mandatory.

Keywords: Clostridium septicum; Clostridium spp; Neoplasms; Digestive System Neoplasms; Gas gangrene.

Acknowledgments:

Not applicable.

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24134 | Stability Study of a New Compounded Medicine for the Treatment of Oral Mucositis

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Background & Aim: Oral mucositis (OM) is an inflammatory and ulcerative disease that affects patients undergoing cancer treatment, namely chemotherapy and radiotherapy, significantly compromising their quality of life. Despite its high incidence and clinical impact, current therapeutic options remain limited, reinforcing the need for new treatment approaches. In this context, the Instituto Português de Oncologia do Porto FG, EPE (IPO-Porto) developed an innovative mouthwash containing dexamethasone sodium phosphate (DSP), ascorbic acid, and sodium hyaluronate for the treatment of OM. In this work the physicochemical stability of this formulation was evaluated to ensure its quality and safety as compounded medicine. **Methods:** Exploratory studies were performed under accelerated aging conditions (40 ± 2 °C), followed by stability studies at real time (20 ± 2 °C) and refrigeration conditions (4 ± 2 °C), as well as in-use stability studies simulating daily patient handling. At each sampling time, physicochemical characterization was carried out (pH, color, odor, and precipitate formation) as well as the DSP quantification using high-performance liquid chromatography. The acceptable range of 90.0–110.0% of DSP strength, was used as the primary stability criterion. **Results:** Results showed that the formulation remained stable at high temperatures (40 °C) on short exposure times (≤6 h), and for 30 days at 4 °C in closed amber glass bottles. In-use stability studies performed during the 7 days after opening (considering three daily applications) showed that the DSP concentration remained above 90%. **Conclusions:** The results obtained enabled the definition of storage conditions and beyond-use date, since they showed that the prepared formulation is stable when stored under refrigeration. Further studies are currently being developed with a

view to its clinical use as a compounded mouthwash in a hospital setting for the treatment of oral mucositis.

Keywords: Cancer therapy; oral mucositis; dexamethasone; mouthwash; stability studies, compounded medicines

24910 | CD5L as a key Regulator of Inflammation in Bacterial Pneumonia Sepsis

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Background & Aim: Sepsis triggered by bacterial pneumonia is a leading cause of death worldwide, yet the molecular regulators orchestrating immune defense remain largely unknown [1]. CD5L, an immunoregulatory protein, has emerged as a key modulator of inflammation [2], but its role in pneumonia-induced sepsis is still largely unexplored. In this project, we aim to define how CD5L shapes immune responses during *Klebsiella pneumoniae*-induced pneumonia, one of the major bacterial causes of pneumonia that can progress to sepsis. **Methods:** We established a mouse model of primary pneumonia through intratracheal inoculation of *K. pneumoniae*. Following infection, wild-type (WT) C57BL/6 mice were treated with recombinant CD5L and monitored for survival and disease progression, including weight loss. In addition, alveolar macrophages from WT and CD5L knockout mice (CD5L KO) were analyzed by flow cytometry and immunohistochemistry to characterize the kinetics of CD5L production upon infection. **Results:** Administration of recombinant CD5L resulted in a measurable improvement in both survival and weight loss in mice infected with *Klebsiella pneumoniae*, indicating a protective effect of CD5L during pneumonia-induced sepsis. Although these findings support a beneficial role for CD5L in modulating the host response to infection, the degree of protection observed remains limited and is not yet sufficient to fully control disease progression. To address this limitation and improve the translational relevance of the model, we are currently refining the experimental design to better reflect the clinical management of bacterial pneumonia. Given that CD5L is predominantly produced by macrophages, we investigated whether tissue-resident alveolar macrophages contribute to the local pool of CD5L and demonstrated that CD5L levels rise in the lung following infection, supporting a macrophage-derived source. To further explore this finding, we are analyzing CD5L expression in lung tissue sections by immunohistochemistry (IHC). **Conclusions:** Together, these findings support a protective role for CD5L during bacterial pneumonia and highlight its potential as an immunomodulatory therapeutic strategy in sepsis.

However, the modest improvement observed indicates that CD5L alone may not be sufficient to fully control infection. By integrating clinically relevant interventions, such as antibiotic therapy, this model will allow a more accurate assessment of the therapeutic potential of CD5L and help determine how immune modulation can complement standard antimicrobial treatment in pneumonia-associated sepsis.

Keywords: Sepsis; Pneumonia; CD5L.

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24259 | Defining tumor-associated glycans as molecular drivers of malignancy and therapeutic resistance in ErbB2-positive advanced gastric cancer

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Background & Aim: Advanced/metastatic gastric cancer (GC) remains a global health concern depicting poor patient prognosis, due to late diagnosis and lack of efficient therapies.[1] Comprehensive molecular profiling of tumors has unraveled possible therapeutic targets, particularly the cell surface receptor ErbB2. ErbB2 is overexpressed in 10-20% of advanced GC cases and therapeutically targeted with the ErbB2-specific trastuzumab (TTZ) monoclonal antibody (mAb).[2] However, most TTZ-treated patients experience molecular resistance, disease progression or relapse. Notably, ErbB2's extracellular region undergoes extensive glycosylation, which is significantly disrupted in cancer cells.[3] GlycoDisplay aims to understand whether ErbB2 glycosylation actively tunes TTZ accessibility, binding and its therapeutic efficacy. **Methods:** *In vitro* characterization of the ErbB2 expression and activation levels in a panel of GC cell lines and their glycosylation profiles by Western blot (WB) and immunofluorescence (IF) to select cell lines for CRISPR/Cas9-based precise glycogene editing.[4] Establishment of ErbB2-positive glycoengineered GC cell lines depicting selected cancer-associated glycosylation profiles. Molecular characterization of the glycoengineered cell lines by WB, IF and flow cytometry. Establishment of two ERBB2-amplified TTZ-resistant (TTZ-R) GC cell lines through their continuous exposure to incremental dosages of TTZ, and characterization of the glycosylation profile. **Results:** NCI-N87 and OE-19 cell lines, showing endogenous ErbB2 expression and activation. These were selected for CRISPR/Cas9 genetic silencing of the glycosyltransferases ST6Gal1, ST3Gal3, ST3Gal4 and FUT3 which can affect various cancer-associated glycan structures. TTZ-R cell lines depict a reshaping of their glyco-transcriptome and increased ErbB2 activation. **Conclusions:** We are setting up a comprehensive cell-based library as an important pre-clinical tool to address glycan-mediated resistance to TTZ in ErbB2-positive GC.

Keywords: Gastric Cancer; Glycans; ErbB2; Glycosylation; Trastuzumab

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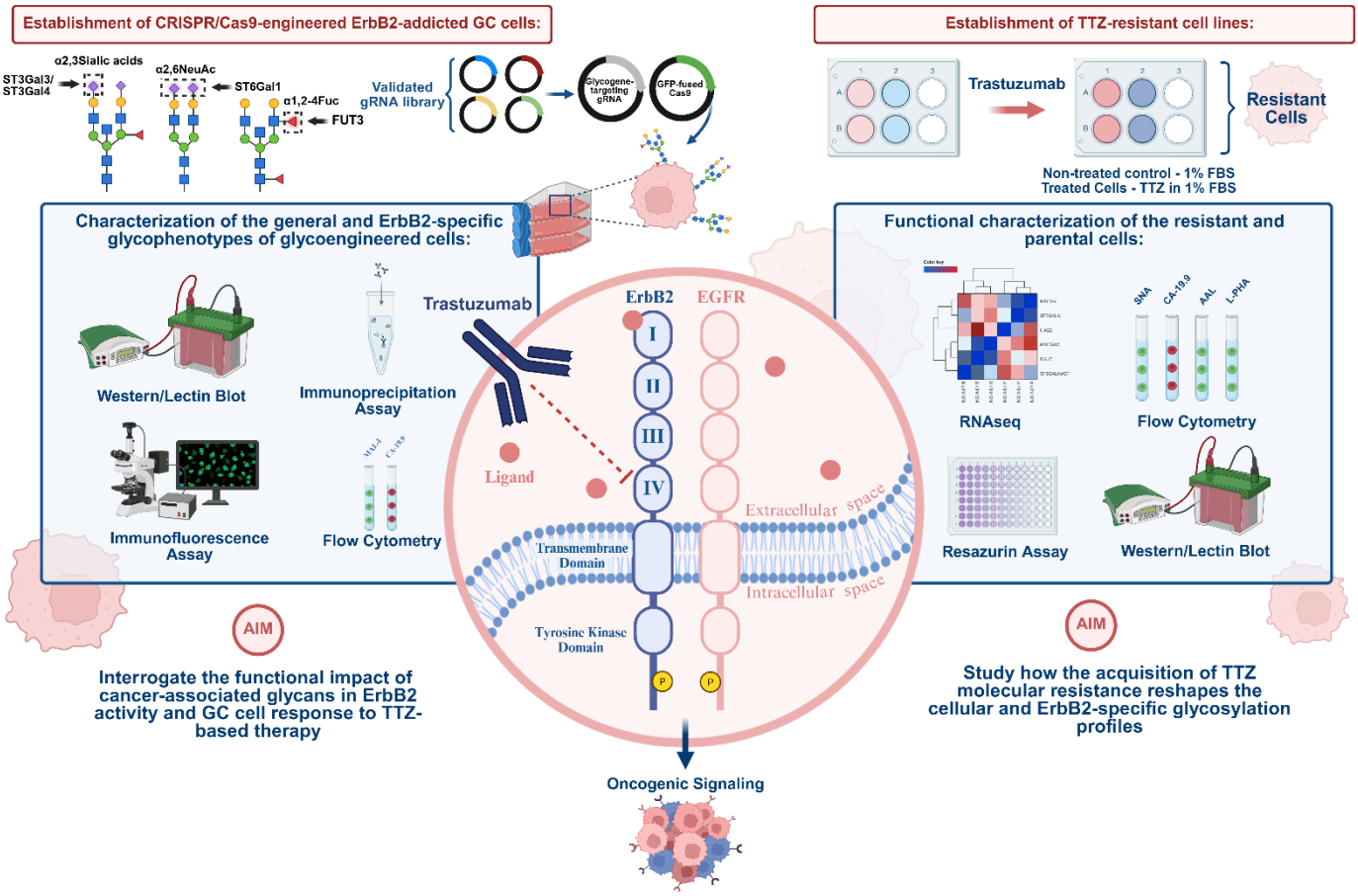

Figure 1: Representation of the scientific strategy to obtain the GlycoDisplay cell-based platform, a tool for the functional dissection of specific ErbB2 glycosylation profiles and their impact on the cellular response to Trastuzumab therapy.

24275 | Pool Management in Kidney Exchange Programs

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Background & Aim: Kidney Exchange Programs (KEP) facilitate transplantation for incompatible donor-recipient pairs [1]. Half-compatible pairs—who can opt for immunosuppression—can be included in KEPs adding complexity to pairing decisions [2] with the balance between maximizing transplants and ensuring equity remaining a central challenge [3]. This work compares prioritization strategies in KEP and assesses immunosuppression acceptance impacts on system outcomes and equity. **Methods:** We developed a 24-year dynamic simulation with compatible, half-compatible, incompatible pairs, and altruist donors, with match runs every 90 days. Three integer programming solvers were compared: (1) Hierarchical with Waiting Time (prioritizing Incompatible over half-Compatible and compatible, and breaking ties by wait time) [4]; (2) Hierarchical without Waiting Time; (3) Maximum Cardinality. We simulated five scenarios where unmatched half-compatible pairs can accept immunosuppression with varying probabilities (0%, 25%, 50%, 75%, 100%) [2, 5]. Each scenario was evaluated across 100 instances to ensure statistical robustness. **Results:** Analysis of 1500 simulations reveal distinct efficiency-equity trade-offs. Maximum Cardinality achieved the maximum number of transplants but caused excessive waiting times for hard-to-match pairs. In contrast, hierarchical models—especially the waiting time variant—improved equity by significantly reducing timeouts and waiting time for incompatible pairs. Crucially, while higher immunosuppression acceptance rates increased the total number of transplants (via half-compatible pairs), it yielded no significant benefits for incompatible pairs. **Conclusions:** Only considering cardinality maximization harms vulnerable groups, whereas prioritization strategies considering compatibility and waiting time improve fairness without drastically reducing the number of transplants. Since immunosuppression acceptance rates offer no benefits for incompatible pairs, algorithmic prioritization remains essential for incompatible patients.

Keywords: Kidney Exchange Program; Optimization; Prioritization Strategies; Immunosuppression; Healthcare Equity

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24276 | Characterization of a Novel CGRP-Induced Rat Model of Chronic Migraine with Gut–Brain Axis Features

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Background & Aim: Chronic Migraine is a disabling neurological disorder, marked by intense headaches. Activation of the trigeminovascular system by CGRP is the most studied mechanism. CGRP induces vasodilation and neurogenic inflammation, sensitizing trigeminal nerves, which promotes peripheral and central sensitization. Altered pain modulation causes cephalic and extra-cephalic hypersensitivity, manifested as cutaneous allodynia[1]. Altered gut–brain communication is increasingly implicated, with CGRP being involved in this axis[2]. We aimed to characterize a new chronic migraine model in rats induced by two-cycles of repeated CGRP injection, assessing central sensitization and the intestinal component. **Methods:** Time-course nociceptive behavior was evaluated by the Von Frey test, and descending pain modulation by the DNIC paradigm. Histology of colon and rectum assessed crypt depth, goblet cells, and gut-associated lymphoid tissue. **Results:** In females, CGRP induced transient, cyclic peripheral sensitization, with periorbital allodynia at each cycle's end and sustained hind paw allodynia. In males, CGRP induced brief peripheral sensitization, shown by periorbital allodynia after the first cycle. DNIC elicited greater analgesia in CGRP-treated males than females, indicating altered pain modulation in females. No differences between groups were observed in the crypt length. CGRP–increased goblet cells in males compared to the vehicle group (14.0±2.1 vs 8.0±1.1). Lymphocytic aggregates were more prevalent in controls, though larger in CGRP-treated females. Lastly, preliminary data show CD4 lymphocyte infiltration was higher in treated females compared to the controls (44.9%±28.7 vs 29.4%±23.7). **Conclusions:** Our results indicate that CGRP induces sex-dependent hypersensitivity and central sensitization. The data support altered gut histology, consistent with the recruitment of the immune system. This suggests that this model might represent a good tool to approach the gut–brain axis in chronic migraine.

Keywords: Migraine; CGRP; Peripheral sensitization; Central sensitization; Gut.

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24279 | Novel molecular approaches to profile microbial enzymes driving oncometabolite synthesis in gastric cancer

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Background & Aim: The gastric microbiome contributes to gastric cancer (GC), and microbial-derived metabolites may mediate host-microbiome interactions that drive tumor initiation and progression. Using machine learning, we identified 112 microbial-derived metabolites, including kynurenine, benzoquinone and diaminopimelic acid, linked to specific microbial enzymatic pathways. Measuring the corresponding enzymes in gastric specimens may provide a functional readout of microbial metabolic activity in the tumor microenvironment. We aim to develop and validate quantitative PCR (qPCR) for large cohorts as a practical and cost-effective alternative to next-generation sequencing. **Methods:** qPCR assays (two primers and a probe) were designed with PrimerQuest™ Tool (IDT, USA) using multiple reference sequences. Performance was assessed *in silico* with an in-house bioinformatics pipeline that tests target detection in a bacterial genome database (114,218 genomes). The best assays were selected for *in vitro* testing based on sensitivity, specificity, precision and F1 Score metrics. **Results:** We designed 469 qPCR assays, including 38 for Kyn, 50 for tryptophan catabolism, 240 for diaminopimelic acid, and 139 for benzoquinone. Top assays (F1 score between 0.5 and 1), covered 2 Kynurenine, 1 tryptophan, 2 diaminopimelic acid, and 1 benzoquinone-related enzymes. We built 3 multiplex assays, each targeting 4 genes per reaction through using unique 5' fluorescent probe dyes for simultaneous, independent detection. Each multiplex includes two microbial enzyme assays, one 16S *rRNA* assays (total abundance of bacteria), and one host *ACTB* assay (for data normalization). Multiplex assays are being validated in 40 GC specimens. **Conclusions:** Targeted molecular assays of microbial metabolic capacity will advance understanding of microbiome-cancer interactions and provide insight into how microbial metabolites contribute to GC development.

Keywords: Microbiome; Metabolites; Microbial enzymes; Gastric cancer

24287 | Knowledge assessment on the rational use of oral antibacterials in Dentistry

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Background & Aim: In 2019, infections caused by resistant bacteria contributed to over 6 million deaths globally, with a significant increase projected by 2050[1, 2, 3]. Antibiotic use is associated with adverse effects, drug interactions, and, crucially, bacterial resistance, compromising the efficacy of future treatments [4, 5]. In light of these concerns, this study aims to assess dentists' perceptions, knowledge, and attitudes regarding the rational use of antibiotics, identifying clinical contexts that generate the most uncertainty in prescribing. **Methods:** A 35-question survey was administered to dentists currently in clinical practice in Portugal. The questionnaire comprised multiple-choice questions and Likert-scale items and was organized into four main sections: (1) sociodemographic and professional profile; (2) general knowledge regarding antibacterial agents; (3) antibacterial prescribing practices; and (4) uncertainties in clinical decision-making. **Results:** The survey was completed by 34 dentists. Preventing bacterial resistance was the main reason for restricting antibiotic use (88%). Amoxicillin was the preferred first-line agent (53%), and most dentists prescribed 7-day regimens (67%). While 70% correctly followed guidelines for infective endocarditis prophylaxis, half reported insufficient knowledge regarding immunocompromised patients, although 88% stated they contact the attending physician in such cases. Additionally, 47% indicated that incomplete patient medical histories moderately influence prescribing decisions. **Conclusions:** Dentists exhibit satisfactory knowledge about antibiotic use, despite persistent gaps in the selection of first-line drugs and the duration of treatment. Some uncertainty was noted in the treatment of immunocompromised patients. Difficulties in learning, practical application, and obtaining patients' medical histories were also reported, highlighting the need for continuous, evidence-based education.

Keywords: Dental Medicine; pharmacological prescription; antibacterials; bacterial infection; immunocompromised; bacterial resistance.

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24970 | Rewired to Resist: Targeting Metabolic Adaptations of Rectal Cancer Radioresistance

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Background & Aim: Rectal cancer (RC) accounts for up to 40% of the global gastrointestinal cancer burden, with over half of the patients presenting locally advanced disease at diagnosis. The current standard of care relies on neoadjuvant radiotherapy (RT), alone or combined with chemotherapy, yet up to 40% of patients are resistant to RT. Therefore, clarifying the mechanisms driving radioresistance in RC is crucial. To address this challenge, we are evaluating whether targeting radiation-induced metabolic adaptations can improve the response of radioresistant tumour-immune-spheroids (TIS) to RT using a panel of pharmacological inhibitors directed to lipid droplet, cholesterol, and iron metabolism. **Methods:** Rectal TIS, consisting of a co-culture of SW837 cells, macrophages, and T cells, were exposed to a clinical short-course scheme of RT (SCRT; 5Gy for 5 days). An initial IC50 assay was performed for selective inhibitors, targeting lipid and metabolic pathways, which were then combined with the SCRT. Cell viability in all co-culture populations, as well as the dissection of the targeted pathways (lipid and ferroptosis), was assessed 72 hours after the last fraction of RT. **Results:** Our preliminary proteomic analysis data of irradiated TIS revealed a higher abundance of proteins involved in metabolic rewiring, particularly in lipid biosynthesis, which likely enables adaptation to RT-induced damage and oxidative stress. Pharmacological agents from the assembled panel seem

to compromise cell viability, especially when combined with a SCRT regimen. **Conclusions:** Combining the SCRT regimen with the inhibition of key metabolic players of radioresistance may be a promising personalized treatment strategy for rectal tumours that exhibit radioresistance.

Keywords: rectal cancer; tumour-immune spheroid model; radioresistance; short-course radiotherapy; metabolism

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24702 | Clinical Impact of American Joint Committee on Cancer 8th Edition Prognostic Staging for Breast Cancer: a certified Breast Unit 5-year Survival Comparison

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Background & Aim: In 2018, the American Joint Committee on Cancer (AJCC) harmonised the TNM staging system for breast cancer (BC) with contemporary clinical practice. This study aimed to evaluate stage migration promoted by the integration of tumour biology into anatomical criteria. Five-year overall survival (OS) was benchmarked against estimates from AJCC 8th edition and previous published studies to assess the 'real-world' performance of the pathological prognostic staging system compared with the traditional anatomical one. **Methods:** A retrospective analysis was conducted on a cohort of 1,075 patients diagnosed with BC at the Breast Centre of the São João University Hospital between 2015 and 2020. Clinicopathological data were obtained from a database certified by the European Society of Breast Cancer Specialists (EUSOMA). The primary endpoint was five-year OS. Survival analyses were performed using Kaplan–Meier estimates and log-rank tests. **Results:** As in earlier studies, stage migration occurred in 42.1% when switching from the anatomical to the pathological prognostic staging system, with 37.5% being reclassified to a lower stage and 4.6% to a higher stage. A downstaging tendency was observed at all stages except IA, and for all BC molecular subtypes except triple-negative tumours, which showed a 53.0% upstaging balance. Consistent with published series, 5-year OS rates were equivalent for both staging systems. There were no significant differences in survival rates between patients who remained in a given stage and those who migrated to that particular stage. **Conclusions:** Our 'real-world' benchmarked analysis shows that AJCC 8th edition pathological prognostic staging system substantially promotes stage migration while

refining survival prediction accuracy. Nevertheless, anatomic staging remains a highly reliable staging system that is practical and reasonable for areas with more limited resources.

Keywords: Breast Neoplasm, Prognosis, Survival, Tumor Staging

24586 | Gastric Cancer Sialoglycoproteome Reveals Distinct Serological Glycoproteomic and Cytokine Signatures

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Background & Aim: Aberrant α2,6-sialylation has emerged as a hallmark of malignancy and a driver of gastric cancer (GC) aggressiveness, supporting its potential as biomarker. Sialylated glycans, including both *O*- and *N*-linked sialoglycans, have been implicated in tumour development, progression, immune evasion and aggressiveness[1-3]. However, most evidence derives from primary tumours, with limited attention to minimally invasive liquid biopsies and the systemic regulation mediated by these structures. Here, we address this gap by integrating serum glycoproteomics, cytokine profiling and clinical data to identify biologically relevant tumour-associated carriers of α2,6-sialylated glycans and their translational potential. **Methods:** Serum α2,6-sialylated glycans were analyzed in both healthy donors and gastric tumours by immunoblotting. Targeted glycoproteomics and cytokine profiling in selected cases identified the main sialylated carriers and associated systemic immune signatures. **Results:** Serum α2,6-sialylation levels were increased in GC compared with healthy donors. Notably, aggressive tumours with poorer prognosis were enriched in α2,6-sialylated structures. Lectin-based glycoprotein enrichment revealed extensive overlap between healthy donors and GC, with more than 497 proteins showing no significant differences in abundance. Only 33 proteins displayed altered expression in GC relative to healthy donors, several of which are associated with systemic response pathways previously implicated in cancer development. Cytokine profiling further revealed marked alterations associated with α2,6-sialylated glycans, highlighting a potential role for α2,6-sialylation in shaping the cancer-associated systemic immune response. **Conclusions:** These findings position α2,6-sialylation as a key feature of GC and support sialoglycan-based strategies for GC management.

Keywords: Gastric Cancer; Cancer Glycobiology; Sialylation; Serum Glycoproteomics; Precision Medicine

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24979 | Localized Delivery of BRAF Inhibitor via Microfluidic-based Lipid Nanoparticles as a Safer Option for Cutaneous Melanoma Management

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Background & Aim: Cutaneous melanoma causes 90% of skin cancer deaths[1]. While surgery treats localized cases, effective local neoadjuvant/adjuvant therapies are needed for unresectable tumors. To avoid the systemic toxicity of oral Vemurafenib (VEM)[2], a BRAFV600E inhibitor, we developed a pH-responsive lipid nanoparticle (LNP) for local delivery. **Methods:** LNPs (DOPE:chol:DSPC:DSPE-mPEG2k; ratio 7:7:3:3 and 7:7:4:2) were synthetized by microfluidics with 500 µg or 1 mg VEM. Characterization included particle size determination, distribution of size, charge, VEM payload, and morphology. In A375 melanoma cells (BRAFV600E positive), 2D viability and uptake (confocal/flow cytometry) were assessed, and *ex vivo* human skin permeation was performed using Franz cells. Evaluation extended to 3D spheroids[3,4], measuring metabolic activity, 3D architecture by H&E staining, and live/dead (Calcein-AM/Hoechst/DRAQ7 – Confocal) distribution over 72 h. **Results:** LNPs showed narrow size distribution (polydispersion index 0.3) of about 151-189 nm and negative zeta potential. The 7:7:3:3 1 mg VEM achieved the highest payload (78.6%). The morphology imaging confirmed rounded liposomal-like structure in the same range of size. In A375 cells, LNPs control were safe while VEM@LNPs 200 µM were able to reduced viability to 30.4%, with flow cytometry showing >88% uptake. Skin permeation showed LNPs mainly into epidermis and dermis in a 24 h window. In 3D spheroids, free VEM caused the most disruption, but VEM@LNPs significantly reduced metabolic activity and structural integrity compared to controls. H&E and live/dead imaging confirmed dose-dependent cytotoxicity and deeper penetration of LNPs over time. **Conclusions:** pH-responsive LNPs capable of efficiently encapsulating VEM, penetrating human skin over time and achieving high cellular uptake were successfully developed. These systems produced

controlled antitumor effects with low intrinsic toxicity, supporting their potential as a safer local therapy for early-stage melanoma.

Keywords: Lipid nanoparticles; cutaneous melanoma; 3D models; spheroids; nanomedicines.

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24298 | Molecular and Immune Profiling of Melanoma: Implications for Prognosis Assessment

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Background & Aim: Melanoma is a malignant tumor that arises from the transformation of melanocytes. In 2022, it was the 17th cancer with higher incident and the 22nd more deadly. The MAPK pathway regulates cell differentiation, proliferation, and survival, and is often dysregulated in melanoma, mostly through *BRAF* or *NRAS* mutations. Mutations in *TERT* promoter (*TERTp*) are also common, potentiating cell immortalization. Although *BRAF* status is an established biomarker for target therapy response, the role of molecular alterations and PD-L1 expression in prognosis determination in melanoma is still not consensual. This study aims to improve melanoma prognosis determination, guided by the molecular and immune profile of the tumors. **Methods:** The current cohort is composed of 75 FFPE melanoma samples from Capuchos Hospital (EC INV195) and IPO-Lisbon (EC UIC/1504). The samples were subjected to a histopathological evaluation, DNA extraction, PCR amplification and Sanger Sequencing to identify hotspot mutations in *BRAF*, *NRAS* and *TERTp*. PD-L1 expression is being accessed in ~140 samples through an automatized immunohistochemistry assay. Statistical analysis will be performed to understand the correlation between the molecular and immune profile and clinicopathological data. **Results:** In the already sequenced cohort, 80% of the cases are superficial spreading subtype, and 48% were diagnosed at >69 years (median age). Hotspot mutations in *BRAF, NRAS* and *TERTp* were found in 45%, 14% and 53% of the cases, respectively, and 50% of *TERTp* mutated cases presented concurrent mutation in *BRAF.* Around 70% of *BRAF* mutated and 67% of *TERTp* mutated cases presented mitotic index ≥1/mm^2 and Breslow index >1mm. **Conclusions:** This study offers insights into the significance of molecular and immune

profiling in predicting patient's prognosis and can support further investigations for prognosis biomarkers determination.

Keywords: Melanoma; *BRAF; NRAS; TERTp;* PD-L1

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24977 | Deciphering Inflammatory microRNAs in Osteosarcoma and in Osteoclasts

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Background & Aim: Osteosarcoma (OS) is the most common primary bone tumor, predominantly affecting children and adolescents[1,2]. Survival remains poor in metastatic and recurrent cases[3]. Progression is influenced by interactions with the bone microenvironment, including osteoclasts (OCs)[4]. MicroRNAs (miRNAs) regulate OCs differentiation and inflammatory signaling[5–8]. This study aims to investigate the levels of miRNAs in OS and OCs, focusing on those involved in inflammation, and to assess the effect of TNF-α on OCs. **Methods:** MiRNA expression was profiled during 11 days of OCs differentiation from monocytes isolated from buffy coats (5 donors) and across distinct OS cell lines (SAOS-2, 143-B and U2OS) and the non-malignant osteoblast cell line hFOB1.19. TNF-α effects were tested under optimal (25ng/mL) and suboptimal (1 and 5ng/mL) RANKL conditions[9]. To optimize the OS–OC co-culture system, RANKL and TNF-α expression were evaluated in OS by RT-qPCR and ELISA. **Results:** miR-342 and -125 were upregulated in SAOS-2 and -29c in U2OS, while miR-155 was downregulated in SAOS-2 and U2OS, and miR-16 and -15a were reduced in all tested OS cell lines, compared with hFOB1.19. During OC differentiation, miR-21, -146a, -342, -29c and -125 levels were increased, whereas miR-223 decreased. TNF-α did not significantly affect the expression profile of most miRNAs (miR-21, -29c, -125a, -146a, -155, -200c, -223 and -342) in OCs, except for the upregulation of miR-155. Results are from at least 4 independent experiments and/or donors. OS monocultures showed low and/or undetectable levels of RANKL and TNF-α, by qPCR (mRNA) and ELISA (secreted proteins). **Conclusions:** This study shows that OS cells have a distinct miRNA profile and do not secrete detectable levels of the osteoclastogenic mediator RANKL in monoculture. Moreover, TNF-α showed minimal effects on the miRNA profile during OC differentiation. These findings lay the groundwork for future co-culture studies on miRNA modulation in the tumor microenvironment.

Keywords: Osteosarcoma; tumor microenvironment; microRNAs; osteoclasts; RNA therapeutics

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24947 | Modulating Tumor–Associated Macrophage Interactions to Improve Radiotherapy Efficacy in Triple-Negative Breast Cancer

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Background & Aim: Triple-negative breast cancer (TNBC) is the most aggressive subtype with limited targeted therapies (1, 2). Radiotherapy (RT) is used in TNBC patients to reduce local relapse. However, radioresistance frequently limits its effectiveness (3). Treatment response is influenced not only by tumor-intrinsic mechanisms but also by the tumor microenvironment (TME) (4). Macrophages are highly abundant in TNBC and may promote tumor survival and limit RT-induced cell death (5). This project aims to investigate macrophage-driven radioresistance using 3D tumor spheroids (TS) and tumor-immune spheroids (TiS), that better recapitulate TME architecture and cell-cell interactions. Using MDA-MB-231 and MDA-MB-468 TNBC cell lines co-cultured with human monocytes, we evaluated how macrophages influence RT-induced DNA damage and tumor cell survival. Pharmacological inhibitors targeting RT-altered pathways will

be tested as potential radiosensitizers. **Methods:** MDA-MB-231 and MDA-MB-468 TNBC cell lines were cultured alone (TS) or co-cultured with human macrophages to generate TiS. Spheroids were exposed to a clinically relevant fractionated RT protocol (5 x 5.2 Gy). DNA damage (γH2AX) and cell viability were assessed at 24h and 72h after the final RT fraction. **Results:** Consistent with previous data from our group, results show that macrophages protected tumor cells from death caused by RT, still sustaining cancer cell proliferation. Interestingly, and despite at 24h after RT the same level of γH2AX was found on cancer cells cultured or not in the presence of macrophages, at 72h post irradiation, a significant decrease in DNA damage was found only on cancer cells co-cultured with immune cells. These findings suggest that macrophages do not prevent the induction of RT-mediated DNA damage; rather, they appear to promote a more efficient DNA repair process. **Conclusions:** Our data suggest that macrophages contribute to TNBC radioresistance and represent a potential therapeutic target to enhance RT response.

Keywords: Triple-negative breast cancer; Radiotherapy; Tumor Microenvironment; Macrophages; 3D spheroid model.

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24794 | Effects of BRAF-Targeted Therapy on Telomerase Expression in Thyroid Cancer

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Background & Aim: Thyroid cancer (TC) is the most common malignancy of the endocrine system. Over the past decades, specific somatic mutations have been associated with tumorigenesis in TC [1]. *TERT* promoter mutations are primarily described in more aggressive thyroid cancers and lead to telomerase reactivation, conferring replicative immortality, one of the cancer hallmarks [2,3]. In addition, the *BRAF* V600E mutation, one of the most prevalent alterations in TC, has been shown to be correlated with the upregulation of ETS transcription factors, which can bind to mutant *TERT* promoters in TC cells, thereby enhancing *TERT* transcription [1,4]. This study aims to evaluate the effect of using a BRAF inhibitor (Vemurafenib) to indirectly reduce *TERT* expression, as illustrated in Figure 1. **Methods:** We used an immortalized normal thyroid cell line (Nthy-Ori 3-1) alongside two TC-derived cell lines (TPC-1 and B-CPAP). TPC-1 harbours the *RET/PTC1* rearrangement, whereas B-CPAP has the *BRAF* V600E mutation, with both presenting the *TERT* promoter mutation (-124 G>A) [5]. Cell viability was evaluated using the Presto Blue assay, followed by the assessment of cell proliferation via the EdU assay and apoptosis using the TUNEL assay. To investigate the impact of Vemurafenib on BRAF signalling, BRAF protein expression and ERK phosphorylation levels were quantified by Western blot. Finally, RT-qPCR was used to evaluate the effects of Vemurafenib on *TERT* transcription. **Results:** Vemurafenib exerted a pronounced effect on cell viability and proliferation in B-CPAP cells, for which the IC_{50} value was reached. In TPC-1 cells, *TERT* expression remained unchanged, and Western blot analysis suggested that the MAPK pathway was not modulated after Vemurafenib treatment. **Conclusions:** Vemurafenib's effect on cell proliferation and viability seems to depend on cell genotype, being stronger in the B-CPAP cell line, which harbours the *BRAF* V600E mutation.

Keywords: Thyroid carcinoma, *TERT* promoter mutation, *BRAF* V600E, Vemurafenib

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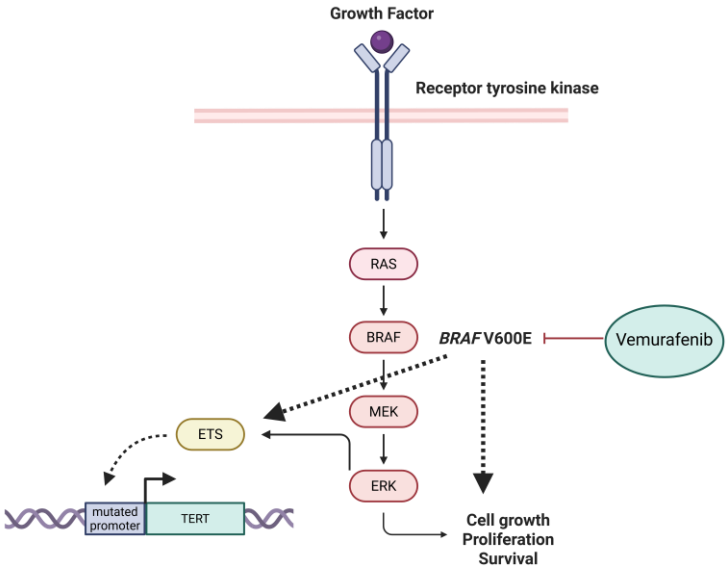

Figure 1: Association between BRAF inhibitors and *TERT* expression. Adapted from: Susanne Horn et al.,TERT Promoter Mutations in Familial and Sporadic Melanoma.Science339,959-961(2013). DOI:10.1126/science.1230062 Created with BioRender.com.

24455 | Investigating the role of LAMP-2 in Autophagy-Related Metabolism and Its Impact on Thymic Epithelial Cell Function and T Cell Development

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Background & Aim: Within the thymus, thymic epithelial cells (TECs) provide key microenvironments for T cell development[1]. Particularly, the selection of functionally diverse CD4 T cells depends on interactions between TCR expressed on T cell precursors with self-peptides:MHC II complexes presented by cortical TECs (cTECs). Although autophagy and lysosomal protein processing have been implicated on CD4 T cell selection, the molecular mechanism that controls the generation of MHC II ligands in cTECs remains elusive[2,3]. Previous observations suggest that Lysosomal-Associated Membrane Protein 2 (LAMP-2) controls the processing of MHC-II-bound positive selecting peptides and contributes to the diversification of CD4 TCR repertoire in the thymus[4]. Nonetheless, the TEC-specific role of LAMP-2 has not been yet thoroughly addressed. Hence, we have generated a novel LAMP-2 cKO mouse model to further explore the role of this protein in autophagic-lysosomal-related pathways and its impact on CD4 T-cell development and immune function. **Methods:** In this project, we will cross LAMP-2 cKO mice with transgenic (HY, OT-I, OT-II) and reporter (LC3-GFP/RFP) animals. Flow cytometry, immunofluorescence and confocal microscopy will be employed to analyse distinct TEC subpopulations and T cell developmental stages. **Results:** Given the role of TECs in T cell selection, we infer that the LAMP2-mediated autophagy-lysosomal pathway regulates the self-peptide:MHC II ligandome in TEC, impacting CD4 T cell selection and subsequent T cell-dependent responses. Our preliminary analysis suggests that LAMP-2 in TECs is required for CD4 selection, which suggests a possible role of this protein for the efficient display of MHC-II-bound positive selecting peptides by cTECs. **Conclusions:** This project will provide critical insights to understand how T cell-mediated immunity is established *in vivo* and a crucial rationale for new therapies to treat T cell-related disorders.

Keywords: T cell Selection; Thymic Epithelial Cells; Autophagy; Lysosome; Antigen Presentation

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24594 | Anthracycline-Induced Toxicity in Human Cardiac Cells: An In Vitro Cardio-Oncology Study

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Background & Aim: Cancer patients face increased risk of cardiotoxicity, largely due to the use of anticancer agents that can damage cardiac tissue [1]. Although anthracyclines are widely used and highly effective, cardiotoxicity is frequent and may progress to heart failure. This study aims to compare the cytotoxic profiles of two anthracyclines (doxorubicin and daunorubicin) in the human cardiomyocyte cell line AC16 and to evaluate whether caspase inhibition can attenuate or reverse drug-induced toxicity. **Methods:** AC16 cells were seeded and maintained in medium supplemented with 12.5% fetal bovine serum and differentiated for 24 h with 2% horse serum [2]. Differentiated cells were exposed to doxorubicin and daunorubicin at concentrations ranging from 0.05 to 20 µM. Cytotoxicity was assessed at 24 and 48 h using neutral red uptake and MTT reduction assays. Cell morphology was evaluated by phase-contrast microscopy. To examine potential prevention of toxicity, a caspase-9 inhibitor was applied to cells treated with 1 and 2.5 µM of each drug. **Results:** Both anthracyclines caused time- and concentration-dependent cytotoxicity, with more pronounced effects in the MTT reduction assay. At 48 h, greatest cytotoxic effects occurred with 2.5 µM doxorubicin and with 2.5, 10, and 20 µM daunorubicin, in both assays. In contrast, 0.05 µM doxorubicin and 0.1 µM daunorubicin did not produce detectable toxicity at either time point in any of the assays performed. Cell density and morphology were visibly altered at concentrations from 2.5 to 20 µM after 24 h and from 1 to 20 µM after 48 h, indicating progressive structural damage over time and with increasing concentrations. Daunorubicin induced earlier and more marked morphological alterations. Treatment with a caspase-9 inhibitor did not attenuate the cytotoxicity induced by either anthracycline. **Conclusions:** Both anthracyclines exerted cytotoxicity in AC16 cells, although daunorubicin appears to induce a more pronounced toxic response under the tested conditions.

Keywords: Doxorubicin, Daunorubicin, AC16 cells, Cardio-oncology.

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24310 | Effects of isotretinoin on human bone marrow mesenchymal stem cells (BM-MSCs)

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Background & Aim: Isotretinoin, a synthetic retinoid derived from vitamin A, revolutionized the treatment of severe acne by targeting key pathogenic factors such as sebum production, inflammation, and abnormal keratinization. While its dermatologic efficacy is well established, growing evidence highlights systemic effects, particularly on bone metabolism. Preclinical studies demonstrate that retinoic acid influences osteogenic differentiation, bone formation, and remodeling. In vitro experiments reveal enhanced expression of osteogenic transcription factors and extracellular matrix mineralization under isotretinoin exposure. Animal models corroborate these findings, showing altered bone healing and remodeling. Clinically, concerns include reduced bone density and risk of osteopenia or osteoporosis, particularly with long-term or high-dose therapy. Thus, the objective of this study is to analyze the effects of isotretinoin on mesenchymal stem cells from bone marrow (BM-MSCs). **Methods:** Human BM-MSCs were cultured in basal and osteogenic media, being exposed for 28 days to retinoic acid (RA) at two concentrations (1µM and 2.5 µM). Basal and osteogenic media were used as controls. The cells were assessed for metabolic activity, morphology, alkaline phosphatase (ALP) activity and gene expression of osteoblastic markers. Comparison between groups was performed using analysis of variance and Tukey post-hoc test ($p < 0.05$). **Results:** RA (1 and 2.5 µM) maintained BM-MSCs viability and sustained higher proliferation (DNA levels) over 28 days compared to the osteogenic control. However, RA significantly reduced ALP activity, severely impairing osteogenic differentiation even in osteoinductive media. No significant dose-dependent differences were found. **Conclusions:** Isotretinoin inhibits osteogenic differentiation, maintaining BM-MSCs in a proliferative, undifferentiated state rather than allowing maturation. These in vitro results elucidate the cellular mechanisms behind the altered bone remodeling and orthodontic tooth movement induced by isotretinoin previously observed *in vivo*.

Keywords: Isotretinoin; retinoic acid; Bone marrow mesenchymal stem cells (BMSCs); Bone tissue engineering.

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24316 | Sustainable optimization of pre-methylenomycin C lactone: Strategies to enhance its antibacterial potential

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Background & Aim: A Problem-Based Learning (PBL) approach in the Optional Curricular Unit "Drug Design and Sustainable Synthesis of Drugs" (4th year, MICF-FFUP) [1] challenged students to propose a new, viable antimicrobial agent. On October 29, 2025, Science Daily reported that scientists from the University of Warwick and Monash University discovered a "hidden" antibiotic 100x more potent against drug-resistant Gram-positive pathogens, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant enterococcus (VRE) [2]. This molecule, pre-methylenomycin C lactone, is a precursor with important antibiotic properties. However, its clinical application is hindered by an unknown biological target, potential toxicity, and limited stability. To solve problems involving the original molecule, this work used Green Chemistry principles and computer-assisted drug design to identify the target and propose sustainable modifications to optimize the molecule and its synthesis. **Methods:** To identify potential targets, we used SwissTargetPrediction and selected DNA gyrase by homology with the targets indicated for both pre-methylenomycin C lactone and methylenomycin A. We proposed an analog with structural modifications to increase stability and the spectrum of action. We used CB-Dock2 to study derivatives interactions with the target, using a fluoroquinolone and the original lead as controls. Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties were studied using SwissADME and pkCSM. The synthesis was formulated in accordance with Green Chemistry principles. **Results:** The proposed analog showed better interaction with the target than pre-methylenomycin C lactone and improved ADMET properties, including the absence of Michael acceptor alerts. **Conclusion:** This analog is promising due to its increased affinity to the potential target, higher stability, and better ADMET properties. Future studies are needed to validate this proposal.

Keywords: Computer-Assisted Drug Design; Pre-Methylenomycin C Lactone; Green Chemistry; Structure–Activity Optimization

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24495 | The Role of Social Capital in the Association of Socioeconomic Inequalities and Periodontitis: A population-based cohort

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Background & Aim: Periodontitis is a chronic inflammatory disease that may lead to tooth loss and reduced quality of life. Although socioeconomic inequalities are associated with periodontitis, the role of social capital, particularly among individuals with type 2 diabetes, remains unclear. This study examined pathways linking socioeconomic position, social capital, and periodontitis in people with and without type 2 diabetes. **Methods:** Data from the Health in Central Denmark Cohort (HICD) were analysed using structural equation modelling to estimate direct and indirect pathways from education and income to periodontitis via social capital. Social capital was modelled as a latent construct including social networks and perceived reciprocity. Models were stratified by diabetes status and adjusted for age, sex, smoking, physical activity, BMI, and mental disorders. Sensitivity analyses included multiple imputation and survey weights. **Results:** The study included 41,370 participants (mean age 61.2 years; 41% women), comprising 20,685 individuals with type 2 diabetes and 20,685 matched controls. Lower education and income were associated with higher levels of periodontitis in both groups. Among individuals without diabetes, smoking, higher BMI, physical inactivity, and lower social capital were directly associated with periodontitis, with indirect effects of socioeconomic position mainly through smoking, BMI, and social capital. Among those with type 2 diabetes, smoking, higher BMI, physical inactivity, mental disorders, and lower social capital were directly associated with periodontitis. Indirect effects operated through smoking, BMI, mental disorders, and social capital, with stronger mediation via social capital than in those without diabetes. **Conclusions:** Periodontitis reflects socioeconomic inequalities operating through behavioural and psychosocial pathways. Social capital appears to be an important mediator, particularly among individuals with type 2 diabetes.

Keywords: Diabetes Mellitus, Type 2; Periodontitis; Social Capital; Socioeconomic Factors.

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24641 | Mineral plasmatic status in children with Attention-Deficit and Hyperactivity Disorder

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Background & Aim: Attention-deficit/hyperactivity disorder (ADHD) is a paediatric neurodevelopmental disorder whose pathophysiology remains unclear [1]. Mineral status has emerged as a potential modulating factor, as minerals such as zinc and magnesium (Mg) are vital for brain functioning and metabolism [2,3]. Despite inconsistent findings, some studies report that children with ADHD exhibit lower plasma levels of zinc, iron, and Mg [4-7], potentially associated with symptom severity [6-8]. This study compared the plasma mineral status of children with ADHD and neurotypical (NT) peers and examined its association with ADHD symptom severity. **Methods:** A case-control study included 158 children aged 5-12 years (ADHD: *n*=97; NT: *n*=61). ADHD diagnosis followed DSM-5 criteria. Blood samples were analysed by routine lab procedures. Mineral ratios (Ca:Mg and Na:K) were calculated. Group comparisons were assessed according to variables' normality, and regression models used for confounders' adjustment. Partial correlations with the Conners ADHD Index were performed, adjusting for inter-group key confounders. **Results:** After age and gender adjustment, and False-Discovery Rate (FDR) correction, children with ADHD showed significantly lower levels of inorganic phosphorus (Pi) (4.75±0.46 vs. 4.99±0.40 mg/dL, *p*=0.002), and Mg (1.67±0.12 vs. 1.74±0.11 mmol/L, *p*=0.001) compared to NT. Mg levels were negatively correlated with the ADHD Index (*r*=-0.19, *p*=0.03), while the Ca:Mg ratio was positively correlated (*r*=0.17, *p*=0.04). Logistic regression showed that higher Mg and Pi levels were associated with lower odds of ADHD (OR=0.000, p<0.001 and OR=0.27, 95% CI: 0.11–0.63, p=0.003, respectively), independently of

age, gender, and FDR correction. **Conclusions:** Children with ADHD showed reduced Mg and Pi concentrations, and higher levels of these were associated with lower ADHD odds. These findings support a potential role of mineral status in ADHD and warrant further investigation.

Keywords: ADHD, Neurodevelopment, Minerals, Plasma

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24919 | Fatty Liver Index and Adiposity Indices in Bariatric Surgery Individuals

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Introduction: Hepatic fat accumulation is closely linked to central/visceral obesity, both negatively affecting metabolic health (MetH). Bariatric surgery improves these parameters. In this longitudinal study, associations between fatty liver index (FLI) and adiposity indices, stratified by MetH phenotype, surgical procedure, and body mass index (BMI), were determined. **Methods:** Metabolically healthy/unhealthy (MH/MUH) phenotypes were defined as in Plümacher *et al.* (2026) in 1,001 adults (CRI-O, Portugal; 81.2% women; 42.6±5.3 kg/m²; 43.7±10.4 y) undergoing Roux-en-Y gastric bypass or sleeve gastrectomy. Correlations between FLI (Chung *et al*. 2022) and lipid accumulation product (LAP, anthropometric–metabolic) or abdominal volume index (AVI, purely anthropometric) (Li *et al*. 2022) were assessed at baseline (B_0; n=754, 771, and 730, respectively) and annually up to 2 y post-surgery ($F_{1\text{-}2}$). Logistic regression (age/sex adjusted) evaluated one-unit LAP and AVI increases by phenotype on odds of FLI>60. **Results:** FLI significantly correlated with LAP and AVI for both MetH phenotypes and BMI [overweight+obese (baseline and post-surgery) and normal-weight (post-surgery)] groups ($p \leq 0.002$), except for MUH from F_2 normal-weight group. FLI significantly correlated with LAP and AVI for both surgical procedures and BMI groups ($p<0.001$) at all time points. At baseline, FLI *vs* AVI correlations were consistently stronger than FLI *vs* LAP correlations. When restricting the analysis to individuals with FLI>60 across all time points, a similar pattern was observed. For

the MH phenotype, at F_1 and F_2, a one-unit increase in LAP and AVI significantly increased the odds of high-risk hepatic steatosis: OR were 1.090 (p=0.005) and 1.071 (p=0.007) for LAP, and 1.788 (p<0.001) and 2.172 (p=0.022) for AVI, respectively. **Conclusion:** FLI is strongly associated with the adiposity indices LAP and AVI, particularly with AVI. AVI may serve as a clinical practice marker of high-risk hepatic steatosis (FLI>60) in MH individuals.

Keywords: obesity; metabolic phenotypes; adiposity index; fatty liver index; bariatric surgery

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24713 | Challenges of Artificial Intelligence to the Legal Framework of Intellectual Property in Healthcare

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Background & Aim: The growing integration of Artificial Intelligence (AI) in scientific research and healthcare has significantly transformed this sector, particularly in drug discovery, medical devices and clinical data analysis. Reflecting this trend, in 2024 the total number of patent applications worldwide exceeded 3.7 million, representing the highest growth since 2018 [1][2]. This evolution raises challenges to intellectual property frameworks, traditionally based on human creativity and intervention [3]. This study aims to analyze the main challenges posed by AI to intellectual property rules in healthcare, focusing on patents and copyright, including issues related to the concept of "invention", inventorship, inventive step and sufficiency of disclosure [4]. **Methods:** A qualitative analysis was conducted based on the review of relevant legislation, namely the Portuguese Industrial Property Code and Copyright Law. Guidelines and decisions from institutions such as the European Patent Office (EPO), United States Patent and Trademark Office (USPTO) and World Intellectual Property Organization (WIPO) were also analyzed. Additionally, a literature search was carried out in Google Scholar using terms such as "AI inventorship", "AI drug discovery intellectual property", "Artificial intelligence reproducibility" and "Sufficiency AI patent". Sources were critically analyzed to identify key legal challenges associated with AI in healthcare. **Results:** The findings indicate that, although current frameworks require human intervention for inventorship, the increasing use of AI places pressure on patentability criteria, particularly in pharmaceutical and biomedical fields [5]. The reliance on large datasets and complex models challenges transparency and sufficiency of disclosure. Issues related to reproducibility and the use of protected data also emerge, raising concerns in copyright and data governance [6][2]. **Conclusions:** Artificial Intelligence does not replace the patent system but requires reinterpretation of key criteria, including invention, inventive step and disclosure. Adapting intellectual property systems is essential to ensure continued innovation in healthcare while safeguarding transparency, reproducibility and equitable access.

Keywords: Artificial Intelligence, Patents, Healthcare.

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24904 | Impact of Pre-Transplant Dialysis Modality on Clinical Outcomes in Patients with Kidney Allograft Failure

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Background & Aim: The impact of pre-transplant dialysis modality, Hemodialysis (HD) or Peritoneal Dialysis (PD), on post-transplant outcomes remains uncertain. While studies suggest a survival advantage for PD, evidence on the clinical course of recipients who develop graft failure is scarce. This study compares graft survival and clinical complications, from transplantation to failure, stratified by pre-transplant modality. **Methods:** A retrospective single-center study evaluated 76 adult kidney recipients with graft failure (2014-2024). The 76 recipients were stratified by pre-transplant modality (HD=66; PD=10). The primary outcome was graft survival time. Secondary outcomes included failure etiology, hospitalizations and major complications. Statistical analysis was performed using SPSS (significance: $p<0.05$). **Results:** PD recipients and donors were significantly younger than the HD group (PD: 49.8±5.1 vs HD: 60.3±4.2 years, p=0.028; PD: 41.8±2.3 vs HD: 51.1±3.1, p=0.039). No differences were found regarding gender, donor type, or comorbidities like diabetes and hypertension ($p>0.05$). Median graft survival was similar (HD: 9.0±12.0 vs PD: 8.0±4.0 years, p=0.460). Complication rates showed no significant differences, including delayed graft function (HD: 28.8% vs PD: 30.0%, p=1.000), urological (HD: 0% vs PD: 10.0%, p=0.132) or vascular events (HD: 3.0% vs PD: 0%, p=1.000). Annual hospitalizations (HD: 0.61±1.01 vs. PD: 0.65±1.21, p=0.763) and cardiovascular events (HD: 0.00±0.03 vs. PD: 0.00±0.00, p=0.330) were comparable. Chronic rejection was the primary failure etiology in both groups (HD: 78.79% PD: 90.00%). **Conclusions:** Pre-transplant modality did not influence long-term graft survival. Despite the demographic advantage of younger recipients and donors in the PD group, this did not translate into superior graft longevity

or lower complication rates. This finding requires further analysis. Chronic rejection remains the primary cause of failure, regardless of prior modality.

Keywords: Kidney Transplantation; Graft Failure; Hemodialysis; Peritoneal Dialysis; Graft Survival.

24662 | Access of migrant and native populations to the Portuguese National Health Service: a secret shopper approach

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Background & Aim: Portugal's National Health Service (SNS) guarantees universal healthcare access regardless of legal status(1). Yet, migrants face significant challenges in accessing and navigating the system, suggesting potential inequities in healthcare access(2-5). This study explores migrants' accessibility to the SNS and whether it differs from natives', in order to understand the underlying healthcare-related barriers. **Methods:** Using a "secret shopper" design, 939 emails were sent to all SNS primary healthcare units from ten fictitious profiles (five migrant nationalities and five natives). Responses were analyzed using descriptive and inferential analyses, including binomial logistic regression, and thematic and content analyses. **Results:** Almost 80% of 939 emails received a reply, and 71.8% were non-automatic. Migrant profiles were significantly less likely to receive tailored replies compared to natives (59.6% vs. 84.1%), particularly the Indian, Bangladeshi, and Ukrainian cases. Portuguese-written emails had higher reply rates than those written in English (81.5% vs. 48.6%, p<.001), and English requests were often answered in Portuguese. Qualitative analysis identified five themes: "Identification and documentation requirements"; "Administrative procedures for access"; "Guidance on navigating the SNS"; "Refusal to provide access in the healthcare unit"; and "Communication and Support". Compared with natives, migrants were more often asked for extra documentation and more frequently redirected to emergency departments or other units. Contrasting the results with Levesque's framework, barriers appeared across all access dimensions. **Conclusions:** Findings suggest differences in healthcare access between the two groups, driven by linguistic, bureaucratic and administrative barriers. Policy improvements should include cultural/linguistic training, clearer national guidelines for healthcare staff, and training on migrants' health rights to promote equitable access.

Keywords: Healthcare access, inequalities, migrant, native, *National Health Service*

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24662 | Access of migrant and native populations to the Portuguese National Health Service: a secret shopper approach

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24865 | Indoor Environmental Quality in a Portuguese Medical School: A Multi-Domain Assessment of Student Health, Comfort and Well-being

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Background & Aim: Students spend considerable time in educational buildings where indoor environmental quality (IEQ) conditions may be inadequate. IEQ is recognized as an important determinant of occupants' health, well-being, comfort, and performance. Since evidence is limited in Portuguese university settings, this study aims to enhance the understanding of IEQ, perception and outcomes among medical students. **Methods:** This cross-sectional study assessed IEQ in lecture rooms (LRs), library and study rooms (SRs) at the Faculty of Medicine of the University of Porto, continuously monitoring carbon monoxide and dioxide (CO_2), temperature, relative humidity, particulate matter (PM), ultrafine particles (UFP), ozone (O_3), volatile organic compounds (VOC), illuminance and noise. 626 students completed questionnaires on IEQ perception, well-being, and health conditions and symptoms. **Results:** LRs exhibited higher $PM_{2.5}$, PM_{10}, O_3, and noise levels and lower illuminance, whereas SRs presented elevated CO_2, VOC, and UFP concentrations but better lighting. The library, the only mechanically ventilated space, consistently showed the lowest CO_2, $PM_{2.5}$, PM_{10}, and UFP levels, also with better lighting. Satisfaction with different IEQ domains was generally positive, with the least positive for noise. $PM_{2.5}$ was significantly associated with diagnosed allergic rhinitis (OR=1.573) and sneezing (OR=5.374), and UFP were positively associated with other skin problems (OR=1.115) and environmental allergies (OR=1.095), and negatively with the absence of reported health conditions (OR=0.934). Student well-being (WHO-5) declined from class to exam season and showed no significant association with IEQ parameters. **Conclusions:** Mechanical ventilation in the library appears to ensure better IAQ conditions. Furthermore,

$PM_{2.5}$ and UFP levels associations with health conditions highlight the importance of effective IEQ management to support students' health and well-being.

Keywords: Indoor environmental quality, Indoor air quality, Medicine students' health, Perceived IEQ, Well-being, Sick-Building syndrome.

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24330 | Receptor-Specific Transcriptional Reprogramming Following A2- And B2- Adrenergic Activation in Glioblastoma Cells

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Background & Aim: Adrenergic α_2- and β_2-receptors regulate proliferation of glioblastoma multiforme (GBM) cell lines of classical and mesenchymal molecular subtypes via non-canonical signaling mechanisms. This study investigated receptor internalization and transcriptional reprogramming triggered by selective adrenergic activation. **Methods:** Two patient-derived GBM cell lines from the Human Glioblastoma Cell Culture (HGCC) resource were used: U3009 (classical) and U3046 (mesenchymal). Cells were cultured in defined serum-free medium [1]. Receptor internalization was assessed after 30 min incubation with solvent, brimonidine (BRI; 10 nM; α_2-agonist), or formoterol (FOR; 1 µM; β_2-agonist). Surface receptor levels were quantified by flow cytometry using anti-β_2-AR (AAR-016-F), anti-α_2A-AR (AAR-020), and anti-α_2B-AR (ARR-021) antibodies (Alomone Labs). Data from 4–5 independent experiments were expressed as percentage of control and analyzed by ANOVA with Tukey's post-test. For transcriptomics, cells were treated with solvent, BRI, or FOR for 48 h, followed by RNA sequencing (Illumina NovaSeq X Plus, paired-end 150 bp; Novogene, UK). **Results:** FOR significantly decreased β_2-AR surface density in U3046 cells (74 ± 15 %; $n = 5$; $P < 0.01$) compared with solvent (100 ± 8 %), with similar effects in U3009. BRI did not alter α_2A-AR or α_2B-AR surface levels. Transcriptomic analysis showed greater overlap of differentially expressed genes between treatments than between cell lines. Both agonists upregulated proliferative (MYC, E2F), angiogenic, and epithelial–mesenchymal transition (EMT) genes, while downregulating tumor-suppressive pathways. However, α_2- and β_2-activation modulated distinct signaling cascades and unique gene subsets within shared pathways. **Conclusions:** α_2- and β_2-adrenergic stimulation induces receptor-specific transcriptional programs in GBM cells,

reflecting subtype- and context-dependent reprogramming that may underlie divergent functional outcomes in classical and mesenchymal tumors.

Keywords: Glioblastoma multiforme; Adrenergic receptors; Molecular subtypes; Receptor internalization; Transcriptomic reprogramming

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24339 | Mesenchymal Stromal Cells-Derived Extracellular Vesicles (MSC-EVs) as Vehicles for Bioflavonoid Hesperidin: A Potential Cell-Free Therapy for IVD Degeneration

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Low back pain is a leading cause of disability, often linked to intervertebral disc (IVD) degeneration [1], which remains unaddressed by current therapies [2]. Minimally invasive therapies targeting inflammation reduction and IVD regeneration/repair, such as mesenchymal stromal cells-derived extracellular vesicles (MSC-EVs), are promising due to their regenerative and anti-inflammatory effects, while avoiding cell transplantation risks [3]. Recently, EVs have also been proposed as drug delivery platforms [4]. Here, we aimed to load Hesperidin into EVs derived from immortalized MSCs (iMSCs), to develop an anti-inflammatory therapy. iMSC-EVs were isolated by differential centrifugation and characterized by NTA, TEM, and BCA. Hesperidin was loaded using mixing or sonication methods at 20-100µg/mL, with loading efficiency assessed by HPLC and UV/Vis and morphology analysed by TEM. Moreover, a chitosan coating on EVs was explored to enhance loading. The stability of Hesperidin in coated-loaded EVs was assessed after 1 week storage at 3 temperatures (4, -20, -80°C). Finally, biological assays are being conducted in a bovine organ culture model of IVD degeneration [5] and human macrophages. These assays include cytotoxicity testing and detection of inflammatory mediators prostaglandin E2 (PGE_2) and IL-1β in conditioned media, which are increased in pro-inflammatory microenvironments. iMSCs were a reliable source for EV production (123±23nm; $5.8\pm1.4\times10^5$ particles/cell). Maximum Hesperidin encapsulation efficiency (EE) was achieved for an initial concentration of 20µg/mL, with 11±4% (mixing) and 22±20% (sonication). Chitosan coating did not enhance EE but appeared to better preserve Hesperidin retention in the EVs at 4ºC. Furthermore, the preliminary biological tests support that loaded-EVs are not cytotoxic and reduce PGE_2 levels. Collectively, our findings provide proof-of-concept for iMSC-EVs as a platform for Hesperidin loading, paving the way for novel cell-free therapies for IVD degeneration.

Keywords: Intervertebral disc degeneration, chronic low back pain, mesenchymal stem/stromal cells, extracellular vesicles, drug delivery, hesperidin

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24313 | Plant-Based Diets and Body Image: A Systematic Review

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Background & Aim: While Plant-based (PB) Diets are increasingly adopted for health and sustainability reasons, their association with Body Image (BI) remains unclear. We aimed to systematically review the evidence on differences in BI between individuals adhering to PB Diets and those following other dietary patterns. **Methods:** A systematic search was conducted in Medline, Web of Science, Scopus and EBSCO, yielding 18 eligible observational studies. **Results:** Findings were heterogeneous, with most studies reporting non-significant or mixed results. However, some trends emerged: vegans showed a higher drive for thinness, and semi-vegetarians appeared to be at higher risk of self-objectification. The motivation for dietary adherence - particularly weight control versus ethical reasons - emerged as a key moderating factor. **Conclusions:** Current evidence does not support a consistent association between PB diets and BI. Further research is needed, using standardised definitions and methodologies to clarify this relationship and its psychological implications.

Keywords: Plant-based Diets; Body Image; Systematic Review

24768 | Neurobehavioral Effects of Blackberry Flavonoids During Alcohol Withdrawal in Male Rats

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Background & Aim: Chronic alcohol exposure induces persistent neuroinflammation driven by microglial activation and increased pro-inflammatory cytokines, contributing to hippocampal dysfunction and impaired neurogenesis (1,2). Alcohol withdrawal can further amplify inflammatory signaling and neuronal vulnerability (3). Flavonoids found in blackberries, particularly anthocyanins, possess antioxidant and neuroprotective properties and can modulate microglial activation (4,5). We investigated whether blackberry flavonoid supplementation during alcohol withdrawal improves behavioral outcomes. **Methods:** Male Wistar rats were exposed to 20% ethanol for 6 months, followed by 2 months of withdrawal. Animals were assigned to Withdrawal (n=8) and Withdrawal+Flavonoids (25 mg/kg/day; n=9). Anxiety-like and exploratory behavior were assessed using the open field (OF) and elevated plus maze (EPM), and spatial learning using the Morris water maze (MWM). As an exploratory approach, we quantified the numerical density of DCX-immunoreactive neurons in the dentate gyrus. Group comparisons were performed using predetermined tests, and Hedges' g was calculated. **Results:** Behavioral analyses revealed that OF, EPM, and MWM outcomes did not differ between groups. The exploratory study suggests that DCX+ neuronal density may change in response to flavonoid supplementation. **Conclusions:** Blackberry flavonoid supplementation during alcohol withdrawal did not significantly alter behavioral outcomes under the present experimental conditions. However, the exploratory findings regarding DCX expression suggest potential modulation of hippocampal neurogenesis, supporting further refinement of the model, including optimization of flavonoid dosage and/or selection of more sensitive timepoints.

Keywords: Withdrawal, Flavonoids, Behavior, Neurogenesis

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24490 | Chemical modulation of lipid-based nanoparticles surfaces to enhance intranasal delivery of Curcumin

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Background & Aim: Delivering therapeutic agents to the brain is hindered by the restrictive blood-brain barrier1. Intranasal administration (IN) offers a non-invasive route capable of enabling direct nose-to-brain transport and rapid access to the central nervous system2. This work aimed to develop lipid-based nanoparticles with distinct surface properties to improve IN drug delivery and assess their potential for brain-targeted applications. **Methods:** Solid-lipid nanoparticles, nanostructured lipid carriers, and liposomes were produced with anionic and cationic surface modifications. Cationic systems were designed using chitosan, octadecylamine, or cetyltrimethylammonium bromide (CTAB) as coating agents and chitosan was also applied to enhance cellular uptake. Curcumin was selected as a model drug due to its anticancer and photosensitizing properties. Nanoparticles were characterized using complementary physicochemical techniques, and their biological activity evaluated in vitro using an in vitro nasal epithelium model and a glioblastoma cell line. Cytotoxicity, nasal permeability, and phototherapeutic activity under irradiation were assessed. **Results:** Permeability assay indicated that SLN and liposomes were the most suited for intranasal administration, showing higher transport across the nasal barrier compared to other formulations, while Curcumin-loaded nanoparticles exhibited significant phototherapy activity in glioblastoma cells, supporting their relevance for brain-targeted therapies. **Conclusions:** Surface-modulated lipid-based nanoparticles demonstrate promising characteristics for intranasal drug delivery and may contribute to more effective therapeutic strategies targeting the brain. The results support the continued development of tailored lipid-based nanoparticles as viable carriers for central nervous system-oriented treatments and reinforce the potential for IN drug delivery.

Keywords: Intranasal delivery, Lipid nanoparticles, Surface modifications, Curcumin, Brain targeting

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24462 | Pruning of grapevines: Protein content and amino acid profile of leaves and stems

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Background & Aim: "Vine thieves" are small shoots that grow along the vine and compete for the plant's nutrients. These shoots are pruned to redirect the plant's resources toward grape development [1] and constitute a substantial portion of the biomass discarded [2]. Their valorization fits within a circular-economy strategy, promoting greater sustainability in the sector. To explore this possibility, the protein content and amino acid profiles of leaves and stems from 3 red grapevine varieties (Vinhão, Touriga Nacional and Padeiro de Basto) were analyzed. **Methods:** Total nitrogen (TN) and protein nitrogen (PN) (after precipitation of the protein fraction with trichloroacetic acid) were determined using the Kjeldahl method (AOAC, method 984.13). Non-protein nitrogen (NPN) was calculated as the difference between TN and PN contents. Protein content was determined based on the conversion factor PN × 6.25 [3]. Samples were further subjected to acid and alkaline hydrolysis to release total amino acids; amino acids were automatically derivatized with OPA/3-MPA + FMOC and quantified by RP-HPLC-FLD [4]. The analyses were performed in triplicate and the results expressed in g/100 g dry weight. **Results:** Protein content in leaves ranged from 19 to 22% with Touriga Nacional showing the highest amount; in stems protein levels ranged from 12 to 13%, and Vinhão had the highest value. True protein represents 80-90% of the total protein in leaves and 50-70% in stems. 18 amino acids, including all essential ones, were detected in both leaves and stems, with a diverse composition. Glutamic and aspartic acids were the most abundant in both types of samples. **Conclusions:** This preliminary study shows the upcycling of vine thieves as promising due to the variety of amino acids present and protein contents. More studies should be done to confirm this biomass as an alternative source of proteins improving the sustainability of the vineyard sector.

Keywords: *Vitis vinifera* L.; amino acids profile; protein content; non-protein nitrogen; sustainability.

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24349 | Toxicological and neuroactive characterization of the psychoactive plant *Tagetes lucida*

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Background & Aim: *Tagetes lucida* Cav., vernacularly known as **'**Mexican tarragon', is commonly used by Mesoamerican tribes for ritualistic, dietary and medicinal purposes [1], being primarily valued for its effects on the central nervous and gastrointestinal systems [2]. As the plant gained popularity in recent years for recreational use and its safety profile remains speculative, this study aimed to elucidate the bioactive compounds present in the aerial parts and to assess its neuropharmacological and toxicological properties. **Methods:** Chemical characterization was performed by HPLC-DAD. Cytotoxic potential was evaluated through the MTT, LDH, NR, glutamate, and MMP assays (up to 1000 mg mL^{-1}) in SH-SY5Y cells. Neuromodulatory effects were analyzed via AchE and MAO-A inhibition [3]. **Results:** The aqueous extract of the aerial parts contained 46 secondary metabolites, predominantly phenolic acid derivates, followed by coumarins and flavonoids, with scoparone and herniarin identified as the main constituents. Cytotoxicity was noted with mitochondrial depolarization from 250 µg mL^{-1} onwards, while impairment of metabolic competence and loss of membrane integrity with LDH leakage was observed at the highest tested concentration. Paradoxically, neuroprotection was observed from 62.5 to 250 µg mL^{-1} regarding glutamate excitotoxicity. Additionally, neuromodulation was achieved through AChE and MAO-A inhibition, starting at 31.25 and 15.625 µg mL^{-1}, respectively. **Conclusions:** Despite increasing the currently limited data, our results highlight the ambiguous effects promoted by this psychoactive plant, since neurotoxicity, indicated by loss of cell competence and integrity, and neuroprotection upon glutamate exacerbation were both found. Moreover, the remarkable inhibition of both neurotransmitter-degrading enzymes suggests

apparent pharmacological properties. Nonetheless, this work recalls further studies to unravel the underlying mechanisms adjacent to our results.

Keywords: Oneirogenic; Entheogen; Neurotoxicity; Chemical profiling; Mexican tarragon.

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24428 | Therapeutic potencial of Urocortins in Heart Failure: A Systematic Review of Clinical and Preclinical Evidence

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Background & Aim: Heart failure (HF) remains a leading and increasing cause of morbidity and mortality worldwide despite effective therapies (1). Its diverse etiology is driven by a cycle of maladaptive neurohormonal activation, impaired cardiac function, and structural remodeling (2). Consequently, urocortins (Ucn1, Ucn2, and Ucn3), members of the corticotropin-releasing factor family (CRF), have been proposed as potential therapeutic targets based on their cardiovascular effects primarily via CRF2 receptors (3). Experimental and clinical studies suggest that urocortins may induce vasodilation, improve cardiac performance, and modulate inflammatory pathways in healthy subjects and HF models (4, 5). However, evidence remains dispersed across heterogeneous studies and has not been systematically synthesized. Therefore, this systematic review aims to critically appraise and integrate clinical and preclinical evidence regarding the cardiovascular effects of urocortins in HF. **Methods:** PubMed, Scopus, Web of Science, and ClinicalTrials.gov were searched from inception to date, following PRISMA 2020 guidelines (6). Eligible studies included original clinical studies and mammalian *in vivo* models investigating the cardiovascular effects of urocortins in HF. Study screening and data extraction were performed independently by two reviewers. Risk of bias and reporting quality were assessed using RoB-2 (7), ROBINS-I (8), SYRCLE (9), and ARRIVE (10) tools. Due to study heterogeneity, findings were qualitatively synthesized. **Results:** Preliminary evidence suggests that the urocortin system, particularly urocortin 2, is associated with beneficial cardiovascular effects across clinical studies and animal models of cardiovascular disease relevant to HF. Substantial heterogeneity was observed across study design and outcome measures. **Conclusions:** Urocortins demonstrated therapeutic potential in HF, highlighting the need for further translational and clinical investigation.

Keywords: Heart failure; Urocortins; Cardiovascular effects; Systematic review

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24628 | Kidney-Heart Axis in ATTRv Amyloidosis: exploring treatment dynamics

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Background & Aim: Hereditary transthyretin amyloidosis (ATTRv) is a multisystem disease by the extracellular deposition of amyloid. Cardiomyopathy and kidney disease compromise long-term survival, unsurpassed by amyloid drugs; there is a gap of knowledge with use of non-steroidal mineralocorticoid receptor antagonists (nsMRAs) and SGLT2 inhibitors (SGLT2i) for kidney-heart axis. Aims: evaluate finerenone and SGLT2i added to standard of care in ATTRv; clarify if renal-cardiac parameters/biomarkers evolve in parallel post-therapy. **Methods:** 12-month retrospective study of fifty ATTRV30M patients; therapy finerenone 10 mg (30), SGLT2 inhibitor (13) or combination therapy (7), at baseline, 6 and 12 months. Biomarkers: albuminuria, creatinine, cystatin C, eGFR creatinine (eGFRcr) and cystatin C (eGFRcys), eGFRcys/eGFRcr ratio, NT-proBNP. Albuminuria, NT-proBNP: log-transformed. Temporal effects: linear mixed-effects models - time, treatment group, interaction. **Results:** Albuminuria decreased over 12 months (mean change: -4.4%; β_time =-0.045, 95% CI -0.113 to 0.022, p=0.030), with similar reductions across treatment groups and no time-by-treatment interaction (p=0.613). Baseline eGFRcys/eGFRcr was inversely associated with albuminuria reduction (r=-0.402, p=0.023), indicating attenuated response in patients with higher baseline ratios. Renal function: marker-dependent evolution. eGFRcys changed significantly (-5.32 mL/min/1.73m², p=0.001), eGFRcr remained stable (-2.89 mL/min/1.73m², p=0.17), highlighting differential sensitivity of filtration markers; NT-proBNP did not change over follow-up in all cohort or within treatment groups (β_time =-0.004, 95% CI −0.052 to 0.044, p=0.862). **Conclusions:** Finerenone and SGLT2i were associated with early renal improvement without parallel cardiac biomarker changes, suggesting asynchronous adaptation. Targeting kidney fibrosis with nsMRAs/SGLT2i is

a dynamic to adopt. Baseline eGFRcys/eGFRcr was associated with renal response magnitude, refining monitoring.

Keywords: ATTR amyloidosis; Kidney-heart axis; biomarkers; finerenone; SGLT2 inhibitors

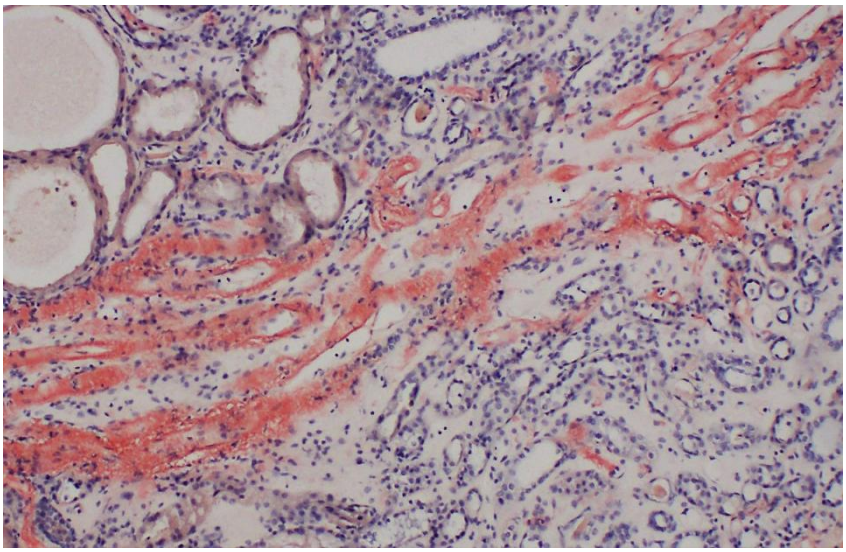

Figure 1: ATTR amyloidosis (ATTRV30M). Kidney biopsy showed medullary interstitial deposits with Congo red affinity *(original magnification x100*). The progression of fibrosis in surrounding tissues may be attenuated by non-steroidal mineralocorticoid receptor antagonists/SGLT2 inhibitors, justifying a paired kidney therapy with the amyloid modifying drugs for cardiomyopathy.

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24849 | Gestational Hyperglycemia: Effects on Maternal Behavior and Hippocampal Plasticity

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Background & Aim Gestational Diabetes (GDM) induces metabolic and inflammatory disorders that may persist beyond pregnancy and influence maternal brain plasticity. Experimental diabetes is associated with hippocampal dysfunction and altered neurotrophic signaling (1,2) which can lead to cognitive disfunction (3). Also, GDM is associated with depression and anxiety (4). However, the long-term effects of gestational hyperglycemia on maternal hippocampus remain unclear. We aimed to determine whether STZ-induced gestational hyperglycemia produces lasting behavioral and hippocampal alterations in dams. **Methods** Female Wistar rats were assigned to Control and STZ groups. After weaning, dams were tested in elevated plus maze (EPM), open field (OF), novel object recognition (NOR) and Morris water maze (MWM). Hippocampal mRNA levels of CDK5, RELN, BDNF and GAD1 were quantified by RT-qPCR. As an exploratory analysis, we evaluated adult neurogenesis by estimating the numerical density of DCX-immunoreactive neurons in the dentate gyrus. ANOVA was used for group comparisons and Hedges' g values were calculated. **Results:** STZ dams showed a slightly increased anxiety-like behavior in EPM, altered exploratory activity in OF and cognitive changes in NOR, although none of these were statistically significant. MVM showed comparable learning rates in control and STZ dams without differences between groups. Exploratory molecular analyses revealed patterns consistent with the modulation of BDNF, GAD1, RELN, and CDK5 expression. However, differences in BDNF expression were the only statistically significant ones. Furthermore, the DCX+ neuronal density was altered in the STZ dams. **Conclusions**: Although our study did not show statistically significant changes in the behavior of STZ dams compared to the control group or in the genetic expression of GAD1, RELN, and CDK5, differences were found in BDNF expression and DCX+ neuronal density, suggesting that gestational hyperglycemia may induce changes in hippocampal neurogenesis.

Keywords: Gestational hyperglycemia, Diabetes mellitus, Streptozocin, Behavior, Neurogenesis

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24924 | In vivo functional validation of stress and hyperglycemia – responsive enhancers in the hypothalamus

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Background & Aim: Metabolic disorders such as diabetes, characterized by chronic hyperglycemia, are associated with increased vulnerability to stress and mood disturbances. Stress and metabolic dysfunction interact bidirectionally, as stress alters glucose homeostasis while hyperglycemia may increase stress sensitivity. The hypothalamus integrates these responses likely through transcriptional changes. This project will investigate how chronic stress and hyperglycemia affect behavior and whether these effects are associated with alterations in stress- and hyperglycemia-responsive transcriptional enhancers, using the zebrafish hypothalamus as a model. **Methods:** Adult zebrafish were assigned to 4 experimental groups: control, overfeeding (OF), chronic stress (S), and overfeeding plus stress (OF+S). Behavioral responses were assessed using the Novel Tank test (NTT). ATAC-seq and RNA-seq were integrated to identify putative enhancer sequences in the hypothalamus. To validate enhancer sequences *in vivo*, we are performing enhancer reporter assays using Tol2 transgenesis. **Results:** NTT analysis revealed statistically significant differences between the control group and the experimental conditions in a behavioral parameter (maximum speed), with additional trends in other behavioral metrics. These differences were observed in males but not in females, suggesting a sex-specific behavioral response. Integrative RNA-seq and ATAC-seq analysis identified 38 genes with correlated decreased expression and chromatin accessibility in hypothalamus under stress. Lepa (leptin a) emerged as a candidate for functional enhancer validation, due to its sensitivity to metabolic and stress signals. **Conclusions:** This work contributes to understanding the molecular mechanisms in the brain that link metabolic dysfunction and stress responses. Functional validation of hypothalamic regulatory elements may reveal pathways linking hyperglycemia and stress.

Keywords: Zebrafish, Chronic Stress, Hypothalamus; Chromatin accessibility.

24932 | Hippocampal Neuroplasticity and Cognitive Impairment in a Metabolic Risk-Related Rat Model of Heart Failure with Preserved Ejection Fraction

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Background & Aim: Heart failure (HF) is increasingly associated with cognitive impairment and hippocampal vulnerability (1–2). Although nearly 50% of the cases of HF fall under the category of HFpEF, research on hippocampal neuroplasticity and cognitive impairment on this category remains limited (3). HFpEF is usually a result of chronic cardiometabolic diseases (4), and the ZSF1-Obese rat is a validated preclinical model of HFpEF that reproduces cardiometabolic dysfunction observed in patients (5). We aimed to determine whether the frequent clinical presentation of HFpEF with metabolic syndrome is associated with behavioral deficits and impaired neurogenesis. **Methods:** Adult male ZSF1 Obese (n=7) and Lean (n=6) rats were evaluated in novel object recognition (NOR) and object location recognition (OLR), paradigms sensitive to hippocampal integrity (6). Anxiety-like and locomotor behavior were assessed using the open field (OF) and elevated plus maze (EPM) (7). Doublecortin (DCX)-immunoreactive cells were quantified in the dentate gyrus (8). Hippocampal mRNA levels of BDNF, CDK5, GAD1 and RELn were measured by qPCR. Group comparisons were performed using Welch's t-test or Wilcoxon tests when appropriate, and Hedges' g was calculated. **Results:** Obese rats showed deficits in recognition and spatial memory, with reduced discrimination index in NOR and OLR. Obese animals also exhibited reduced locomotor activity, while anxiety-related indices showed no consistent differences. No differences were found in DCX+ cell density. Exploratory outcomes showed possible downregulation of GAD1 mRNA, whereas BDNF, RELn, and CDK5 mRNA

expression were unchanged. **Conclusions:** The combination of HFpEF and metabolic syndrome in ZSF1-Obese rats is associated with hippocampal-dependent memory impairment and dysregulation of plasticity-related genes. Although DCX+ cell numbers were unchanged, indicating preserved proliferation of newborn granule neurons, this does not imply preserved neuronal survival or functional integration.

Keywords: HFpEF, Metabolic Syndrome, Hippocampal formation, Neurogenesis, Behavior

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24356 | Angiotensin-converting enzyme (ACE) activity in colonic tissue, colonic content, and cage fecal pellets in the TNBS-induced experimental rat model of colitis

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Background & Aim: We previously showed that angiotensin-converting enzyme (ACE) activity is higher in the healthy rat intestinal content than in the corresponding intestinal tissue[1]. Rats with 2,4,6-Trinitrobenzenesulfonic acid (TNBS)-induced colitis show alterations in ACE activity along the gut[2]. We aimed to assess ACE activity association between colonic content and corresponding tissue, and in fecal pellets throughout the protocol. **Methods:** Protocols were approved by institutional and national authorities (ORBEA-ICBAS-UP and DGAV). Male Wistar Han rats (10–12 weeks) were used. Colitis was induced by a single rectal instillation of a 21% ethanolic solution of TNBS (15 mg/rat; 250 µL). Non-manipulated rats were used as controls (CTRL). Samples of serum, distal colonic tissue, and corresponding content were collected at day (d) 7; fecal pellets at d0, d3, d5, and d7. ACE activity was quantified by a fluorometric enzymatic assay (substrate: Z-Phe-His-Leu). Statistical analysis was performed by unpaired t-test, Mann-Whitney test, paired t-test, or Wilcoxon test, as appropriate. **Results:** Serum ACE activity was similar between TNBS and CTRL (nmol/min/mL: 237.4[201.9-284.5] vs 253.1[192.5-272.9]; $p>0.05$). In the distal colonic tissue, ACE activity was similar between TNBS and CTRL (nmol/min/mg of total proteins: 4.58[2.84-7.84] vs 4.50[2.57-6.57]; $p>0.05$), while in the distal colonic content, ACE activity was lower in TNBS compared to CTRL (nmol/min/mg of total proteins: 7.44[3.25-17.66] vs 76.53[43.13-129.30]; $p<0.05$). Paired analysis showed higher ACE

activity in the distal colon content than in the corresponding tissue in CTRL (p<0.01) but not in TNBS rats (p>0.05). In TNBS rats, fecal ACE activity decreased on d3 and d5 compared to d0 (nmol/min/mg of total proteins: 29.32[8.12-66.03]; 5.77[1.64-27.22]; 8.88[5.10-19.41]; p<0.05). **Conclusions:** The luminal environment may be a sensitive indicator of intestinal inflammation, and fecal ACE activity exhibits potential as a non-invasive biomarker.

Keywords: Angiotensin-converting enzyme (ACE), colitis, intestinal content, feces, 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced model

Acknowledgments:

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24360 | Aquatic-Based Exercise in Adults with Type 2 Diabetes: Clinical and Metabolic Effects and the Emerging Role of Wearable Technologies

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Background & Aim: Type 2 Diabetes Mellitus (T2DM) is a major global health challenge. Although physical activity is central to metabolic control, adherence to land-based exercise is often limited by obesity and musculoskeletal constraints. Aquatic exercise may offer a low-impact alternative. Wearable technologies allow real-time monitoring of exercise load and glycaemic responses, yet their role in aquatic settings remains unclear. This study aimed to synthesize the clinical and metabolic effects of aquatic exercise, with or without wearable integration, in adults with T2DM. **Methods:** A systematic review was conducted according to PRISMA guidelines and registered in PROSPERO (CRD420251169973). MEDLINE, Cochrane CENTRAL, Web of Science, Scopus, and IEEE Xplore were searched from inception to December 2025. Randomized and non-randomized longitudinal studies evaluating structured aquatic exercise in adults (≥ 35 years) with T2DM were included. Two reviewers independently performed study selection and risk-of-bias assessment (RoB 2; RoBANS 2), with disagreements being resolved by a third reviewer. The primary outcome is glycaemic control assessed by HbA1c. Evidence certainty was evaluated using GRADE. **Results:** Of 2,781 records identified, 11 studies were included in the review. Aquatic exercise was associated with improved glycaemic control, particularly reductions in HbA1c, though effect estimates varied across studies. Secondary outcomes suggested benefits in body composition and physical fitness. Considerable clinical and methodological heterogeneity was observed across intervention characteristics and outcome reporting. Wearable device integration was inconsistently investigated. **Conclusion:** Aquatic exercise may improve metabolic outcomes in adults with T2DM. However, variability in study design and reporting limits the certainty of current evidence. High-quality randomized trials with standardized protocols are needed to better define its clinical impact and the role of wearable technologies.

Keywords: Type 2 Diabetes Mellitus; Aquatic Exercise; Land-based Exercise; Glycaemic Control; Wearable Devices

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Table 1: Systematic Review Population, Intervention, Comparator, and Outcome (PICO) Criteria

PICO criterion	Details
Population of interest	Adults (≥ 35 years) diagnosed with Type 2 Diabetes Mellitus
Intervention of interest	Aquatic exercise interventions
Comparator or control group	Land-based exercise or sedentary/standard care
Outcomes of interest	Primary: HbA1c Secondary: Fasting blood glucose, body composition, physical conditioning, quality of life, exercise adherence, and wearable device data.

24380 | Estimating the environmental and health impacts of small reductions in food intake

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Background & Aim: Food production accounts for 70% of global freshwater use and one-third of greenhouse gas emissions (GHGE). Concurrently, Europe faces rising rates of obesity and overweight, suggesting an ingestion above the physiological requirements, placing unwarranted pressure on food, environmental and health systems. This highlights the urgent need to promote healthier and more sustainable eating habits. This study aimed to assess the environmental and health impacts of reductions in total food intake while maintaining the individual's eating pattern. **Methods:** We used data from the National Food and Physical Activity Survey, including participants aged between 18-64 years and with two dietary interviews (n=3102). To estimate the impact of intake reduction, we analyzed three scenarios of energy restriction (never below the physiological requirements): major (20%), moderate (10%) and minor (5%). For each scenario two versions were tested, one considering the reduction in all food items proportionally and one where the intake of fiber and dairy remained without reduction. Environmental impact of each scenario was assessed through the reduction of GHGE and land use (LU) based on estimates of the SHARP-ID[1]. Regarding health, the impact was estimated by the reduction in the incidence of colorectal cancer (CRC) and of type 2 diabetes (DM2). **Results:** The environmental benefits will be 0.19 kg. eqCO2 and 0.37 for GHGE and 0.23 m^2 and 0.47 for LU, respectively for moderate and major scenarios. Concerning health, the major scenario is the one with major benefits, better if the ingestion of dairy and fiber remained constant: 34 vs 103 prevented cases for CRC and 316 vs 620 for DM2 cases. **Conclusions:** Reducing food intake, even without changing the usual food pattern, brings meaningful benefits to both the environment and health. However, betting on a message to reduce global food intake while maintaining the consumption of fruit, vegetables and dairy is the one that provides the greatest gains.

Keywords: Energy reduction; Environmental impact; Cancer incidence; Diabetes incidence

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24384 | 4F-Bu$_3$GalNAc as a Novel Cancer Drug that Inhibits the Biosynthesis of SLeX

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Background & Aim: Glycosylation is frequently dysregulated in cancer, leading to the expression of tumor-associated glycans, such as Sialyl Lewis X (SLeX), that is involved in tumor progression, invasion, and metastasis[1]. Fluorinated monosaccharide analogs have emerged as promising inhibitors of glycosylation[2], and recently we identified the fluorinated N-acetylgalactosamine, 4F-GalNAc, as an inhibitor of SLeX biosynthesis. In this work, we aimed to test novel 4F-GalNAc formulas by introducing additional chemical groups as inhibitors of SLeX biosynthesis. **Methods:** The inhibitory capacity of the newly synthesized compounds was assessed by a dose-response assay, and the biological impact of the most promising compound was evaluated both *in vitro* and *in vivo*. To determine transcriptional changes, we performed RNA-seq analysis and further validated these changes using mass spectrometry and enzymatic assay of polypeptide N-acetylgalactosaminyltransferase 2 (ppGalNAcT2) activity. **Results:** Addition of butyryl groups to 4F-GalNAc (4F-Bu$_3$GalNAc) yielded a stronger inhibitory effect on SLeX biosynthesis than 4F-GalNAc. Additionally, using lower concentrations, 4F-Bu$_3$GalNAc markedly reduced cell migration, invasion, and E-Selectin binding. Western blot analysis revealed alterations in receptor tyrosine kinase (RTK) activation and a reduction in RTK molecular weight, pointing to impaired glycosylation after treatment. RNAseq analysis showed alterations in N-glycan biosynthesis and sugar donor metabolic pathways. Validation analysis showed that 4F-GalNAc analogs depleted the biosynthesis of natural sugar donors and impaired ppGalNAcT2 activity. *In vivo* assay showed that 4F-Bu$_3$GalNAc treatment inhibited tumor growth more than 4F-GalNAc. **Conclusions:** 4F-Bu$_3$GalNAc acts as a sugar metabolic inhibitor, impairing cellular glycosylation. This leads to reduced cell-malignant traits, modulation of signaling pathways, and suppression of tumor growth *in vivo,* highlighting its potential as a novel anticancer drug.

Keywords: Fluorinated GalNAc analogs; Cancer; Glycosylation; Sialyl Lewis X (SLeX).

Acknowledgments: This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through the research project PTDC/MED-QUI/2335/2021. AFC was supported by an FCT PhD grant (UI/BD/150829/2021), and CG by the stimulus of Scientific Employment (2022. 04678.CEECIND).

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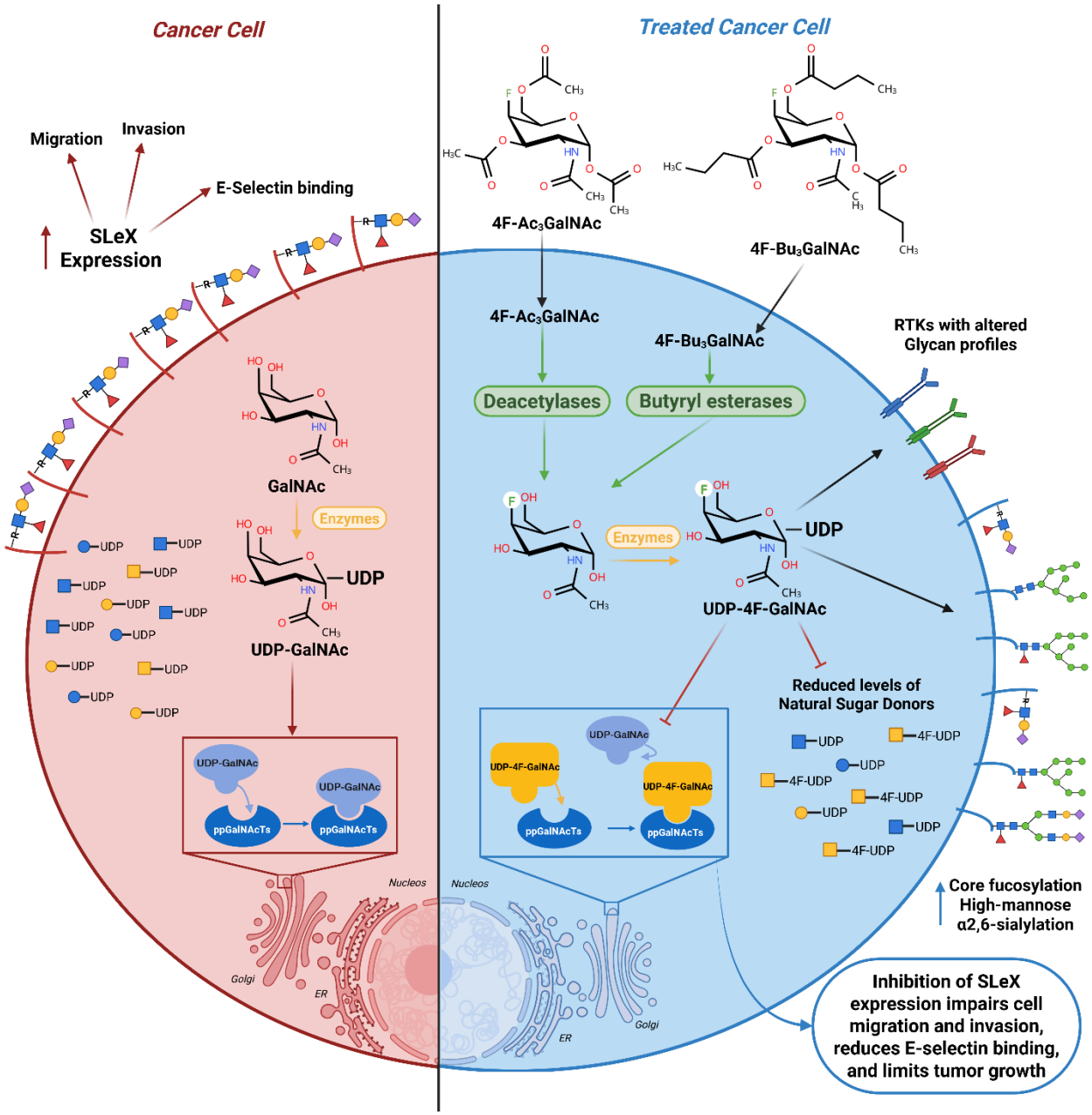

Figure 1: Schematic representation of the effect of fluorinated GalNAc analogs on cancer cell glycosylation and cancer cell behavior.

24385 | Unveiling the mechanisms underlying Foxn1 expression in Thymic Epithelial Cells

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Background & Aim: The thymus is crucial for the generation of functional T cells via specialized niches provided by thymic epithelial cells (TECs) [1]. The transcription factor Forkhead box protein N1 (Foxn1) is the master regulator of TEC differentiation and its gradual decline with age causes thymic atrophy [2]. Thus, understanding the mechanisms that regulate Foxn1 expression holds significant therapeutic potential [3,4]. Nonetheless, the molecular network that controls its induction in TECs remains unknown. Identifying these regulators *in vivo* is challenging because, once expressed, Foxn1 triggers a complex transcriptional program that masks its upstream inducing signals [5]. To circumvent this, we developed a novel thymus-specific Foxn1 deficient mice ($AR3^{KO}$). **Methods:** We intercrossed $AR3^{KO}$ mice with $Foxn1^{GFP}$ reporter [6] ($AR3^{KO}$:$Foxn1^{GFP}$) and lineage-tracing animals ($AR3^{KO}$:$Foxn1.Cre^{EYFP}$), which allow us to isolate primitive Foxn1-committed TECs (pro-TECs) that are blocked in their differentiation due to the Foxn1 mutation but can respond to Foxn1-inducing signals. The dynamics of $AR3^{KO}$ pro-TECs were monitored by flow cytometry across embryonic, postnatal, and adult stages. **Results:** Our results confirmed that $AR3^{KO}$ mice are athymic and devoid of T cells, while maintaining a normal hair fur coat. In $AR3^{KO}$:$Foxn1^{GFP}$ mice, GFP-expressing pro-TECs are detected in the embryonic and postnatal life, despite their progressive decline over time. However, fate-mapping analysis revealed that pro-TECs persisted in adult $AR3^{KO}$:$Foxn1.Cre^{EYFP}$ mice, indicating that they do not disappear with age but are rather unable to maintain the activation of the transgenic $Foxn1^{GFP}$ promoter. **Conclusions:** Our data suggest that Foxn1-deficient TEC^{GFP^+} cells represent primitive TEC progenitors that have received Foxn1-inducing signals but remain developmentally arrested. Hence, these cells will provide a unique framework to capture and decipher the regulatory circuits controlling Foxn1 expression in TECs.

Keywords: Thymus; Organogenesis; Thymic Epithelial Cells; Foxn1; T cell development

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24611 | Impact of intestinal biomarkers in cardiac reverse remodelling induced by pregnancy

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Background & Aim: Pregnancy induces cardiovascular (CV) adaptations that normaly regress postpartum through cardiac reverse remodeling (CRR). Inflammatory and gut-derived factors may influence this process. This study aimed to evaluated associations between faecal calprotectin (FC), serum zonulin (Zn), *Akkermansia muciniphila* and CRR. **Methods:** This study assessed women in the 3rd trimester (3T), (30–35 weeks) and 6 months postpartum (6MP) from the PERIMYR/OralBioBorn cohort study Clinical, dietary (MEDAS questionnaire), echocardiographic, anthropometric and biological samples (stool and blood) were collected. **Results:** Thirty-four pregnant women (median age 33 years) with at least one cardiovascular (CV) risk factor were included. From 3T to 6MP, FC, Zn, and *A. muciniphila* significantly decreased (all $p<0.05$). FC correlated with left ventricular (LV) mass (LVM) in the 3T ($\rho=0.405$; $p=0.024$) and with E/e' at 6MP ($\rho=0.424$; $p=0.017$). Zn correlated with diastolic blood pressure ($\rho=0.468$; $p=0.021$), LVM ($\rho=0.517$; $p=0.010$), and LV volume ($\rho=0.484$; $p=0.019$). *A. muciniphila* showed no significant CV associations. Greater adherence to the Mediterranean diet at 6MP was correlated with better LV diastolic function ($\rho=–0.510$; $p=0.015$) and lower LV relative wall thickness ($\rho=–0.560$; $p=0.007$). Higher red meat intake was associated with increased Zn levels ($p=0.004$) and PP2 weight ($\rho=0.637$, $p=0.020$). Olive oil as the main fat source was associated with lower LVM, whereas higher saturated fat intake at 6MP was linked to adverse echocardiographic findings. **Conclusions:** Maternal inflammation and intestinal permeability biomarkers are associated with CRR and may indicate early CV risk. Mediterranean diet adherence supports intestinal and CV health in the perinatal period, reinforcing integrated maternal CV prevention strategies.

Keywords

Pregnancy, cardiac reverse remodelling, intestinal biomarkers, Mediterranean diet

Acknowledgments:

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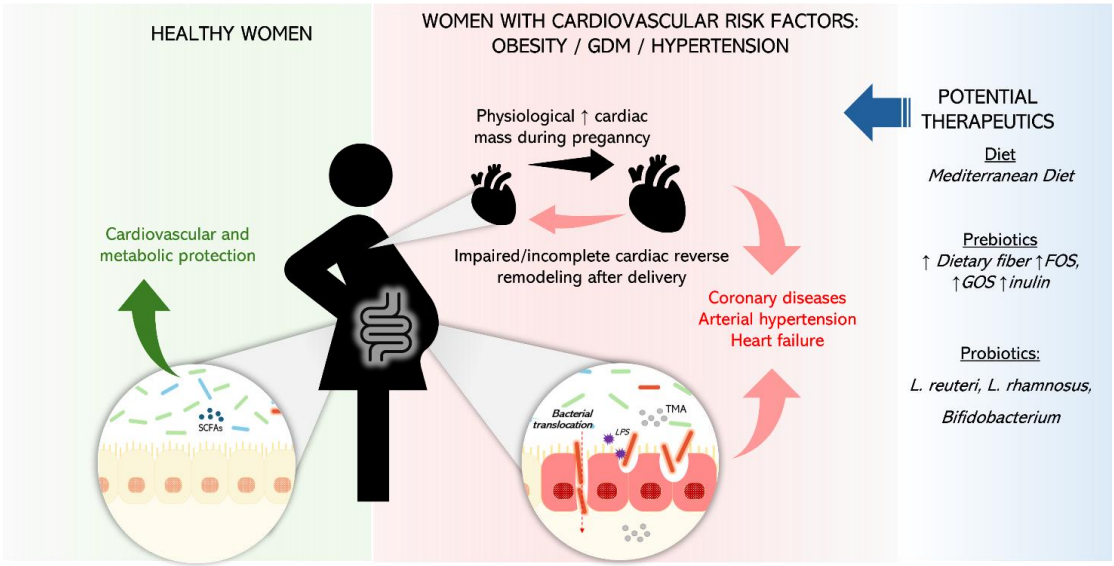

Figure 1: Schematic overview of the proposed gut–heart axis during pregnancy. Maternal gut dysbiosis and increased gut permeability may promote systemic inflammation by translocating bacterial components, such as lipopolysaccharide, while reduced production of short-chain fatty acids impairs anti-inflammatory signalling. These processes, influenced by dietary patterns (e.g., Mediterranean diet) and probiotic intake, may modulate cardiovascular adaptation and cardiac remodelling in pregnancy, especially in the presence of comorbidities. Image originally submitted as part of a review article to the journal *Acta Portuguesa de Nutrição* (submitted, decision pending).

24940 | First-Trimester Free β-hCG as a Marker for Pathogenic Copy Number Variants: a retrospective cohort study

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Background & Aim: First-trimester combined screening (cFTS) estimates the risk of fetal aneuploidies using maternal age, nuchal translucency (NT), nasal bone and other ultrasound markers, and maternal serum markers including pregnancy-associated plasma protein A (PAPP-A) and free beta-human chorionic gonadotropin (free β-hCG). Chromosomal microarray analysis (CMA) identifies submicroscopic copy number variants (CNVs). Although increased NT has been associated with pathogenic CNVs, the relationship between abnormal β-hCG and CNVs remains unclear. We aimed to evaluate the association between abnormal first-trimester β-hCG levels and pathogenic CNVs detected by CMA. **Methods:** We performed a retrospective cohort study including pregnancies that underwent cFTS between 11+0 and 13+6 weeks of gestation and CMA, covering the period between 2013 and 2025 at a tertiary center in Porto, Portugal. This study was approved by the Ethics Committee of the hospital. Cases with multiple gestations or common aneuploidies were excluded. Free β-hCG values below the 5th percentile and above the 95th percentile were compared with the middle 90% of the distribution. Logistic regression was used to estimate odds ratios (OR) adjusted for maternal age and NT. Statistical significance was set at $p < 0.05$. **Results:** Among 24134 cFTS results, 367 singleton pregnancies with CMA were included. Pathogenic CNVs were identified in 41 cases (11.2%). The prevalence of pathogenic CNVs was highest among pregnancies with β-hCG values in the lowest 5% of the distribution (27.8%). Low β-hCG values were significantly associated with pathogenic CNVs after adjustment for maternal age and NT (adjusted OR 3.465, 95% CI 1.156–10.389, p=0.027). **Conclusions:** Extremely low first-trimester β-hCG levels are associated with an increased risk of pathogenic CNVs and may represent a useful marker for identifying pregnancies at higher risk of genomic abnormalities, thereby supporting consideration of further genetic evaluation in selected cases.

Keywords: First-trimester screening; β-hCG; Copy number variants; Chromosomal microarray; Prenatal diagnosis

24444 | Telomere Regulation in PitNETs: ATRX/DAXX Expression and pTERT Mutation Status

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Background & Aim: Pituitary neuroendocrine tumours (PitNETs) are common intracranial neoplasms, and a subset exhibits clinically aggressive behaviour that is not fully explained by known genetic and epigenetic alterations. Telomere maintenance, through telomerase reactivation or alternative lengthening of telomeres (ALT), may contribute to PitNET progression, through loss of ATRX/DAXX expression. **Methods:** A total of 209 formalin-fixed, paraffin-embedded PitNETs from 181 patients treated between 1988 and 2021 were analysed. pTERT genotyping, quantitative real-time PCR for relative telomere length, and immunohistochemistry for ATRX and DAXX were performed. ATRX expression was quantified in 92 cases using QuPath and classified as negative, mosaic or positive. **Results:** No pTERT promoter mutations were identified. Among the 92 tumours with quantified ATRX expression, 9 (9.8%) were negative, 32 (34.8%) mosaic and 51 (55.4%) positive, with ATRX loss occurring predominantly in acromegaly. Statistically significant differences in relative telomere length were observed between ATRX groups, particularly between positive and mosaic cases, with ATRX-positive tumours displaying relatively longer telomeres. Similar findings have been reported in the context of Alpha-thalassaemia/ mental retardation syndrome. No significant associations were found between relative telomere length and tumour invasion or recurrence. However, patients in remission exhibited lower relative telomere expression compared with those with active disease. **Conclusions:** ATRX expression abnormalities are relatively frequent in PitNETs and associate with differences in telomere length and disease status, supporting a role

for telomere regulation in PitNET biology. Further studies are needed to clarify how ATRX/DAXX loss drives telomere dysregulation and aggressiveness and to refine molecular classification and targeted treatment strategies.

Keywords: PitNETs, telomere length, ATRX/DAXX, pTERT

Acknowledgments:

Bolsa SPEDM - Hra Pharma Iberia Na área de. Síndrome de Cushing

Bolsa da Fundação para a Ciência e a Tecnologia com a referência 2021.05640.BD

24391 | Cyclodextrins and Approved Drugs for Niemann–Pick Type C Disease in ARPE-19 Cells expressing *NPC1* missense variants

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Background & Aim: Niemann-Pick type C disease (NPC), mostly caused by pathogenic *NPC1* variants, is characterized by accumulation of unesterified cholesterol and other lipids in Late endosomes/Lysosomes (Vanier, 2010). Two of the most frequent pathogenic variants associated with NPC are the missense p.Pro1007Ala, associated with adult and less severe form of the disease, and p.Ala1035Val, when combined with the SNP p.Ile858Val, has been associated with earlier onset and more severe clinical manifestations. Despite availability of approved therapies in some countries - Arimoclomol, Miglustat and N-acetyl-L-leucine (NALL) - therapeutic responses can vary depending on the specific *NPC1* variant. Accordingly, this study compares next-generation cyclodextrins with the classical 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) in transduced *NPC1*$^{-/-}$ ARPE-19 cells expressing either wild-type or pathogenic variants (p.Pro1007Ala; p.Ala1035Val+p.Ile858Val). Despite NPC disease is predominantly a neurological and visceral disorder, this immortalized retinal pigment epithelial line constitutes a validated cellular model for NPC drug screening (unpublished data; David H et al.). **Methods:** *NPC1*$^{-/-}$ ARPE-19 transduced cell lines were treated for 48h with HP-β-CD (50 or 100 µM), next-generation CD (100 µM), Miglustat (50 µM), or NALL (5mM). Cell lysates were harvested for Western blot analysis (n=2) with data quantified and normalized. Additionally, lysosomal distribution and clustering was evaluated through LAMP1 immunofluorescence. **Results:** HP-β-CD and next-generation cyclodextrin ameliorated lysosomal clustering in *NPC1*$^{-/-}$ ARPE-19 and transduced lines, but in *NPC1*$^{-/-}$ ARPE-19 + p.Pro1007Ala the results are more

pronounced. NALL showed comparatively less evident effects. **Conclusions:** These findings support the therapeutic potential of next-generation cyclodextrins, in the treatment of NPC disease associated with these specific missense *NPC1* variants, highlighting a strong genotype-dependent response in these cells.

Keywords: Niemann-Pick type C; cholesterol; lysosomes; cyclodextrin.

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24646 | Exploring biological effects of fisetin derivatives in human keratinocytes

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Background & Aim: The skin is constantly exposed to environmental stressors, such as pollutants and radiation, which induce oxidative stress and inflammatory processes, leading to cutaneous damage. The identification of new compounds with beneficial biological activities, particularly antioxidant and anti-inflammatory effects, is therefore of great relevance. Fisetin, a flavonol present in several plant species relevant to the human diet, has demonstrated promising biological properties. This study aimed to evaluate the biological potential of semi-synthetic fisetin derivatives in human keratinocytes (Figure 1) [1]. **Methods**: Eight samples were evaluated for their antioxidant potential using the DPPH radical scavenging assay. In parallel, cell viability was assessed in the human keratinocyte HaCaT cell line, and the impact on intracellular reactive oxygen species (ROS) generation was determined. Preliminary enzyme inhibition assays, including tyrosinase and collagenase, were also performed. **Results:** Four compounds showed significant antioxidant activity, with an inhibition range between 20% and 80% across different concentrations. Cell viability assays indicated low cytotoxicity of the derivatives compared to fisetin. Additionally, some compounds exhibited strong tyrosinase inhibitory activity, with inhibition values ranging from 80% to 90%. **Conclusions:** The studied fisetin derivatives demonstrated relevant antioxidant and enzymatic inhibitory properties, with low cytotoxicity, highlighting their potential for application in cosmetic formulations aimed at protecting against environmental aggressions and cutaneous oxidative stress.

Keywords: Oxidative stress; tyrosinase; fisetin; and keratinocytes

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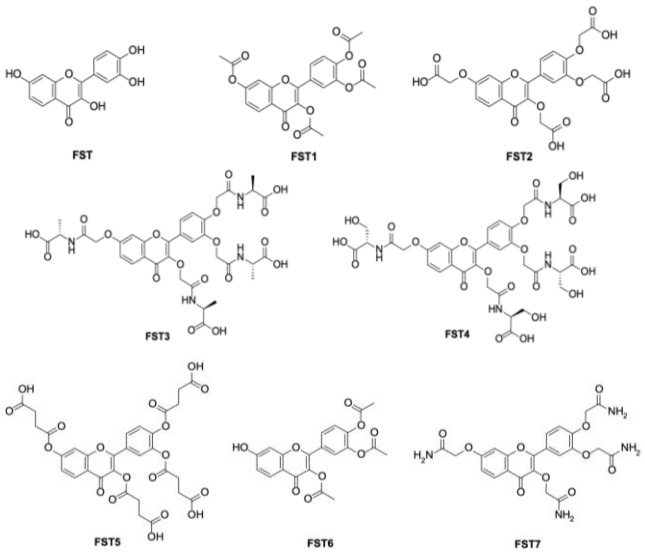

Figure 1 Chemical structures of fisetin (FST) and its semi-synthetic derivatives evaluated in this study.

24605 | Left Atrial Appendage Occlusion during Cardiac Surgery:

a retrospective single-center study

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Background & Aim: Left atrial appendage occlusion (LAAO) has emerged as an effective, non-pharmacological alternative for stroke prevention in patients with atrial fibrillation (AF) [1]. **Methods:** A retrospective single-center cohort study comprising all patients who underwent valvular and/or coronary heart surgery (CABG) with concomitant LAAO, between January 2015 and December 2017, was conducted at a tertiary university hospital. In this analysis, 142 patients were included. Data regarding demographics, surgical details and post-operative outcomes were collected from paper files and Electronic Health Records. Primary outcomes were procedural safety and post-operative complications, including stroke, re-operation rate and mortality during a ten-year follow-up. **Results:** The cohort included 142 patients (mean age of 66 ± 11 years; mean EuroSCORE II of 5.5 ± 7.4); 39% were women. Pre-operatively, 87% had AF and the median left atrial volume was 89 mL (min 30, max 219). Valvular surgery involved the mitral valve in 86.3% of cases, predominantly combined with tricuspid (53.4%) or aortic and tricuspid (21.9%) procedures. Mitral disease was frequently rheumatic (46.6%) and regurgitant (50%), while aortic pathology was degenerative (36.3%) with regurgitation (44.5%); tricuspid disease was mostly regurgitant (85.6%). Regarding surgery procedures, beyond LAAO, 84% underwent valvular surgery and 15% valvular surgery + CABG. In mitral surgery, 31.5% underwent valve repair, 28.8% received biological and 24.7% mechanical prosthesis. For LAAO, the most used technique was continuous suture (68.6%), followed by stapler (31.4%). Post-operative stroke occurred in three participants (2.1%), two of whom underwent valvular replacement and one concomitant CABG and valvular replacement. Re-operation was required in 14 cases (9.9%), six immediate due to bleeding or incision issues and eight late mitral replacements during the follow-up period. Hospital mortality rate was 3.5%. There were no significant differences in post-operative stroke ($p>0.999$) or hospital mortality ($p>0.999$) between the stapler and suture groups. **Conclusions:** This single-center experience showed that

concomitant LAAO during cardiac surgery is a safe procedure for thromboembolism risk reduction in AF patients.

Keywords: Cardiac Surgical Procedures; Atrial Fibrillation; Left Atrial Appendage closure; Stroke.

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24449 | Engineering an Organ-on-a-Chip System to Probe an Early Diffuse Gastric Cancer Profile

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Hereditary Diffuse Gastric Cancer (HDGC) is associated with mutations in the CDH1 gene. Its multifocal and often asymptomatic nature complicates early diagnosis. Prophylactic total gastrectomy (PTG) is the most effective preventive measure, but it has significant long-term consequences. Predicting which HDGC patients will develop invasive disease is currently difficult. We propose identifying biomarkers linked to early-onset disease to improve risk stratification and clinical decision-making. To address the challenge of studying cancer initiation, we aim to develop a biomimetic tool that replicates the gastric environment, facilitating the discovery of predictive secreted biomarkers. Our team developed a biomimetic tool, the DGC-on-a-chip, that replicates the stomach's physiological environment using soft lithography and xurography to mimic the gastric mucosa layers. We employed the MKN74 cell line for cohesive gastric epithelium and KATOIII cell line as a model of DGC. The chip's performance was evaluated under long-term cell culture. For biomarker profiling, we will analyze secretomes from patient-derived gastric organoids and KATOIII cultures using liquid chromatography tandem mass spectrometry and smallRNAseq. Our results indicate that the DGC-on-chip is resistant to delamination. We successfully established a co-culture of KATOIII and MKN74 cells, with KATOIII foci identifiable for up to 16 days using FarRed tracer labeling. Proteomic analysis revealed a pool of 2,232 relevant proteins, of which 1,708 were uniquely secreted by KATOIII, and 660

proteins were shared between both groups. This pool of 2232 molecules will be matched with the proteome of patient-derived DGC organoids. Our results highlight the potential of organ-on-a-chip technology to address a clinically relevant limitation and its application in biomarker discovery. Further validation of our model in a clinical setting could improve risk stratification and lead to more informed clinical decision-making.

Keywords: Gastric Cancer, microfluidics, Organ-on-chip, Biomarkers, Secretome.

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24686 | Polymer-modified lipid nanoparticles for targeted colon delivery

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Background & Aim: Oral drug administration offers advantages for cancer therapy due to its convenience and patient compliance.[1] Colorectal cancer is the third most prevalent and second most deadly type of cancer, making the development of novel treatments crucial.[2] Nanoparticle-based drug delivery is very promising, as these systems can be designed with specific properties for site-targeted delivery.[3] This study aimed to explore surface modifications of lipid nanoparticles to enhance their properties and enable colon-targeted drug delivery through an enzyme-responsive strategy designed to react with pectinase in the colonic microflora. **Methods:** Solid lipid nanoparticles and nanostructured lipid carriers were produced and coated with octadecylamine (ODA) and pectin, followed by encapsulation of doxorubicin (DOX). Particle size, distribution and zeta potential were characterized by dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). Surface chemistry was examined by FTIR, and incorporation efficiency was determined by UV-vis spectroscopy. Stability was evaluated under simulated gastrointestinal conditions (FaSSGF, FaSSIF and simulated colonic fluid). Enzyme-triggered drug release was assessed in simulated colonic fluid containing pectinase. Cytocompatibility was tested using L929 fibroblastic cell line (ISO 10993-5). **Results**: DLS and NTA confirmed that pectin-coated formulations had suitable particle size, polydispersity and zeta potential for oral use, and validated a successful coating. FTIR also verified effective surface modifications with ODA and pectin. DOX encapsulation reached 100% efficiency. Formulations remained stable in all simulated fluids. In a microflora-triggered release assay, DOX was released. Cytocompatibility tests showed safety at lipid concentrations between 0.0625-0.25 mg/mL. **Conclusions:** The lipid nano-formulations produced proved to be stable, functional and suitable for the desired application, showing potential as carriers for colon-targeted drug delivery.

Keywords: Colon Targeting; Oral Drug Administration; Lipid Nanoparticles; Gastrointestinal Stability.

Acknowledgments:

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24873 | Design and development of potential CBD nanodelivery systems for the treatment of anxiety

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Background & Aim: Anxiety disorders are highly prevalent mental health conditions, characterized by excessive fear, worry, and nervousness. Conventional treatments present limitations, including adverse side effects and the risk of dependence. Cannabidiol (CBD) has shown promising anxiolytic effects with few side effects; however, its clinical use is limited by poor solubility, low stability, and reduced bioavailability. This study aims to develop novel CBD nanodelivery systems for the treatment of anxiety disorders with optimized physicochemical properties and safety profiles. **Methods:** Two CBD-loaded nanodelivery systems, nanomicelles and self-nanoemulsifying drug delivery systems – SNEDDS, were developed to improve the physicochemical properties of CBD. The developed formulations were characterized by particle size, polydispersity index, and zeta potential using dynamic light scattering (DLS). These parameters were evaluated to assess particle distribution, dispersion quality, and colloidal stability. To confirm the particle morphology and size distribution obtained by DLS measurements, TEM analysis was performed. **Results:** The developed systems showed nanoscale particle sizes with relatively narrow size distributions, indicating a homogeneous formulation. The PDI values suggested acceptable dispersion uniformity, and the measured zeta potential indicated potential colloidal stability of the systems. TEM imaging confirmed the nanoscale dimensions and morphology of the particles, supporting the size measurements obtained through DLS analysis. **Conclusions:** The delivery systems developed demonstrated suitable particle size and dispersion properties. The results suggest that these systems may help overcome some limitations of CBD. However, further *in vivo* studies and clinical trials are needed to confirm its long-term efficacy and safety.

Keywords: Cannabidiol; anxiety disorders; nanodelivery systems; bioavailability; dynamic light scattering.

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24933 | Unraveling Layer-Specific Response to Radiotherapy in Triple Negative Breast Cancer Spheroids

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Background & Aim: Reliable *in vitro* models to study radiotherapy (RT) response in triple negative breast cancer (TNBC) remain elusive. Traditional 2D cultures fail to reproduce the complex architecture and heterogeneity of solid tumors, which in contrast, 3D tumor spheroids better mimic key features of the tumor microenvironment, as cell-cell interactions and gradients of oxygen and nutrients. These features contribute to the formation of distinct zones within the spheroid closely resembling the *in vivo* tumor structure and affecting RT efficacy.[1] Herein, we present a 3D TNBC spheroid platform that enables spatial characterization of tumor cell responses to a clinically relevant RT scheme across distinct spheroid regions. **Methods:** MDA-MB-231 spheroids were established and submitted to 5 fractions of 5.2Gy or left without irradiation (CTR). After they were analyzed by transcriptomics, proteomics, transmission electron microscopy (TEM) and immunohistochemistry (IHC). Imaging data was after quantified through ImageJ and QuPath and statistical analyzed through GraphPad. (Figure 1) **Results:** RT decreased spheroids viability, while increasing cell size and the number of organelles, such as mitochondria, lipid droplets, rough endoplasmic reticulum and autophagic vesicles in surviving

cells. The peripheral layer of the spheroids exhibited the most pronounced organelle alterations and the highest levels of DNA damage, indicated by increased γ-H2AX and elevated CAIX expression, following irradiation. Proteomic profiling revealed upregulated pathways linked to cell cycle progression and organelle fission, supporting G2/M cell cycle arrest. **Conclusions:** We hypothesize that, to balance the DNA damage and oxidative stress induced by RT, cancer cells undergo cell cycle arrest, leading to increased cell size and organelle content. Overall, this TNBC 3D model represents a promising platform for radiobiology research and for high-throughput screening of combinatory treatments to overcome radioresistance

Keywords: Breast Cancer; Radiotherapy; Radioresistance; Spheroids

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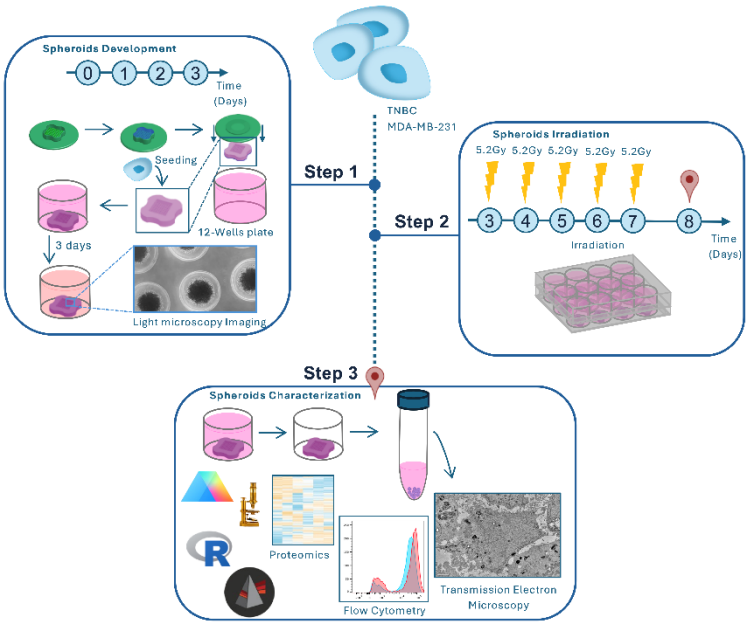
Figure 1:



MDA-MB-231 Spheroids formation and characterization to a clinically relevant scheme in triple negative breast cancer.

24948 | Hand Function, Tethered Swimming Force, and Performance in Masters Swimmers

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Background & Aim: Swimming is a well-studied cyclic sport in which performance is influenced by several factors [1]. However, evidence remains limited in Masters swimmers. It is known that manual dexterity [2] and grip strength [3] decline with age, which may contribute to reduced swimming performance; however, no studies have examined age-related changes in tethered swimming force. Therefore, this study examined the associations between manual dexterity, handgrip strength, tethered swimming force, and swimming performance in Masters swimmers. **Methods:** Twenty-four male (74.23±9.28 kg; 44.54±12.42 years) and sixteen female Masters swimmers (61.90±10.38 kg; 41.19±14.51 years) voluntarily participated in this study. Participants performed a maximal handgrip strength test using a calibrated dynamometer (*Takei model 5101 TKK*), a motor coordination test using the Minnesota Manual Dexterity Test (*USA*), and a 30-s all-out tethered front crawl test using an isokinetic resistance device (*Swim-Spektro, Italy*). Swimming performance was assessed through a maximal 200-m front crawl swim, a common event swam by those swimmers. **Results:** Compared with females, male swimmers showed higher maximal and mean propulsive force, handgrip strength, and faster 200-m performance: 354.20±62.66 vs 232.59±33.74 N (p=0.051); 147.04±32.62 vs 101.68±16.47 N (p=0.03) and 42.24±6.51 vs 29.72±3.71 kg (p=0.01), respectively. Mean propulsive force was negatively correlated with 200-m performance time in women (r=−0.631, p=0.009) and men (r=−0.656, p<0.01). Manual dexterity was positively correlated with 200-m time in women (r=0.558, p=0.025) and men (r=0.473, p=0.020). **Conclusions:** Mean propulsive force was associated with swimming performance in Masters swimmers. Handgrip strength did not correlate with performance, whereas manual dexterity showed a moderate association with performance in both groups, suggesting that manual coordination may contribute to Masters swimmers' performance.

Keywords: Swimming; manual dexterity; handgrip strength; tethered swimming force; Performance

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24690 | Bio-Inspired Lipid Nanoplatforms for Brain Tumor Targeting

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Background & Aim: Glioblastoma Multiforme (GBM) is the most common malignant brain tumor in adults [1]. However, the current standard of care fails to effectively improve disease prognosis as patients have a median survival time below 15 months [2]. Nanotechnology represents a potential alternative to conventional GBM treatments by overcoming challenges imposed by brain drug delivery, specifically the Blood-Brain Barrier (BBB) [3]. Particularly, cubosomes are non-lamellar lipid-based nanoparticles characterized by their high biocompatibility, stability, and complex cubic structures [4]. Therefore, the aim of this work was to develop biomimetic cubosomes that selectively target GBM. **Methods:** The cubosomes were produced by thin-film hydration and optimized by a Box-Benken Design. The nanoparticles were characterized and their stability and drug release profile were assessed. *In vitro* studies included the evaluation of hemo- and cytocompatibility, anti-cancer efficacy, BBB permeability, and cell uptake. **Results:** The cubosomes exhibited adequate sizes for IV administration and brain drug delivery with drug encapsulation exceeding 70%. Stability was maintained for at least 4 weeks upon storage at 4 °C and for 24 h in protein-rich biological media. A slower release of drug from cubosomes was achieved in Simulated Body Fluid pH 7.4 when compared with a free drug solution. The nanoparticles were non-hemolytic and demonstrated high cytocompatibility in both healthy and cancer cells. Drug-loaded cubosomes exhibited dose-dependent anti-cancer activity in GBM cells, which was higher than free drug. Additionally, cubosomes exhibited good permeation capacity across an *in vitro* BBB model and higher cellular internalization in GBM than in non-cancer cells. **Conclusions:** Overall, these findings suggest that the developed biomimetic cubosomes are promising nanocarriers with tumor homing capabilities for targeted GBM therapy.

Keywords: cubosomes, glioblastoma multiforme, brain drug delivery, mitoxantrone

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Acknowledgments:

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24404 | Tumor organoid-based 3D models to uncover predictive biomarkers for breast cancer progression

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Background & Aim: Breast cancer (BC) is the most common cancer in women and, despite continuous research, its relatively high mortality due to metastasis remains a major concern [1,2]. Prognosis varies by subtype: Luminal A and B, HER2-overexpression and Triple-Negative (TN, including Basal-like and Claudin-low), the latter two having the worst outcomes [2]. Identifying predictive biomarkers of metastasis is crucial to improve prognosis. While traditional 2D models fail to replicate tumor microenvironment complexity, 3D tumor-derived organoids mimic better the *in vivo* conditions. Thus, we aimed to develop and optimize a protocol to generate BC organoids from xenografted tumors representing different subtypes and then to adapt it for patient samples. **Methods:** We conducted a pre-clinical study by injecting BC cell lines representing each subtype (MCF-7/Az and T47D for Luminal A; BT474 for Luminal B; SKBR3 and MDA-IBC-3 for HER2+; BT20 and MDA-MB-468 for Basal-like; MDA-MB-231 and SUM149PT for Claudin-low) into immunosuppressed female N: NIH(S)II-nu/nu mice. After tumor growth, tumors were excised and processed to establish tumor-derived organoids. A non-tumorigenic human cell line (MCF10A) and mouse breast samples were used as healthy controls. After optimization, the protocol was applied to primary breast tumor samples from IPO-Porto to generate patient-derived organoids (PDOs). **Results:** Both cell line-derived and patient-derived organoids showed success rates consistent with literature (~70% and ~55%, respectively) [3]. Furthermore, organoids retained the histological profile of their original tumors, supporting their value for studying metastatic predictive biomarkers. While further analyses are ongoing, this study lays groundwork for future research. **Conclusions:** Organoid cultures provide a more biologically relevant model for tumor biology. Their successful establishment opens

opportunities to study BC progression, potentially improving early detection, treatment and patient prognosis.

Keywords: Breast Cancer, Metastasis, Organoid, Biomarkers

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24416 | Metabolic Reprogramming and Adipose Tissue Contribution to Peritoneal Metastasis in Diffuse Gastric Adenocarcinoma

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Background & Aim: Gastric cancer comprises biologically distinct subtypes with different metastatic patterns. Diffuse gastric cancer (DGC) preferentially spreads within the peritoneal cavity, whereas intestinal gastric cancer (IGC) metastasizes mainly via the bloodstream. Peritoneal adipose tissue may help DGC dissemination through tumor-induced adipocyte browning, enhancing lipolysis and fatty acid release to fuel tumor growth. However, the relationship between subtype-specific metabolic traits and metastatic tropism remains unclear. The aim is to metabolically distinguish DGC from IGC and determine whether tumor–adipocyte crosstalk promotes adipocyte browning, lipid metabolic reprogramming and immune microenvironment modulation, supporting peritoneal metastasis. **Methods:** Mitochondrial organization, metabolic activity, redox homeostasis, antioxidant defenses and cellular architecture were compared in DGC and IGC cell lines using TMRM, resazurin, ROS quantification, H_2O_2 challenge, Western blot and E-cadherin assessment. Co-cultures with adipose tissue from bariatric and gastric cancer patients enabled multi-omics analyses focusing on lipid droplets (LD). **Results:** Preliminary data show similar relative mitochondrial networks across models but higher mitochondrial polarization in diffuse-type cells (MKN45, KATOIII). IGC cells (MKN74) displayed stronger cell–cell adhesion and structural organization. Although basal ROS was markedly higher in MKN74 (~11×), all lines responded similarly to H_2O_2, indicating distinct antioxidant profiles: MKN45 showed higher catalase and SOD1, whereas KATOIII showed elevated thioredoxin. KATOIII had higher baseline LD content (~4× vs. MKN74; ~10× vs. MKN45).

Co-culture increased LD accumulation in all lines, particularly with cancer-derived and omental adipose tissue. **Conclusions:** Gastric cancer cell lines exhibit subtype-specific mitochondrial, redox, antioxidant and structural profiles that may underlie differences in biological behavior and metastatic dissemination.

Keywords: Diffuse gastric cancer; Tumor–adipocyte crosstalk; Peritoneal metastasis; Lipid metabolic reprogramming.

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HUMANITIES AND SOCIAL SCIENCES

IJUP

24521 | All for One and One for All: A Methodology for Linked Open Data Curation

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Background & Aim: In information science, the post-custodian paradigm's schism with its predecessor, over the push and pull between access and curation, often has its casualties in the materials kept within the collections of libraries, archives, and museums, which are now slowly becoming available beyond institutional systems, including across cultural and national boundaries. As increasing volumes of data and information from these institutions are published online, issues of structuring and scale grow more acute. Within one of the largest collaborative knowledge bases' Linked Open Data initiatives, which enables metadata interoperability (Barbosa, 2025), the need for robust approaches to managing large-scale uploading and editing is evident. Accordingly, since operational obstacles may arise when using such software, this methodology seeks to provide future collaborators with an overview of its use, functionalities, and challenges, in line with prior work (Faria & Correia, 2022). **Methods:** The PDF of a joint manuscript was processed using PDF Arranger to organize its contents and Pattypan to upload the items along with their triple-structured metadata. **Results:** Previously confined to the Évora Public Library, the item is now available under open access, indexed by international search engines, and accessible to any internet user through the infrastructure established with Wikimedia, ensuring its information is findable, accessible, interoperable, and reusable. **Conclusions:** By functioning as both a backup repository and a linking mechanism for diverse sources, this method was able to mitigate the access limitations caused by outdated axioms and proprietary systems in order to enable use by artificial intelligence systems and global and interconnected databases (Correia, 2023). Additionally, it allows educational institutions to reaffirm their commitment to actively disseminate open knowledge in the current digital era, promoting universal access to informational heritage and culture.

Keywords: Linked Open Data; Metadata; Open Knowledge; Wikimedia; Web Platform for Social Sciences

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24689 | From Library to Linked Open Data: Metadata Structuring of Old Manuscripts in Wikidata

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Background & Aim: Historically, custodian institutions have kept their data confidential and restricted access to their systems, limiting the visibility and reuse of information held by public libraries, which, as public heritage, are essential for scientific research and international communities. The central challenge lies in achieving interoperability between different platforms used in the these institutions. The proposed strategy involves transitioning to a Linked Open Data model, using Wikidata, a project of the Wikimedia ecosystem, as the central platform. Wikidata is one of the largest collaborative knowledge bases, allowing the storage, access and reuse of metadata (Barbosa, 2025). As such, it ensures information integration into linked data networks, enabling their use by artificial intelligence systems and global and interconnected databases (Correia, 2023). **Methods:** The methodology centered on the bulk upload of metadata to Wikidata. This required mapping fields and properties between the source format and the data model of this platform, ensuring semantic alignment between both systems. Data cleaning, mapping, and ingestion were performed using OpenRefine, enabling a structured and efficient workflow throughout the process. **Results:** As a result, the metadata from the "Compendio dos Misterios da Fee" and 50 more manuscripts, previously confined to Évora Public Library, is now available in Open Access and indexed by global search engines, accessible to any internet user through the Wikimedia infrastructure. **Conclusions:** This project presents a replicable workflow for custodian institutions seeking to broaden access to their collections, within FAIR and Linked Open Data principles. Wikidata serves not only as a backup repository, but as a tool for linking different sources, which allows Public Libraries to reaffirm themselves as active agents in the dissemination of free knowledge in the current digital scenario, promoting universal access to culture and ancestral information.

Keywords: Linked Open Data; Metadata; Open Knowledge; Old Manuscripts; Wikimedia

Acknowledgments: We would like to express our sincere gratitude to Dr. Zélia Parreira, Director of Biblioteca Pública de Évora, for graciously welcoming us into her library, and to André Barbosa, Director of Wikimedia Portugal, for introducing us to this project opportunity.

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24320 | Who gets to sing America? Nationalism, Race, and the Securitization of Reproduction under the Trump Administration

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Mainstream scholarship on nationalism often treats the nation as a gender-neutral and natural organizing principle of the international system. Feminist literature, however, challenges this gender blindness, arguing that women occupy a central role in the biological, cultural, political, and symbolic reproduction of the nation. Using Donald Trump's presidency (2017-2021) as a case study, this article employs a critical discourse analysis of legislation related to reproductive rights to identify the specific mechanisms through which women's sexuality becomes securitized and thereby associated with the continuity of the nation. The findings reveal that reproductive governance in the US increasingly operates as a security practice that polices citizenship, racializes belonging, and legitimizes intensified intervention in women's bodily autonomy. These results expand current feminist scholarship by foregrounding race as a central component of contemporary reproductive nationalism. This becomes especially relevant in the study of American nationalism, given that Trump built his presidency on populist anti-immigration stances. Therefore, understanding nationalist reproduction through the intersection of gender and race problematizes Trump's nationalist aspirations and, in the words of Langston Hughes, a Black American activist and poet, his notion of who best belongs to and represents America.

Keywords: women, race, nationalism, securitization, reproductive rights

24370 | Overuse Of The Term "Terrorism"? A Study Of Yemen's Red Sea Blockade

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On 19 October 2023, Yemen's Ansar Allah (commonly known as the Houthis), who have controlled the capital Sanaa since 2014, announced a naval blockade in the Red Sea targeting vessels en route to or connected with the State of Israel. The group framed this action as a response to what it characterized as genocide in Gaza, invoking Article 1 of the Convention on the Prevention and Punishment of the Crime of Genocide, to which Yemen is a signatory, and claiming a duty to prevent and punish genocide. These operations were widely categorized as "terrorist" acts, prompting condemnation by the UN Security Council and a campaign of air strikes by a coalition of states. This article adopts a postcolonial perspective to examine whether the Red Sea blockade should be understood within the conventional "terrorism" framework, or whether it represents a phenomenon that challenges and exceeds that dominant classification. While the concept of terrorism is frequently employed in mainstream political and media discourse, its analytical adequacy remains contested. This case provides an opportunity to assess the limits and potential overextension of the term, particularly in contexts where non-state actors employ force while invoking international legal norms. It also raises broader questions about the presumed monopoly of legitimate force attributed to nation-states in the international system. Methodologically, the article combines a literature review on the Red Sea blockade with a discourse analysis of official statements by Ansar Allah and its leader, Abdul Malik al-Houthi, following 7 October 2023. The analysis focuses on the group's stated objectives, its legal and political justifications, and the blockade's positioning within international law and debates on terrorism. The research ultimately seeks to determine whether the prevailing "terrorist" framing adequately captures the nature of the Red Sea blockade, or whether it obscures important political, legal, and normative dimensions of the case.

Keywords: Red Sea; Yemen; Terrorism

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24718 | Parity for Whom? An Intersectional Analysis of Gender Quotas in Brazil, France, Portugal, and Sweden

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Background & Aim: Gender quota literature has predominantly emphasized descriptive representation, often overlooking how quota design interacts with race and class. Emerging debates suggest that parity mechanisms, when applied in isolation, may reproduce structural inequalities. This study examines whether the effectiveness of gender quota laws depends on the incorporation of an intersectional lens. It analyzes Brazil, France, Portugal, and Sweden to assess how legal frameworks and political culture shape intersectional substantive representation, challenging universalist assumptions in global governance. **Methods:** This research adopts a comparative mixed-methods Most Different Systems Design, combining qualitative analysis of primary legal sources with quantitative data from the Inter-Parliamentary Union and the International Institute for Democracy and Electoral Assistance. The analytical framework evaluates quota mechanisms, sanction regimes, scope of application, and the inclusion of race and class variables. **Results:** The findings suggest that universalist models in Portugal and France primarily benefit established elites. In Portugal, thresholds function as minimalist requirements rather than as a foundation for equality. In contrast, Brazil demonstrates intersectional innovation, particularly in campaign financing for Black women, while Sweden shows that consolidated party norms can foster substantive change without rigid legal imposition. The results highlight how different institutional trajectories require distinct intersectional strategies. **Conclusions:** The efficacy of quotas relies on institutionalized intersectional mechanisms beyond formal enforcement. Political culture and institutional context remain decisive for substantive inclusion. An intersectional analysis uncovers inclusion dynamics overlooked by gender-only approaches, demonstrating that gender, race, and class are central to political transformation across diverse institutional models.

Keywords: Gender Parity; Intersectionality; Political Representation; Electoral Quotas.

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24775 | Violence beyond the battlefield: Militarized occupation and planetary justice in Western Sahara

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Background & Aim: Western Sahara remains one of the longest unresolved cases of colonial occupation and territorial militarization in the contemporary international system. Since Spain's withdrawal in 1975 and the subsequent Moroccan occupation, violence has been sustained not only through direct repression but through permanent military infrastructures that reconfigure space and conditions of life in the desert. This paper analyzes the separation wall (Berm) and surrounding minefields as central devices through which colonial violence is enacted and reproduced. **Methods:** This article adopts a qualitative, interdisciplinary approach grounded in critical conflict analysis, political ecology, and decolonial scholarship. It examines the Berm and minefields as infrastructures of occupation, focusing on effects on territory, mobility, and ecological relations. The analysis draws on academic literature, NGO and humanitarian reports, United Nations documentation, environmental studies, medical reports, and qualitative interviews to trace their multiscalar and multispecies consequences. **Results:** The analysis demonstrates that the Berm and minefields operate far beyond their original military purpose. They fragment the territory, restrict human and non-human mobility, disrupt hydrological dynamics in a fragile desert ecosystem, and undermine Saharawi pastoral and nomadic practices. These infrastructures operate across local, regional, and systemic scales, generating prolonged violence that affects livelihoods, ecological balance, and intergenerational knowledge transmission. **Conclusions:** The article argues that the Berm and minefields reveal how colonial occupation materializes as a durable, multitemporal form of violence inscribed into the landscape. Approaching the Western Sahara conflict through these infrastructures allows it to be understood not merely as a political or legal dispute, but as an ongoing colonial process producing enduring harms across bodies, ecosystems, and temporalities.

Keywords: Western Sahara; Enviromental Justice; Multiespecie Violence; Landmines; Colonial Ocupation

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24846 | Entangled Vitality: Reconceptualising Sino-Chilean Lithium Diplomacy through a Multispecies Lens

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Background and Aim: Recognising the increasing relevance of lithium extraction for the global transition to renewable energy sources, and given that the Salar de Atacama in Chile hosts the world's largest brine-based lithium mining operations, this article draws on a Multispecies Justice framework to explore the relationship between Lithium extraction in Chile and the country's diplomatic relations with China. It examines the impacts of these dynamics on local multispecies communities. Thus, this article presents a situated, multispecies account of commercial diplomacy, reframing it as embedded in more-than-human communities, where human and other-than-human lives are jointly shaped by neo-extractivist arrangements. **Methods:** The study is based on a comprehensive analysis of the literature on neoextractivism in Latin America, the socio-ecological impacts of the lithium supply chain, Chinese foreign policy, official declarations and reports issued by international organisations, as well as relevant public declarations. **Results:** The findings reveal that the "green" Sino-Chilean diplomatic rhetoric prioritises industrial decarbonisation while obscuring severe multispecies costs. Existing studies show that lithium mining in this area exerts unsustainable pressure on aquifers, leading to water depletion and salinisation, impacting more-than-human communities, disrupting habitats and risking the lives of several species, triggering migratory imbalances and further intensifying tensions between the government, companies, and more-than-human communities. **Conclusions:** While increasing attention has been paid to the impacts of lithium extraction, the literature has largely treated commercial diplomacy and socio-ecological impacts as separate domains. This article reconceptualises diplomacy by foregrounding the entanglements intrinsic to the new geological epoch and the planetary violence inherent in neo-extractivist approaches, highlighting the embeddedness of diplomatic practices' within the Earth system.

Keywords: Chile, China, Lithium, Interspecies Diplomacy, Multispecies Justice.

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24918 | "Inside Out" Diplomacy: How emotions affect the Israeli-Palestinian Conflict"

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Background & Aim: In the context of the Israel-Palestine conflict, this article draws on recent approaches to emotions in International Relations to explore how emotional expressions in political communication shape perceptions of the conflict among external audiences, often contributing to the securitization of certain actors involved. Traditionally, the role of emotions in international politics has been marginalized by rationalist approaches portraying political behavior as primarily driven by reason and strategic calculation. However, emotional scholarship in IR challenges this view, emphasizing that emotions are not merely irrational deviations but socially and politically meaningful phenomena shaping political practices. **Methods:** Building on the framework of emotional diplomacy, the analysis examines how emotional cues, such as anger, fear or compassion, are communicated through images, videos, speeches and mediated appearances of political actors. The study employs qualitative discourse and visual analysis of political communication related to the Israel–Palestine conflict, examining how emotional narratives are constructed and circulated. It compares the positioning of three European countries (Germany, Spain and the United Kingdom) to assess how these emotional framings shape external responses to the conflict. **Results:** Findings suggest that emotional representations play a significant role in structuring how the conflict is interpreted beyond its immediate geopolitical context. In this case study, emotions appear instrumentalized by political actors to legitimize policy choices and courses of action, contributing to narratives that justify violence against Palestinians. **Conclusions:** By highlighting the communicative and representational dimensions of emotion, this study contributes to scholarship on emotions in IR and shows how emotional expressions function as political resources in highly mediatized conflicts, shaping international legitimacy and diplomatic responses.

Keywords: Israel-Palestine conflict; Emotions; Diplomacy; Media

24927 | A Seat at the Table: Portugal's Identity Construction in its 2027-28 UNSC Campaign

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Background & Aim: This article analyses how Portugal actively projects an identity through its campaign for a non-permanent seat at the United Nations Security Council (UNSC) for the 2027-2028 term. While the existing literature on campaigns for non-permanent seats on the Security Council tends to address states' ambitions and strategic motivations, this article seeks to explore Portugal's campaign as a site of international identity construction. **Methods:** The article draws on a relational constructivist approach to identity, linking it with small states literature, while addressing the limited attention given to Portugal's current UNSC candidacy. Analytically, it employs the notion of "acts of identification" designed by Bernd Bucher and Ursula Jasper, characterized by its challenge of traditional, static notions of state identity. Hence, Portugal's identity claims are analyzed across three dimensions: narratives of the self, relations towards others, and policy choices. Methodologically, the study uses discourse analysis of official campaign materials, including speeches, statements, and official government communications. **Results:** We conclude that Portugal, throughout its campaign, has presented itself as a bridge builder, and an indispensable mediator in the international system, projecting an identity centered on multilateralism, cooperation and credibility. **Conclusions:** Thus, the article suggests that UNSC candidacies should be understood not only as electoral initiatives, but also as exercises in constructivist identity building. It also demonstrates how a small state uses multilateral discourse to acquire recognition in the global arena.

Keywords: Portugal, United Nations Security Council, Identity, Constructivism, Campaign

24972 | Strategic Communication and Coercive Diplomacy: A Postcolonial Analysis of Trump 2.0's Impact on Mexico and Colombia

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Background & Aim: The second Trump Administration has brought a volatile shift in U.S. foreign policy toward Latin America, through transactional diplomacy and coercive measures. This study analyzes the strategic discourse of the Trump 2.0 administration in the political landscapes of Mexico and Colombia, examining how these countries navigate U.S. pressure. It aims to understand the emergence of a "neo-colonial" logic of fear and its role in reshaping regional political dynamics. **Methods**: We use Critical Discourse Analysis and Corpus Linguistics. Using AntConc software, we analyze a specialized corpus of official statements from the Trump 2.0 administration. The methodology combines the study of strategic intent and crisis communication with an analysis of diplomatic language in both the U.S. and the subject states. This helps us decode how specific linguistic choices act as tools of coercive public diplomacy in relations with Mexico and Colombia, whose case studies are examined through Congressional Research Service reports and regional policy changes regarding migration, trade, security, and drug trafficking. **Results**: Findings indicate a pattern of "diplomacy by threat." Mexico's President, Sheinbaum, has navigated high volatility, responding to tariff threats with concessions such as pausing oil shipments to Cuba. In Colombia, the security partnership has strained under President Petro's rhetoric and Trump's retaliatory measures, including aid suspension and visa restrictions. The results demonstrate that U.S. discourse effectively coerces targeted states into aligning with Washington's unilateral priorities. **Conclusions**: The Trump 2.0 administration's discourse imposes a logic of submission that destabilizes the political and social fabric of Mexico and Colombia. This "neo-colonial" approach cultivates a state of fear, prioritizing U.S. demands over domestic goals. This shift weakens regional autonomy and stability, signaling a regressive trend in Inter-American relations.

Keywords: Trump; Latin America; Political Discourse; Mexico; Colombia

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24312 | The Stained-Glass Windows of the Side Chapels Of the Church of Our Lady of the Conception, Porto

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Background & Aim: Recognizing the need to study the importance of stained glass in a sacred space, this research aims to explore the stained glass windows of the side chapels of the Church of Our Lady of the Conception, Porto. The historical and social context, the construction process, architectural and urban planning issues of this church were approached by Virgílio Seisdedos (1997) and Domingas Vasconcelos (2004). Regarding the stained glass windows, the only known study is the theological reflection and brief visual description by Carlos Azevedo (2007). Up until now, our study is the only one that explores a holistic reading. In relation to the art of stained glass, we highlight the reflection of Denise Michelotti (2011) and the detailed research of Fernando Quintas (2014). **Methods:** Our research has a basis on authors that study the Church of Our Lady of Conception, Porto and the stained glass theme. We also performed various field analysis on the stained glass windows alongside their formal analysis and a review of documentary materials held at the parish church. **Results:** The literature review and the analysis of the stained glass windows allowed us to explore material, technical, formal, functional, symbolic, iconographic, craft, and social contexts. **Conclusions:** The research reveals that the art of stained glass is more than decorative, it is profoundly functional, symbolic, and evocative, capable of protecting spaces, shaping atmospheres, communicating symbolic values, and producing sensory and seductive effects that invite the faithful to establish a close connection with the Divine. This analysis also reveals that the study of the stained glass is essential for a holistic and diachronic reading of a sacred space, as we can see on the Marian representation of the studied stained glass windows, which have a harmonious dialogue with the spirit that lives in this church dedicated to the Immaculate Conception.

Keywords: 20th Century; Church of Our Lady of the Conception, Porto; Stained Glass; Marian Representation; Mauméjean

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Figure 1: Vitral da Natividade de Jesus, presente na capela de Santa Teresinha do Menino Jesus da Igreja de Nossa Senhora da Conceição, Porto. 2024. Fonte: Fotografia da autora

24890 | ”They Want You”: The impact of the Vietnam War on American veterans in Oliver Stone’s *Born on the Fourth of July*

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Background & Aim: Despite Vietnam War-related films being a significant theme within Cinema Studies around the world, this work aims to analyze how the 1989 Oliver Stone film *Born on the Fourth of July* depicts trauma, disillusionment and alienation in American Vietnam veterans. **Methods:** In order to reach this objective, scenes of the film will be cross-read with real life events at the time of (and prior to) the film’s setting from a cultural lens. As a starting point, an exploration of the historical context behind the Vietnam War will be assembled, so as to better frame the film’s storyline. Secondly, a deep examination of the film’s most crucial scenes will be made, focused mainly on scenes which speak to the real-life consequences of the Vietnam War on a physical, psychological and social level. Lastly, a reflection will be made on how this particular film chooses to portray the aftermath of the political quarrel, and how it still lingers in American history in contemporary times. **Results:** The research presented in this work shows how Oliver Stone’s film delves into often-overlooked aspects of war, such as the consequences of taught nationalism and patriotism during war times, the betrayal soldiers frequently feel towards the government, and other gruesome aspects that are regularly overlooked in other films regarding the Vietnam War. **Conclusions:** In conclusion, this scrutiny delves into how cinema can help illustrate the perhaps unspeakable sides of war, along with the unveiling of the mass manipulation of the youth during periods of political tension. While a number of filmic analysis have been performed in this theme, the addition of a cultural perspective to the study of film broadens its outcomes.

Keywords: Oliver Stone; Vietnam; America; Cultural Studies; Cinema Studies

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24144 | *Nunc est cadendum*. Baudelaire and Pessoa: Aesthetics of the Fall

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Background & Aim: Manipulating Horace's verse, *Nunc est bibendum*, we take the liberty of stating *Nunc est cadendum*. Now that we have fallen definitively into the realm of self-absorption, of the discord between the subject and reality, it is important to sublimate the pain of the fall. When permanently separated from the transcendent, let us praise the immanent, let us be apologists for a metaphysics or an anti-metaphysics. In opposition to supreme values (The Good, the Beauty and the Truth), the Evil, the Ugly and the Deceitful insinuate themselves. In the dialectic between Good, the morally positive and original, and Evil, the negative and derivative, choose the latter. To the extent that *God is dead*, that the supersensible no longer influences the sensible world, that the divine (no longer) serves us, let us not serve it and prefer the immanent, the low, the palpable that serves us and that we will serve. It is the devaluation of supreme values, metaphysical nihilism, or rather, a staunch metaphysical naturalism, which clearly signifies decadence, that pervades the literary works. **Methods:** *Since all literary texts have an intertextual dimension (...) and can be related to other texts that dialogue with them and are projected onto them*, a comparative analysis is made between the chapter *Revolta* in Charles Baudelaire's *Flores do Mal*, *Poema em Linha Recta* by Álvaro de Campos, and *Fausto* by Fernando Pessoa, based on a philosophical foundation. Although all intertextuality is considered artificial and experimental, an attempt is made to establish a connection with philosophical *corpora* and to assume the literary art of these authors as a non-systematized philosophy. The writing begins with a preliminary reflection on the convergence and divergence of literary and philosophical texts. This is followed by a literary and philosophical theorization: an exploration of the Baudelaire-Nietzschean concept of the Aesthetics of the Fall, and an association of the works analyzed with this concept of literary art**.** **Results:** The joint analysis highlights the profound continuity between three distinct ways of facing the modern crisis of subjectivity and transcendence. In Pessoa, both in the metaphysical figure of Fausto and in the confessional expression of Campos, we find consciousness taken to its extreme: the man who seeks the absolute and finds Nothingness, the individual who seeks authenticity and finds only the social mask, respectively. The existentialism latent in Pessoa, although prior to the systematized

philosophical movement, manifests itself in anguish, in the fragmentation of the self. The confrontation between these works reveals, therefore, that literary and philosophical modernity is not only a crisis of meaning, but also a multiplicity of possible responses to the collapse of a metaphysical center. Human existence arises without guaranteed transcendence, and it is in this empty space that these authors erect their works, transforming helplessness into aesthetic lucidity, symbolic rebellion and tragic consciousness. **Conclusions:** these works embody an attempt to express the mystical theory of the *superman*. Indeed, if we examine these works and the evolutionary framework of European poetry in general, it is the *superman* that we find pulsating in every verse, a Nietzschean superman who is essentially a failure, unable to find the musculature and nervous system best suited to his function. The idea that man, being a rope between the animal and the divine, the incarnation of the tightrope walker in *Thus Spoke Zarathustra*, falls into the abyss of religion, in Baudelaire's case, into the abyss of Nothingness, in Ortónimo's case, and into the abyss of the Other in Campos, and must sublimate the pain of the fall. In short, the emulation of Lucifer's decline: a prince of metaphysical exile, a humiliated figure, sometimes arrogant, sometimes resigned.

Keywords: Flores do Mal, Charles Baudelaire, Fernando Pessoa, Aesthetic of the Fall, Nietzsche

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The Good: that which is of interest to everything in general	The Evil: that which is of no interest in any context
The Beautiful: the order and unity (harmony) of the entity	The Ugly: the absence of order and unity of the entity
The True: the entity that is effectively real	The False, Deceptive: illusion, illusory entity, the entity that does not fit in with reality.

Figure 1: Duality established based on *Nietzsche's words 'God is dead'* from *Heidegger's Paths in the Forest*. The foundation of the essay resides in and depends on this dichotomy.

24576 | Evaluation of the Performance of Water Erosion Models as a Function of DTM Resolution: Study in the Semi-Arid Region of Paraíba

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Background & Aim: In the upper Paraíba River basin, a combination of environmental factors contributes to the accelerated development of linear soil erosion. In soils derived from crystalline rocks, surface runoff predominates, intensifying erosive dynamics within the basin. In Brazil, several studies have focused on predicting soil loss across different regions, however, there remains a lack of investigations addressing this issue in the Brazilian semi-arid region using the Simulated Water Erosion (SIMWE) model. In this context, this study, developed as part of a research initiative at the University of Porto within the framework of international mobility, aims to assess the influence of Digital Terrain Model (DTM) spatial resolution on the hydrological response of the SIMWE model in terms of erosion and deposition. **Methods:** The Digital Surface Model (DSM) was generated from a Unmanned Aerial Vehicle (UAV)-based aerial survey conducted on July 1, 2025, resulting in a point cloud with a spatial resolution of 1 m. The point cloud was classified and filtered, including the removal of points corresponding to vegetation. Subsequently, kriging was applied for the spatial interpolation of elevation, producing DTMs with resolutions of 10 m, 5 m, and 2 m. Surface hydrological flow simulations considered an extreme rainfall event of 53.3 mm h^{-1}. Infiltration rates, determined in the field, were differentiated between depositional areas and slopes. Manning's roughness coefficients were assigned to three land use and land cover classes based on a supervised classification of the orthomosaic. Sediment transport parameters followed the recommendations of the *Water Erosion Prediction Project* (WEPP) manual. Model validation was performed by cross-referencing simulated erosion–deposition patterns with mapped erosive features, using the Area Under the Curve (AUC) metric. **Results:** The results indicate that DTM resolution significantly affects the

magnitude and spatial organization of erosion and deposition processes, controlling hydrological connectivity and the erosion–deposition balance. **Conclusions:** The 2 m spatial resolution showed the best results with 79.6% prediction AUC.

Keywords: Water Erosion; Digital Terrain Model; Semi-Arid Region.

24311 | Material culture and funeral shrouds in the early eighteenth-century *Santa Marinha de Oleiros*

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In the Catholic faith of the early modern period, death was a pivotal transition defined by the duality of salvation or eternal damnation. This theological certainty required meticulous preparation, manifesting in a complex "material culture of death" which served as a bridge to the divine, such as the treatment of the physical body (Moraes, 2021, p. 293). Centering on the funeral shroud, a pivotal interface between the deceased and the community, this study examines how these garments functioned as vessels of both spiritual identity and socioeconomic status. By analyzing the expression of material culture through these shrouds, this research identifies implicit meanings linked to Catholic dogma alongside external socioeconomic factors[3]. Drawing on 93 death records (1725–1735) from the parish of Santa Marinha de Oleiros, currently in Vila Verde, Braga, this research studies the implicit and explicit meanings embedded in burial attire. By cross-referencing primary archival data with literature (among others, Ferraz, 2016; Lopes, 2023; Rodrigues, 2015), the findings reveal that shroud selection was governed by a dual logic: a religious criterion linked to the concept of being "mature in the faith," and a pragmatic economic criterion dictated by the monetary value of the textiles. While preliminary, this study underscores the crucial role of funerary material culture in the exercise of imagining the past and understanding the mentalities of the eighteenth century, offering a nuanced lens through which to view the intersection of piety and property in the early modern period.

Keywords: funeral shrouds; material culture of death; parish records; Santa Marinha de Oleiros; sin.

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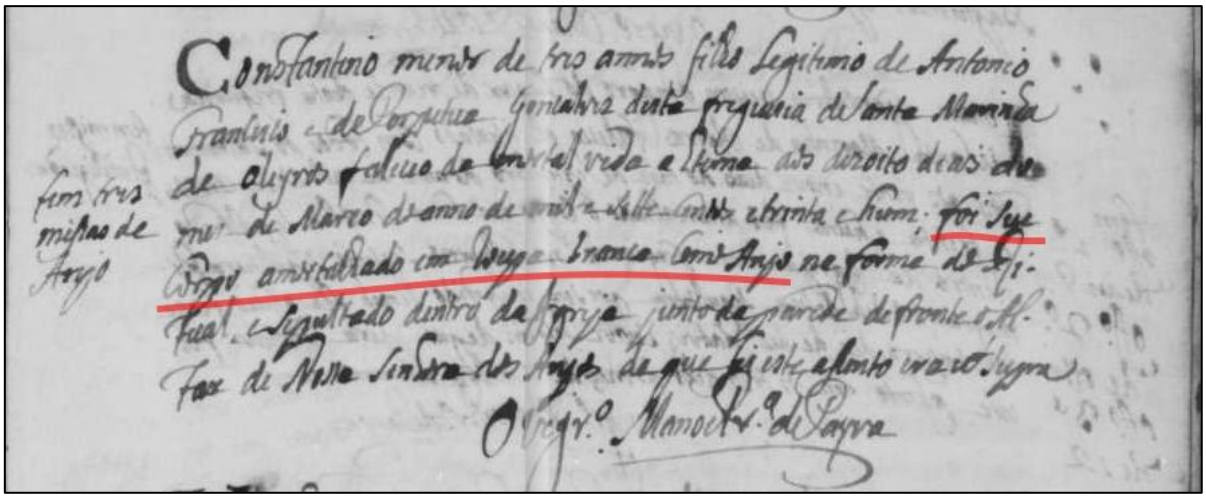
Figure 1: Death record of *Constantino* (March 18, 1731; author's emphasis on the record)[4].


Constantino menor de tres annos filho Legitimo de Antonio
Francisco de Porqueira Freguezia de Santa Marinha
de Oleiros faleceo de mal de vida e forma ass direito dias de
fum tres mezes de mor de Março de anno de mil e sette centos e trinta e hum foi sepultado com Cruz branca com Anjo na forma de Sepultura e sepultado dentro da Igreja junto da parede de fronte da Porta de Nossa Senhora dos Anjos de que fiz este assento era ut supra
O Vigr.o Manoel Rz.o de Sayra

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24317 | Between emotional communities: expressions of tolerance in Lisbon's inquisitorial records (1587-1626)

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Background & Aim: This project aims to reconceptualize the study of tolerance in Early Modern Portugal as a process rooted in individual emotion. Moving beyond the 20th-century traditional interpretation of tolerance as a pragmatic concession to avoid conflict (Lecler, 1953), this study argues that it functioned as an active social behavior. Following the idea that tolerance is not merely a political instrument but an attitude among common people (Schwartz, 2010), this study posits that individuals, shaped by their emotional communities (Rosenwein, 2002), express emotions that can cultivate a culture of acceptance toward the Other. **Methods:** The analysis employs a dual methodology: first, an etymological deconstruction of the term and its semantic field, drawing on Frevert's (2011) and Gonçalves' (2019) contributions; second, the application of theoretical models of emotional expression to the Portuguese historical context. This framework is applied in the Lisbon inquisitorial records, with a focus on cases involving transgressive practices (Betthencourt, 2000) by Old Christians, which might reveal permissive attitudes towards minorities within a society where the control of conscience held paramount importance over physical coercion. **Results:** So far, the results suggest that the primary sources used in this project, such as the Caderno do Promotor and the Livro das Confissões e Ratificações, reveal an undisputed existence of individual behaviors that may be interpreted as expressions of tolerance, although not as a dominant social norm. **Conclusions:** In doing so, this application follows a historiographical trend advanced by Kaplan (2007) and Walsham (2009), who examine tolerance as a subject of Early Modern social history, but aims to go even further by also integrating the history of emotions as a key analytical framework to historicize and reinterpret tolerance and its expressions.

Keywords: *tolerance, emotions, early modern, Portugal, Lisbon*

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24465 | The Commodification of Culture: Free Museum Admission and the Democratization of Heritage at the Museu Nacional de Soares dos Reis (Portugal) and the Museu Paranaense (Brazil)

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This investigation examines the museums free admission policies in Portugal and Brazil, focusing on the Museu Nacional de Soares dos Reis and the Museu Paranaense. Based on the paradigm of Cultural Democracy (Chauí, 2009), as well as on the Brazilian and Portuguese Federal Constitutions and international conventions on heritage. This study highlights the importance of debating how free access contributes to the democratization of cultural heritage and reinforces culture as a right rather than a commodity. The comparative analysis between the Museu Nacional de Soares dos Reis (Portugal) and the Museu Paranaense (Brazil) demonstrates that free admission policies significantly contribute to increasing visitor numbers and consolidating museums as democratic spaces for cultural access. Even with full free admission, the Museu Paranaense maintains a high visitor numbers, with a consistent return of students and tourists driven by the initiatives of its Educational Department. In this case, free admission does not devalue the exhibited works; rather, it brings the population closer to the museum space. Thus, the provision of free access should not be regarded as a public expense, but as an investment in the collective construction of cultural identity and in the democratization of knowledge. However, free admission alone is not sufficient. It must be combined with communication, education, and cultural mediation policies that foster public engagement and the development of critical thinking. While this scenario may seem unrealistic given the prevalence of market-oriented perspectives, the combination of paid and free admission days emerges as a viable solution: it ensures economic sustainability while broadening access.

Keywords: Free admission; Cultural Democracy; National Museum Soares dos Reis; Paranaense Museum

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24500 | The Empire in Postcards: Colonial Propaganda and the Imagery of the Estado Novo

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Background & Aim: The Estado Novo regime sought to promote a colonialist discourse rooted in the "civilizing mission" myth, which framed colonial rule as a paternalistic necessity to lead "non-civilized" peoples toward Western modernity. This study analyses the illustrated postcards produced by Eduardo Malta featuring participants from the 1st Portuguese Colonial Exhibition (Porto, 1934). The aim is to examine how these objects functioned as propaganda for the regime. **Methods:** Using a qualitative, iconographic methodology, the work analyses twelve postcards as primary sources. The analysis is structured around three dimensions: Imperialism, Culture, and Gender. It explores how visual elements were deployed to reproduce relations of domination and subordinate the colonized "other" within the imperial hierarchy. **Results:** The investigation reveals a carefully staged "vertical empire". Postcards of "assimilated" figures like *Régulo* Mamadú Sissé, often depicted in European or ceremonial dress, were used to demonstrate the success of Portuguese moral and educational policies. Culturally, a hierarchy was established where Asian people (e.g., Macau and Timor) were portrayed as "sophisticated" and "closer to Europe" while African people were infantilized and depicted as "primitive". Crucially, the representation of African women, such as Rosinha, involved deliberate eroticization and nudity. These images served a dual purpose: they represented the colonies as feminized spaces open to white male conquest and encouraged Portuguese migration to Africa. **Conclusions:** The study concludes that these postcards were a "first lesson in colonialism" for the Portuguese population. They successfully consolidated a cohesive colonialist national identity by normalizing their overseas presence as an intrinsic part of the nation's civilizational legacy. By disseminating these images domestically and internationally, the regime legitimized its "right to colonize" and effectively integrated the colonial imagery into the daily lives of the masses.

Keywords: Estado Novo; Colonial propaganda; Visual imagery; Civilizing Mission.

Acknowledgments:

I would like to thank the professors of the "Issues of the History of Europe" module for their feedback and guidance.

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24632 | *Expose to Charity and Mercy*: The *Casa da Roda* of Porto as an observatory of family and affective dynamics (1757-1778)

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Background & Aim: The *Casa da Roda* of Porto was responsible for receiving and caring for foundlings, in a time when child abandonment was an accepted practice. The research done so far around this institution has focused on its organization, how it provided aid, and on the volume of children in its care, however, in our work, we take a different approach and look at the *Casa da Roda* of Porto from the lense of the history of emotions. In our research, we aimed to extrapolate the meanings and importance of this institution and the children that it cared for, and, by using the *Casa da Roda* as an observatory for emotional and family dynamics, we tried to understand the relationships around these children, their families, and caregivers. **Methods:** Using the information preserved in the archive of Porto's *Roda*, from institutional documents about administrative issues, to individual records of each child, and focusing on a sample situated between 1757 and 1778, we tried to decipher emotional elements hidden in actions, objects, words, and other details that could enlighten the views surrounding this institution and the emotional experiences of those involved in it. **Results:** The reality we found points to a complex web of emotions and experiences. Even though some sources point to a familiarity with the abandonment of children, this doesn't, however, mean indifference or carelessness towards them. The data collected shows that, in different ways and save some exceptions, those involved in this reality cared about the lives of these foundlings, were concerned about their wellbeing, and developed connections and relationships with them. **Conclusions:** By shining some light into the complexity of the emotional lives around the *Casa da Roda* of Porto, this work reinforces the importance of bringing a conscious approach to the affective dimension of this topic, as well as to reconsider some incomplete and unfounded assumptions that have been previously made and replace them with a more informed and nuanced view.

Keywords: Casa da Roda; foundlings; history of emotions; Porto.

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This work was developed under the guidance of Prof. Inês Amorim and Prof. Paula Menino Homem, whose help and advice I'm very grateful for.

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24717 | From Father to Son: A Case Study of the 15th Century Public Notaries of Porto, Gonçalo Anes Barbosinho and João Barbosa.

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Background & Aim: In the second half of the 15th century, there was a growing bureaucratization of the royal apparatus, with the public notary becoming a key figure. This study analyses the documentary registry of the public notary Gonçalo Anes Barbosinho and the transfer of his office to his son João Barbosa, allowing us to understand how family relationships could affect public notaries and the impact of forgery on their careers. Historiography on medieval notaries has grown significantly in recent decades, with names such as Bernardo Sá Nogueira[5], Maria Cristina Cunha[6], and Maria Helena da Cruz Coelho[7] standing out. **Methods:** The research was conducted following the model of another case study by Ricardo Seabra[8], through consultation of primary sources mentioning the two public notaries under analysis. Information was gathered regarding the grantors and their social status, the location where the deeds were drawn up, and the nature of the legal act. **Results:** Gonçalo Anes Barbosinho had a troubled career, marked by document forgery, which resulted in his imprisonment and exile. However, he continued his career after escaping, performing essential services for the mercantile and ecclesiastical elites of Porto. The premise that kinship facilitated access to the profession would not be straightforward in this case, as João Barbosa was haunted by his father's bad reputation, which resulted in conflicts with the *Vereação Portuense*, even though this public notary has produced, due to royal authorization, a registry of thirty-five documents. **Conclusions:** The main contribution lies in the systematization and analysis of Gonçalo Anes Barbosinho's work. Although there were already studies by Ricardo Seabra[9] that pointed to the notary and his crimes of forgery, there was still a need to study his documentary production and the transfer of the profession to his son João Barbosa.

Keywords: Medieval Notary Public; Documents Forgery; Professional Succession

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24782 | The analysis of trauma through the letters of former combatants of the Portuguese Colonial War in Angola, 1965-1972

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Background & Aim: The historiography of the Portuguese Colonial War has increasingly emphasized memory, identity, and postcolonial legacies. Trauma studies show that war can produce deep identity ruptures that are difficult to verbalize, and the clinical recognition of PTSD in 1980 introduced a category that did not exist during the conflict, complicating retrospective analysis. Despite a solid theoretical framework on memory, trauma, and war writing, the diachronic study of soldiers' letters as a space to detect progressive manifestations compatible with PTSD remains underexplored. This study hypothesizes that letters written by former combatants in Angola (1965–1972) reveal increasing psychological distress through tonal shifts, repetitions, fragmentation, silences, and indirect references. The main objective is to examine how neurosis and psychological suffering are expressed or silenced in wartime letters, identifying temporal and linguistic patterns of trauma while contributing to the dialogue between memory studies, war experience, and trauma theory. **Methods:** A qualitative analysis model of epistolary writing was adopted, inspired by Liz Stanley's[10] theoretical approach to the dialogical, perspectival, and contextual nature of letters. Thus, the analysis focused on the temporal evolution of emotional tone, affective lexicon, and expressions of distress and suffering, repetitions, erasures, and graphic irregularities, as well as silences and discursive gaps. **Results:** The results indicate that a qualitative and diachronic reading of the letters reveals growing psychological distress and identity fragmentation, offering a more nuanced understanding of former combatants' experiences and advancing historiographical debates on memory and trauma in the Portuguese colonial context. **Conclusions:** This study aims to demonstrate that epistolary writing played a dual role: simultaneously a mechanism for symbolic survival and a space for the involuntary exposure of psychological fragility.

Keywords: *Trauma, Memory, Correspondence, Portuguese Colonial War.*

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24810 | Between a brigade of migrants and a brigade that never was: the portuguese volunteers in the Spanish Civil War

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Background and Aim: Although several authors have identified the presence of no more than 150 portuguese *brigadistas* in the International Brigades (IB) (Skoutelsky, 2006), the absence of a Portuguese Brigade during the Spanish Civil War has generated debate in Portuguese historiography and in studies of the Brigades as a global expression of antifascist solidarity. Traditional interpretations argue that no autonomous Portuguese unit was created because most Portuguese who fought for the Spanish Republic had been living in Spain for years. When the war broke out, they joined workers' militias and later the Popular Army rather than the IB. **Methods:** Explaining why one of Spain's two neighboring countries, sharing a long land border, did not give rise to an autonomous Brigade requires consideration of political, military, sociological and diplomatic factors. **Results:** Political and military conditions were decisive, as the entire border quickly fell under Francoist control in the first weeks of the war, and the Portuguese resistance in exile on Spanish territory, which conditioned their overall intervention in the war. Above all, sociological factors were crucial: in the 1930s, the Portuguese formed the largest foreign migrant community in Spain. Understanding these combined factors clarifies both the scale and the character of Portuguese intervention on the Republican side during the Spanish Civil War, offering a more complex picture of the portuguese volunteers. **Conclusion:** This paper argues that the absence of a Portuguese Brigade resulted from the interaction of political, diplomatic, and socio-cultural factors which conditioned the formation of an autonomous unit.

Keywords: antifascism; International Brigades; Spanish Civil War; inmigration;

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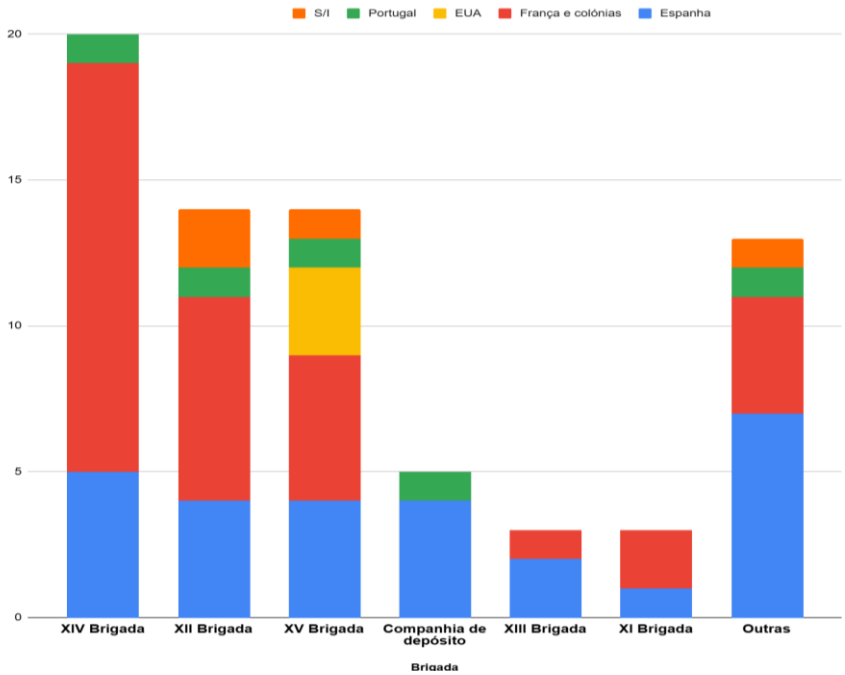
Figure 1: **Origin of the Portuguese by brigade**



24880 | Women's roles: Eduardo Teixeira Pinto's photographical perspective during the Estado Novo period

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Background & Aim: During the Estado Novo regime (1933-1974), Portuguese society underwent a Regeneration Project thought to reshape its political, economic, social and cultural structures. In this sense, the regime tried to build and propagate a feminine model where women were expected to be totally dedicated to family and housework. The law accompanied this tendency, restricting women's rights and freedoms and imposing a lifestyle of submission to the "head of the household" (a man). However, this ideal model would only be found among upper- and middle-class women, as the remaining needed to work outside their homes. This article intends to use Eduardo Teixeira Pinto's photographic work created during this period to explore the relation between the normative discourse and the roles women have in the selected photographs. The aim is to evaluate the level of continuity and discontinuity between the two dimensions. **Methods:** Eight photographs featuring the presence of women were selected. Through a qualitative analysis, this article seeks to understand how they were presented (what they were doing), how this relates to the broader economic and social context and, lastly, the level of divergence and/or continuity when compared to the ideal woman model created by the regime. Two photographs where women are not present were also selected to reflect on how their absence from certain moments might relate to the normative framework. **Results:** Through this work, it was possible to understand that women appear aligned with the regime's ideal in certain contexts (when taking care of children) but also misaligned with the propagated ideal by taking an active part in economic activities. **Conclusions:** This article concludes and reinforces the notion that women's roles in Estado Novo society, when compared to the ideal constructed by the regime, should be seen as ambiguous and dynamic rather than static. Through these images, women appear to defy this model while simultaneously complying with it.

Keywords: women; Estado Novo; ideal; normative; roles.

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24915 | “Jesus is here!”: The Oratories of the Holy Family of the Parish of Peroselo

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Background & Aim: The circulation of the Holy Family oratories in the parish of Peroselo, municipality of Penafiel, is a religious practice experienced in a rural context that remains poorly documented. There are continuous changes in forms of participation and intergenerational transmission reinforce the need for systematic recording. This study aims to analyze and document this practice, seeking to understand how it works in the local context, the agents involved, the associated devotional gestures, and the meanings attributed to it by the faithful. **Methods:** The research uses a qualitative methodology that combines bibliographic research and fieldwork, developed through direct observation and interviews with faithful members of the community. It describes the functioning of the circulation of the oratories, the associated objects and gestures, as well as the role of the caretakers in organizing the practice. **Results:** The empirical analysis reveals reconfigurations in its practice, namely the adaptation of its temporality, the reduction of collective family prayer, and the growing centrality of objects as support for individual devotion and affective memory. There is also a decrease in participation, particularly among younger generations, with the continuity of the practice often justified by family ties and childhood memories. **Conclusions:** This research contributes to the documentation and understanding of a changing local religious practice, highlighting the role of devotional objects and the mediation of caretakers in preserving forms of religiosity experienced in community contexts.

Keywords: Oratories of the Holy Family; Domestic Devotion; Affective Memory; Community; Devotional Practice.

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This research was oriented, within the course of “Dynamics of the Sacred Space” in the Art History, Heritage and Visual Culture Masters, by Professor Marisa Santos.

Figure 1: Oratory of the Infant Jesus of Prague. Parish of Peroselo, Quintã. Photo by Beatriz R. Moreira.

24978 | The “antas” (dolmens) of Alvão (Vila Pouca de Aguiar) and Their Engraved Assemblage. A History of an Obliterated Collection

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The first archaeological explorations of the “antas” of Alvão Mountain, conducted by the priests R. Rodrigues and I. Brenha, took place between 1894 and 1899. During this fieldwork, approximately 150 dolmens were identified, 56 of which were excavated in Vila Pouca de Aguiar. These investigations yielded a substantial corpus of lithic artefacts, as well as stones bearing engravings or sculptures. Initially attributed to the Neolithic, the assemblage attracted the attention of prominent archaeologists of the late 19th century and, subsequently, of the early 20th century. A few pieces bear incised signs that Brenha interpreted as a possible Neolithic writing system, later referred to as the “Alvão alphabet”. Mendes Correia classified them as “Iberian script” — a characterization that sparked considerable scholarly debate regarding the authenticity, chronology, and cultural attribution of the incised signs. Over the course of the 20th century, these pieces gradually lost their academic standing and credibility, ultimately being relegated to the margins of the history of archaeology. This paper seeks to reassess the authenticity of these pieces, revise their chronological attribution, and investigate the raw materials and production techniques involved. To this end, it presents findings based on written documentation concerning the reconstruction of the collection's historical and archaeological record, the excavation methodologies employed, and the strategies used to establish the authenticity of the pieces. The overarching objective is to situate both the artefacts and the scholarly activities associated with them within the broader history of archaeology — particularly in relation to the emergence of prehistoric art as a field of study — and to account for the near-total erasure of this collection from academic discourse throughout most of the 20th century and into the early 21st century. Scientifically annotated 3D models of the 29 pieces held at MHNC-UP will also be presented.

Keywords: “Antas” of Alvão; History of archaeology; Portable Art; Prehistory; “Alvão alphabet”.

Acknowledgments: Special thanks are due to Professor Maria de Jesus Sanches for her supervision of this study, conducted in the context of the author's master's dissertation.

24984 | ABC Magazine: Jorge Barradas, the "Artist of Women"

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Background & Aim: In the 20th century, the press played a fundamental role in cultural dissemination and the construction of social ideas, with magazines serving as mirrors of society. In Portugal, the magazine ABC – Revista Portuguesa stood out as an important means of disseminating news and ideas, even becoming a means of social dissemination between the First Republic and the Military Dictatorship. Despite the importance of this periodical at the time, studies are still limited, especially when it comes to its illustrations. In this context, Jorge Barradas was one of the main artists who stood out for his representation of femininity. The aim of this research is to analyse the illustrated covers of ABC magazine by Jorge Barradas, seeking to understand the representations of women in the city and women in the countryside, reflecting Portuguese society in the 1920s and 1930s. **Methods:** The research is based on a comparative analysis between the two types of representation, using the collection of the Lisbon Municipal Newspaper Library, with a total of 543 covers published between 1920 and 1931, of which Jorge Barradas illustrated 111 covers**. Results:** The results show two main representations: the representation of the cosmopolitan and modern woman, linked to French and English models, which declined after the military coup of 1926, and the rural woman, represented in a romanticised and idealised way, linked to the traditional and rural world. **Conclusions:** It can be concluded that Jorge Barradas' illustrations in ABC magazine prove to be an important source for understanding the society of the 1920s and 1930s, allowing us to identify cultural, social and political transformations. Through the representation of femininity, it is possible to reflect not only on the aesthetic ideas of the era, but also on the mentality associated with the period under study. Demonstrating how illustrations in periodicals are important sources for historical and social study, I understand the values and dynamics of Portuguese society.

Keywords: Magazine; ABC – Revista Portuguesa; Jorge Barradas; Illustration; Woman

Acknowledgments:

This work was oriented by Professor Ana Cristina Sousa within the course of Image and Contexts I, in the Art History, Heritage and Visual Culture Masters.

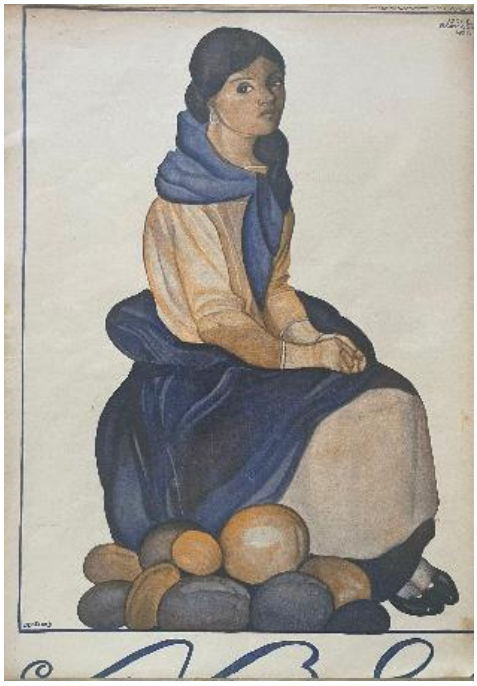

Figure 1: ABC, nº 328, 28 de outubro de 1926, Illustration Jorge Barradas, Photo by Luana Carneiro

24985 | The *Portrait of Dom Pedro IV* (594 A PIN MNSR): Visualizing a Transnational Monarch

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Background & Aim: The *Portrait of Dom Pedro IV,* or *Portrait of King Pedro IV* (594 A PIN MNSR), painted by John Simpson between 1832 and 1834 and currently part of the Soares dos Reis National Museum (MNSR) itinerary, serves as a point of departure for understanding Dom Pedro I, the first emperor of Brazil, who later became Dom Pedro IV, King of Portugal. As a transnational figure whose actions shaped the trajectories of both countries, this study examines how Dom Pedro was represented in the visual arts over time in Portugal and Brazil, within the contexts of nineteenth-century portraiture and Romanticism. **Methods:** A field visit to the Soares dos Reis Museum enabled the collection of photographs of the portrait for formal analysis. This was complemented by an iconographic comparison with coeval portraits produced in Portugal and Brazil, alongside a state-of-the-art review of the monarch's life and visual representations. **Results:** The research highlights the painters responsible for portraying Dom Pedro, examines the institutions associated with his reign, and analyzes his role in promoting the fine arts in Brazil and Portugal. It also considers the enduring impact of his image on artistic production and architecture, especially in the cities of Rio de Janeiro (Brazil) and Porto (Portugal). **Conclusions:** The study advocates a multicultural and transnational perspective on the historical events linking Brazil and Portugal in the nineteenth-century, considering both the period itself and the present-day interpretations through representations of Dom Pedro IV. By problematizing issues of dating and artistic models, it contributes to the historiography of nineteenth-century royal portraiture and supports research carried out in dialogue between the two countries on Dom Pedro's lasting visual legacy.

Keywords: Brazil; Dom Pedro IV; Portraiture; Portugal; Transnacionalism

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Figure 1: John Simpson [1782-1847], *Portrait of King Pedro IV* (594 A Pin MNSR), 1832-1834. Soares dos Reis National Museum, Portugal. Photo: Letícia Dantas, 2025.

24123 | From the Troubadour Love to The Struggle for Freedom: The Symbolism of D. Dinis's Pine Green to Manuel Alegre

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Background & Aim: The poet Manuel Alegre and King D. Dinis may seem to have nothing in common. However, a closer examina>on reveals that both were poets and poli>cians, despite the eight centuries separa>ng them. Moreover, it is widely acknowledged that Manuel Alegre was inspired by D. Dinis's poetry, especially the *verde pino* mo>f. The main aim of this study is to compare the two authors through the poems *Ai flores, ai flore do verde pino* and *Uma flor de verde pinho.* **Methods:** To support this comparison, the analysis begins with the two poems and supported by the selected bibliography we were able to find several similari>es regarding the style, symbols and their meanings. ALerwards, the concept of neotrovadorism was analysed to iden>fy the differences and understand how Alegre used a 13th century love poem to explore his lack of freedom. **Results:** The comparison proves to be relevant as there are both similari>es and differences that deepen the analysis. It was possible to understand the reasons that led Alegre to reinterpret the poem *Ai flores, ai flores do verde pino*, par>cularly in the light of the historical context in which he was wri>ng it. **Conclusions:** This study represents one of the first comparisons between these two specific poems, highligh>ng their importance and relevance to literary studies.

Keywords: Manuel Alegre; D. Dinis; verde pino; neotrovadorismo

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24795 | "...The Ruthless Pursuit of Blood with All a Child's Demanding": A comparative analysis of Octavia E. Butler's and Anne Rice's representations of vampirism

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Background & Aim: While comparative analyses of Octavia E. Butler and Anne Rice's vampires already exist, particularly regarding Butler's subversion of Gothic tropes, this study aims to expand that dialogue by delving deeper into themes of queerness, morality, aging, and the overall human condition. Furthermore, to complement this perspective, AMC's eponymous adaptation of *Interview with the Vampire* will be explored, focusing specifically on the narrative shifts and updates presented in the book adaptation. **Methods:** To achieve this, a close reading of Butler's novel, key scenes from Rice's *The Vampire Chronicles*, and AMC's *Interview with the Vampire* will be conducted to provide an in-depth comparison of these works. **Results:** Beyond existing scholarship, this research shows that these works deal with similar and contrasting aspects, including the human-vampire dynamic, the duality of vampiric communities and vampire isolation, each author's unique understanding of race, or even instances where the AMC television show aligns more closely with Butler's vampires than with its own source material. **Conclusions:** In conclusion, while this study expands on pre-existing research, the scope for further work remains vast, as future analyses could benefit from examining a broader range of vampire media through a singular lens, such as the representation of women, for instance.

Keywords: Anne Rice; Octavia E. Butler; Vampirism; Comparative studies

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24796 | The Truth, The Whole Truth, and Nothing but the Truth: Fact and Fiction in the Works of Elizabeth Smart

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Background & Aim: Previous research about Elizabeth Smart's *By Grand Central Station I Sat Down and Wept* (1945) rarely fails to mention the context in which it was written and the impact this setting had on it. The dialogue surrounding Smart's love affair with poet George Barker—documented in her journals and correspondence—and the search for its reflection in Smart's *By Grand Central Station* has led to it being labelled as "autobiographical fiction" or "novel-journal". The study of literary works with autobiographical implications is even more extensive and polemical; life-writing's place in the literary canon has also been the target of much contention. This research is not preoccupied with issuing Smart's work a specific label but rather aims to explore how the autobiographical elements of the text may affect its analysis. **Methods:** For this purpose, a close reading of *By Grand Central Station I Sat Down and Wept* was performed through Smith and Watson's adaptation of Genette's theory on the metalepsis. **Results:** By employing a metaleptic analysis of autobiographical texts, the realms of fact and fiction are allowed to coexist in Smart's short "novel", and its multiple layers gain further depth and meaning. **Conclusions:** Instead of performing an analysis of *By Grand Central Station* that presupposes a specific label, this research allows for a conflation of reality and fabrication. It would also be interesting to apply this lens to other works by Smart such as "Dig a Grave and Let Us Bury Our Mother".

Keywords: Elizabeth Smart; Life-Writing; *By Grand Central Station I Sat Down and Wept*; Canadian Literature; Metalepsis.

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24968 | The Dark Side of Justice: Catiline's voice in Sophia de Mello Breyner Andresen and Cesare Maccari

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Background & Aim: This paper is based on a comparative reading between Sophia de Mello Breyner Andresen's poem "Catilina" (1947) and Cesare Maccari's painting "Cicero denounces Catiline" (1880), which aims to analyse the way that both artists represent the *Catiline Orations*, a group of four speechs given by Cicero against Catiline in 63 b.C. **Methods:** The connection proposed before was only possible by bibliographical readings of theoreticalcritical essays about Cicero's speeches and Sophia's literary work focused on the political and social inequalities. Futhermore, to highlight this connection, it was imperative to do a close-reading of *Catiline Orations* and "Catilina" and analyze "Cicero denounces Catiline" through two different perspectives: Cicero's and Catiline's. Some of the bibliographical references which were fundamental to sustain this paper include NAHAS (2011), PINHO (2022) and CALHEIROS (2022). **Results:** All the four speeches of Cicero aim to condemn Catiline for crimes he allegedly committed. However, in no part we find Catiline's perspective on the accusations made against him. In this way, Catiline represents those who are economically weak and powerless in a system driven by money and political influence. Sophia's poem looks to Catiline not as a criminal but as someone who fights against these injustices, denouncing the Roman political system and representing those whose voices were never heard. There is the possibility to interpret Maccari's painting either through Cicero's point of view or through the poem's perspective which results in an ambiguous interpretation. **Conclusions:** The essay concludes that the painting and the poem can be related and united in a single voice that confronts political injustices, although we have no evidence that Sophia wrote the poem based on Maccari's painting. In this way, both arts serve the function of exposing the true reality, often stifled by those who have more power.

Keywords: Interarts Literature; Catilina; Sophia de Mello Breyner Andresen; Justice; Politics.

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Figure 1: *Cicero denounces Catilina*, (1880), a*fresco* by Cesare Maccari, 400 x 900 cm

24976 | The Crisis of Representation in Modernity: Resistance to Theory and the Refusal of Interpretation

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Background & Aim: The Western aesthetic paradigm was consolidated through Aristotelian mimesis and the transitive dimension of linguistic signs. The advent of modernity fragmented this mirage, exposing the opaque nature of language. Paul de Man posits this rupture as a semantic aporia, while Susan Sontag criticises hermeneutic fixation, suggesting an "eroticism of art". This paper aims to demonstrate that the failure of meaning theorised by De Man and Sontag's sensory refusal constitute the foundations of modern aesthetics, corroborating the crisis of representation as an inherent condition of literary and artistic creation. **Methods:** This research is based on a qualitative methodological approach of a theoretical-critical nature, providing an intersection between post-structuralist reflection (through the contributions of De Man, Sontag, Derrida, Foucault, and Barthes) and the rigorous analysis of two aesthetic objects: the pictorial surrealism of René Magritte (in the work *Ceci n'est pas une pipe*) and the sensationalist poetry of Álvaro de Campos (in the compositions *Tabacaria* and *Ode Triunfal*). **Results:** The analysis reveals that Magritte materialises the betrayal of the sign on a visual level. By deconstructing the calligram model, the painting proves the failure of the referentiality theorised by De Man. In literature, Álvaro de Campos illustrates a simultaneous transformation. In *Tabacaria*, the lyrical subject stages a rhetorical autophagy and the dissolution of identity, while in *Ode Triunfal*, the aggressiveness of syntax and onomatopoeia converts the text into a sensory machine. In this way, Campos dismisses canonical exegesis, achieving the *jouissance* proposed by Barthes and materialising Sontag's demand for sensory order, a scenario in which the content dissolves. **Conclusions:** Aesthetic modernity inhabits the fissure between representative fallacy and the visceral desire to experience it. It can be concluded that the resolution to this aporia lies in the re-education of sensitivity. This study has repercussions on the state of contemporary art by demonstrating that the permanence of the artistic object requires the rejection of intellectual domestication, thus rescuing art in its purest state.

Keywords: Crises of Representation; Hermeneutics; Erotics of Art; Aporia; Modern Aesthetics

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Figure 1:

René Magritte [Ceci n'est pas une pipe]

24247 | Equality and non-discrimination in local development: boosting the business sector

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Background & Aim: Debates on equality and non-discrimination have intensified due to persistent inequalities and the need for fairer organizational environments. National and international frameworks, including the National Strategy for Equality and EU/UN guidelines, reinforce the duty to mainstream gender equality across public and organizational action. Organizations play a key role in reproducing or changing inequalities and transforming environments and cultures. At the local level, Portuguese law requires municipalities to integrate gender perspectives through Municipal Equality Plans. This study examines how companies in Penafiel respond to expectations of social responsibility in this domain and how national guidelines translate into local organizational practices. **Methods:** Developed during an internship within the Municipal Plan for Equality and Non-Discrimination (PMIND), the study adopts an action-research approach suited to real organizational contexts. The ongoing assessment of PMIND is the starting point, using document analysis. Then, methods include questionnaires, interviews and field notes with emphasis on interviews with business leaders and the local Business Association **Results:** Analysis of PMIND activities in 2025 shows strong investment in education, health, social solidarity and municipal workers' well-being, but a lack of measures directed at the business sector. Fieldwork revealed clear disparities between large companies and small family businesses regarding equality policies, training and awareness. Interviews indicate that most companies follow traditional efficiency-driven management models, with equality rarely seen as a strategic concern. **Conclusions:** Findings reveal a gap between national equality commitments and their implementation in local organizational practices. The business sector remains insufficiently engaged, and inequalities persist, particularly in smaller enterprises. A more structured municipal strategy to support companies in integrating equality measures is essential to align local development with national and European goals.

Keywords: Organizational Practices; Equality; Non-discrimination; Local Governance; Corporate Social Responsibility

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PSYCHOLOGY AND EDUCATION SCIENCES

24220 | How do graduates in Educational Sciences apply the knowledge acquire during their training in professional contexts?

"I believe that all areas through which people pass and where relationships are established should be considered areas of Educational Sciences." – excerpt from the empirical study.

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Background & Aim: This study addresses the guiding question: "How do graduates in Educational Sciences mobilise the knowledge acquired in their degree in different work contexts?" It arises from the interest of researchers and the wider academic community in Educational Sciences, aiming to understand how these graduates draw on their training to meet professional demands and challenge common-sense views about this degree and its employability. **Methods:** The study was conducted within the course Research Methodologies – Laboratory of Qualitative Methodologies, using a problem-based approach. The research question guided the theoretical problem and research process, informed by the paradigm of complexity [1]. A qualitative approach was adopted, using semi-structured interviews to collect Educational Sciences graduates' perspectives and participant observation to describe their professional contexts: schools, industry, and higher education institutions. Field notes and interviews underwent content analysis, and an analytical model was developed to interpret the empirical material, bridging theory and data. **Results:** Findings show that graduates mobilise a wide range of competences in different areas, such as selecting conceptual lenses to interpret educational challenges and using an inquiry-based approach to develop situated solutions. Professionals mention benefits of the interdisciplinary nature of their "broad-band" [2] training and internships as gateways to the labour market. Results also indicate that these graduates can work in various professional contexts, adapting and reconfiguring their knowledge to situational demands. **Conclusions:** This research moved beyond common-sense representations, showing

how Educational Sciences graduates mobilise and transfer their knowledge across contexts. It highlights the main challenges they face and the strategies developed to overcome them, contributing to a deeper understanding of the social relevance of Educational Sciences training.

Keywords: Educational Sciences degree and graduates; mobilization; diversity of contexts.

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24324 | Linguistic Inclusion as a Pathway to Social Integration:

An Educational Intervention with Migrants at Porta Solidária (Porto, Portugal)

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Background & Aim: Although language learning is increasingly recognised as a key condition for migrants' social integration, empirical evidence on participatory community-based models that combine language, empowerment, and citizenship remains limited. Grounded in critical pedagogy (Freire, 1970, 1996), education as the practice of freedom (hooks, 1994), community intervention (Menezes, 2002), and liberation psychology (Martín-Baró, 1998), this study evaluated the educational, social, and relational impacts of a linguistic inclusion intervention implemented at Porta Solidária (Porto) as a device for emancipation and social integration. Porta Solidária is a parish-led hub for vulnerable migrants. **Methods:** A participatory action-research design was adopted (Barbier, 2001). Adult migrants from multiple countries of origin and with heterogeneous levels of proficiency participated. A mixed-methods evaluation combined quantitative data from a satisfaction scale with qualitative data from participant observation and open-ended written messages. The intervention was dialogical, situated, and flexible, fostering peer mediation and collaborative learning. **Results:** Findings showed high satisfaction, engagement, and retention (mean 4.8/5), alongside gains in communicative confidence, social interaction, and sense of belonging. Peer mediation emerged as a structuring outcome, strengthening empowerment processes (Rowlands, 1997) and group cohesion. Beyond language learning, relevant social and emotional effects were reported, including increased self-esteem, participation, and perceived recognition. **Conclusions:** Community-based linguistic education, conceived as a critical, participatory, and ethical practice, can be an effective and transferable model with strong potential for continuity. This study contributes empirical evidence of language learning as a lever for social inclusion, empowerment, and citizenship, with implications for adult education, community intervention, and inclusive public policies.

Keywords: Linguistic Inclusion; Migration; Adult Education; Critical Pedagogy; Socio-Educational Interventi

Acknowledgments:

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24512 | How are the educational strategies of graduates in Education Sciences present in their practices?

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Background & Aim: Educational Sciences establish themselves as a plural and interdisciplinary scientific field, whose specificity lies in the critical analysis of educational processes in diverse contexts. It is therefore considered relevant to understand the practices and strategies adopted by graduates in Educational Sciences in what constitutes their professional performance. This study is part of the curricular unit Research Methodologies of Qualitative, and it aims to investigate the practices of professionals in Educational Sciences to understand how the educational strategies are present in their professional contexts. **Methods:** Based on a qualitative approach, this study includes semi-structure interviews with 5 graduates in Education Sciences who work in diverse professional contexts. The data collected was processed through content analysis. Five key categories related with models and strategies in Educational Sciences, the relationship between education and training, reflective practice, ethics in professional practice, and professional contexts and practices were identified. A total of 7 semi-structured interviews were conducted, and 23 field notes. Field notes and interviews underwent content analysis, and an analytical model was developed to interpret the empirical material, bridging theory and data.**Results:**With flexible adaptation to the needs of individuals and to work in multidisciplinary teams prevailing. Professional practice is guided by a microethics and constant reflexivity, essential for managing emotional tensions and defining limits of intervention. The transition to the job market is marked by uncertainty and social invisibility in the field, with internships proving to be decisive for its clarification. **Conclusions:** This study contributes to the understanding of the professional practices of graduates in Educational Sciences, highlighting the diversity of models of action in professional practice, as well as the challenges of entering the labor market.

Keywords: Educational Sciences, Research, Professional Practices, Reflexivity.

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24538 | Perceptions of integration and sense of belonging in higher education: a case study of Education Sciences students

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Background & Aim: Over the past 50 years, Portuguese higher education has evolved from an elitist system to a mass system, shaped by the political and social transformations that followed the 25 April 1974 Revolution. However, massification has not ensured full democratisation, and socioeconomic and cultural inequalities persist as barriers to the right to belong. This study analyses first-year Education Sciences student´s perceptions of integration and how they construct their place within a massified system, examining differences between first-generation and non-first-generation students. **Methods:** Using a single-case, exploratory qualitative design within a phenomenological-interpretative paradigm, the study analyses responses from 23 students at a Portuguese public higher education institution, collected through an open-ended questionnaire survey. Data were analysed using categorical content analysis, organised around four *a priori* dimensions: motivations; massification/democratisation; integration structure and processes, and expectations. **Results:** Findings identified a motivational triad (pragmatism, personal development, and social influence) alongside critical perceptions regarding economic barriers and selective admission criteria. The relevance of institutional support, particularly peer mentoring, emerged as a key mitigating factor for the lack of familial cultural capital, especially among first-generation students. Cross-cutting analysis produced a typology comprising four integration profiles, with the "Rooted" profile predominating; notably, this profile was composed mainly of first-generation students, which contrasts with patterns commonly reported in the research literature. **Conclusions:** The study concludes that integration and sense of belonging are multidimensional, relational processes that ensure persistence, success, and belonging. The study recommends institutional policies oriented towards inclusion, continuous student support, and the meaningful evaluation of impact.

Keywords: Higher education; Democratisation; Integration; Sense of belonging; First-generation students

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This work was developed under the supervision of Amélia Veiga, PhD (Centre for Research and Intervention in Education (CIIE) | Faculty of Psychology and Educational Sciences, University of Porto, Portugal).

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24659 | Is medical students' pathway associated with their academic performance?

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Background & Aim: Academic performance in medical students is shaped by multiple intrinsic and extrinsic factors, yet Portuguese data remain limited. This study evaluated academic performance among undergraduate medical students at ICBAS (University of Porto) and its association with sociodemographic and academic variables. **Methods:** This observational, retrospective, and analytical study was based on data available from the academic records system. It included students who enrolled for the first time in the medical degree at ICBAS during the academic years 2018/2019 (MIM 2018) and 2019/2020 (MIM 2019). Academic performance was assessed using the weighted average of final course grades (MPCF). Its association with sex, type of secondary school, admission pathway, admission grade, and academic status was analysed. To assess these associations, Student's t-tests, analysis of variance, and simple and multiple linear regressions were performed. **Results:** Data from 365 students were analysed (184 from MIM 2018 and 181 from MIM 2019). MPCF was higher among students from public secondary schools (B = 0.60 IC95%:[0.26; 0.94] for MIM 2018 and B = 0.58 IC95%:[0.26; 0.90] for MIM 2019) and showed a positive correlation with the admission grade ($p \leq 0.001$ for both), with stronger associations observed in pre-clinical years. The relationship between the admission pathway and MPCF proved inconsistent when stratified by curricular year; however, overall, students enrolled through special contingents or access regimes had significantly lower grades. No statistically significant differences in MPCF were generally observed based on sex or academic status. **Conclusions:** These findings may contribute to the development of more effective and tailored academic support strategies. However, further research is needed to explore additional relevant variables and to strengthen the applicability of these results within Portuguese medical education system.

Keywords: Academic performance; medical students; undergraduate medical education; sociodemographic factors; medical education.

24708 | Ecological Mentoring as a Socio-Educational Mediator: Reconfiguring School Relational Ecologies

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Background & Aim Grounded in the ecological systems theory of Bronfenbrenner [1], development is shaped by dynamic interactions across nested systems. In school contexts, peer mentoring operates along a continuum between structured programmes [2] and informal relational dynamics [3] ,[4], with evidence suggesting that informal mentoring may potentiate stronger developmental gains [5]. This study proposes Ecological Mentoring as a Socio-Educational Mediator (EMSM) as a socio-educational mediation model emerging from action research in a 5th-grade class within a TEIP school. **Methods** A qualitative action-research [5] design integrates structured mentoring sessions, participant observation andcollaborative reflection. The researcher assumes the role of Ecological Mediator, intentionally organising relational spaces and developmental opportunities across systems. **Results (Preliminary)** Cycle 1 findings indicate that formal mentoring structures catalyse informal peer-support networks within classroom and playground microsystems. These dynamics extend to mesosystemic articulations among teacher, researcher and peers, contributing to improved classroom climate, strengthened learning attitudes, enhanced peer relationships and observable personal and social growth. **Conclusions** EMSM advances the state of the art by reconceptualising peer mentoring as a systemic socio-educational mediation process rather than solely a formal programme or dyadic relationship. By integrating mentoring theory with ecological developmental theory, the model shifts analysis from individual matches to relational ecologies. Preliminary evidence suggests that mentoring can function as an ecological regulatory mechanism capable of reorganising school relational climates and promoting socio-academic development in vulnerable contexts. Further cycles will refine and test the model's transferability. **Keywords:** peer mentoring; socio-educational mediation; ecological systems theory; action research; school climate.

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24973 | Movement-Based Learning in Geoscience Education: Teaching the Rock Cycle in 7th Grade

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Background & Aim: Understanding the Rock Cycle is challenging for students due to the abstract, dynamic and multiscale nature of geological processes. Research shows that students often develop fragmented or linear interpretations of the Rock Cycle and struggle to understand its systemic relationships and temporal scales[1-3]. Embodied learning approaches have been suggested to support conceptual understanding of complex scientific phenomena[4]. This study explores the potential of movement-based activities for teaching the Rock Cycle in 7th-grade Natural Sciences within a Project-Based Learning framework, aiming to analyse how embodied modelling can support students' understanding of geological processes. **Methods:** The study follows an action-research design within a qualitative interpretative framework. A five-lesson teaching sequence was implemented in a 7th-grade Natural Sciences class (n = 18, aged 11–13), integrating Project-Based Learning and embodied modelling based on Laban Movement Analysis. Data were collected through classroom observations, teacher field notes, students' learning diaries and pre- and post-tests. Qualitative content analysis and data triangulation were used to examine students' conceptual understanding of the Rock Cycle and transversal competencies. **Results:** Preliminary results indicate that embodied activities facilitated the visualisation of non-observable geological processes and helped students move beyond linear representations of the Rock Cycle. The activities also supported students' understanding of geological timescales, a concept frequently identified as challenging in geoscience education. This approach promoted collaboration, scientific communication and student autonomy, while contributing to more positive peer interactions. **Conclusions:** These findings suggest that integrating movement-based approaches in geoscience education can support systemic understanding of geological processes while fostering transversal competencies. The results highlight the potential of embodied approaches for addressing conceptual difficulties in Earth science learning.

Keywords: Rock Cycle; movement-based learning; Laban Movement Analysis; Project-Based Learning; geoscience education.

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24139 | Adaptation and Preliminary Validation of the Empathy Scale for Portuguese Teachers

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Background & Aim: As noted by Eklund and Meranius (2020), empathy is the capacity "to understand, feel and share other person's feeling". Divided into cognitive and affective subtypes, the latter encompasses positive and negative emotions. Teachers' empathy is linked to supportive learning environments and prosocial behaviors (Cuff et al., 2014). The study aimed to translate and adapt Empathy Scale for Teachers (EST) by Wang et al. (2022) into Portuguese and examine associations between teacher's empathy and their empathy-enhancing practices in the classroom. We hypothesized that (1) teachers with higher empathy would adopt more empathic practices in their classrooms and that (2) cognitive empathy, being not linked to emotional distress, could be a stronger predictor of these practices. **Methods:** The study followed Gudmundsson's (2009) adaptation guidelines. An online survey was conducted with 53 Portuguese 1st and 2nd cycles teachers in the Spring of 2025, using also the Basic Empathy Scale (BES) by Pechorro et al. (2018) and the Self-Reflection Scale on Promoting Empathy in the Classroom (SSPEC) (Schools2030, n.d.). Analyses included internal consistency, correlations, and regressions. **Results:** EST showed good consistency but presented lower reliability for the negative affective subscale. Cognitive factors of both empathy scales predicted empathic practices better than affective ones. EST negative affective remained marginally significant in multiple regression. **Conclusions:** Results confirmed both hypotheses. The lower internal consistency of the negative affective and its correlation with the affective factor of BES, may be due to item bias caused by translation issues. Cultural and occupational factors among Portuguese teachers, such as high stress and the value placed on maintaining emotional distance, could have amplified these effects. Future studies should address translation bias statistically, use larger samples, and include external evaluations to minimize social desirability bias.

Keywords: Empathy; Teachers; Cognitive; Affective

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24204 | The Transition from Foster Care to Adoption: A Qualitative Study of Adoptive Parents' Experiences

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This qualitative study explores adoptive parents' experiences of the transition of children from foster care to adoption within the *Family to Family* programme. The sample comprised 11 adoptive families, and data were collected through semi-structured interviews specifically developed for this study and conducted with adoptive mothers and fathers. The interviews focused on parental perceptions of the transition process, associated emotional challenges, facilitating and hindering factors, and the child's adjustment. Thematic analysis identified two overarching themes encompassing dimensions related to the emotional experience of the transition, the phased nature of the process, relational continuity for the child, and professional support. Overall, parents described the transition as emotionally demanding, highlighting the importance of consistent professional support, gradual and individualised transition planning, and the maintenance of relational continuity. Participants perceived that carefully planned processes tailored to the child's needs promoted emotional security and positive adjustment. This study is particularly relevant as the first study examining the *Family to Family* program, contributing to a better understanding of transition processes from foster care to adoption and to the development of informed and sensitive practices that address the needs of the families involved.

Keywords: adoption, foster care, transition, adoptive parents, qualitative study, *Family to Family* programme

24217 | Emotional Control in Chess: a thematic analysis of coping strategies among chess player-coaches

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Background & Aim: This study investigates chess players' perceptions regarding emotions in the competitive context of chess, seeking to understand the emotional control strategies developed by practitioners. The central objective was to explore and analyze how different chess enthusiasts face their emotions and manage their emotional control. **Methods:** A qualitative exploratory methodology was adopted, utilizing thematic analysis of semi-structured interviews conducted with two male chess players (a 39-year-old Ukrainian and a 41-year-old Brazilian), who act primarily as players and coaches. **Results:** The results revealed three central categories: (1) Emotions in chess, highlighting the relevance of self-confidence; (2) Emotional control strategies, where rationalization and dissociation emerge as coping mechanisms for stressful situations; and (3) Pressure in chess, evidencing perceptions of the differences between individual and team play, as well as the impact on mental health. **Conclusions:** It is concluded that, although athletes develop intuitive coping strategies with maturity, there is a lack of professional psychological support and robust techniques, suggesting the need for the validation of specialized interventions, such as psychological skills training and the use of tools like biofeedback, applied to chess.

Keywords: Sport psychology; Chess; Emotional Control; Coping;

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24248 | Heroes at Heart: The Role of Hospital Volunteering from the Perspective of Families with Hospitalized Children

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Background & Aim: Pediatric hospitalization entails numerous challenges, representing a major source of psychological stress not only for the hospitalized child but for the entire family, especially caregivers, often leading to heightened levels of exhaustion and emotional burden. Hospital volunteering emerges as a source of support and relief and thus, as a valuable resource to mitigate the negative impact of this experience and contribute to improvements in well-being. In this sense, the present study aimed to examine the levels of parental exhaustion among caregivers of hospitalized children and explore their perceptions of hospital volunteer support, intending to characterize their contributions to the experience. **Methods:** A mixed methods approach was employed, including individual interviews and the administration of a questionnaire to caregivers, comprising the Portuguese version of the *Parental Burnout Assessment* and the translation of the *Family, Infant, and Preschool Program Loyalty Scale*. A total of 103 caregivers completed the questionnaire and 10 participated in individual interviews. **Results:** Results indicated low levels of parental exhaustion, which varied according to some sociodemographic factors, such as gender, employment, and social support. Despite those low levels, significant emotional impacts were reported. At the same time, caregivers were very satisfied with the volunteer project, especially the ones who reported higher burnout, suggesting a potential stress-buffering role of volunteer support. Caregivers highlighted various contributions, from logistical to emotional, social, and relational. **Conclusions:** These findings underscore the importance of addressing not only the needs of hospitalized children, but also of their caregivers, reinforcing the importance of investing in family support measures, such as volunteer initiatives, which mitigate caregiver stress and promote more humanized and family-centered care.

Keywords: pediatric hospitalization; parental burnout; hospital volunteering; family-centered care

Acknowledgments:

São João University Hospital

Ronald McDonald House Charities

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24256 | Experiences through barriers and facilitators of the acculturation process in elite migrant female soccer players

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Background & Aim: Transnational mobility has become a central feature of athletic careers, demanding continuous psychological adjustment to new sociocultural environments. Within sport psychology, acculturation is understood as a dynamic and non-linear process shaped by everyday negotiations of meaning and relationships (Ryba et al. 2018). Grounded in the cultural transition model (Ryba et al. 2016), this study aimed to explore perceptions of the acculturation process among elite migrant female soccer players during the initial phase of their relocation (first 2-3 months). **Methods:** A qualitative design was adopted. Three North American professional players competing in Portugal participated in a semi-structured focus group. Data were transcribed verbatim and examined through thematic analysis to explore barriers, facilitators, and the psychological experiences associated with early cultural adaptation. **Results:** Language was consistently described as the most pervasive barrier, constraining communication, autonomy, social participation, and intensifying feelings of uncertainty. Athletes reported experiences of mental overload and heightened emotional fluctuations, typical of acute cultural adaptation. Social support from staff, teammates, and particularly from fellow migrant players functioned as a protective resource, fostering emotional regulation, shared understanding, and progressive feelings of belonging. **Conclusions:** Findings reinforce acculturation as a relational and meaning-making process rather than an individual outcome. Early transition is marked by vulnerability but can be facilitated by supportive environments that promote social repositioning. The study contributes to the literature by situating cultural transition within elite women's football and highlighting the relevance of club and peers support to migrant athletes' psychological needs.

Keywords: acculturation; cultural transition; women's football; migrant athletes; adaptation

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24283 | Adolescent Perspectives on Conspicuous Consumption: A Qualitative Study in Portugal

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Background & Aim: Conspicuous consumption, defined as the display of goods to signal status and identity, remains under-explored regarding its impact on adolescence, a developmental stage characterized by personality formation and heightened sensitivity to social influence. This study aims to investigate how Portuguese adolescents understand the logic of conspicuous consumption within their social environments. **Methods:** The study employed a qualitative thematic analysis involving 14 adolescents (aged 12–18) from Lisbon, Porto, and Madeira. Data were collected through cognitive interviews based on the Conspicuous Consumption Scale. The analysis was facilitated by Requalify software, utilizing word cloud generation, exploratory tagging for emerging categories, and knowledge network mapping to identify recurring patterns in the participants' discourse. **Results:** The findings indicate a high level of critical awareness among adolescents regarding the mechanisms of conspicuous consumption. Participants recognized its role in organizing social hierarchies; however, they expressed discomfort with this dynamic and a desire to remain autonomous from it. Despite this explicit rejection of ostentation, their narratives revealed a central ambivalence: even choices favoring simplicity operated as symbolic markers. This suggests that adolescents remain situated within a field where consumption, even in its understated forms, continues to function as a tool for social positioning. **Conclusions:** These findings highlight the complexity of how adolescents articulate consumption, image, and identity in an era dominated by digital visibility. The study contributes to the state of the art by demonstrating that even "anti-consumption" attitudes in youth can be a form of status signaling, suggesting that social intervention and educational programs must address the subtle, symbolic layers of modern consumer culture.

Keywords: Conspicuous consumption; Adolescence; Social status; Identity formation.

24352 | Writing Motivation in the Digital Age: Comparing Paper and Digital Writing among Students in Grades 5, 7, and 9

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Background & Aim: Motivation is a predictor of writing performance in school. However, this drive can vary across different contexts[1-2], and in the digital era more traditional writing methods may not be as engaging for students[3]. In Portugal, research on motivation towards different writing modalities is scarce, which does not reflect the transition of educational assessments to a digital format. Using a sample of Portuguese students enrolled in Grades 5, 7, and 9 (22 classrooms), this study aimed to: a) analyze text quality and motivational levels in paper and digital writing modalities; and b) examine whether text quality and motivational levels across modalities vary depending on gender and grade level. **Methods**: We expanded the data on Grades 5 and 7 from two schools collected by Silveira (2025)[4] and partially presented in IJUP 2025. We added seven Grade 9 classes from the same schools. Data for all grades were collected simultaneously during the winter of 2025, using a cross-sectional design (two 50-minute sessions) in which students' writing motivation was assessed via self-report scales and text quality was evaluated based on two opinion essays—one handwritten and one typed on a computer. Text quality was assessed by three independent raters following a scoring rubric[4]. **Results**: Regarding text quality, 5th graders performed better when writing on paper than on a computer, whereas among 7th graders no differences emerged depending on the modality. We also found a decline in writing motivation throughout schooling for both modalities, with boys reporting lower motivational levels than girls. For both modalities, alphabet writing fluency and self-efficacy significantly predicted text quality. Grade 9 data is currently being coded and will also be presented. **Conclusions**: The present study sheds light on Portuguese students' writing motivation and performance, particularly in digital contexts, contributing to the development of adequate pedagogical strategies.

Keywords: Digital writing; Writing motivation; Writing performance; Adolescent students

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24420 | Motivation and Emotion in Sport: Relationships Between Motivational Climate, Competitive Anxiety, and Coping Strategies in Acrobatic Gymnasts

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Background & Aim: Motivational climate has been associated with athletes' psychological well-being in sport, namely competitive anxiety. However, the mechanisms underlying this association, particularly the possible mediating role of coping strategies, remain insufficiently explored. Therefore, this study aimed to examine whether coping strategies mediate the relationship between perceived motivational climate and competitive anxiety in acrobatic gymnastics, a sport with unique cross-age and technical structure. **Methods:** A cross-sectional design was employed on a sample of 106 Portuguese acrobatic gymnasts (Mage=14.08, SDage=1.99; 90.6% female), which completed self-report measures previously adapted to the Portuguese context (PMCSQ-2, SAS-2, and Brief COPE). Correlational analyses were conducted, followed by mediation models that tested the study's hypothesis. Lastly, moderation analyses were done to explore age and competitive division effects. **Results:** Results partially supported the study's hypothesis as maladaptive coping fully mediated the effect of an ego-oriented climate on competitive anxiety, indicating that these environments exacerbate anxiety by promoting less effective coping. In contrast, adaptive coping did not mediate the association between task-oriented climate and anxiety, suggesting alternative protective mechanisms. Age was proven to moderate the relationship between ego-oriented climate and competitive anxiety, whereas competitive division moderates the association between task-oriented climate and competitive anxiety. **Conclusions:** Findings highlight coping processes as a key explanatory mechanism linking ego-oriented climates and competitive anxiety. Practical implications focus on the reduction of environmental cues that reinforce avoidance and self-blame, and the importance of promoting cooperation and mastery-oriented feedback within training contexts, to support athletes' psychological well-being and performance.

Keywords: Motivational climate, competitive anxiety, coping strategies, acrobatic gymnastics, sports

24509 | Development of the Dyscalculia Assessment Battery of University of Porto

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Background & Aim: Dyscalculia is a specific learning disorder that affects the development of numerical skills and impacts daily functioning. Given the scarcity of standardized assessment tools in Portugal, we developed the *Dyscalculia Assessment Battery of the University of Porto (BAD-UP)*, which includes ten performance-based tasks for children (Number Recognition, Symbolic and Non-Symbolic Magnitude Comparison, Numerical Stroop, Ordinality, Dot Enumeration, Arithmetic, Written and Oral Transcoding, Problem Solving, Rapid Digit Naming and Digit Span) and two questionnaires for parents assessing both their children's and their own learning difficulties. **Methods:** The BAD-UP was administered to 97 second-grade children and their parents (72 mothers, 63 fathers). Children's performance was measured through accuracy and efficiency. The questionnaires were adapted from the Colorado Learning Difficulties Questionnaire and the Adult Arithmetic History Questionnaire. **Results:** Higher performance was found in more automatic tasks, while cognitively demanding tasks revealed greater difficulties. No significant sex differences emerged. Parents' self-reported mathematical difficulties were associated with reported difficulties in their children. The first questionnaire showed good psychometric properties, whereas the second demonstrated weaker but developing validity. **Conclusions:** Overall, the BAD-UP demonstrates good psychometric properties and addresses a critical gap in the national context, where standardized tools for dyscalculia are scarce. By enabling earlier and more precise identification of numerical difficulties, the BAD-UP supports more targeted educational and clinical responses, promotes children's academic, emotional well-being and provides evidence that can inform public policies aimed at inclusive education as well as equitable access to learning opportunities.

Keywords: Dyscalculia; Psychological Assessment; Assessment Questionnaires; Children; Parents.

24793 | Emerging Adults' Social Media Use: Exploring the protective role of Self-Efficacy, Critical Thinking and Digital Literacy.

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Background & Aim: Social media has become ubiquitous in our lives, yet excessive use has been linked to problematic patterns and negative mental health outcomes (Shannon et al., 2022). While risk factors have been extensively studied, less attention has been paid to protective factors. This study examined whether self-efficacy, critical thinking, and digital literacy protect against problematic social media use (PSMU) across five dimensions: preference for online interaction, mood regulation, cognitive preoccupation, compulsive use, and negative outcomes. We also investigated whether digital literacy moderates the protective links of self-efficacy and critical thinking. **Methods:** A cross-sectional study was conducted with 311 emerging adults (*M*=23.2 years, *SD*=3.6; 65.9% female). Participants completed online self-report measures assessing PSMU dimensions and the three independent variables. Structural equation modelling tested direct associations and moderation link. **Results:** Self-efficacy was negatively associated with negative outcomes (β=-.13, *p*=.049). Critical thinking was negatively associated with cognitive preoccupation (β=-.24, *p*<.001) and compulsive use (β=-.19, *p*<.001). Digital literacy significantly moderated the self-efficacy-negative outcomes relationship (β=-.15, *p*=.008), with protective link strongest at high digital literacy levels. Earlier social media initiation was linked to a higher use of platforms for mood regulation (β=-.17, *p*=.014). **Conclusions:** Findings showed PSMU's multidimensional nature and revealed that the three independent variables target distinct problematic use dimensions. Digital literacy functions primarily as an enabler that amplifies self-efficacy's protective capacity rather than as an independent protective factor. Prevention programs should integrate digital literacy education with self-efficacy and critical thinking development, address early social media exposure, and adopt multidimensional approaches tailored to specific PSMU dimensions.

Keywords: Problematic use, social media, self-efficacy, critical thinking, digital literacy.

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24822 | Oleksiy, the concrete trapeze artist: Mental health and immigration

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Background & Aim: What if we combine a literary text with an academic text? This was the proposal carried out in the "Escrita e Ciência Psicológica" for a first degree in psychology, by making connections between the content of a short story and a scientific article. Migration leads us to attachment theory, migratory grief, institutional void, depression, and suicide. These themes are explored in a literary manner in the short story "Oleksiy, o trapezista do betão", which tells the story of an immigrant from Ukraine who suffers from the distance of his nuclear family, the fear of being forgotten by his children, while waiting for the bureaucratic process to grant him authorization to practice as a doctor in the new country, his untreated clinical depression, and ultimately, his suicide, as well as the loss of his principles and life itself. At the end of the course, with the proposal of the EUGLOH project, we worked on literacy in artificial intelligence, reflecting on learning with and without it. **Methods:** The selection of the short story (literary text) was carried out; subsequently, a search was conducted in indexed databases for a scientific article that relates to the theme addressed in the first reading. After that, we identified psychological concepts present in a narrative form. **Results:** The value of this work was to demonstrate how literary genres can serve as a rich source for psychology students to practice their understanding of psychological constructs in texts that are not necessarily academic, while still grounding their discourse in science, facts, and texts within a scientific context. **Conclusions:** We thus conclude that this type of work is an example of how art and science can go hand in hand and serve as a means of exploring abstract concepts in more concrete ways.

Keywords: Mental health; Immigration; Literary text; Scientific text

24893 | Gender and Age Differences in the Division of Household and Caregiving Tasks and Perceived Fairness

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Background & Aim: The division of household and caregiving tasks remains a central dimension of gender inequality within families that may influence individuals' perceptions of fairness and psychological adjustment. While contemporary narratives promote gender equality, it is unclear whether these changes have translated into more balanced family practices. This study aims to analyze gender and age group differences in the distribution of paid work, household labor and caregiving tasks, as well as associated perceptions of fairness. **Methods:** A questionnaire was administered to a stratified sample of 871 participants, (53.5% women and 46.5% men). Most participants were married (42.7%) and 66.6% reported having children. Regarding age distribution, 21.4% were young adults (18-34 years), 35.1% were adults (35-54 years), and 43.5% were older adults (55+ years). Participants reported their involvement in household chores, childcare tasks and paid professional work. Perceptions of fairness regarding the division of these activities were also evaluated. **Results:** Significant gender differences were found in most household and caregiving tasks: women reported significantly more involvement in these tasks, whereas men reported higher involvement in household repairs and maintenance tasks. No gender differences were found regarding paid work. Age differences were observed in most domestic tasks, except for childcare activities. Women aged 55+ showed the highest involvement in house cleaning. Overall, women perceive the division of tasks as less fair than men. **Conclusions:** These findings highlight persistent gender and age inequalities in task division as well as enduring differences in the perceptions of fairness. Understanding these patterns is important for research on family dynamics, gender attitudes and well-being.

Keywords: Gender differences; Household labor; Caregiving tasks; Perceived fairness; Family well-being

24941 | Making sense of nonsense: Is there a link between illusory perception and paranormal beliefs?

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Background & Aim: Our brain is not a passive receiver of information. It tries to decipher patterns from fragmented input so that we can anticipate and act more effectively in the world. This process is usually adaptive but can also lead to perceptual illusions. Previous research suggests that a bias to detect signals in ambiguous sensory stimuli may underlie the tendency to believe in paranormal events (e.g., Seymor et al., 2022). The present study aimed to replicate Seymor et al. (2022) by assessing whether the tendency to detect faces in ambiguous images is correlated with paranormal beliefs. **Methods:** Participants (N = 166; 114 women, 51 men; mean age = 35 years) were recruited via social media to complete a 10-min online study examining face-detection ability. Mooney faces (n = 30) and control black-and-white images (n = 30) were randomly presented. After each image, participants indicated whether it contained a face. At the end, participants answered four questions about paranormal beliefs using a 6-point Likert scale and demographic questions. **Results:** Figure 1 shows the distribution of performance in the face detection task (Panel A), the variability of paranormal belief scores among participants (Panel B), and their correlation (Panel C). Face detection (M = 0.756, SD = 0.101) was well above chance. A paranormal belief score was created by summing responses across the four items (M = 11.325, SD = 4.453). Against our hypothesis, the correlation between performance and paranormal beliefs was non-significant and close to zero (r = −0.064, p = .413). **Conclusions:** The current study failed to replicate Seymour et al (2022) despite having more than double their sample size. These findings suggest that tendency for illusory perception does not necessarily predict the tendency to hold paranormal beliefs.

Keywords: Mooney faces; Facial pareidolia; Paranormal beliefs; Face detection; Replication

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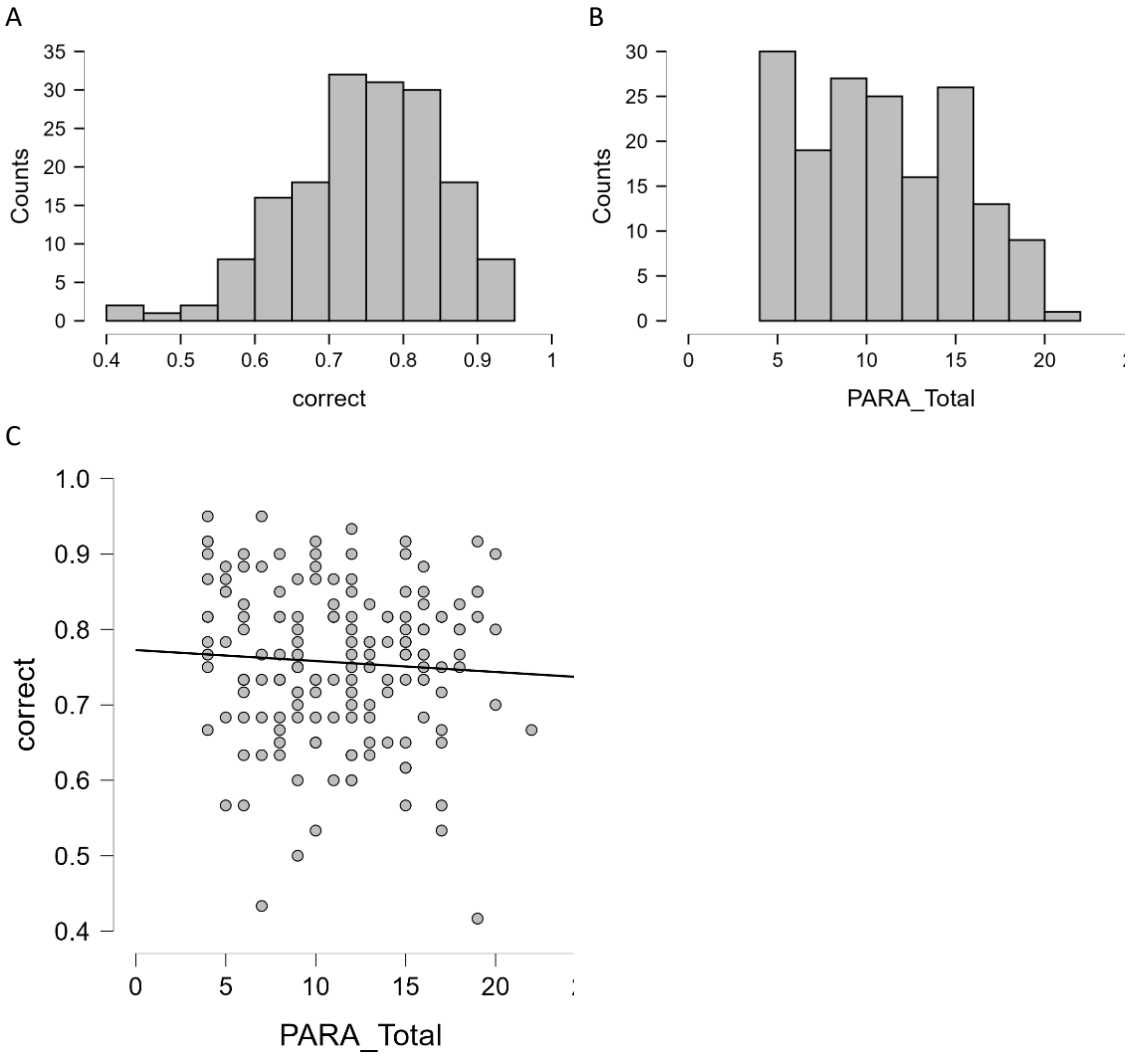

Figure 1: Panel A shows the distribution of scores in the face-detection task across participants. Panel B shows the distribution of paranormal scores (min 4; max = 24) among participants. Panel C shows the correlation between face-detection accuracy and paranormal scores. If a bias to detect faces in ambiguous stimuli was associated with paranormal beliefs, a negative correlation between these variables was expected.

SPORT SCIENCES

24226 | The Relative Age Effect in Portuguese Women's National Football Teams: Associations with Age Groups, Playing Position, And Playing Time

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Background & Aim: Despite being extensively studied over recent decades, the Relative Age Effect (RAE) continues to influence talent identification and development models, particularly in team sports[1]. Given the growing relevance of women's football and limited evidence[2], this study aimed to analyse the presence and evolution of the RAE in Portuguese women's football, focusing on players selected for national teams across different competitive age groups. **Methods:** The study was conducted using open-access data from the Portuguese Football Federation and comprising the historical records of female players with international appearances from U15 to the Senior National Team. Birth dates were grouped into quartiles to assess the RAE. Chi-square tests and Odds Ratios (95% CI) were applied to examine quartile distributions, complemented by analyses stratified by playing position. Binomial and Poisson regression models were used to evaluate the association between birth quartiles and minutes played. **Results:** The RAE was most evident in younger age groups, favouring players born in the first quartile of the year. This effect decreased in intermediate categories and reversed at the senior level. Greater vulnerability to the RAE was observed among goalkeepers, defenders, and midfielders, whereas forwards displayed an opposite pattern. Birth quartile did not significantly influence playing time, with competitive level emerging as the main determinant of participation. **Conclusions:** The findings suggest that the RAE functions primarily as an initial selection filter in Portuguese women's football but diminishes with age as performance-related factors gain prominence. These results align with international literature and highlight the importance of implementing mitigating strategies in talent identification and selection processes to promote greater equity and support the sustainable development of women's football, specifically in Portugal.

Keywords: Relative Age Effect; Women's Football; Talent Identification; Talent Development.

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24244 | Do Players' Voices Matter? An Exploratory Study in Angolan Futsal

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Background & Aim: The growth of futsal in Angola highlights the need to better understand coaches' pedagogical practices to inform the design of future coach education programmes. Contemporary sports pedagogy views the coach as a facilitator of learning, capable of promoting meaningful learning through active, reflective, and contextualised methodologies which aligns with a player-centred approach (PCA) (Bowles & O'Dwyer, 2020). Within this framework, listening to players and managing their autonomy in a balanced manner are particularly relevant for their technical, tactical, and human development. Considering the Angolan futsal context, this study aimed to explore why coaches listen to their players and how they incorporate players' opinions in the coaching process. **Methodology:** This exploratory study adopted a qualitative approach grounded on an interpretative paradigm. Data were collected through semi-structured interviews to five Angolan futsal coaches and then analysed through thematic analysis. **Results:** The findings revealed that all coaches considered listening to players to be crucial for understanding their difficulties in both training and competition. Coaches reported that players' insights helped them introduce new tactical solutions and encouraged players to take greater responsibility for their own learning and training processes. **Conclusions:** Data interpretation suggested that coaches adopt a PCA, representing a pedagogical paradigm shift in Angolan futsal context. Coaches demonstrated a more humanistic and critical pedagogical orientation, focused on the development of active, aware, and competent individuals both within and beyond the competitive environment. However, coaches also reported difficulties in deliberately creating opportunities for players to express their views. From a practical perspective, these findings support the development of future coach education programmes that emphasise what and how pedagogical strategies can foster players' voice.

Keywords: Coach Education; Sports Pedagogy; Player-centered Approach; Exploratory Research; Futsal

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24265 | Immigrant Students' Perceptions of their Inclusion in Physical Education: A Qualitative Case Study

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Background & Aim: Research-informed reflective practice is central to contemporary teacher professional development, particularly within culturally diverse educational contexts. Developed as part of a supervised teaching internship, this study aimed to explore immigrant students' perceptions of their inclusion in Physical Education (PE), examining how pedagogical practices shaped experiences of participation, belonging and equity. **Methods:** A qualitative case study was conducted in a Portuguese lower secondary school located in a socioeconomically vulnerable area. Six immigrant students participating in PE classes were invited to take part in semi-structured interviews designed to elicit in-depth accounts of their experiences. Data were analysed using thematic analysis procedures. **Results:** Participants generally described PE as positive and engaging; however, their sense of inclusion was influenced by interconnected factors. The pedagogical relationship with the teacher emerged as central, particularly regarding clarity of instruction, emotional support and sensitivity to cultural differences. Peer dynamics significantly affected participation, either facilitating integration or reinforcing marginalisation. The diversity of activities and cooperative tasks enhanced engagement, whereas language barriers and limited instructional adaptation occasionally constrained meaningful involvement. Students emphasised that inclusion extends beyond physical presence, requiring recognition, active participation and a genuine sense of belonging. **Conclusions:** Inclusive PE depends on intentional pedagogical strategies grounded in intercultural competence, relational awareness and differentiated instruction. Conducting practitioner research strengthened reflective capacity and deepened professional understanding of diversity-responsive teaching. The findings highlight inquiry-based practice as a catalyst for professional growth and more equitable educational environments.

Keywords: Physical Education; Inclusion; Immigrant Students; Intercultural Education; Pedagogical Practices

24288 | Dual Career Experiences of Portuguese Youth National Team Football Players: A Mixed-Methods Study of Perceived Pedagogical Support and Structural Barriers

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Background & Aim: The dual career framework, strongly promoted by the European Commission, emphasizes the need to balance high-performance sport and education. In football, however, structural constraints often challenge this balance. This study aimed to explore how Portuguese youth national team football players perceive pedagogical strategies and school support in managing their dual careers. **Methods:** A mixed-method exploratory-descriptive design was adopted. Participants were male and female Portuguese football players aged 15–18 years, representing national teams in their respective age groups. Quantitative data were analyzed using descriptive statistics (means and percentages). Open-ended responses were examined through content analysis to identify recurring themes regarding school support, flexibility, and academic adaptation. **Results:** Most participants intended to pursue higher education, with 43.37% aiming to complete a bachelor's degree and 36.14% a master's degree. Although 69.85% reported that schools value their role as student-athletes, 65.06% indicated receiving no specific academic support, and only 18.07% had access to a school mentor. Content analysis revealed institutional efforts to adapt curricula in some cases. Nevertheless, participants reported limited schedule flexibility, excessive workload, insufficient learning materials, and rare opportunities to reschedule assessments. Overall, athletes perceived a lack of understanding of dual career demands. **Conclusions:** Despite strong academic aspirations, Portuguese youth football players perceive insufficient structural and pedagogical support for dual career development. These findings highlight the need for flexible policies, tailored academic resources, and enhanced institutional awareness to ensure sustainable educational pathways alongside elite sport.

Keywords: dual career; youth football; student-athletes; educational support; mixed methods

24297 | Is technical skill performance between 11 and 16 years associated with senior competitive level in basketball?

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Background & Aim: Evidence indicates that technical skills play a crucial role in distinguishing top-level players from their lower-level peers [1,2]. However, it remains unclear whether technical proficiency during early career stages is related to high performance in adulthood. This study aimed to investigate the extent to which skill performance across adolescence is linked to senior competitive level. **Methods:** From the original sample of 293 male basketballers enrolled in the INEX study, the present analysis includes 46 players (age = 12.53 ± 1.15 years; training experience = 5.30 ± 1.93 years at baseline) who were still competing at senior level during the 2025/2026 season. Four technical skills – shooting, passing, control dribble, and slalom dribble – were assessed biannually over three consecutive years. Mean values for each skill were calculated across the assessment period. Senior competitive level was classified according to the male divisions of the Portuguese Basketball Federation: $1^{st}/2^{nd}$ divisions (group 1) and $3^{rd}/4^{th}$ divisions (group 2). Associations were analyzed using ordinal logistic regression. **Results:** Better mean performance in shooting (OR = 1.41; 95% CI: 1.15–1.74; p = 0.001), passing (OR = 1.10; 95% CI: 1.03–1.18; p = 0.004) and control dribble (OR = 0.50; 95% CI: 0.26–0.96; p = 0.037) between ages 11–16 was associated with a greater likelihood of attaining higher competitive senior levels. Slalom dribble also presented a marginal association with the competitive level (OR = 0.45; 95% CI: 0.20–1.00; p = 0.050). **Conclusions:** These findings suggest that technical skills are important prerequisites for attaining high performance in adult basketball. Given that senior-level competition typically relies on shooting efficiency, collective play, and rapid yet intentional dribbling, coaches working with youth players should prioritize the early development of these skills, as they appear to be essential for future success.

Keywords: Technical Skills; Long-term Development; Youth Players; Basketball

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24789 | Multicomponent Training With Dual-Task in Older Adults: a Protocol Study

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Background & Aim: Aging is associated with declines in physical performance and cognition, increasing the risk of functional dependence and dementia. Multicomponent training (MT) is recommended for older adults; however, adding structured dual-task cognitive exercises may enhance motor-cognitive interaction and executive functioning. Here, the aim is to describe the protocol for a multicomponent dual-task intervention study examining its effects on physical and cognitive outcomes in community-dwelling older adults. **Methods:** This two-arm cluster randomized controlled trial involves 72 community-dwelling older adults (83.3% women) recruited from four pre-existing exercise classes. These classes were randomly assigned to either a Multicomponent Training (MT) group (n=37) or a Dual-Task (DT) group (n=35). To minimize potential bias, both interventions follow identical training schedules and exercise structures, differing only in the systematic inclusion of cognitive tasks in the DT group. The intervention will be conducted over 24 weeks, consisting of two 45-minute sessions per week, divided into three progressive mesocycles ranging from moderate (RPE 9–11) to vigorous intensity (RPE 14–17). Specifically, the DT group will perform exercises targeting inhibitory control, cognitive flexibility, and working memory. Outcomes will be assessed at baseline and after 24 weeks. Physical performance will be evaluated using the 6-Minute Walk Test, handgrip strength, 5-time and 30-second sit-to-stand tests, gait speed, and the Timed Up and Go. Executive function will be measured through the Stroop, Corsi Block-Tapping, and Trail Making Tests (A and B). Data will be analyzed using a repeated-measures ANOVA to test for group × time interactions, with $p<0.05$ as the significance level. **Results:** Data collection is ongoing. Both groups are expected to improve physical performance, with greater gains in inhibitory control and mobility-related tasks in the dual-task group. **Conclusions:** Integrating progressive dual-task stimulation into MT may enhance executive and functional outcomes in older adults, supporting autonomy and fall prevention.

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Keywords: Aging; Dual-task; Multicomponent training; Executive function; Functional performance

24787 | Screening Utility of Handgrip Strength for Depression Symptoms in Older Adults

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Background & Aim: Depression is a common disease later in life and is associated with low physical fitness. Handgrip strength (HGS) is a widely used biomarker of functional health in older adults and has been associated with depressive symptoms [1]. Nonetheless, its ability to identify depressive symptoms is not sufficiently explored in the literature. Therefore, this study aimed to evaluate the association between handgrip strength and depressive symptoms, as well as to explore its screening performance in older adults. **Methods:** This is an observational cross-sectional study conducted on 195 older adults (age: 73.6±8.1 years; 87.7% women). Depressive symptoms were assessed with the Geriatric Depression Scale-15 (GDS-15) [2], with GDS-15≥5 indicating the presence of depressive symptoms. HGS was measured according to the American Society of Hand Therapists [3], and is reported as the highest value and allometric scaling by height [4]. Statistical procedures included Spearman correlations, multivariate ANCOVA, and ROC curve analysis. **Results:** The mean highest HGS in the sample was 21.47±5.63, and the mean allometric HGS was 16.06±3.93. Depressive symptoms were negatively correlated with highest HGS ($\rho=-0.158$; $p=0.009$) and allometric HGS ($\rho=-0.150$; $p<0.015$), although the strength of these associations was weak. ROC curve analysis did not demonstrate good discriminative ability for HGS indices (AUCs < 0.80), considering commonly accepted thresholds for acceptable discrimination (AUC ≥ 0.80). Allometric scaling showed high sensitivity (85.5%). **Conclusion:** A negative association was observed between depressive symptoms and both HGS measures, although of weak magnitude. Neither HGS index demonstrated strong discriminative power. Allometric HGS may have potential as a preliminary screening indicator for identifying individuals at risk, but it should not be considered a standalone diagnostic tool.

Keywords: Handgrip strength, Physical fitness, Depression, Allometric scaling

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24345 | Conditional and Coordinative Abilities in Youth Football Athletes Across Elite and Recreational Contexts

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Background & Aim: Differences in training environment and competitive demands during childhood may influence motor performance in youth football [1,2]. Elite programmes generally involve more frequent and structured practice, whereas recreational settings emphasize participation and enjoyment. Previous research indicates that physical and motor capacities vary according to competitive level in youth soccer [3]. However, direct comparisons between elite and recreational settings at early ages remain scarce. Therefore, this study aimed to examine conditional and coordinative abilities in youth football players across elite and recreational contexts. **Methods:** Twenty-six male football players (elite: n = 14; recreational: n = 12), all with ≥3 years of practice, completed standardized tests assessing agility (T-test), sprint speed (40-m), explosive strength (horizontal jump), bilateral coordination (Ladder Test), and reaction time (Two-Choice Response in Motion). Between-group comparisons were conducted using the Mann–Whitney U test ($p \leq 0.05$). **Results:** Significant between-group differences were observed in agility ($p < 0.001$), sprint speed ($p = 0.017$), explosive strength ($p = 0.002$), bilateral coordination ($p = 0.011$), and reaction time (right: $p = 0.002$; left: $p < 0.001$). The elite group demonstrated lower time-based values in agility, sprint speed, and reaction time, alongside higher scores in explosive strength and bilateral coordination. No significant differences were found for reaction time asymmetry ($p = 0.178$). **Conclusions:** Exposure to a more structured and performance-oriented training context appears to be associated with more advanced conditional and coordinative performance profiles in early football development.

Keywords: Youth football; Conditional abilities; Coordinative abilities; Motor performance; Talent identification

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24432 | Differences in Manual and Pedal Dexterity Between Young Female Artistic Gymnasts and Volleyball Players

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Background & Aim: Dexterity represents a fundamental component of motor performance, encompassing speed, precision, and coordination of limb movements [1]. During childhood and early adolescence, sport-specific training may influence neuromuscular development and functional lateralization [2]. Artistic gymnastics and volleyball impose distinct motor demands, potentially leading to differences in manual and pedal dexterity as well as functional asymmetries [3,4]. However, comparative evidence between these modalities in young female athletes remains limited. This study aimed to compare manual and pedal dexterity between artistic gymnasts and volleyball players, examining potential differences between preferred and non-preferred limbs. **Methods:** This study included 36 female athletes (20 artistic gymnasts and 16 volleyball players) aged 10–14 years (12.33 ± 1.20). Manual and pedal dexterity were assessed using the Manual and Pedal Tapping Tests. Participants performed two trials per limb under standardized conditions, with the best performance retained for analysis. Data were analyzed using IBM SPSS Statistics. Normality was assessed with the Shapiro–Wilk test, and between-group and between-limb comparisons were performed using the Mann–Whitney U test. Statistical significance was set at $p \leq 0.05$. **Results:** No statistically significant differences were observed between sports in manual or pedal dexterity ($p > 0.05$). Similarly, no significant differences were found between preferred and non-preferred limbs ($p > 0.05$). Within-sport analyses also revealed no significant functional motor asymmetries. **Conclusions:** Manual and pedal dexterity did not differ between artistic gymnastics and volleyball athletes during early adolescence. Additionally, no relevant functional asymmetries were identified. These findings suggest that, at this developmental stage, sport-specific demands in these modalities may not substantially influence dexterity performance.

Keywords: Manual dexterity; Pedal dexterity; Artistic gymnastics; Volleyball; Functional asymmetry

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24374 | Structural pathways linking sociodemographic characteristics, air pollution exposure, 24-hour movement behaviours, and health-related outcomes in children: An exploratory study using network science.

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Background and Aims: Air pollution exposure is a major global health concern, particularly for children, given their susceptibility to systemic inflammation and oxidative stress. While physical activity is known to benefit health, its interaction with APE and other co-dependant behaviours within a 24-hour continuum remains understudied. Therefore, this study utilizes network science to investigate the structural pathways linking sociodemographic characteristics, individual air pollution exposure, 24-hour movement behaviours, and health-related outcomes in Portuguese primary school children. **Methods:** A pilot cross-sectional exploratory study was conducted with 14 children (aged 8-11) from Valongo, Portugal. Sociodemographic data and household characteristics were assessed via questionnaire. Individual APE ($PM_{2.5}$, PM_{10}, and CO_2) was measured using portable sensors over 48 hours, while movement behaviours were assessed via accelerometry over five weekdays. Health outcomes included cardiorespiratory fitness, body mass index, total oxidant status, and total antioxidant status. An extended structural impact model was employed for data analysis in Python (3.14). **Results:** Sociodemographic factors exerted the strongest impact on movement behaviours (normalized impact = 0.96). Movement behaviours emerged as a partial structural bottleneck, mediating the impact of environmental exposure on health outcomes (indirect impact = 0.66) more significantly than the direct environmental-health pathway (impact = 0.43). **Conclusion:** 24-hour movement behaviours presented a mediating impact on the relationship between air pollutants and health-related outcomes in the evaluated children.

Keywords: Air pollution exposure; 24-hour movement behaviours, Network science, Cardiorespiratory fitness; Oxidative stress.

24457 | Relationship Between Napping and Working Memory Performance in Preschool Children

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Background & Aim: Daytime sleep is recognized as a facilitator of memory consolidation and information processing efficiency in early childhood. Recent studies suggest that the cessation of napping reflects hippocampal maturation and information processing efficiency. However, literature still presents gaps regarding how the persistence or absence of napping specifically influences working memory across varied developmental contexts. This study aimed to investigate the influence of napping habits on working memory performance in preschool children. **Methods:** A total of 8 preschools in the Porto district (4 rural and 4 urban) were conveniently invited to participate in this study. All children aged 3–4 years whose parents provided consent were included, totaling 120 participants (62 boys; 3.64 ± 0.4 years old). To measure nap duration, the Actigraph GT3X accelerometer was used, with data processing conducted via ActiLife software. Sample was categorized into those who do or do not nap. Working memory was measured using the Mr. Ant test. Simple linear regression analysis was conducted using SPSS (version 29.0.2.0(20)). **Results:** The analysis highlights that child naps did associate Mr. Ant scores (***F*** (1, 100) = 2.023$; p = 0.158$). The coefficient of determination (***R^2*** = 0.020) indicated that only 2% of the variance in the dependent variable was explained by the proposed model. An unstandardized coefficient of ***B*** = -0.254 was observed, suggesting a negative relationship trend. **Conclusions:** These preliminary findings suggest that napping status did not predict Mr. Ant scores. Other unanalyzed factors may exert a greater influence on the outcome studied.

Keywords: Working memory; napping; preschoolers.

24607 | Effects of laps and disciplines on Supertri E performance

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Background & Aim: Supertri E is a recent triathlon race format and has attracted growing interest. It requires high-intensity efforts across continuous laps of pool swimming, virtual cycling and running. While conventional triathlon performance has been studied [1], this format remains unexplored. This study aimed to understand whether triathletes' performance varies across laps and within each discipline of Supertri E. **Methods:** 11 competitive male triathletes (21.0±2.9 years; 71.2±8.9 kg of body mass) performed a non-official race of three laps (L) of 200m swim (pool), 4km bike (turbo trainer) and 1km run (treadmill). Performance time (s) was recorded using a stopwatch, excluding transitions. A linear mixed-effects model (LMM) was employed and Cohen's d was used as an effect size measure. **Results:** The mean performance in L1, L2 and L3 was 830.4±85.4, 877.6±98.1 and 862.1±82.4 s, respectively. Performance differed across laps ($F(2,20)=10.72$, $p=0.001$). L1 was faster than L2 (β=-47.20, $p=0.001$, d=0.74), while L2 did not differ from L3 (β=15.56). For each discipline, performance in swim (L1: 169.5±9.2 s, L2: 182.9±9.2 s, L3: 185.2±9.2 s; $F(2,20)=26.65$, $p<0.001$), bike (L1: 393.2±28.6 s, L2: 416.9±35.7 s, L3: 413.2±29.0 s; $F(2,20)=7.06$, $p=0.005$) and run (L1: 267.7±12.8 s, L2: 277.7±12.8 s, L3: 263.7±12.8 s; $F(2,20)=5.46$, $p=0.013$) changed throughout laps. In swim and bike, L1 was faster than L2 (swim: β=-13.39, $p<0.001$, d=1.45; bike: β=-23.75, $p=0.007$, d=0.73), but no difference was found between L2 and L3 (swim: β=2.29; bike: β=3.80). For run, while L2 and L3 differed (β=14.03, $p=0.013$, d=1.10), no difference was found between L1 and L2 (β=-10.05; d=0.79). **Conclusions:** Performance in Supertri E seems to change across laps. Swim and bike performance declined from L1 to L2, while run decreased only from L2 to L3. These findings highlight the effects of laps and discipline on fatigue-related performance changes. The slower L2 may also reflect poor race strategy management, possibly related to the triathletes' competitive level, which not allow to generalize results to higher competitive levels.

Keywords: E-sports; Triathlon; Performance; Laps; Disciplines

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24676 | Comparison of Static and Dynamic Balance in Female Gymnasts from Different Disciplines (Artistic, Rhythmic, and Trampoline)

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Background & Aim: Balance is a fundamental motor capacity in gymnastics, contributing to technical execution and injury prevention. Given the discipline-specific demands of Olympic gymnastics, balance performance may vary according to training characteristics [1,2]. This study aimed to compare static and dynamic balance among female gymnasts from Artistic, Rhythmic, and Trampoline Gymnastics, and to examine inter-limb asymmetry and associations between preferred and non-preferred limb performance. **Methods:** Thirty female gymnasts (n = 10 per discipline), aged 8–12 years, were included. Static balance was assessed using the Flamingo Test, and dynamic balance using the Y Balance Test (normalized composite score). Inter-limb asymmetry indices were calculated. Differences between disciplines were analyzed using the Kruskal–Wallis test and Mann–Whitney pairwise comparisons. Associations between limbs were examined using Spearman's correlation. Statistical significance was set at $p \leq 0.05$. **Results:** Significant differences were observed in static balance for the preferred ($p < 0.001$) and non-preferred limb ($p = 0.003$), with Artistic Gymnastics demonstrating higher values, followed by Rhythmic and Trampoline Gymnastics. In dynamic balance, significant differences were found for both limbs ($p < 0.001$), with Artistic and Rhythmic Gymnastics showing higher scores than Trampoline Gymnastics. No significant differences were observed in inter-limb asymmetry. Correlation analyses revealed strong positive associations between preferred and non-preferred limbs in both static balance ($r = 0.785$, $p < 0.001$) and dynamic balance ($r = 0.881$, $p < 0.001$). **Conclusions:** Balance performance varies across gymnastics disciplines, likely reflecting training specificity. Artistic Gymnastics exhibited higher static balance, while Artistic and Rhythmic Gymnastics showed higher dynamic balance than Trampoline Gymnastics. No differences were observed in inter-limb asymmetry between disciplines. These findings may inform discipline-specific training strategies.

Keywords: Static Balance; Dynamic balance; Gymnastics disciplines; Inter-limb asymmetry; Female gymnasts

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24742 | Association Between Dynamic Balance and Agility in Youth Football Players

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Background & Aim: Agility and dynamic balance are key components of motor performance in youth football, where frequent accelerations and changes of direction are required [1]. Dynamic balance may contribute to change-of-direction ability [2], however, evidence regarding this association in young football players, particularly considering sex differences, remains unclear. This study aimed to examine the relationship between dynamic balance and agility in youth football players and to compare performance between boys and girls. **Methods:** Our sample comprised thirty federated youth football players (16 boys, 14 girls; 11.57 ± 0.73 years). Dynamic balance was assessed using the Y-Balance Test (anterior, posteromedial, and posterolateral directions), and agility was measured using the T-Test. The best performance was retained for analysis. Due to non-normal data distribution, between-sex comparisons were performed using the Mann–Whitney U test, and associations were assessed using Spearman's rank correlations ($p < 0.05$). **Results:** Significant sex differences were observed in the anterior ($p < 0.001$) and posterolateral ($p = 0.039$) directions of the Y-Balance Test, with boys demonstrating greater reach distances. No significant differences were found in the posteromedial direction ($p = 0.677$). In the T-Test, boys showed lower mean completion times; however, differences were not statistically significant ($p = 0.183$). Correlation analyses revealed significant negative associations between T-Test time and Y-Balance scores, indicating that greater dynamic balance was associated with better agility performance. **Conclusions:** Dynamic balance is associated with agility performance in youth football players, particularly in anterior and lateral reach components. Although sex differences were observed in specific balance measures, agility performance did not significantly differ between boys and girls. These findings support the inclusion of dynamic balance training within youth football development programs.

Keywords: Dynamic balance; Agility; Motor performance; Youth football players; Sex differences

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24785 | Cognitive Function and Curved Path Walking Performance in Older Adults

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Background & Aim: Curved path walking requires anticipatory postural control, directional adjustments, and cognitive flexibility, making it particularly sensitive to cognitive decline. However, the interaction between curved walking performance and cognitive impairment severity remains insufficiently investigated (1). This study examined whether curved path walking performance differs according to cognitive impairment severity defined by Montreal Cognitive Assessment (MoCA) classification (2). **Methods:** In this cross-sectional study, 358 older adults (mean age = 75.15 years; 70.7% women) were categorized into three groups based on MoCA scores: preserved cognition (PC = 18–25 points), mild cognitive impairment (MCI = 10–17 points), and cognitive impairment (CI = 0–10 points). Curved path walking performance was assessed using the 2.44m Timed Up and Go test. Between-group differences were analyzed using the Kruskal–Wallis test with post-hoc pairwise comparisons. Associations between MoCA scores and walking performance were examined using Spearman correlation. **Results:** Non-parametric analysis revealed significant differences across cognitive impairment groups (Kruskal–Wallis $p < 0.001$), with progressively slower performance as impairment severity increased (PC: median = 5.65 s, IQR = 2.39; MCI: median = 7.66 s, IQR = 4.34; CI: median = 10.35 s, IQR = 8.88). Pairwise comparisons showed significant differences between PC and MCI groups ($p < 0.001$) and between PC and CI groups ($p < 0.001$). The difference between the MCI and CI groups was smaller and not significant. MoCA scores were moderately and negatively correlated with the Timed Up and Go test (Spearman $r = -0.461$; $p < 0.001$). **Conclusions:** Curved-path walking performance closely reflects the severity of cognitive impairment. These findings support the potential of curved walking assessment as a clinically meaningful marker of functional decline in dementia and reinforce its relevance for functional screening across cognitive impairment stages.

Keywords: Curved path walking; Cognitive impairment; Montreal Cognitive Assessment; Timed Up and Go; Functional mobility; Dementia.

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24792 | What drives movement in childhood? The roles of family sport participation, socioeconomic status, and school resources and infrastructure in physical activity and sedentary behavior

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Background & Aim: Creating movement-friendly environments is crucial to improve childhood physical activity (PA) and reduce sedentary behaviors (SB) [1]. Family and school contexts are important for promoting healthy habits [2,3]. This study examines the associations between family sports participation, socioeconomic status (SES), school resources and infrastructure, and PA and SB in schoolchildren. **Methods:** Data are from the REACT project [4], comprising 648 children (357 girls, 6–10 years) across 25 schools. Wrist-worn ActiGraph wGT3X-BT accelerometers measured moderate-to-vigorous PA (MVPA) and SB over seven consecutive days. Questionnaires assessed family variables (income-based socioeconomic status (A (low), B (medium), and C (high)) and child/parental sports participation) and school environments (recess resources and infrastructure). Separate mixed-effects regressions for MVPA and SED were used to account for children nested within schools, adjusting for age and sex. **Results:** Child sports participation was significantly associated with reduced SB (β=-18.62, p=0.028) and increased MVPA (β=4.61, p=0.034). Parental sports participation had no significant effect on MVPA or SB (p>0.05). Regarding SES, a higher status (level C) was inversely associated with MVPA (β=-7.30, p=0.003) compared with the lowest level, but showed no association with SB. In the school environment, obstacle courses uniquely decreased SB (β=-35.43, p=0.004). Furthermore, available recess materials and outdoor sports fields were both effective in decreasing SB (β=-22.19, p=0.049 and β=-29.06, p=0.009, respectively) and increasing MVPA (β=6.93, p=0.002 and β=9.75, p=0.007, respectively). Other school infrastructures, such as indoor spaces, playground equipment, and covered areas, showed no significant associations with MVPA or SB (p>0.05). **Conclusions:** Dynamic school environments and structured sports are fundamental for promoting PA in childhood.

Keywords: Physical Fitness; Physical Activity; Sedentary Behavior; Family Context; School Context

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24813 | Sex-specific associations between explosive and reactive strength and 40 m sprint performance in early adolescence track and field athletes

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Background & Aim: Peak speed requires maximal, explosive, and reactive strength, which are commonly assessed using the squat jump (SJ), countermovement jump (CMJ), and rebound-continuous jump (RJT) testing protocols [1-3]. These qualities involve force–velocity relationships that vary across sprint phases [4,5]. This study examined the relationships between SJ, CMJ, RJT, and 40 m sprint performance in boys and girls (10–13 years), adjusting for age, height, and body mass. **Methods:** This cross-sectional study included 294 athletes (157 girls, 137 boys) from the EXPERT study [6]. Assessments comprised height, body mass, CMJ, SJ, RSI, and 40 m sprint time. Separate multiple linear regression analyses were conducted by sex, with sprint time as the dependent variable, and each strength measure entered into separate models, adjusted for age, height, and body mass. **Results:** In girls, SJ (β=0.023; R^2 =0.338), CMJ (β=0.029; R^2=0.382), and RSI (β=0.302; R^2=0.328) were significantly associated with 40 m sprint performance (p<0.01), explaining 32.8–38.2% of the total variance. Age, height, and body mass were significant across models, indicating a major contribution from growth-related factors. In boys, SJ (β=0.031; R^2=0.528), CMJ (β=0.035; R^2=0.565), and RSI (β=0.488; R^2=0.535) were significantly associated with performance (p<0.001), explaining 52.8–56.5% of the variance. Height was a significant predictor, whereas age and body mass were not. **Conclusions:** Findings showed that performance in all three vertical jumps was significantly related to 40 m sprint performance in both sexes, with stronger associations in boys. However, growth-related variables accounted for the total variance in sprint (height and body mass in girls; height in boys), highlighting that growth is also a key performance factor. Results underscore the need for integrated strength and sprint training during youth development, in both boys and girls.

Keywords: Sprint performance; Explosive strength; Reactive strength index; Adolescence; Sexual dimorphism

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24854 | Anxiety and Perceived Stress in Young Football Athletes

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Background & Aim: Pre-competitive anxiety and perceived stress are psychological factors that may significantly influence young athletes' performance and well-being. According to the cognitive-motivational-relational theory of stress [1], stress arises from person-environment interactions and cognitive appraisal. Previous research suggests that female athletes tend to report higher levels of competitive anxiety, but evidence focusing specifically on young soccer players remains limited. This study aimed to analyse pre-competitive anxiety and perceived stress in young soccer athletes, examining sex differences and associations between these variables. **Methods:** This cross-sectional study included 61 U12 soccer players (36 boys, 25 girls; aged 11.28±0.66), all with a minimum of one year of competitive experience. Pre-competitive anxiety was assessed using the Sport Anxiety Scale-2 [2], and perceived stress using the Perceived Stress Scale-4 [3]. Mann-Whitney tests were used to compare sexes and Spearman correlations examined associations between variables. **Results:** Although female athletes presented higher values across all anxiety subscales, no statistically significant differences were found between sexes in somatic anxiety, cognitive anxiety (worry, concentration disruption), or total anxiety ($p > .05$). Correlation analyses revealed significant positive associations between perceived stress and all anxiety dimensions. The strongest correlation was observed between perceived stress and cognitive anxiety (concentration disruption, $r_s = .630$, $p < .01$). **Conclusions:** Perceived stress was positively associated with pre-competitive anxiety in young soccer players, particularly with cognitive anxiety linked to concentration difficulties. While female athletes reported higher anxiety levels, sex differences were not statistically significant. These findings highlight the importance of psychological support strategies focused on stress and anxiety management in youth soccer.

Keywords: pre-competitive anxiety; perceived stress; youth soccer; sex differences.

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24930 | Intracycle Drag Assessment Method (IDAM): A novel approach to measuring active drag through small and controlled perturbations

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Background & Aim: Several methods to estimate active drag (Da) in swimming have been proposed since the 1970s [1,2], with reported mean values ranging from 20 to 220 N [3]. This study presents a development of the recent Haskins et al. [4] method, termed the Intracycle Drag Assessment Method (IDAM). With IDAM, intracycle variations of active drag are analysed while considering the force required for positive acceleration of the swimmer, its main novelty. The present work provides a proof of concept for this assessment approach. **Methods:** Four competitive swimmers, two national-level and two Olympic, one male and one female in each group, volunteered. Swimmers were attached to a swimming-specific dynamometer (Swim-Spektro, Talamonti Spa, Ascoli Piceno, Italy) and performed one front crawl maximal tethered test, followed by one semi-tethered test using a resistance of 5% of the maximal tethered force. Active drag was estimated as the difference between the force recorded in full tethered and that obtained during the semi-tethered test, plus the force required to positively accelerate the swimmer during each intracyclic phase of increasing velocity. **Results:** A two-peak active drag profile was observed across the swimming cycle, consistent with upper-limb actions, and expected contralateral asymmetries. Active drag values ranged from 93 to 173 N. This range reflects the intentional inclusion of swimmers of different sex and performance level, while IDAM revealed consistent intracycle patterns across participants. **Conclusions:** IDAM may be considered an upgrade of the Haskins et al. [4] method, allowing active drag characterization throughout the entire swimming cycle while accounting for positive acceleration forces. Mean active drag values were high and aligned with the upper range reported in the literature. Although values are estimated, in the absence of a gold standard, the strength of the approach lies in the internal and external coherence of the results.

Keywords: Swimming, Assessment, Tethered, Semi-tethered, Intracycle Active Drag

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24974 | Rethinking Positional Profiles: Mapping Player Competencies in Male Football Through a Scoping Review

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Background & Aim: Understanding the competencies required for different playing positions in football is essential for player development, talent identification and training design[1, 2]. Although research has examined player performance by playing position, the literature remains fragmented and unevenly distributed across performance domains [3, 4]. Most studies focus on physical indicators, whereas tactical, technical and psychological dimensions receive less attention[5]. This scoping review aimed to map the existing literature on tactical, technical, physical and psychological competencies according to playing position in male football players and to identify gaps in the evidence base. **Methods:** A scoping review was conducted following PRISMA-ScR guidelines[6, 7] and a preregistered protocol in the Open Science Framework[8]. Searches were performed in Scopus, Web of Science, PubMed, SPORTDiscus and LILACS. Studies including male football players from youth to adult levels (U15–senior), classified according to the Participant Classification Framework (Tier system)[9], were eligible. **Results:** The search yielded 21,590 records, of which 14,434 remained after duplicate removal. After screening, 237 studies met the inclusion criteria(Fig.1). Research was strongly dominated by studies examining physical performance indicators in senior elite players. Defenders and goalkeepers accounted for most positional analyses, while forwards were rarely investigated in isolation. Tactical and technical competencies were less frequently explored, and psychological attributes were rarely investigated. Evidence was largely concentrated in Tier 3–4 players and senior age groups. **Conclusions:** Evidence on player competencies by playing position in male football is uneven

across domains, positions, and competitive levels. The predominance of physical performance research highlights important gaps requiring integrated and developmental approaches to inform player development, talent identification and training design.

Keywords: football; playing position; player competencies; scoping review; talent

Acknowledgments:

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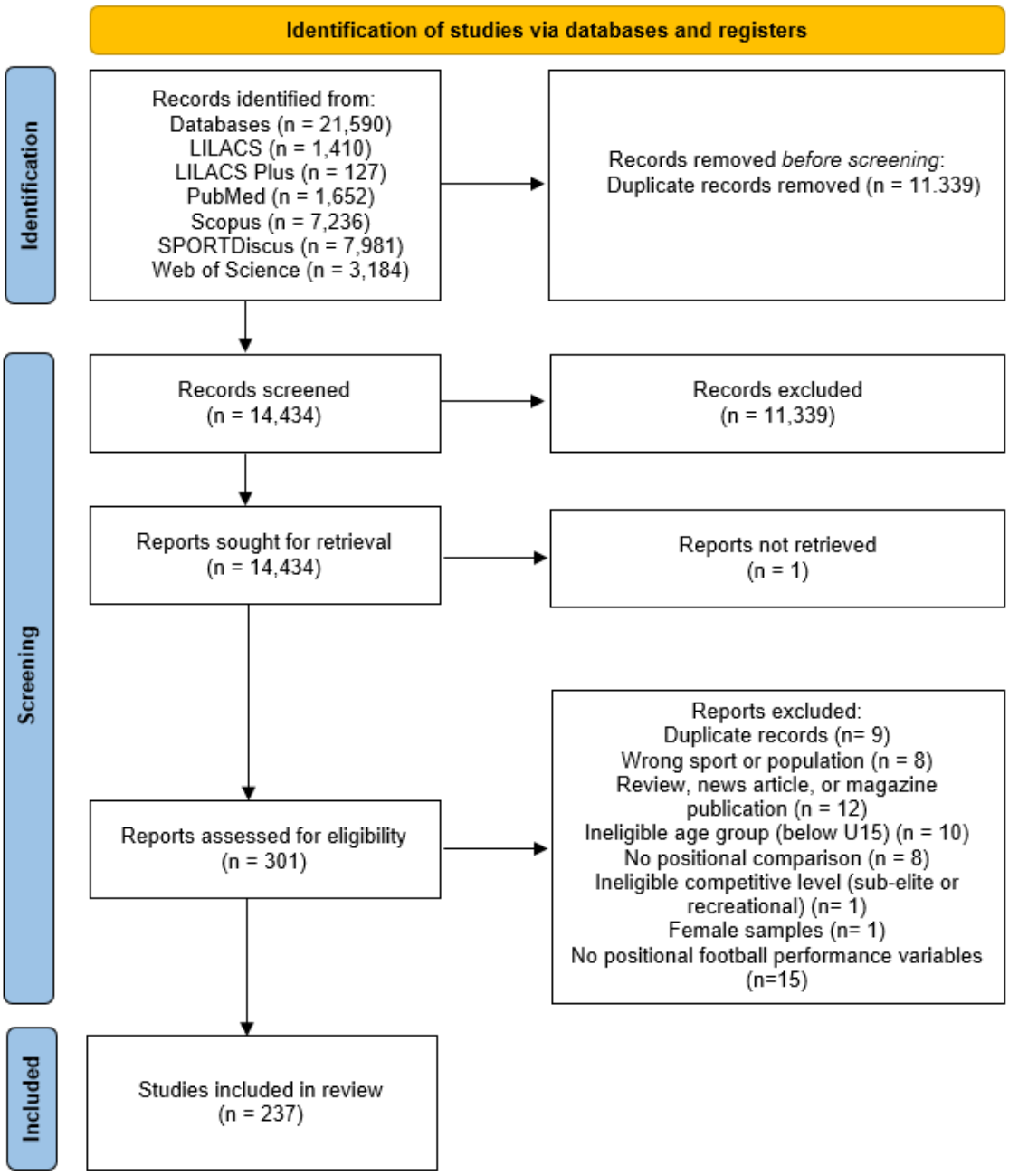

Figure 1: PRISMA flow chart outlining study inclusion process

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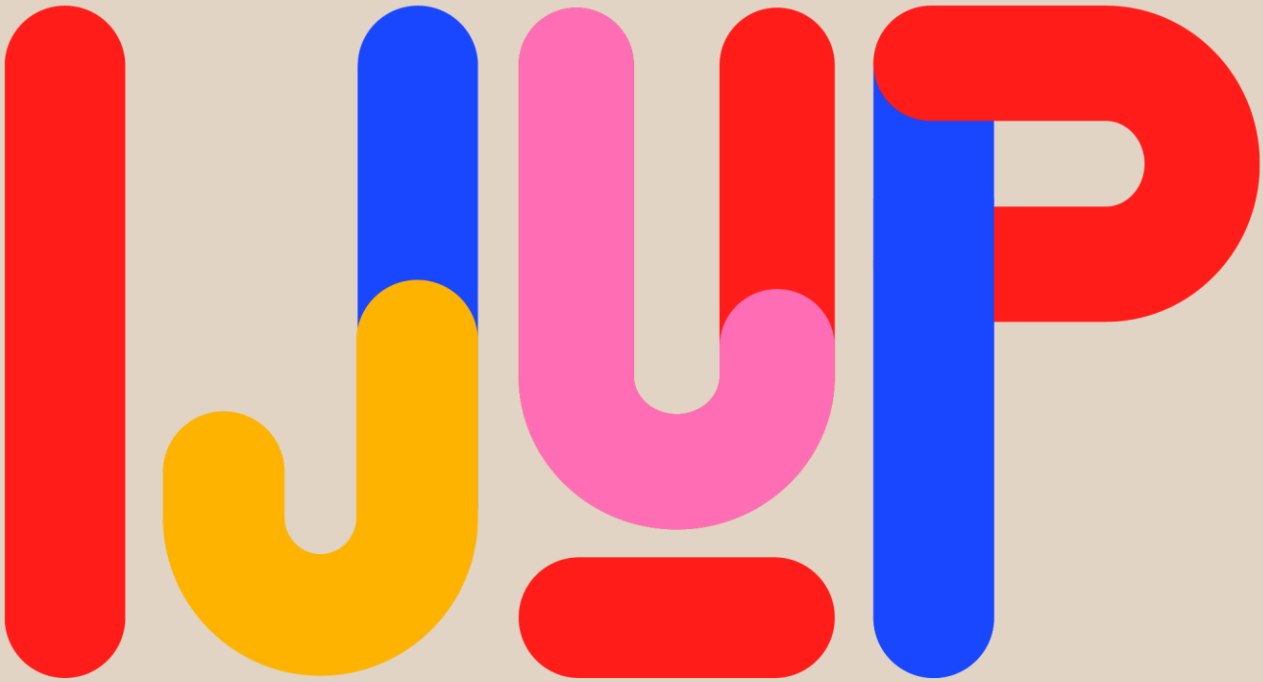

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24212 | Agr@TecVerde Project: Promoting Environmental Protection through Sustainable Agriculture

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Background & Aim: Sustainable agriculture is essential to ensure food production while minimizing environmental impacts on soil and aquatic ecosystems. However, concepts such as nutrient leaching and integrated food production systems are often difficult for students to fully understand through theory alone. This study evaluated the use of hands-on experimental microcosms to enhance secondary school students' understanding of sustainable agriculture, with a focus on nutrient leaching and aquaponics. **Methods**: Two low-cost experimental microcosms were developed. The leaching microcosm simulated different soil management practices (bare soil, cultivated soil, and cultivated soil with fertilizer) and their effects on water quality and aquatic organisms (*Daphnia magna* and *Danio rerio*). The aquaponics microcosm illustrated the integration of fish, plants, and water in a closed-loop production system. Both experiments were implemented during interactive science sessions, and students completed identical pre- and post-activity questionnaires assessing their knowledge of agronomy, sustainable agriculture, nutrient leaching, and aquaponics. Learning gains were evaluated by comparing responses before and after the activities. **Results**: Students demonstrated improved understanding following the activities. While baseline knowledge of general sustainable agriculture concepts was high (>90%), the hands-on microcosms significantly enhanced comprehension of specific topics. Correct responses regarding aquaponics components increased from 43% to 78%, understanding of plant functions from 42% to 69%, and knowledge of nutrient leaching from 71% to 85%. **Conclusions**: Experimental microcosms enhance strengthen students' understanding of sustainable agriculture, particularly complex processes. By linking agricultural practices to water quality and ecosystem responses, these activities promote experiential learning, environmental awareness, and offer a low-cost, easily replicable approach for sustainability education.

Keywords: Agronomics; Environmental education; Experimental microcosms; Leaching; Aquaponics

Acknowledgments: This work was developed within the Agro@TecVerde project and benefited from the collaboration of teachers, students, and facilitators involved in the experimental sessions.

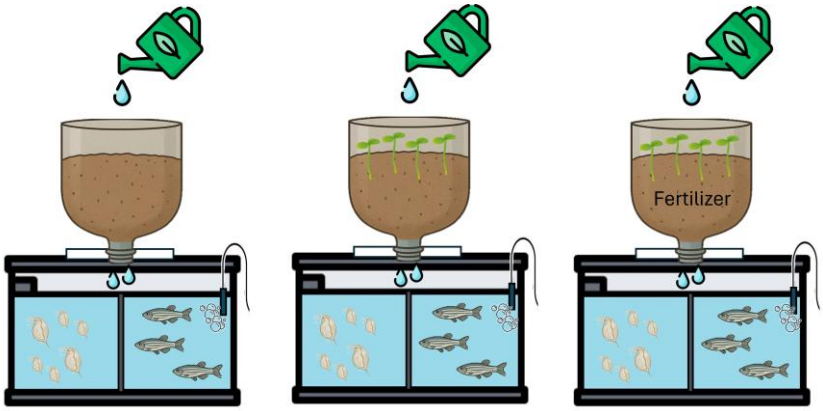

Figure 1: Leaching Experiment

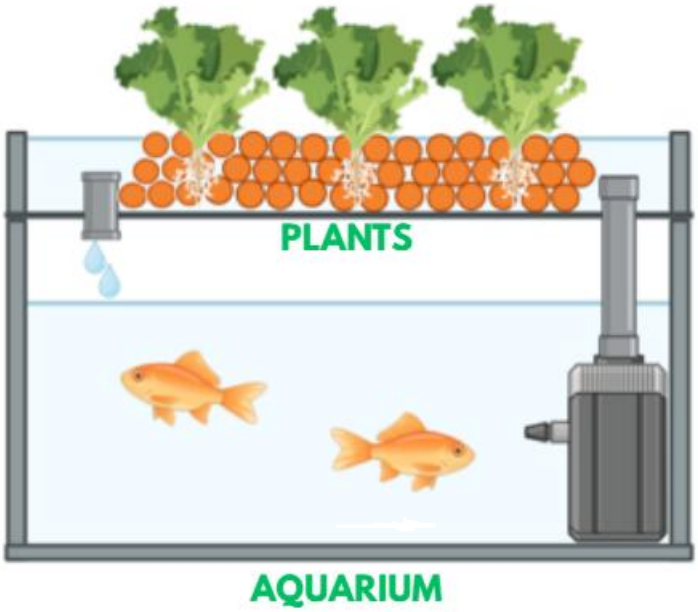

Figure 2: Aquaponics experiment

24547 | Assessment of Thermal Imaging as a Tool to Monitor Heat Stress in Dairy Cows- Preliminary results

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Background & Aim: Heat stress negatively affects dairy cow welfare and productivity, leading to physiological imbalances and economic losses in livestock systems [1, 2]. Traditional measurements of core body temperature, such as rumen or rectal monitoring, are often invasive and labor-intensive. Infrared thermography has emerged as a non-invasive technique to assess surface temperature changes related to thermal stress, particularly in the ocular region [3]**.** This study aims to evaluate the potential of thermal imaging to monitor heat stress in dairy cows and explore its relationship with physiological and environmental parameters. **Methods:** A preliminary dataset was collected from four dairy cows, including ruminal temperature, rectal temperature, and eye surface temperature obtained through thermal imaging. Environmental conditions such as ambient temperature (*T*, in °C) and relative humidity (*RH*, in %) were recorded and used to calculate the Temperature-Humidity Index (*THI*) as an indicator of heat stress, according to equation 1 [4].

$$THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH)(1.8 \times T - 26) \quad \text{Eq. 1}$$

The procedure for calculating the inner canthus temperature was optimized to reduce discrepancies between the area delineated in the reference (non-infrared) image and the corresponding area identified in the infrared image. A multivariable correlation analysis was conducted using Pearson's product–moment correlation coefficient. **Results:** Eye temperature was moderately correlated with rectal temperature and strongly correlated with ruminal temperature. The latter also showed strong positive associations with ambient temperature and the *THI* index. **Conclusions:** Infrared thermography shows promise as a non-invasive tool for monitoring heat stress in dairy cows. However, some variables were based on very small sample sizes, so these findings should be considered preliminary and confirmed with additional analyses.

Keywords: Heat stress; Dairy cows; Infrared thermography; Eye temperature; Animal welfare

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24570 | Biofortification of microgreens with zinc, selenium, iodine and magnesium: efficacy and optimization of the 'nutri-priming' approach

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Background & Aim: Nutrient deficiencies remain a major global nutritional challenge, impairing immune competence, cellular homeostasis, and regulation of oxidative stress. Agronomic biofortification is a sustainable strategy to enhance micronutrient levels in edible plant organs, particularly in horticultural crops. In controlled environments, such as vertical farming systems, seed nutri-priming can be a tool to improve mineral accumulation and quality, yet studies are scarce. Within the framework of the R&D project Refarming, co-financed by Norte2030, this study aims to optimize and assess the effectiveness of seed nutri-priming for the biofortification of microgreen with zinc (Zn), iodine (I), magnesium (Mg), and selenium (Se). **Methods:** The experimental design includes three microgreen species/varieties (Mustard Radd, Mustard White, and Pak-choi White) and four concentrations of each mineral. Microgreens were sown in trays (n= 6) and grown in a climate chamber (22± 1 °C; 70% RH; 16 h photoperiod). Seeds were primed with increasing concentrations of $ZnSO_4$, Na_2SeO_4, KI, and $MgSO_4$ to identify optimal doses based on germination, biomass, pigment content, and mineral accumulation. Anthocyanins, total phenolics, antioxidant activity (e.g. DPPH), and mineral content will be evaluated. Data will be analyzed using multifactorial ANOVA, Tukey's test ($p < 0.05$), Pearson correlations, and PCA. **Results:** Microgreens are expected to accumulate micronutrients without compromising seed germinate on, with this biofortification efficiency strategy depending on the species and the concentration of the target mineral. Nutri-priming is anticipated to induce species-specific physiological responses, supporting the development of optimized biofortification protocols for vertical farming systems. **Conclusions:** Based on preliminary results, nutri-priming shows strong potential to improve mineral density and nutritional quality of microgreens in vertical farming, supporting optimized and sustainable biofortification strategies to mitigate micronutrient deficiencies.

Keywords: Mustard Radd; Mustard White; Nutri-priming; Pak-choi; Vertical farming

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Figure 1: Microgreens optimization trial

24291 | Development of thermotolerant portuguese rice: genetic and reproductive analysis

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Background & aims: The increase in global temperature has had serious adverse effects on agricultural production worldwide, with high temperatures particularly affecting plant reproductive processes [1]. *Oryza sativa* (rice) is one of the most important food crops for human consumption. This work focuses on the analysis of the genetic and reproductive performance of four rice varieties cultivated in Portugal (Caravela, Ariete, OP2115 line, and Ceres) under heat stress (HS) conditions. **Methods:** Reproductive and agronomic performance (pollen tube growth, pollen viability, no. of grains, and grain weight) were assessed under different field cultivation conditions, namely flooded irrigation and sprinkler irrigation (HS condition). In parallel, the same rice varieties were grown in growth chambers, either at control (28°CDay/24°C night) and HS (38°C Day/32°C night) conditions. A genetic analysis was conducted focusing on a target gene involved in reproductive heat tolerance in rice, *ACC OXIDASE 1* (*OsACO1*) [2]. To enable a faster and more detailed functional analysis, *Arabidopsis thaliana* orthologs of *OsACO1* (*AtACO1* and *AtACO4*) were studied. T-DNA knockout insertion lines were used, and a double mutant (*aco1aco4*) was obtained. Wild-type and *aco1aco4* plants were exposed to control and HS conditions (38°C for 4h) during the reproductive phase. Reproductive performance was evaluated under both conditions through silique clearing, seed set analysis, and pollen tube growth visualization. **Results:** Preliminary data show reduced pollen deposition under HS compared to the control condition. Under field sprinkler irrigation, Caravela, Ceres, and OP2115 exhibited improved reproductive and agronomic performance. Evaluation of the reproductive performance of *aco1aco4* Arabidopsis plants is ongoing. **Conclusions:** This integrated genetic and reproductive approach provides valuable insights into the mechanisms underlying reproductive heat tolerance in rice, using *Arabidopsis* as a proof of concept.

Keywords: *Arabidopsis thaliana*, *Oryza sativa*, Heat Stress, Rice varieties, Reproductive performance

Acknowledgments:

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24551 | Evaluation of *Chrysoperla carnea* predatory potential for the biological control of Phthorimaea absoluta

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Background & Aim: *Phthorimaea absoluta* (Lepidoptera: Gelechiidae) is a major global tomato pest, with chemical control increasingly compromised by populations' resistance to most active substances. Biological control seems to be a viable and sustainable alternative against *P.absoluta*; however, the role of common generalist predators, as *Chrysoperla carnea* (Neuroptera: Chrysopidae), remains insufficiently validated. This study aimed to evaluate the potential of *C. carnea* as a biological control agent through field co-occurrence assessment and laboratory predation assays. **Methods:** Field co-occurrence was assessed using a 12 m^2 greenhouse placed within a 600 m^2 multispecies flowering meadow. From a total of 24 tomato plants placed inside the greenhouse, half were infested with *P. absoluta* larvae. To record *C. carnea* presence, two samples were conducted bimonthly from June to September 2025: (i) In meadow: four 20 m transects, were sampled using sweep-netting (at 5m linear intervals); (ii) In greenhouse: all plants were observed for counting *C. carnea* occurrences, also the number of *P. absoluta* larvae were registered. Predation was tested under controlled laboratory conditions using sealed arenas (n=10 replicates/treatment). Three prey treatments were evaluated: early-instar *P. absoluta*, late-instar *P. absoluta*, and *Toxoptera citricidus* (positive control). Following a fasting period, individual *C. carnea* larvae were introduced into the arena. After an 18 h exposure period (25 °C, 80% RH, 14L:4D), prey were classified as consumed, dead, or alive. Predator-free controls were included for each treatment to assess prey's natural mortality. **Results:** Preliminary results indicate that *C. carnea* co-occurs with *P. absoluta* on field. Laboratory arena assays demonstrate that *C. carnea* actively preys on *P. absoluta*. **Conclusions:** These findings

support the potential of *C. carnea* as a biological control agent of *P. absoluta,* contributing to the development of integrated pest management strategies.

Keywords: Green lacewing; Sustainable pest management; Tomato leafminer; Trophic interaction; *Tuta absoluta*

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24239 | Expression patterns of eight Hydroxyproline-O-Galactosyltransferases essential for reproductive development in Arabidopsis

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Background & Aim: In angiosperms, during the reproductive process, cell walls play a pivotal role, especially Arabinogalactan-Proteins (AGPs) [1]. AGPs are highly glycosylated proteins decorated by arabinogalactan side chains representing 90% of the AGP molecule [2]. AGP glycosylation is initiated by a reaction catalysed by hydroxyproline-O-galactosyltransferases (Hyp-GALTs), specifically eight of them (GALT2-9), which add the first galactose to Hyp residues [3-5]. Five Hyp-GALTs (GALT2,5-9) were already described as essential for AGP functions in pollen development, ovule development, pollen-pistil interactions and seed morphology [4]. **Methods:** In this study, we obtained one construct for the development of a reporter line through the fusion of yellow fluorescent protein (YFP) with the promoter region of Hyp-GALT4. In parallel, expression patterns of seven Hyp-GALTs were analysed in reproductive tissues of *Arabidopsis thaliana* using previously developed reporter lines *(GALT2:NLS3xYFP, GALT3:NLS3xYFP, GALT5:NLS3xYFP, GALT6:NLS3xYFP, GALT7:NLS3xYFP, GALT8:NLS3xYFP, and GALT9:NLS3xYFP)* and Confocal Laser Scanning Microscopy (CLSM). **Results:** All analysed Hyp-GALTs showed strong expression in reproductive tissues, including female organs (pistil and ovules) and male gametophytes (pollen grains). **Conclusions:** Overall, this work reinforces the crucial importance of AGP carbohydrates in carrying out the biological functions of AGPs during the reproductive process.

Keywords: *Arabidopsis thaliana*; Arabinogalactan Proteins; Galactosyltransferases; Plant reproduction; Expression Patterns

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24615 | From Sugars to HMF: Chemical Changes and Safety Concerns in Commercial Dried Fruits

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Background & Aim: Fruits are essential components of a balanced diet, but their high moisture content makes them highly perishable. Therefore, drying is frequently used to extend shelf life. However, thermal processing can promote non-enzymatic browning reactions, including Maillard and sugar degradation reactions, which may lead to the formation of 5-hydroxymethylfurfural (HMF), a heat-induced compound with potential toxicological effects [1]. Sugar composition, particularly the relative abundance of reducing sugars, strongly influences these reactions. This study aimed to characterize the free sugar profile and HMF content of commercial dried fruits and to explore potential associations between sugar composition, HMF levels, and visual color intensity. **Methods:** Commercial dried apple, pear, and strawberry samples were purchased from local supermarkets in Portugal, ground, and subjected to triple aqueous extraction. Sugars (fructose, glucose, and sucrose) and HMF were quantified by HPLC-ELSD and HPLC-DAD, respectively. **Results:** Sugar profile varied significantly among the fruit types. Fructose predominated in apple and pear samples, whereas sucrose was the main sugar in the strawberry sample. Glucose was present at lower concentrations. A moderate positive correlation was observed between fructose and glucose, while both sugars were negatively correlated with sucrose. HMF content varied substantially across samples and did not consistently correspond to visual color intensity, as some lighter samples showed higher HMF levels. Only weak correlations were observed between individual sugars and HMF, indicating that its formation cannot be explained solely by the initial concentration of a specific sugar. **Conclusions:** The commercial dried fruits differed significantly in their sugar composition and HMF content. HMF formation depends on multiple factors, including fruit type and processing conditions. Visual color intensity alone was not a reliable indicator of HMF formation.

Keywords: Dried fruits; Sugar composition; HMF

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24383 | Molecular Insights from Arabidopsis to Rice: Functional Analysis of Two Candidate Genes Involved in Heat Stress Tolerance

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Background & Aim: Climate change threatens global food security, as rising temperatures and extreme heat events reduce crop survival, yield, and quality. Plant reproductive tissues are highly sensitive to heat stress (HS), and in rice (*Oryza sativa*), this disrupts spikelet development, pollen–pistil interactions, gamete viability, and seed set. To elucidate the molecular mechanisms underlying reproductive HS tolerance, this study uses *Arabidopsis thaliana* as a proof-of-concept model to functionally characterize candidate genes for translation to rice improvement. Candidate genes associated with HS tolerance were identified through RNA-seq analysis of pollinated pistils from the heat-tolerant rice variety Nagina 22 [1]. **Methods:** Two rice genes, *OsMESL* and *OsDMC1* (overexpressed in N22), were selected, and their *Arabidopsis* orthologs (*AtMES11* and *AtDMC1*) were analyzed to investigate their roles in reproductive HS tolerance. T-DNA knock-out and knock-down insertion lines were used: *AtMES11* (SALK_140504C) and *AtDMC1* (SALK_203499C and GK-918E07). Wild-type and mutant plants were exposed to HS conditions (38 °C for 4h) during the reproductive phase, and reproductive performance was evaluated under both control and HS conditions, including silique clearing, seed set analysis, and in vivo pollen tube growth. **Results:** The *dmc1* plants exhibited strong reproductive defects under HS, with reduced silique length and compromised ovule viability, as indicated by callose accumulation in embryo sacs. Analysis of the *mes11* plants is ongoing. In parallel, overexpression constructs were generated using Gateway cloning to assess the effects of increased gene expression on reproductive performance and to complement knock-out phenotypes. **Conclusions:** The results obtained so far highlight the relevance of these genes in reproductive responses to HS. Further functional validation may support their potential use in strategies to improve crop resilience and sustain productivity under HS conditions.

Keywords: *Arabidopsis thaliana*, *Oryza sativa*, Plant Reproduction, Heat Stress

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24648 | Nanoencapsulation of RNA as a biopesticide for controlling crop diseases

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Background & Aim: Climate change is accelerating the global spread of agricultural pests and diseases worldwide, posing serious threats to food production, public health and economic stability [1]. The extensive use of synthetic pesticides has resulted in soil and water contamination, raising serious concerns regarding ecosystem balance and human health [2]. To overcome some of the existing challenges, this project proposes a product based on RNA interference (RNAi), a conserved natural mechanism, which may facilitate regulatory and social acceptance [3]. Despite the growing interest in this approach, the intrinsic RNA instability remains a significant constraint. Therefore, this project aims to develop a biopesticide obtained from the nanoencapsulation of RNAi targeting the fungal plant pathogen *Botrytis cinerea*. **Methods:** Lipid nanoparticles were developed using sustainable compounds and an organic solvent-free approach. An '*in vitro*' assay was performed to evaluate the antifungal activity of generated nanoparticles through determination of fungal colony area (cm^2) on Potato Dextrose Agar (PDA) medium. **Results:** Characterization of nanoparticles demonstrated appropriate physicochemical properties and good stability at different storage temperatures. We are currently conducting '*in vitro*' assays, and preliminary results suggest that the inhibition of *B. cinerea* growth may be influenced by the nanoparticles' shelf life. **Conclusions:** Further studies will be undertaken to evaluate the efficacy of RNAi-loaded nanoparticles in controlling grey mould disease caused by *B. cinerea* once applied to host plants. Overall, this work will contribute to the development of a sustainable strategy to reduce reliance on synthetic pesticides.

Keywords: Biofungicides; Plant pathogens; *Botrytis cinerea*; RNA interference; Sustainability

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24939 | Soil microbial functional diversity as a bioindicator of vineyard management in the Douro Region

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Background & Aim: Vineyard soil microbial communities are shaped by edaphoclimatic conditions and management practices, increasingly recognized as a key component of viticultural *terroir* [1]. Soil bioindicators provide a functional approach to assess microbial activity and evaluate the impact of management strategies on soil health and ecosystem services [2]. This study aims to assess the sensitivity of Biolog EcoPlate™ community-level physiological profiling (CLPP) as a bioindicator, under different management practices. **Methods:** The study was conducted at Quinta do Casal da Granja (Real Companhia Velha, Alijó, Portugal). A vineyard sampling plan was co-developed with growers to evaluate three management practices (1) intensity of plant protection products, compared between two grape varieties with different susceptibility to downy and powdery mildew – Moscatel Galego (more susceptible) and Arinto (less susceptible); (2) type of vegetation cover in the inter-row (spontaneous vs. sown); and (3) period since the last deep soil mobilization in the inter-row space, directly related to vineyard age (older vs. younger vineyards). Soil samples were collected in spring and autumn, and microbial functional diversity was assessed using Biolog EcoPlate™ CLPP. **Results:** Data from both seasons sampling campaigns is available. CLPP integrating average well color development (AWCD), as a measure of the overall metabolic activity of the soil microbial community, substrate richness, and functional diversity indices (Shannon-Wiener) across management practices and seasons are extracted from this analysis. **Conclusions:** These bioindicators are expected to differentiate vineyard plots under different management practices, reflecting variation in metabolic activity and functional diversity of soil microbial communities in response to the different soil management practices. The results allow to evaluate CLPP as a biological indicator for monitoring soil health in viticulture, under integrated production mode.

Keywords: Soil health; Biolog EcoPlate™; Viticulture practices.

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24678 | Sustainable Kombucha formulations using dry and wet coffee husks

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Background & Aim: Kombucha is traditionally produced from *Camellia sinensis* tea using a symbiotic culture of bacteria and yeast. Coffee husks, a by-products from coffee processing, represent a sustainable alternative substrate. This study evaluated targeted phenolic and alkaloid compounds of kombuchas produced from *Coffea arabica* and *Coffea canephora* husks, obtained from the dry and wet coffee processing methods. **Methods:** Kombuchas from coffee husks infusions with black tea (90% + 10%) were fermented for 15 days. Samples collected throughout fermentation (T0, T6, T9, T12 and T15) were analyzed by RP-HPLC-DAD (280 nm) [1]. **Results:** Distinct chromatographic fingerprints were observed between *C. arabica* and *C. canephora* kombuchas, and also between dry- and wet-processed coffee husks. Overall, wet-processed husks yielded kombuchas richer in bioactives, except for chlorogenic acids in *C. canephora*, which were higher with dry husks. The highest caffeine signal was detected in wet *C. canephora* kombucha, while chlorogenic acids were most pronounced in wet *C. arabica* kombuchas. Across fermentation, *C. arabica* showed a consistent decline in caffeine alongside an increase in chlorogenic acids, supported by opposite time correlations. In *C. canephora*, dry-husk kombuchas showed positive time correlations for both caffeine and chlorogenic acids, whereas wet-husk samples showed no clear temporal association. **Conclusions:** Coffee husks from both species are promising substrates for kombucha production, preserving relevant phenolic compounds, and exhibiting fermentation-driven modulation of target bioactives, particularly when produced with husks from wet-processed coffee. These findings support coffee husk valorization for the development of sustainable functional beverages.

Keywords: Kombucha; *Coffea arabica*; *Coffea canephora*; Coffee processing, Phenolic Compounds.

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ARCHITECTURE

24741 | Along the path of the herd. Architecture and communal organization in Covas do Monte

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Background & Aim: Few places across Portugal still preserve the rural identity, traditional ways of life, and community practices that have shaped a significant part of the Portuguese vernacular built heritage. The village of Covas do Monte, located in the Gralheira massif and nestled in the slopes of São Macário mountains, is our case study and one of the remote settlements that has resisted abandonment and total depopulation, with its communal herd standing as a vivid spatial expression of the collective memory it still maintains. We propose a critical analysis of the community dimension in the village's architecture and social-spatial organization, through the cohesive nucleus of traditional buildings and the landscape that unfolds around it. **Methods:** The research methodology focuses on fieldwork using maps, drawings, and photographs, developed from site observations and interactions with the local population. This approach is complemented by relevant bibliography and cartography related to the region's characteristics. The daily path of the goats, grazing communally, was also used as a tool for transitioning between scales and structuring the process. **Results:** This work demonstrates that, in Covas do Monte, spatial organization and patterns of use emerge from the community's everyday collective habits, shaping houses, managing natural resources, and organizing common lands. **Conclusions:** This research reveals that the organization of space in these mountain settlements is not primarily the outcome of formal decisions, but rather an active process through which the community collectively creates, imagines, and continually reshapes housing, the settlement, and the surrounding territory, not just as shared spaces, but as a cultural practice capable of fostering autonomy, identity and to this day continuity. In this sense, understanding the dynamics of Covas do Monte highlights the increasing need to rethink the notions of the common and the collective in contemporary spaces.

Keywords: communal organization; collective memory; territory; traditional architecture; village

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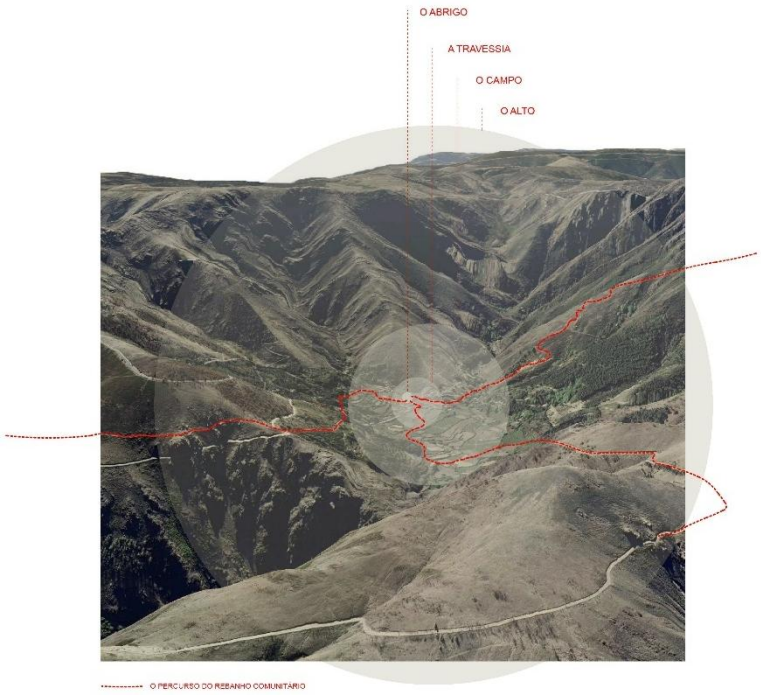

Figure 1: Conceptual scheme of the research structure. Collage developed by the authors over a Google Earth image.

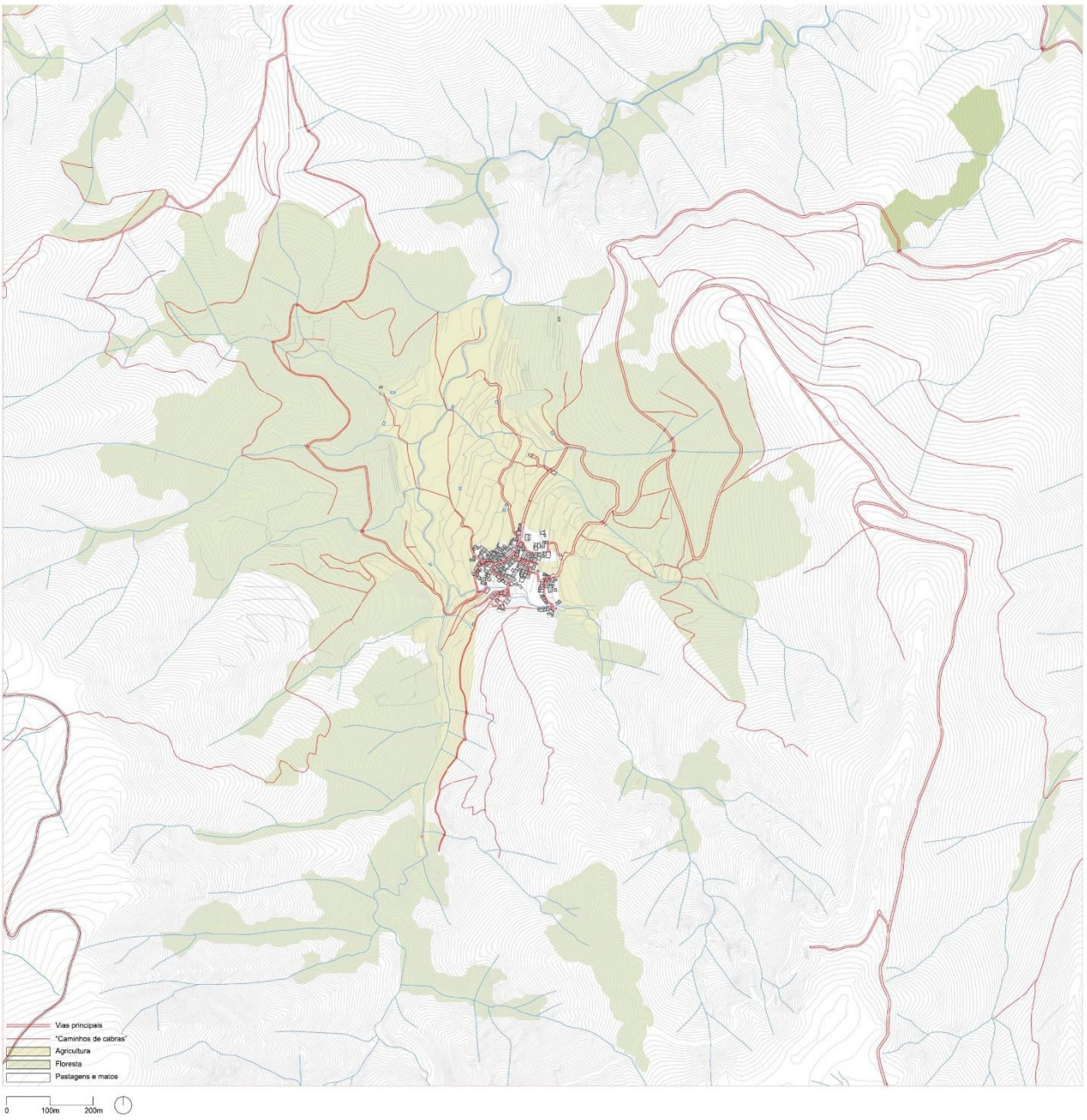

Figure 2: Plan of the Covas do Monte landscape indicating agricultural fields, forest areas, pastures, scrubland, and the paths of the communal herd. Drawing developed by the authors based on cartography provided by the Municipality of São Pedro do Sul.

24625 | Architecture and Therapeutic Experience. Design Principles and Best Practices in Palliative Care and Long-term Care Units

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Background & Aim: Demographic ageing, the increasing incidence of chronic and degenerative diseases, and the worsening of social isolation reflect a scenario of growing vulnerability that has led to a rising demand for more specialized healthcare. In this context, Palliative Care Units and Long-term Care Units gain relevance by integrating, in addition to specific medical care, spatial responses that are coherent with human needs. This study aims to examine these care typologies and contribute to the identification and discussion of architectural strategies that respond to the contemporary challenges of healthcare. **Methods:** A comparative methodology was adopted, based on the analysis of four case studies, two in Portugal and two in the European context, complemented by on-site visits. The research is structured around three key design principles: the interior-exterior relationship, the positive use of circulation spaces, and the potential for transformation and appropriation of space by the patient. These criteria were defined through a review of specialized literature and informed by approaches such as *Evidence-Based Design* and *Psychosocially Supportive Design*. **Results:** The comparative analysis revealed significant differences in architectural approaches. The conscious integration of the identified principles was found not to compromise technical rigour, but rather to enhance the therapeutic quality of the built environment. International examples tend to adopt more holistic and patient-centred strategies, whereas Portuguese models display more institutional characteristics, with scope for evolution towards more flexible and humanised design approaches. **Conclusions:** The structured integration of key design principles enhances patient comfort, wellbeing and autonomy without compromising regulatory demands. Palliative and Long-term Care Units reveal the potential of care-centred architecture to act as an active therapeutic agent, extending its impact across the entire spatial experience of care.

Keywords: Architecture; Health; Healthcare Facilities; Well-being; Recovery

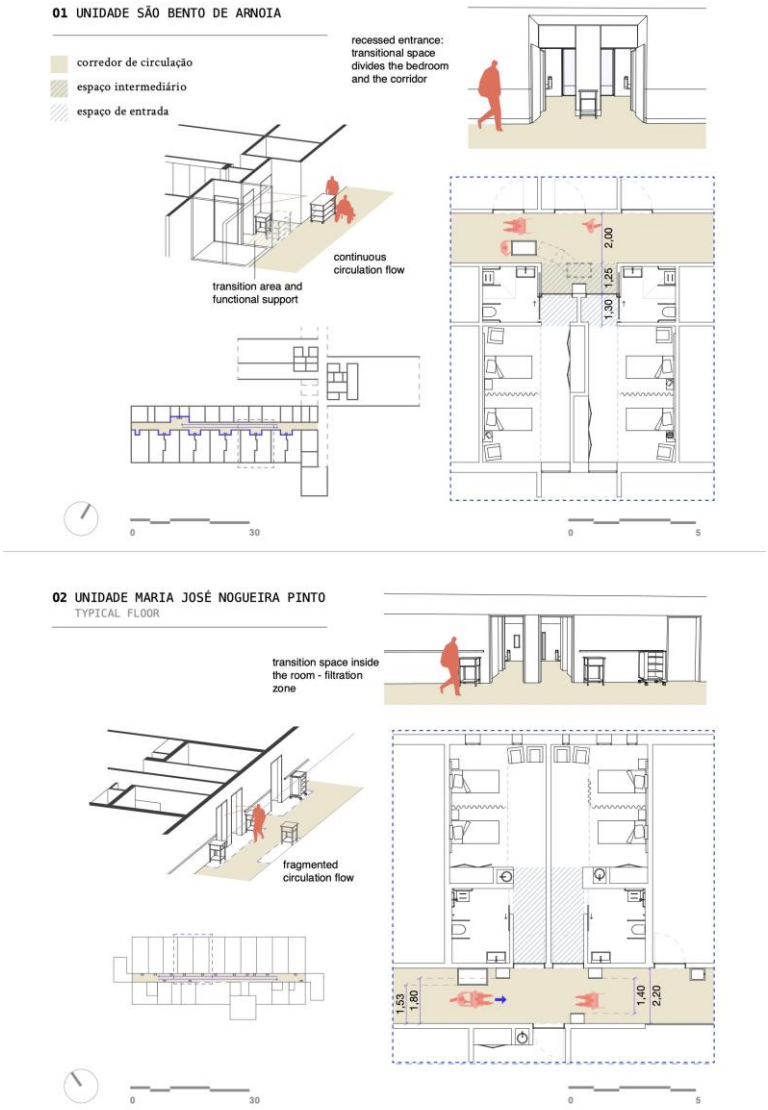

Figure 1: Analysis of solutions for transition between the patient room and circulation space. © Author.

24966 | EXPERIENCES IN ARCHITECTURE BY TÁVORA AND SIZA – (PART III)

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Background & Aim: This communication presents the third phase of an investigation carried out at FAUP. This focuses on the work of Fernando Távora and Álvaro Siza, framing the systematic collection of architecture from different historical periods and distinct styles, in the context of Portuguese interventions. At the same time, a spatial comparison was carried out, using graphic and bibliographic means, of the three case studies authored by each work of the two referenced Portuguese architects. **Methods:** the research focuses on large architectural spaces intended for spiritual enjoyment, exploring the theme of religious architecture based on the works and texts of Fernando Távora and Álvaro Siza, mediated between the modern period and the contemporary era. The aim is to understand how architecture enhances places of gathering and worship, reflecting its historical time, as well as how space, beyond pure architectural conception, is specific to this purpose of living. The objectives consist of studying architectural principles from various studies and the organizational characteristics of spaces, analyzing how they were conceived by reference architects, and identifying relationships between design intention, built form, and type of user experience. **Results** e methodology adopted is based on comparative and critical analysis, using historical reviews, graphic representation, and drawings of projects and works from a specific time period. **Conclusions:** It analyzes how the conception of architectural forms can evoke emotions and sensations, influencing spatial experience and liturgical practices. Styles and architects are addressed, examining key moments in the history of architecture, as well as their intentions and design solutions adopted to achieve intense sensory experiences. The objectives include critical reflection on the creation of harmony in the spatial conception of contemporary religious architecture

Keywords: Religion, Architecture, Siza

24749 | Geopoetics: critical cartography as a representation of urban policy instruments in the Oporto's "Ilhas"

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Background & Aim: Contemporary urban policy reveals a persistent gap between institutional planning instruments and the socio-affective dimensions of everyday life. Despite growing emphasis on participation, technical frameworks still privilege abstract, standardized representations, overlooking lived, perceived and relational space. Grounded in the work of Henri Lefebvre and engaging with critical urban theory (Neil Brenner), this research frames critical cartography as a socio-technical device capable of confronting and rearticulating institutional representations. Focusing on Oporto's "Ilhas", through three Urban Rehabilitation Operations (ORU), it evaluates how socio-affective cartography can mediate between institutional instruments and lived realities, enhancing policy intelligibility and supporting more inclusive interventions. **Methods:** A qualitative, critical approach is adopted, understanding space as socially produced. A multiple case study examines three ORUs, combining document analysis with socio-affective cartography developed through participatory processes with residents and technicians. Cartography is treated as an interdisciplinary socio-technical tool, informed by John F. C. Turner, supported by fieldwork with local initiatives enabling co-produced territorial knowledge. **Results:** Findings reveal a mismatch between institutional representations and residents' perceptions. While ORUs support physical rehabilitation, they neglect lived and relational dimensions. Participatory mapping exposes overlooked needs, spatial conflicts, informal practices and everyday dynamics absent from formal tools. **Conclusions:** Critical cartography helps bridge policy and local realities by integrating socio-affective dimensions. It advances the operationalization of critical urban theory in practice, strengthens participatory approaches, and enhances the intelligibility of housing policies.

Keywords: Critical cartography; Urban policy; Housing; Citizenship

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Figure 1: “Ground mural”, 2025. (photo by Beatriz Freitas)

24673 | Journey to the ''Inquérito'': memories and reenconters (1955-2023). A walk through the Peneda-Gerês National Park

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This dissertation proposes a contemporary revisitation of the *Inquérito à Arquitectura Regional Portuguesa* (1955–1960), reassessing its legacy within current disciplinary debates. Grounded in the hypothesis that travel is a fundamental instrument for architectural knowledge, the research aims to investigate how a direct, embodied encounter with territory can renew knowledge of popular architecture in the Minho region and contribute to a more vital, situated, and contemporary architectural practice.

The study draws upon the travel experiences of Fernando Távora and Le Corbusier to frame travel as a formative tool. Central to the methodology is a six-day pedestrian journey through the Peneda-Gerês National Park, revisiting locations documented in the *Inquérito*. Walking and photography are adopted as primary methodological instruments, enabling observation, documentation, and critical interpretation of transformations in the built environment. Direct dialogue with António Menéres, a key participant in the *Inquérito*, further informed the research.

The enduring value of the *Inquérito* lies not in the preservation of static images of the past, but in its attentive and empathetic engagement with territory. By reactivating travel as a methodological practice, this research contributes to the state of the art by advocating an architectural approach that mediates between memory and transformation, fostering a responsible, territorially grounded practice. The comparison between past documentation and present observation enabled a nuanced reassessment of authenticity, continuity, and adaptation in contemporary rural contexts.

The fieldwork revealed a complex reality: while many vernacular structures have been altered or partially lost, these transformations often reflect legitimate social and economic aspirations. In summary, the research identified both erosion and reinvention, leading to the conclusion that territory is always based on the persistence of cultural patterns within changing material conditions.

Keywords: Travel, *Inquérito*, Vernacular architecture, Memory, Walking

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Figure 1: Vilarinho da Furna Dam, Peneda-Gerês National Park, Portugal. Photograph by the author, 9 October 2023.

24410 | On Culture and Territory: Reactivation Processes at the Convento de Santo Cristo da Barca

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Background &Aim: This study examines the impact of cultural projects on the reactivation of desertified territory in Portugal. The case study, and driver of the design action, is the Convento de Santo Cristo da Barca, in Almeida. By identifying the vulnerabilities of this frequently neglected territory, culture emerges as a catalyst for reactivating the communities' collective memory, through the sustainable rehabilitation and refunctionalisation of architectural heritage which, after years of neglect and underuse, continues to mark the Portuguese interior. The aim is to understand how to align the potential of this place—architecture, culture and community spirit—with the integration and social cohesion of this expectant territory. **Methods:** The methodological approach is multidisciplinary. Resources include the analysis of specific bibliography; the study of projects with similar frameworks developed nationally; and interviews with representatives of the analysed projects and local community agents. Photographic records combined with cartographic and architectural representation will support the Dissertation. An active and direct engagement with the case study is intended, aiming to refine a cultural laboratory proposal suited to the analysed context. **Results:** A site-specific project for the Convento de Santo Cristo da Barca is expected, promoting adaptable uses and functional flexibility. The research carried out in Almeida should ultimately act as a catalyst for the sustainable reuse of other expectant structures in Portugal. This process seeks to contribute to broader debates on cultural policy, territorial equity and heritage-led regeneration in low-density regions. It emphasises long-term sustainability, community participation and replicability. **Conclusions:** Expected results relate to understanding how and to what extent artistic and cultural projects, anchored in proximity-based social structures and a bottom-up logic, can be developed through the reuse of expectant architectural heritage.

Keywords: Culture, Reactivation, Participation, Almeida, Heritage

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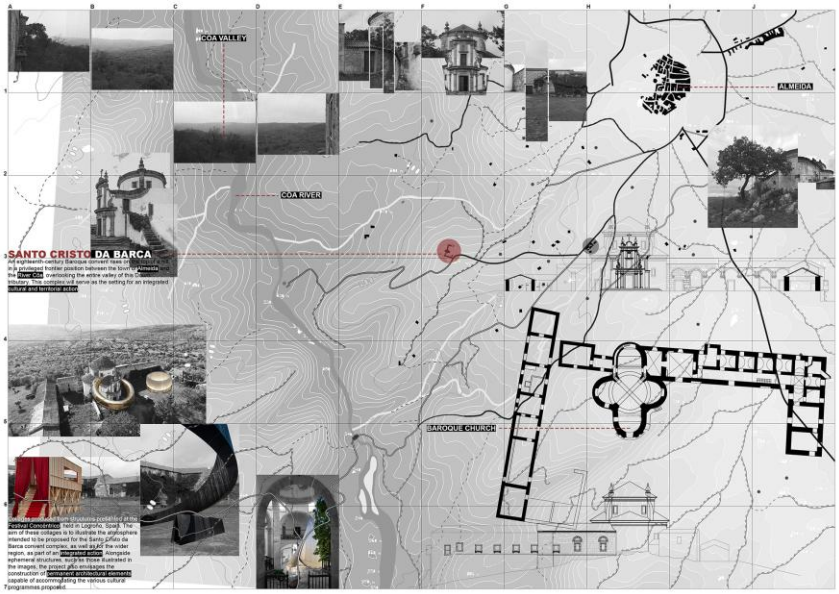

Figure 1: Collage of Santo Cristo da Barca.

24857 | The housing crisis issue: Five Periods

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Background & Aim: Authors such as Bachelard, Pallasmaa, Hertzberger, Forty, Rapoport, etc., prove that housing is a fundamental element in protecting and constructing the self. This urbanisation structure in Portugal represents 90% of building use[1] (80% of urbanised land)[2] and has authors such as M. C. Teixeira[3], N. Portas[4], N. Teotónio Pereira[5], making it possible to define five periods: the industrialisation of the 19th century and the emergence of precarious forms of housing; the Republic (1910) and the undeniable recognition of the housing problem; the "Estado Novo" (1933-1974) and the 'economic houses' and the promotion of ownership; the post-74 democratic transition and investment in public housing; and accession to the EEC (1989) to the present day, highlighting the crisis of the 21st century. The aim is to understand the fifth period from the perspective of architecture in three sub-periods: 1989 to the 2008 crisis, the slight fall in interest rates in 2015, and the current gentrification and continued speculation. **Methods:** Using hybrid statistical analysis methodology (INE, Eurostat, etc.), the decrease in housing construction stands out: from 1.620.106 buildings during the 2nd and 3rd periods to 1.138.816 in the 4th and 110.784 in the 5th [6]. Secondly, we seek to identify the essence of the current crisis (21st century), which shows new indicators of energy poverty (36.1%)[7] overcrowding (11.2%)[8] and an increase in the homeless population (from 1.017 in the AMI in 2002 to 9.403 homeless people in 2024)[9], redefining the concept of deprivation. At the same time, we will analyse the complex network of architectural responses that can be conceived as rentals, cohabitation, credits, etc., using graphical representation of diagrams and critical analysis. **Results:** The research previously initiated presents partial results that require further discussion and critical analysis. **Conclusions:** The conclusion is expected to relate to the current crisis: alongside the reduction in historical physical precariousness, there is the difficulty of access and the poor quality of the building previously built. This can only be addressed based on a complexity of factors.

Keywords: Housing; Portugal; Crisis; Poverty;

References:

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ARTS

24666 | Black Women in Portuguese Colonial Visual Culture

Bruna Silva[1]; Margarida Dias[2]; Cristina Ferreira;

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Background & Aim: Portuguese visual culture retains colonial marks that normalized racial stereotypes, especially in the representation of Black women. Graphic and editorial design lacks pedagogical tools to critically interrogate these images. Based Afro-descendant heritage on the researcher's, this project proposes an editorial artifact as a reflective and critical object, exposing the mechanisms of visual racism in Portuguese colonial illustrations. **Methods:** The project is based on a qualitative methodology, based on the historical and documentary survey of colonial illustrations. The analysis is conducted using critical visual studies [5] and decolonial feminist frameworks [2][1]. The set of colonial illustrations (e.g., Figure 1) is analyzed at three levels: descriptive, formal, and critical, using guiding questions that clarify the interpretative criteria and highlight the distance between the historical context of production and the current context of analysis, allowing the identification of patterns of representation. The artifact is organized at these levels, also integrating the Portuguese historical framework and the contemporary practices of Black artists, establishing a critical counterpoint. To assess how the methodological mediation affects the reading of the image, the artifact is tested at two levels: (1) with Black women and organizations; and (2) with the general public. **Results:** Preliminary analysis identified recurring visual strategies of exoticization, hypersexualization, and subalternization. The three-level structure has proven effective in making interpretive operations transparent and transferable. Initial testing suggests that structured mediation broadens recognition of racialization mechanisms across different reader profiles. **Conclusions:** The study positions graphic and editorial design as an active agent in decolonising visual culture, contributing a replicable, theoretically grounded model for critically reading colonial visual archives in pedagogical contexts.

Keywords: Black Movement; Female Identity; Visual Culture; Illustration; Antidiscrimination

Acknowledgments:

I would like to thank my supervisor Cristina Ferreira and co-supervisor Margarida Dias for their critical guidance and methodological rigor in guiding the development of this project. I would also like to express my gratitude to the black women and organizations who agreed to collaborate in the testing phase, whose availability and sharing are fundamental to the ethical and critical dimension of the work. I would also like to thank the institutions and archives that provided access to the illustrations analyzed, as well as my colleagues and teachers who are contributing with relevant comments and discussions throughout the research process.

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Figure 1: Example of colonial illustration that forms part of the corpus of analysis and will be subject to descriptive, formal and critical reading throughout the project. [“Portugal and the Colonies”, Rafael Bordalo Pinheiro, 1902]

24238 | Dimensional Typography: Variable Font for Extended Reality Interfaces Supporting Social Reconnection of Users with Physical Limitations

Ana Sofia Coelho[1]; Pedro Amado[2]; Eliana Penedos-Santiago[3]; …

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Communication for individuals with Locked-In Syndrome (LIS), partial or total, is limited by the scarcity of graphic solutions adapted to their motor and visual restrictions. While Extended Reality (XR) environments, including Virtual Reality (VR) and Augmented Reality (AR), offer significant potential for inclusive practices, the field of typographic accessibility within the context of developing interaction design solutions is still recent. This research aims to develop a variable typeface for XR interfaces, expanding, through design, communicational possibilities for these users.

The project explores customization of typographic variation axes within a digital font to optimize visual comfort and interaction, enabling users to adjust it according to their individual needs. Validation follows User-Centered Design principles and relies primarily on qualitative methods, including semi-structured interviews and observation before and after prototype use. This approach enables the identification of usage patterns, difficulties, and strategies based on six participants with physical limitations, recruited through the Portuguese Amyotrophic Lateral Sclerosis Association (APELA) as part of the research project Six.

Results suggest that the typeface, primarily designed for three-dimensional planes, transcends purely aesthetic or functional roles, significantly enhancing the communicational experience by promoting comfort, effectiveness, and social and emotional reconnection. Customization proves crucial, as it allows each user and their caregivers to tailor the interface to their abilities, thereby facilitating content perception and overall experience.

These results contribute to making the Extended Reality (XR) experience more accessible to different users, highlighting the importance of typographic and inclusive design for individuals. The interaction design solution, which allows customization through parametric adjustments, in this case, font variation axes, can also be applied to other areas.

Keywords: Variable Typography (3D); Customization of Typographic Axes; Inclusive Interface Design; Typographic Accessibility and Legibility in Extended Reality; Extended Reality (Virtual Reality and Augmented Reality).

Acknowledgments: APELA (Portuguese Amyotrophic Lateral Sclerosis Association), and the participants and researchers of Project Six.

24692 | E-Waste in Product Design: A Designer's Iterative Process for Recycling Solutions

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Background & Aim: Today's rapid growth in electronic waste poses a critical environmental challenge, particularly due to the low recycling rates of the complex mix of materials that make up e-waste. Through a design-led perspective, this study investigates the feasibility of recycling post-consumer Polypropylene (PP) recovered from small domestic appliances into a modular product for workspaces. Thus, this research aims to define how the designer's intervention can transform waste into functional objects. **Methods:** The methodology followed an iterative approach in a collaborative makers space—Viva Lab—using Precious Plastic machinery. The process involved manual dismantling of e-waste and further sorting of materials used in trials from domestic appliances, followed by shredding, thermal fusion, and pressing. Several iterations were conducted first to determine which polymer was best to use among the recovered waste streams, and then to identify the optimal mass-to-volume ratio and temperature for producing high-quality panels. To validate the material's mechanical properties and behavior, specimens were machined and tested at the laboratories of the Faculty of Engineering, University of Porto, and subsequently compared to standardized tensile tests of virgin PP. **Results:** The results showed a significant learning curve regarding thermal shrinkage and mold planarity. Furthermore, early iterations suffered from incomplete fusion and surface burn marks, which were later mitigated. The mechanical tests confirmed the result of a brittle material with a ceramic-like behavior, although having high structural stability for static loads. **Conclusions:** The study shows that localized recycling of e-waste is technically feasible and provides a viable material for a functional organization system. The success of the process was shown to depend on strict control of the fusion-time ratio and the designer's capability to adapt to the material's reduced ductility. The results also show that 'imperfections' tend to be inevitable in artisanal recycling processes. However, these imperfections are characteristics of the process itself, shaping an aesthetic identity related to the material's provenance.

Keywords: Design, E-Waste, Polypropylene Characterization, Local Manufacturing, Precious Plastic, Prototyping, Circular Design.

24249 | Editorial Practice as Reactivation: From Archival Digitisation to Editorial Mediation in the Desmond Morris Archive at MHNC-UP

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Background & Aim: The preservation and enhancement of archives present significant challenges in enabling efficient access for researchers and the general public. This internship focuses on the scientific archive of Desmond Morris at the National Museum of Natural History and Science of the University of Porto (MHNC-UP), specifically addressing unpublished marginal sketches designated by Morris as "biomorphs" **Methods:** The project aims to investigate how editorial practice can reactivate scattered archival materials, transforming them into objects of cultural mediation. The methodology integrates archival techniques (inventorying, cataloging, digitization, and metadata creation), with editorial practice based on the work of Didi-Huberman's and Warburg's concept of the atlas come in, as a form of visual knowledge based on juxtapositions and associations, with a special focus on the "montage" phase. Archival processing and editorial practice are treated as sequential and interconnected, involving selection, digitization, and metadata creation, and then translating this work into editorial mediation aimed at researchers and the general public. **Results:** The results of this investigation are a catalog revealing previously unpublished aspects of Morris's spontaneous artistic work, a database of the documents that were digitized to facilitating future research, and a proposal for a spatial installation extending editorial logic into exhibition contexts. **Conclusions:** In conclusion, the project aims to offer a replicable model for the enhancement of archives and the creation of editorial content, while demonstrating methodologies that can inspire similar projects at other institutions.

Keywords: Scientific Archive; Desmond Morris; Editorial Practice; Reactivation

Acknowledgments:

Desmond Morris

Professora Cristina Ferreira

Supervisora de Estágio Anabela Magalhães

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24400 | From Digital to Material: The integration of Additive Manufacturing with Formative Manufacturing.

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Background & Aim: This study was carried out within the framework of research aimed at understanding the potential impact of Parametric Design as a method on Product Design. Trying to comprehend if it can help us improve products, be better designers or change the way we design. It can also be questioned by how it translates into production methods and what are the better technologies for it. Following it, led to the development of a methodology intersecting Parametric Design (PD), Additive Manufacturing (AM), and Formative Manufacturing (FM) for Product Design. This presentation is focused on the AM with FM synergy. While AM excels in prototyping and remanufacturing, it often lacks the cost-effectiveness required for mass production (Pou et al., 2021). By integrating AM with FM mould-making techniques, these limitations can be mitigated. Thus, the aim is to learn how the integration of these two production methods can help in the workflow of Product Design with PD. **Methods:** Because of financial and infrastructural limitations, the methodology employed AM and FM techniques achievable outside industrial context, as such, these exercises approach what is only achievable in a workshop. Preliminary exercises compared Fused Deposition Modelling (FDM) and Stereolithography (SLA). Small-scale FDM prints were used for initial learning, followed by larger models. A model was then printed via SLA to compare surface quality with FDM one. FM techniques included plaster and silicone moulds intended to replicate prints using wax, silicone, ceramics, glass, plastics, and metals. Due to financial and time constraints, the SLA print was used only for plaster, while FDM models were used across silicone, plaster, and specialized moulds for glass, metal, and plastic. First, a silicone mould was made and used to replicate forms in resin, and it was tested for heated plastic conformation. Then, the two plaster moulds were used for wax, silicone, and ceramics. The last ones were the metal mould, developed from wax replicas, and the glass mould, utilizing plaster moulds and silicone as reference. **Results:** Although the experiments are still ongoing, it can be noted that FDM and SLA can be used as alternative ways to produce reference models for moulds. Avoiding the need to use more time

consuming and costly techniques, like hand-carving wood, clay or wax. Between FDM and SLA, the former is significantly faster while the latter provides superior surface finishes. Regarding FM, silicone, plaster and the moulds for glass and metal can all be created with the 3D printed models, while also capturing well the surface finish obtained in FDM and SLA. However, in some cases, when the weight pressure is above the limit of plaster and silicone, these techniques are not viable. Instead, it was when the materials were poured or injected into the mould using a syringe that the best and most accurate models were produced. As seen in the wax, silicone and metal models. It can also be noted that the different viscosity of materials can also impose a deciding factor into the thickness and shape of the model. **Conclusions:** AM can synergize with FM, particularly when aiming to use materials that can be poured into the moulds. When pairing FDM with reusable silicone or plaster moulds, the limitations of mass production of AM can be mitigated. When high-fidelity aesthetics are required, SLA remains a viable option within these mould workflows. Conversely, non-industrial moulds for glass and metal are generally disadvantageous in large-scale production contexts due to waste, expense and time. However, if the aim is to obtain a prototype in glass or metal, these moulds could still be viable. As a whole, FM techniques can be used to contradict the materials and mass production limitations of FDM and SLA, by allowing the reproduction of the model in various materials and at a faster rate. It can in some cases be used as a production method, or in others, as a prototyping method.

Keywords: Product Design; Additive Manufacturing; Formative Manufacturing

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[1] Pou, J., Riveiro, A., & Davim, J. P. (2021). Additive Manufacturing: A volume in Handbooks in Advanced Manufacturing. In *Additive Manufacturing*. https://doi.org/10.1016/B978-0-12-818411-0.01001-6

24218 | GESTO-ESCRITA: An Asemic Editorial Project from an Artistic and Inclusive Perspective – Rediscovering Handwriting as a Practice of Language in (De)constructio

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[1]Faculty of Fine Arts, University of Porto - Master's student in Graphic Design and Editorial Projects

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This research project is based on the phenomenon of the displacement of handwriting in the digital age, also reflected in educational debates on reduction or suspension cursive writing instruction – yet notes that, as an analogue technology, it faced challenges before digital typing dominated. The study thus focuses on reduced handwriting time due to communication shifts and its expressive potential beyond formal functions. Drawing on Vilém Flusser's[1], it explores handwriting's contemporary relevance amid accelerated communication, proposing its rediscovery as inventive language with artistic and inclusive potential, considering the legacy of 'beautiful letters' – historically tied to classical rigour and calligraphic craft – alongside a hypothesis of subverting calligraphic practice (Benedicto)[2] through asemic writing (Schwenger)[3]. The methodology combines a public survey on handwriting habits and interviews with designers, teachers and calligraphic artists, using a qualitative, *practice-based* (RAE)[4] approach where artistic practice is both object and method of research. The empirical path includes: exploratory calligraphic exercises, sessions of asemic writing, a solo exhibition and a performance, culminating in an inclusive workshop with participants of diverse cognitive profiles and reduced mobility from AEDS[6] in Porto. The research produces an asemic editorial object that integrates the empirical process and workshop materials, focusing on the analysis of writing practices as gesture. This artifact functions as a critical device for synthesis and reflection, suggesting the potential of gesture-writing for inclusion and individual expression without claiming measurable impacts, while opening pathways for future artistic and editorial research. In this context, handwriting emerges as an open language and expressive practice beyond legibility, inviting its rediscovery of the act of writing by hand as a process that reveals the gesture of writing in constant (de)construction.

Keywords: Editorial Project, Handwriting, Gesture-writing, Asemic Writing, Inclusion

Acknowledgments:

The patience and generosity of my supervisors on this journey of “comings and goings”: Professor André Rangel, PhD, and Professor Verónica Metello, PhD.

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[1] Flusser, Vilém (2010). A Escrita: Há Futuro para a Escrita? (Murilo Jardelino da Costa, Trad.). São Paulo: Annablume.

[2] The concept of subversion in calligraphy is a proposal developed in the dissertation ‘Thoughts, Acts, Vibrations, etc. An investigation into calligraphy and calligraphic practice as a calligraphic gambiarra project’ (BENEDICTO, Bianca, 2018), in which the author discusses, based on the practice of a workshop-project, the role of gesture as an act of expressive creation of writing, based on the triad presented by calligrapher John Stevens, which reveals calligraphy as form, movement and rhythm.

[3] Schwenger, Peter. (2019). Asemic: The art of writing. University of Minnesota Press.

[4] Practice-based research regards creation as a means of generating knowledge through doing and experimentation. Within this framework, *research* is defined as “original investigation undertaken in order to gain new knowledge and understanding”(RAE, 2005, as cited in Borgdorff, 2012, p. 41), encompassing artistic practice, ideas, images, performances and artefacts.

[5] During the methodological process, a practical workshop on asemic writing was also adopted as an investigative practice. It was conducted in five online sessions by visual artist Lia Petrelli, who has been developing her research and artistic work on the asemic movement since 2015.

[6] Association for Education and Social Development www-aeds.org.pt

24671 | Internship at CIIMAR: Discovering the marine forest

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Background & Aim: Marine forests play a crucial role in coastal ecosystems, contributing to biodiversity [1], carbon sequestration [2], and environmental balance, among other benefits [5]. Despite their ecological importance, their relevance often remains underrecognized outside the scientific community. Effective communication strategies are needed to bridge the gap between marine research and non-specialist audiences [3]. This project, developed during a five-month internship at CIIMAR (interdisciplinary centre of marine and environmental research), explores how visual storytelling can support marine science communication by translating CIIMAR's research on seaweeds into accessible formats for the general public [6]. **Methods:** The work combined field observation of marine forest research with the development of visual communication strategies. The Benthic Ecology and Environmental Solutions Research Group [4] served as the main point of contact. Activities included participation in field campaigns and meetings, conversations with researchers, visual research, brainstorming sessions, and editorial production. Iterative visual development was carried out through illustration, narrative design, and editorial experimentation. Tested solutions included page layouts, illustrated explanations, and comic-based sequences aimed at simplifying concepts while maintaining scientific accuracy. **Results:** The primary outcome is a book about Marine Forests for a non-scientific audience, integrating illustrations and comics with researcher-produced texts to facilitate comprehension. Secondary outcomes include project logos, a web page design, illustrations for a scientific paper, and a live drawing event. **Conclusions:** The materials produced during the internship were positively received by non-scientific readers involved in testing activities and welcomed by researchers as an alternative communication medium. Illustration and comics emerged as effective languages for engaging broader audiences with marine science.

Keywords: marine science communication; visual storytelling in science; public engagement in marine research; environmental awareness.

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[4] CIIMAR. (n.d.-c). Benthic Ecology and Environmental Solutions Research Group. Interdisciplinary Centre of Marine and Environmental Research. https://www.ciimar.up.pt/teams/benthic-ecology/

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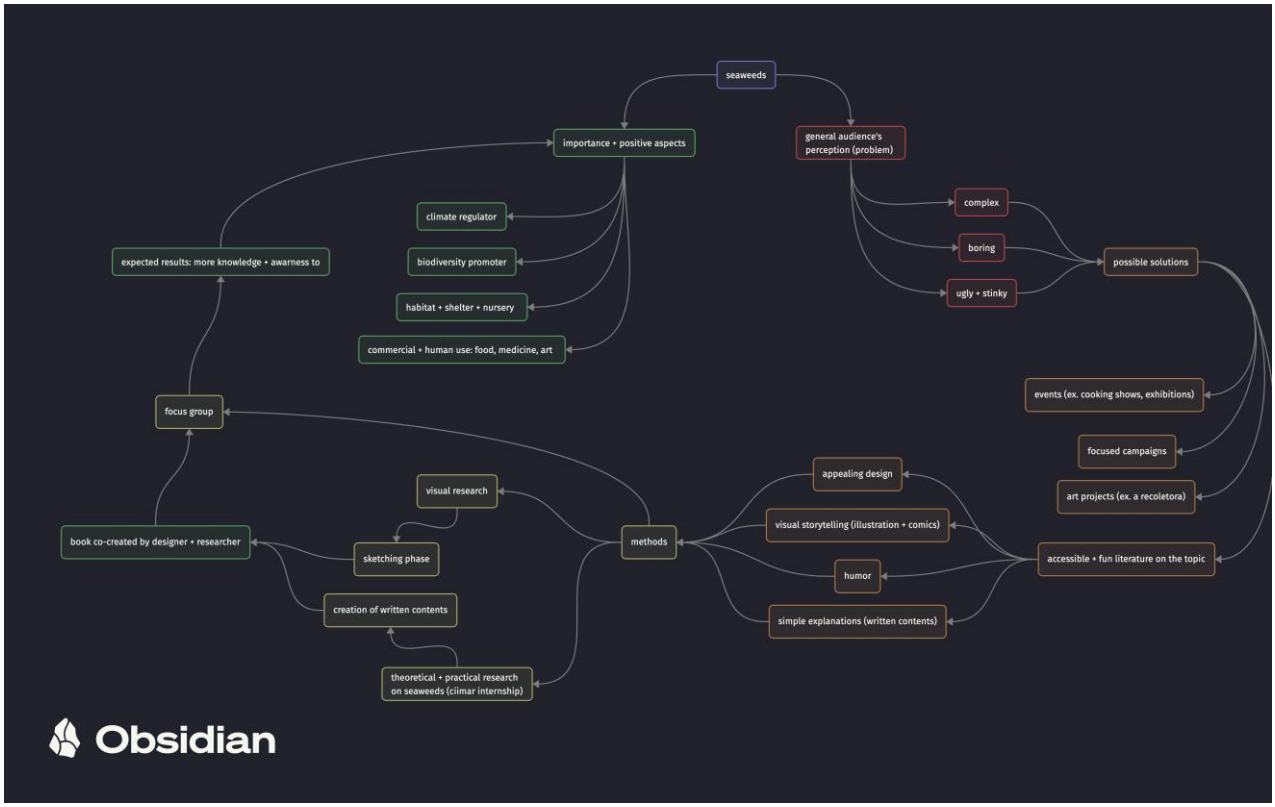
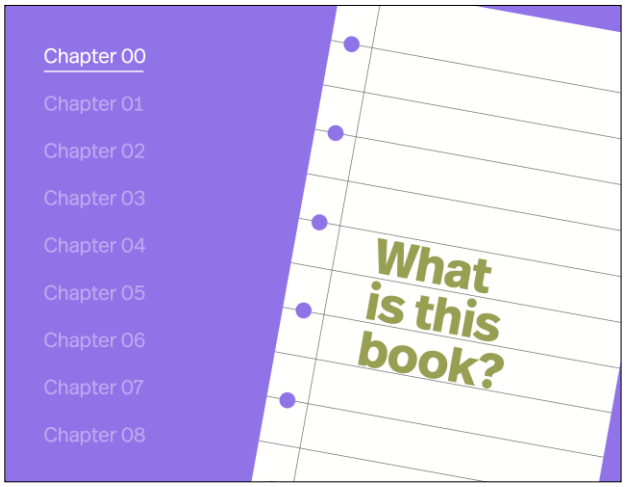
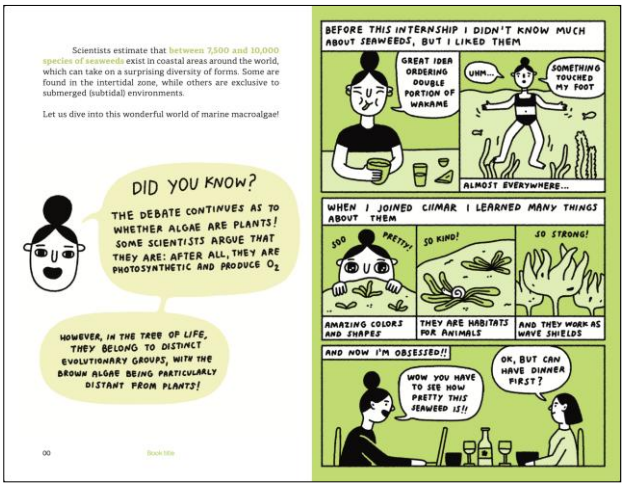
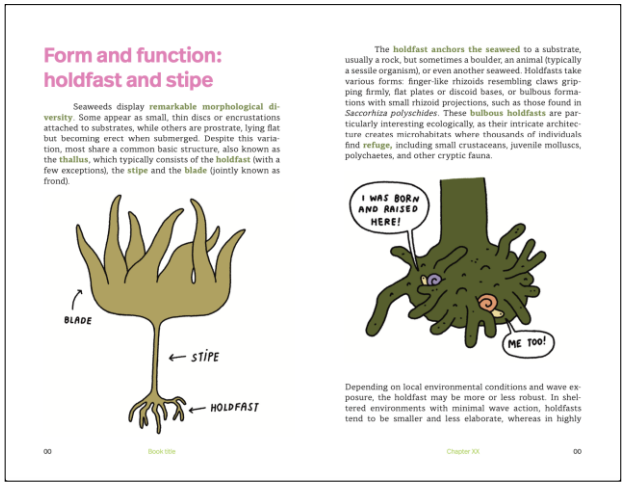
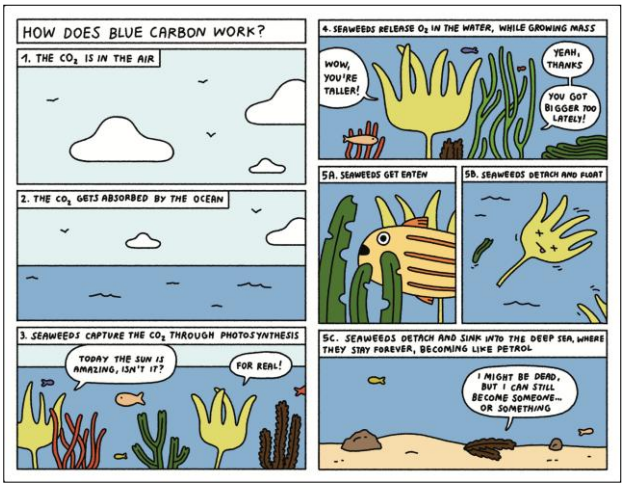


Figure 1: Work plan









Figures 2-5: Excerpts from the Marine Forests book

Figure 6: Fieldwork in Galiza (Sept. 2025)

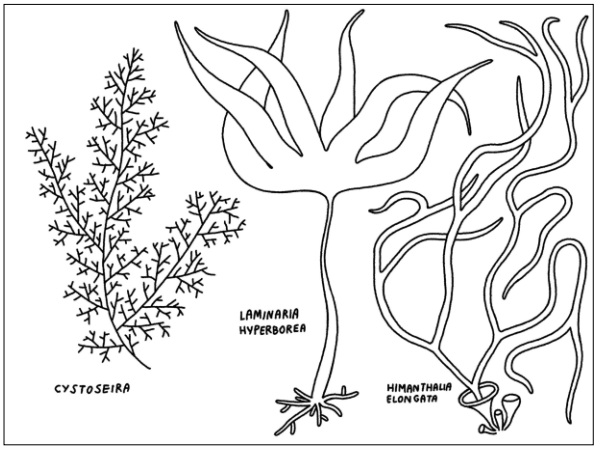


Figure 7: Seaweeds study

Figures 8,9: FUNsea project logo

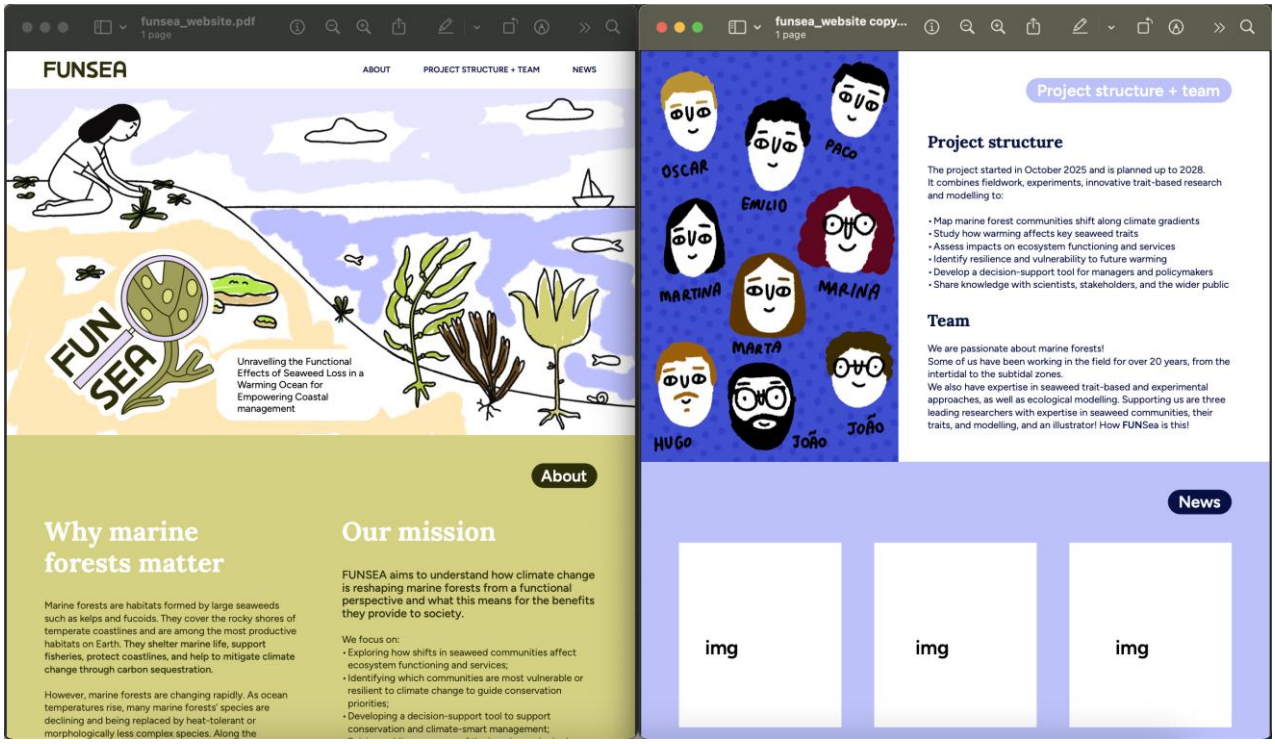


Figure 10: FUNsea project web page design

Figures 11,12: Live drawing event

24237 | New Sound: Analysis of Luso-African Collection from Fonoteca Municipal do Porto (1960–1992)

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Faculdade de Belas Artes da Universidade do Porto

This study analyzes a collection of Luso-African vinyl records, dated between 1960 and 1992, held at the Municipal Sound Archive of Porto (FMP). The project resulted in two editorial artefacts: a visual and analytical catalogue, focusing on the graphic, artistic, and historical qualities of record covers, and a project report, which contextualizes the ideologies and sociocultural movements of the period.

The catalogue identifies and characterizes the collection, including artists, record labels, and associated entities, and selects covers of particular relevance to design and visual studies. It further examines their graphic composition and compares findings to highlight the collection's diversity, musicality, and historical and visual significance. Its development was informed by João Alexandre's (2021) study of the Tropicalist movement in Brazil and the project *"Orfeu: Visual and Analytical Catalogue"* (ESAD-IDEA, 2022).

The report explores key historical and sociocultural frameworks, including *Vocação Imperial* (Silva, 2003), which supported Portuguese colonial expansion; *Luso-tropicalism*, developed by Gilberto Freyre and used by the Estado Novo to justify colonial rule and reinforce social hierarchies (Arenas, 2011; Cardão, 2020); and *Cosmopolitanism*, promoted as an image of cultural unity despite its divergence from reality (Moorman, 2008). It also documents the analytical process, the development of the catalogue, and the role of the FMP in preserving and promoting this archive (FMP, 2025). The project concludes that these editorial artefacts enhance the visibility of the Luso-African collection, demonstrating how it was shaped by both historical contexts and graphic practices, while highlighting the importance of public engagement in sustaining and expanding the archive.

Keywords: Luso-African; Editorial Design; Fonoteca Municipal do Porto; Vinyl Album Covers

Acknowledgments:

To my family, friends and teachers for the support given, to Hugo and Armando (FMP) for the help in the research and to Prof. José Carneiro for the guidance on this project.

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24758 | Out of rhythm - sound, silence, and the stolen time

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[1]FEUP, Porto University, Porto, Portugal;

Background & Aim: The linear model of life - birth, growth, work, reproduction, death—is not natural but a form of social control known as *crononormativity* [1]: the use of time to organize human bodies toward maximum productivity. This regime renders invisible those who cannot conform to its rhythms. This work explores "crip time," an epistemology emerging from chronically ill, disabled, and neurodivergent bodyminds that challenges normative temporality. The aim is to investigate how artistic practice can translate the subjective, often-invalidated experience of living with chronic fatigue and invisible symptoms, proposing the performance installation Atlas (ou Duplo) as a form of embodied resistance against crononormative expectations. Methods: This practice-based research employs an autoethnographic and exploratory artistic methodology. The central method is the creation of the durational performance Atlas (ou Duplo), developed through collaboration and trial and error. The work utilizes a glass sculpture molded from a scintigraphy of the artist's spine, connected to the artist's nude body by a braid of hair collected over two years. An internal light within the spine, modulated by external sound via an Arduino, serves as a metaphor for sensory overload. The future iteration will integrate an electroencephalogram to translate real-time brainwave activity into light intensity, using scientific instrumentation to validate a subjective, non-normative bodily experience. Results: The performance materializes the fissure between crononormativity and lived crip time. The translucent glass spine, fallen hair, and wavering light externalize the invisible exhaustion and neurological labor of a fatigued body differentiating it from common tiredness. The work successfully employs a technical apparatus—co-opting the language of scientific objectivity—to contest the standard metrics imposed on bodies. It visually translates concepts of crip time, such as the *time of illness* [2] and the fragmented experience of a body that no longer adheres to expected life stages. Conclusions: This artistic research concludes that Atlas (ou Duplo) operates as a political and epistemological act. By externalizing the internal experience of crip time, it challenges the hegemonic notion that productivity determines a life's value. The work affirms that rest is not a failure and that survival itself can be a maximum achievement. It contributes to the field by demonstrating how performance art can translate the language of a non-normative bodymind and denounce the violence of social scrutiny diagnostic, ultimately resisting the exclusion of non-normative bodies from the possibility of a future.

Keywords: crononormativity ; crip time ; practice-based; performance; non-normative.

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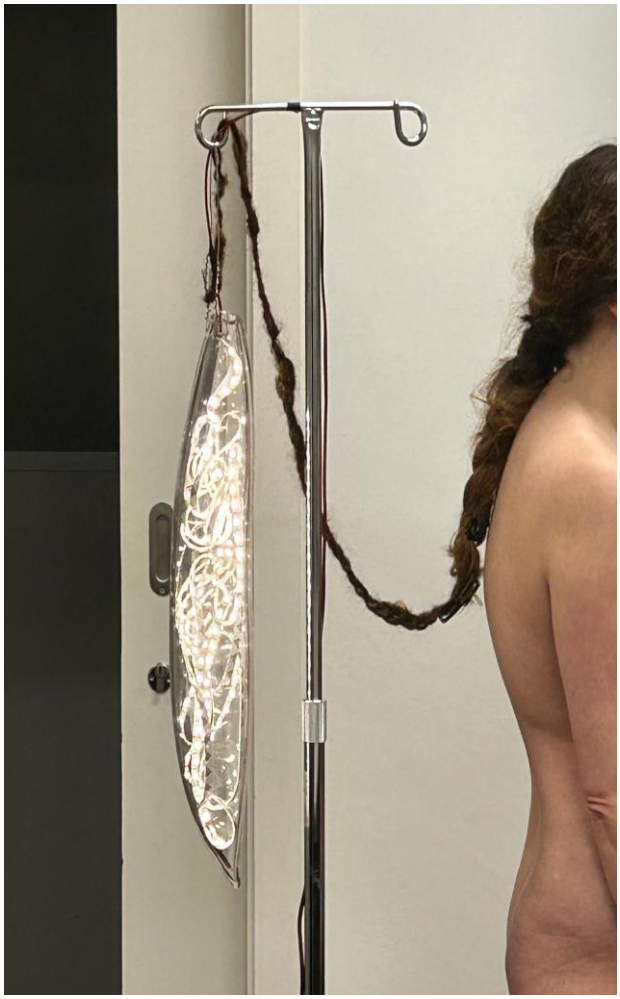

Figure 1: Atlas (ou o Duplo) performance test

24656 | Queer-feminist Graphic Design: A sapphic zine as a tool of resistance and lesbian identity

Claudia Martina; Cristina Ferreira

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Background & Aim: Graphic designers are agents of communication who actively shape cultural representations — and, historically, design has both propagated and resisted oppressive ideologies. This project investigates how queer-feminist theory can inform graphic design practice, using the development of a sapphic zine as a research instrument. The central research question is: *how can queer and feminist frameworks structure editorial design decisions that make lesbian and sapphic identities visible, in a Portuguese-language context where such spaces are scarce?* **Methods:** The research adopts a practice-based design methodology combining three lines of inquiry: (1) bibliographic research across feminist and queer theory, lesbian identity, and zine design history; (2) comparative analysis of existing queer and lesbian zines, examining visual language, editorial strategies, and community-building functions; and (3) qualitative data collection through surveys and interviews with lesbian groups (*Clube Safo*, *Dyke4Dyke zine*, *superFLINTA cineclube*) to identify communicative needs and representational gaps. These findings directly informed the conceptual and visual decision-making process of the zine *estrela de vénus*. **Results:** Analysis of existing zines and the qualitative data revealed recurring patterns in how sapphic communities negotiate visibility, safety, and self-representation in editorial formats. These findings shaped concrete design decisions in *estrela de vénus* — including its collaborative authorship model, the use of open submission structures, and visual strategies that foreground plurality over a unified aesthetic. The development process also surfaced tensions between legibility for a broad audience and specificity for a community-insider readership, a finding with implications for queer editorial design practice more broadly. **Conclusions:** This project contributes to understanding how practice-based design research can function as a site of community knowledge production. The zine operates not merely as a final artefact but as a reflective instrument through which queer-feminist values are tested, negotiated, and materialized in editorial form. The study suggests that design processes informed by community consultation and queer theory can generate both

culturally meaningful objects and transferable methodological insights for feminist design practice.

Keywords: Queer-feminism, LGBTQ+, zines, lesbian identity, graphic design

Acknowledgments:

Cristina Ferreira

24737 | Vida de Artista: The independent illustration work in Portugal in the context of self-publishing fairs and author businesses

Camila Cota[1]; Júlio Dolbeth[1];

[1] Design Department, Faculty of Fine Arts, University of Porto, Porto, Portugal

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Background & Aim: This research focuses on independent publishing in Portugal, examining fanzines, the DIY movement, and art fairs. It involves direct engagement with illustrators working in Portugal and practical experiences in creating and selling illustrated products at fairs. The project titled "Vida de Artista" (“Artists’ Life”) addresses the difficulties independent illustrators face in making a living through their art. It employs several reproduction techniques to create illustrated pieces for sale and distribution. Additionally, the project proposes organizing a self-publishing fair for FBAUP students to encourage independent artistic production and foster a supportive peer network. **Methods:** This research is based on a literature review on illustration and independent publishing, focusing on the illustrator’s role as artist, editor, and producer. It is complemented by semi-structured interviews with independent illustrators in Portugal and organizers of illustration fairs and galleries, analyzed through thematic coding. Fieldwork includes active participation and online forms at independent publishing, illustration, and craft fairs in Porto, with data collected through field notes and survey responses. Practice-based activities are documented through process records and critically analyzed as research material. **Results:** Preliminary results indicate that the practice of independent illustration in Portugal faces challenges with financial sustainability, often making it necessary for illustrators to take on commissioned work. Participation in fairs brings unpredictable and generally unstable economic results, impacted by many different factors. Still, being present at these events and using online promotion through fair organizers can help illustrators gain more visibility. **Conclusions:** It can be concluded, albeit provisionally, that these dynamics reinforce the illustrator's autonomy as an author, editor, and producer, although significant structural challenges persist.

Keywords: Illustration; Sustainability; Author’s work; Independent publishing fairs

References:

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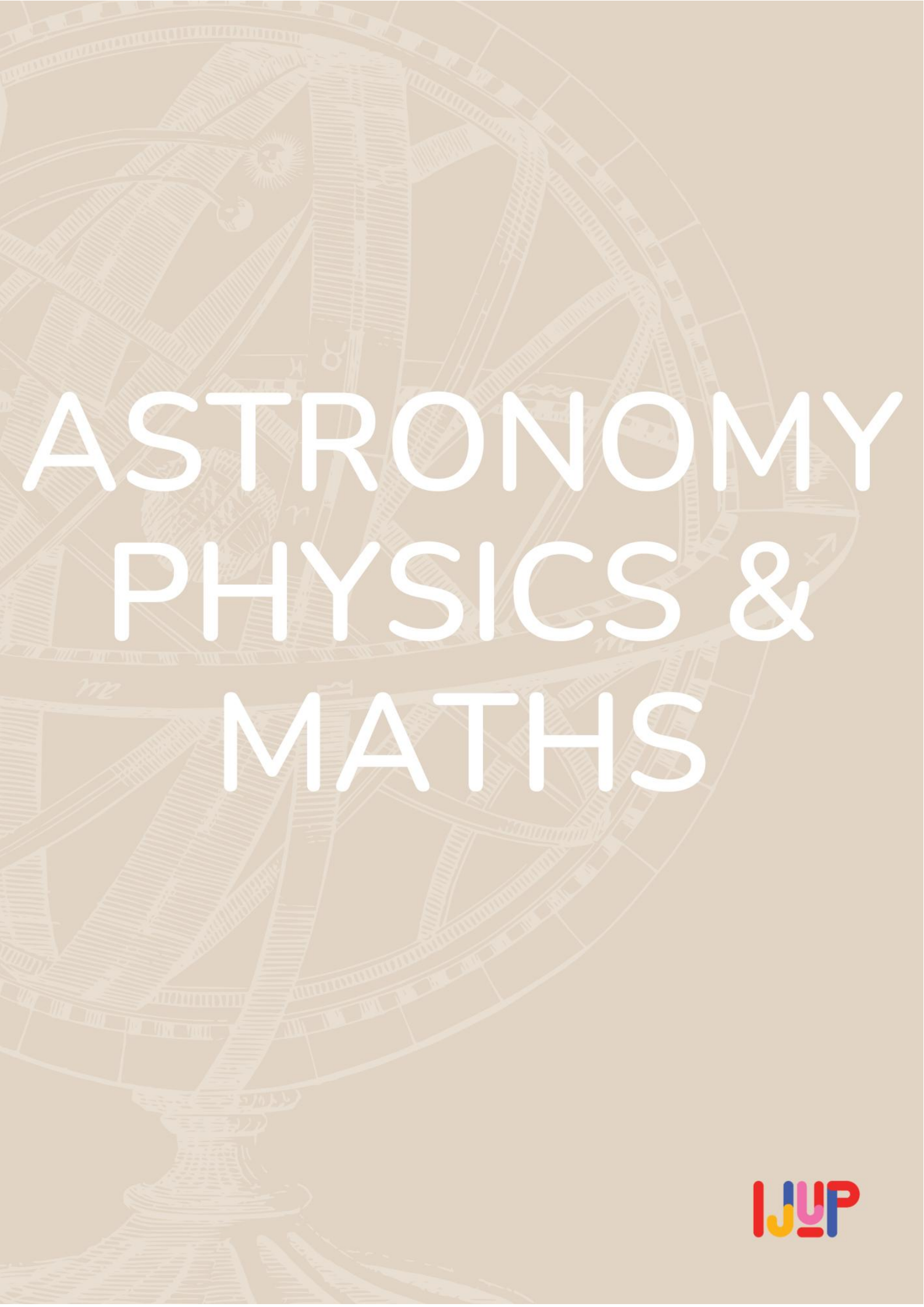
Figure 1: Fair setup for participation at "Mercado Quimera" for fieldwork research, February 2026, Porto, Portugal

Figure 2: Provisional illustrated poster for an illustration and self-publishing fair for FBAUP students to be developed in collaboration with the respective student association.



ASTRONOMY PHYSICS & MATHS

24620 | Conditional Diffusion Models for Probabilistic Spatiotemporal Temperature Generation: Hourly Downscaling from Coarse Daily Fields

Diogo F. Cruz[1,2]; Carla Gonçalves[1,3]; Ricardo Jorge Bessa[1]

[1]Centre for Power and Energy Systems, INESC TEC, Porto, Portugal; [2]Department of Mathematics, Faculty of Science, University of Porto, Porto, Portugal; [3]Department of Informatics Engineering, Faculty of Engineering, University of Porto, Portugal;

Background & Aim: Global Climate Models like CMIP6 [1] provide essential long-term climate projections but at a coarse spatial and temporal resolution (large grid sizes and mostly daily or monthly data), limiting their use in local-scale impact studies. Dynamic downscaling with regional climate models offers physical consistency but is computationally expensive. In contrast, statistical approaches struggle to capture complex non-linear dependencies. This study proposes a diffusion-based generative downscaling framework to produce physically plausible and high-resolution hourly temperature fields from coarse daily climate projections. **Methods:** We develop a Conditional Diffusion Probabilistic Model [2] to generate high-resolution hourly temperature fields from coarse daily condition inputs. A (2+1)D U-Net was trained on 30 years of ERA5 reanalysis data [3] to learn to iteratively denoise corrupted high-resolution temperature fields. The reverse diffusion process is conditioned on low-resolution daily means, static topographic features (orography, land-sea mask), and cyclical temporal encodings (day-of-year, hour-of-day). The loss function combines mean squared error with physics-informed terms that penalize unrealistic spatial and temporal gradients and promote daily mean conservation. **Results:** The proposal generates realistic hourly temperature fields from CMIP6 daily projections, maintaining large-scale coherence with coarse constraints while reproducing key climatological statistics observed in ERA5, e.g., diurnal cycles and extreme behavior, with meaningful inter-sample variability. **Conclusions:** This work offers a scalable and physically coherent approach for climate downscaling and generation, emphasizing the capacity of generative models to capture complex geophysical distributions. Probabilistic high-resolution ensemble generation from deterministic coarse states provides a robust tool for localized climate risk assessment and infrastructure resilience planning.

Keywords: Climate downscaling; Diffusion models; Spatiotemporal Temperature Fields; Ensemble Generation

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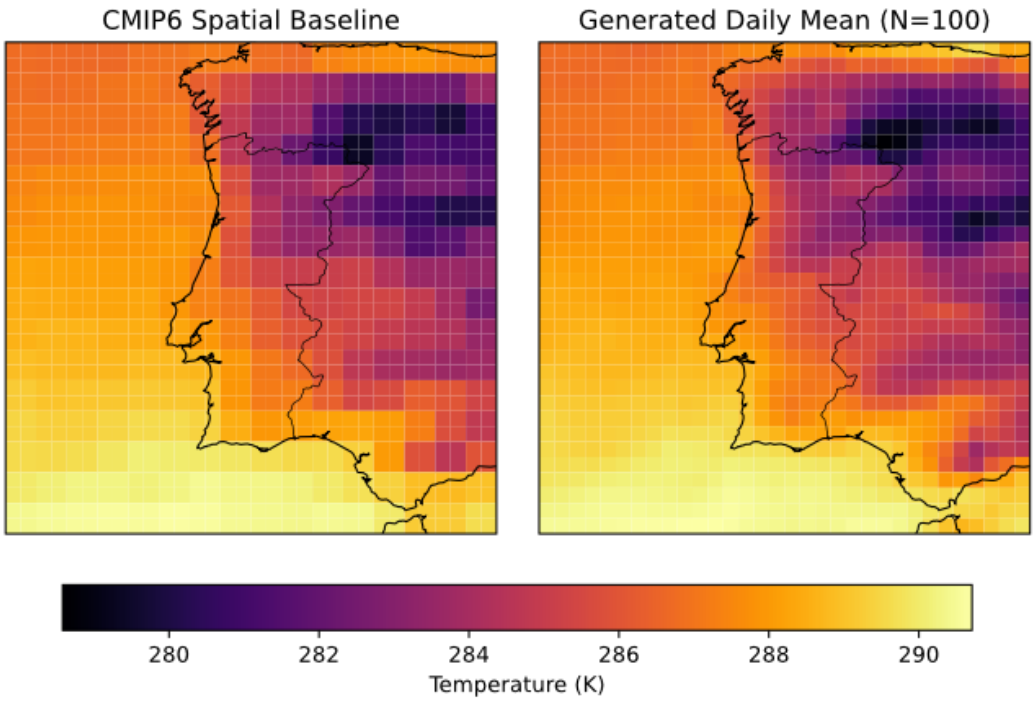

Figure 1: Comparison between daily mean temperature field from CMIP6 (left) and the ensemble mean of 100 generated samples by the proposed model (right), for January 1, 2083.

24736 | The Use of Artificial Intelligence as a Tool in Learning Mathematics

Sousa, Clarisse; Delgado, Fátima;

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Background & Aim: In recent years, Artificial Intelligence (AI) has become more common in students' independent study. It can give explanations, step-by-step solutions, and immediate support in different subjects [1]. In Mathematics, these tools can help students explore strategies, clarify doubts, and build confidence when solving exercises [2]. However, we still know little about how students actually use these technologies and whether they can critically understand and evaluate the answers they receive [3]. This study aims to understand how lower secondary school students use Artificial Intelligence when studying Mathematics and what impact they believe it has on their learning. It also analyses the role of the teacher in guiding this process. **Methods:** This study follows a qualitative and interpretative approach. The intervention takes place in a lower secondary school class and has three main stages. First, an initial questionnaire is applied to identify how often and in what ways students use Artificial Intelligence in their Mathematics study. Then, students complete a classroom task where they analyze a solution produced by Artificial Intelligence. This allows the researcher to observe how students interpret, check, and discuss the given answers. Finally, a final questionnaire is applied, or an audio or video recording is collected, to understand students' views about the usefulness of Artificial Intelligence, its limitations, and its impact on their understanding of the content. Throughout the intervention, observations are made about the teacher's role in guiding the analysis of the AI-generated solutions. **Results:** The research is still at an early stage, and no data has been collected yet. However, it is expected that students will use Artificial Intelligence in different ways, from searching for quick answers to using it in a more exploratory way. Difficulties in evaluating the reliability of the generated answers are also expected, as well as the need for teacher guidance to promote a more critical use of these tools [2][3]. **Conclusion:** The study is expected to help understand the role of Artificial Intelligence in teaching Mathematics, emphasizing the importance of teacher guidance in interpreting AI-generated answers and promoting more critical and independent learning.

Keywords: Artificial Intelligence; learning Mathematics; educational technology; students' perceptions; teacher mediation.

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24619 | Carbon-based Smart Security Labels for Anti-Counterfeiting Textiles

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Background & Aim: One of the biggest problems for companies with a unique product is counterfeiting [1]. Nowadays, counterfeiting technology needs to be evolved, so this work relies on the development of a new coding system for the textile industries [2]. This system is multifunctional based, integrating different features like electrochemical ones, that combined into a QR-based smart label, make it unique and not reproducible for anti-counterfeiting. **Methods:** Commercial carbon nanotubes were used as carbon-based materials and incorporated in a suitable polymer-based conductive ink. The obtained ink was used to print different patterns like QR-codes or code bars, for example, on textile substrates. The samples were submitted to electrochemical measurements, using an impedance bridge analyzer. To facilitate and systematize the processing of the obtained data, was developed a Python program. The program can determine automatically the electrical parameters of interest, which was used to classify the tags as legit or not legit, according to a designed criterion of selection. **Results:** With the data analyzed, it was verified that depending on the preparation conditions, certain labels have a well-defined electrochemical response. Studying the impedance properties associated with the capacity of the system, it was possible to verify a local maximum associated with a specific frequency of resonance proper of the system. **Conclusions:** The experiments in terms of electrochemical features of the labels lead to the conclusion that it was possible to have a feature like the resonance frequency making part of the smart labels. In respect to a certain criterion, it was possible to classify the tags as legit or not legit.

Keywords: carbon-based materials; electrochemical features; smart labels; anticounterfeiting textiles.

Funding:

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Acknowledgments:

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Figure 1: Photograph of a printed code bar in a textile substrate using the developed carbon-based ink.

24916 | Development and Optimization of Dielectric Materials for Triboelectric Nanogenerators

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Background & Aim: This work aims to improve the efficiency of dielectric materials for use in triboelectric nanogenerators (TENGs), which convert mechanical energy into electrical energy. Their performance depends on the dielectric properties of the material and their surface's morphology. Polyvinylidene fluoride (PVDF) and polytetrafluoroethylene (PTFE) are two materials widely used due to high electronegativity and stability, however, the parameters used during the process remain in need of optimization. This study focuses on investigating those parameters, specifically in the production of PVDF thin films and PTFE nanofibers. **Methods:** The PVDF films were produced via spin coating and the resulting samples were tested using a pneumatic triboelectric system, against reference triboelectric materials. For the second phase, currently on-going, PTFE nanofibers were produced using electrospinning and the effect of the processing parameters, such as solution viscosity, applied voltage and curing temperature, was studied through structure characterization and a solid-liquid TENG system, for performance testing. **Results:** In the PVDF films, triboelectric output increased with thickness and average output stability tests showed an initial decrease in peak-to-peak voltage, which stabilizes over time. "Flash" curing processes provided outputs twice as high as longer curing treatments, suggesting a better crystallization of the solution. The PTFE study aims to, similarly, find the trends followed by the output voltage with varying electrospinning and curing parameters. **Conclusions:** The results demonstrate that the TENGs' performance is strongly influenced by the processing parameters. The continued optimization of PTFE nanofibers is expected to improve the solid-liquid TENG efficiency. These findings contribute to the development of increasingly higher performance triboelectric energy devices.

Keywords: Triboelectric Nanogenerators; PVDF thin films; PTFE nanofibers; Dielectric materials; Electrospinning

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24746 | Numerical Modeling of Ultrashort Pulse Dynamics in Laser Oscillators

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Background & Aim: Kerr-lens mode-locked lasers are frequently modeled using the master equation, which assumes only minor pulse parameter changes per round trip [1]. However, this approximation often neglects the discrete nature of cavity elements and higher order dispersion terms, which are critical for characterizing sub-10 fs pulses. This work aims to develop a one-dimensional numerical simulation that models pulse evolution across the laser cavity while providing the flexibility to selectively analyze the impact of second and higher order dispersion on dispersion-managed mode-locking dynamics. These simulations are important for several applications, including frequency-comb metrology, multiphoton microscopy, ultrafast spectroscopy of materials and integrated Ti:sapphire photonics [2]. **Methods:** The simulation is based on an iterative algorithm to track the pulse's spectral amplitude as it propagates through the discrete components of the cavity: the active medium, prism pairs, and mirrors. Propagation within the crystal is modeled by numerically solving the nonlinear Schrödinger equation in the presence of gain and self-phase modulation induced by the Kerr non-linearity. **Results:** Preliminary results demonstrate the code's ability to simulate the build-up of a pulse into a steady-state spectrum, depending on the balance between the gain and distortion profiles. We are currently varying the cavity dispersion and nonlinear material parameters in our numerical model to investigate their impact on the spectral evolution of the pulse. **Conclusions:** Once fully validated, these numerical implementations will become an important tool for predicting and tailoring the output of ultrafast laser oscillators.

Keywords: Ultrafast lasers; Nonlinear optics, Kerr-lens mode-locking; Self-phase modulation

Acknowledgments:

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24777 | Rotating Magnetocaloric Effect in a La(Fe,Mn,Si)13Hy Plate

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Background & Aim: Magnetocaloric materials undergo temperature changes when subjected to varying magnetic fields, a phenomenon known as the magnetocaloric effect (MCE). This property offers a sustainable alternative to traditional vapor-compression cycles that rely on greenhouse gas emitting refrigerants. In samples with rotationally asymmetric shapes, rotating an external field of constant intensity results in a change of the demagnetizing field and consequently rotating magnetocaloric effect (RMCE). Although the RMCE is typically lower than the conventional MCE, it enables simpler and more compact device designs. In this study, we aim to characterize the RMCE in one of the most promising alloys with conventional MCE, the $La(Fe,Si)_{13}$ family of alloys. They exhibit a remarkable MCE due to a first-order phase transition (FOPT). Adjusting the Fe/Si ratio, doping the alloy, or utilizing hydrogenation allows tuning the Curie temperature T_C to specific operational needs, making them interesting materials for active magnetic regenerator (AMR) systems. **Methods:** The RMCE of a $La(Fe,Mn,Si)_{13}H_y$ plate with $T_C \sim$ 302 K was characterized via magnetization (MPMS3 SQUID magnetometer) and adiabatic temperature change measurements. Structural phases were analyzed via SEM-EDX where an ~25% amount of α-Fe phase was estimated and corroborated by magnetization measurements. Additionally, magnetostatic simulations performed using FEMCE and COMSOL Multiphysics were employed in order to understand the response of the material to different applied field intensities in the two orientations. **Results:** A maximum of $-\Delta S_{iso}^{rot} = 3.68$ J kg^{-1} K^{-1} for $\mu_0 H_{ap} = 0.6$ T was observed, which is +194% of what has been observed in Gd for the same magnetic field . The structural analysis confirmed the presence of the α-Fe phase, which is consistent with the magnetization data collected during the characterization process. **Conclusions:** The results characterize the $La(Fe,Mn,Si)_{13}H_y$ plate as a viable material for RMCE applications. By utilizing tuning methods like hydrogenation and doping, these alloys remain promising for the development of efficient AMR systems.

Keywords: Refrigeration; Magnetocaloric Effect; Rotating Magnetocaloric Effect; La(Fe, Si); Magnetism

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24504 | Rotating Magnetocaloric Effect in DyCo2 Due to Shape Anisotropy

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Background & Aim: Magnetic refrigeration is a promising eco-friendly alternative to vapor compression, based on the magnetocaloric effect (MCE) - the temperature change when a magnetocaloric material experiences a varying magnetic field. The rotating magnetocaloric effect (RMCE) arises by rotating a constant-intensity magnetic field around samples with magnetic and/or shape anisotropy. Unlike conventional MCE, RMCE avoids field intensity switching, potentially reducing the amount of permanent magnets and complexity in refrigeration devices [1]. In this work we aim to investigate the feasibility of magnetic refrigeration based on the RMCE by exploiting the shape anisotropy. **Methods:** $DyCo_2$ is a known magnetocaloric material (T_C = 134.9 K) with potential for low-temperature applications, including H2 liquefaction [2,3]. Two bulk polycrystalline samples prepared by arc-melting were cut and polished to achieve anisotropic shapes. For both samples, the MCE and RMCE (Figure 1) were characterized using SQUID magnetometry. Additionally, the sample with the highest aspect ratio (7.1) was further investigated through direct temperature measurements performed in a cryocooler. **Results:** Higher magnetic entropy changes are observed at lower fields ($4.13\ J\ Kg^{-1}K^{-1}$ at $0.6\ T$). This non-intuitive behavior shows that, unlike MCE systems, RMCE devices do not require strong fields to achieve optimal performance. **Conclusions:** The results confirm the feasibility of $DyCo_2$ for RMCE-based magnetic refrigeration at cryogenic temperatures, enabled by shape anisotropy. The RMCE performance is not maximized by stronger fields and with lower fields one can reduce magnet use and device complexity, improving the viability of large-scale RMCE refrigeration.

Keywords: magnetic refrigeration; rotating magnetocaloric effect; shape anisotropy; demagnetizing effect; cryogenic temperatures.

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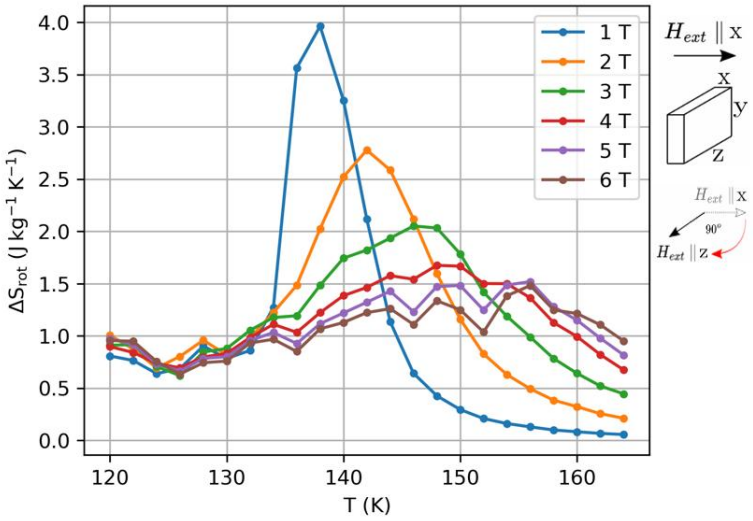

Figure 1: Rotating magnetic entropy change at different applied fields for $DyCo_2$.

24653 | Study of interparticle and polymer interactions in magnetocaloric composites

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Background & Aim: The search for more sustainable alternatives to conventional refrigeration systems has increased interest in magnetocaloric technology, which relies on temperature changes in certain materials when exposed to magnetic fields. One promising approach is the development of polymer-based composites containing magnetocaloric microparticles, combining tunable properties with scalable processing. However, their performance depends strongly on particle–polymer interactions. This work aims to understand how these interactions vary with composition and how they influence the overall behavior of the composite. **Methods:** Computational methods were used to study the interaction dynamics between magnetocaloric microparticles and the polymer matrix. Different particle/polymer ratios were considered to evaluate their effect on particle distribution and interaction behavior, as well as on the resulting physical and magnetocaloric properties. **Results:** Preliminary results show that the particle/polymer ratio significantly affects interaction dynamics within the composite. Simulations indicate that the alignment of magnetic moments between particles occurs on timescales ranging from tens to hundreds of milliseconds, while alignment with an external magnetic field occurs on the order of seconds. These differences suggest distinct mechanisms governing particle–particle and particle–field interactions, with implications for the overall magnetocaloric response. **Conclusions:** This study highlights the importance of particle–polymer interactions in determining the behavior of magnetocaloric composites. Understanding these effects is essential for optimizing material design and supports the development of more efficient and scalable refrigeration technologies.

Keywords: Magnetocaloric effect; Composite materials; Polymer matrix; Particle interactions; Sustainable refrigeration

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24543 | Synthesis and characterization of magnetic nanoparticles for anticounterfeiting textile applications

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Background & Aim: Counterfeit goods are an increasing danger in the global economy, notably in the textile industry, even with the advanced security labels already existent. Nanomaterials with optic, electrochemical or magnetic properties can be incorporated into a functional ink for textile applications, creating smart labels that improve anti-counterfeiting protection [1]. The aim of this work is the synthesis and characterization of nanomaterials with magnetic features, with the goal of developing a practical and reliable method for the measurement of their magnetic anticounterfeiting features, when incorporated in functional inks and coated onto textile substrates. **Methods:** Iron-based oxide magnetic nanoparticles (MNPs) with spinel structure were synthesized through an aqueous coprecipitation route [2]. Powder X-ray diffraction (XRD) was used to study phase composition and crystallinity, and a Superconducting Quantum Interference Device (SQUID) magnetometer was used for advanced magnetic characterization. The prepared MNPs were mechanically grounded and incorporated into a polymer-based mixture to obtain a suitable ink for textile coating by Mayer-rod method. **Results:** In initial tests with a small magnet, the prepared MNPs displayed clear magnetic properties, and preliminary XRD analysis presents the expected diffractogram pattern, indicating the successful preparation of the MNPs. Furthermore, the application of the MNP functional ink onto the textile was successful, resulting in a clearly defined barcode pattern (Figure 1). **Conclusions:** Magnetic nanomaterials were successfully synthesized and applied to textiles, demonstrating potential for the development of configurable and readable smart labels with polymer-based inks.

Keywords: Magnetic nanoparticles, Functional inks, Anticounterfeiting smart labels, Innovative textiles

Funding:

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Acknowledgments:

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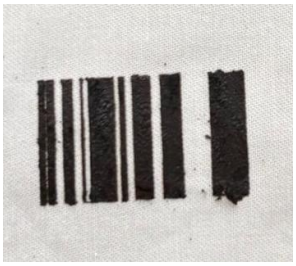

Figure 1: Barcode pattern coated on a textile substrate using a magnetic polymer-based ink.

BIOLOGICAL SCIENCES

24306 | An Inflamed Intestinal Barrier is not necessarily a leaky Barrier: an in vitro study with mycotoxins

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Background & Aim: Intestinal epithelial barrier dysfunction, including altered immune signaling, tight-junction disruption and increased permeability, can be caused by inflammatory processes [1]. Mycotoxins, common food contaminants, worsen this damage and induce oxidative stress [2]. Inflammation is often assumed to increase xenobiotic uptake, yet published data remain inconsistent [3]. This study aimed to validate an inflamed in vitro intestinal model and see if acute inflammation changes the effects and permeability of a mycotoxin mix. **Methods:** Caco-2/HT29-MTX (90:10) monolayers were differentiated for 21 days on 12-well Transwell inserts. Inflammation was induced with IFN-γ (16 h) followed by IL-1β and TNF-α (24h) [4]. After, the apical medium was replaced with DMEM or a non-cytotoxic mix of ochratoxin A (OTA), zearalenone (ZEN) and aflatoxin B1 (AFB1) (500 µg/L each) (3h). Real-time TEER (CellzScope) monitored barrier integrity. Inflammatory, oxidative stress and intestinal-barrier-related gene expression were measured by qPCR and mycotoxin transport was quantified in the basolateral chamber at 30, 60, 120 and 180 min. **Results:** A 23% TEER decrease was shown, along with strong induction of inflammatory (IL-1β up5-fold) and oxidative stress markers (SOD2 up15-fold), confirming the inflammatory state of the model. Exposure to the mycotoxin mix did not further reduce TEER. Barrier-related gene expression remained unchanged between conditions, and mycotoxin exposure did not significantly affect their gene profiles. All mycotoxins crossed the epithelial layer with AFB1 showing the highest permeability (6.6%), followed by ZEN (4.7%) and OTA (0.2%), in normal conditions, matching the absorption patterns known [5]. Transport was similar in normal and inflamed models. **Conclusions:** The model showed a TEER decrease without a rise in mycotoxin transport. These findings refine our understanding of barrier responses, though further validation is needed to assess its relevance under sustained inflammation.

Keywords: In vitro intestinal model; Intestinal absorption; Inflammation; Mycotoxins

Acknowledgments:

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24378 | An Organoid-based Multi-Organ-on-a-Chip Platform for Studying Gastric Cancer Metastasis

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Background & Aim: Gastric cancer (GC) is a leading cause of cancer-related death worldwide due to late diagnosis. The Laurén classification divides GC into intestinal, diffuse, and mixed subtypes with distinct growth and dissemination patterns. Intestinal-type GC forms solid tumors that metastasize to distant organs, whereas diffuse-type GC consists of poorly cohesive cells that rapidly spread through the peritoneum, often causing early mortality. Mechanisms driving organ-specific metastatic tropism remain poorly understood, partly due to limitations of animal models and the inability to analyze the full metastatic process in patients. We aim to develop a human-relevant multi-organ-on-a-chip (MoC) integrating GC organoids/spheroids with target tissues. **Methods:** The device contains four interconnected chambers: one gastric tumor and three metastatic sites. For intestinal-type GC, these include liver, lung, and bone; for diffuse-type GC, bone is replaced by a peritoneal model. A perfusable, endothelial-lined channel mimics vasculature and enables dissemination. Initial validation will use immortalized GC and organ-specific cell lines, followed by patient-derived organoids. Metastasis will be monitored in real-time using immunofluorescence. **Results:** The platform is fabricated by multilayer PDMS microfabrication and plasma bonding, with hydrogel-filled compartments for long-term 3D culture. Validation confirmed hydrogel confinement in the compartment. Fluid exchange into the hydrogel occurs within 2 hours at 3 mbar, confirming transport. GC spheroids generated in agarose microwells form uniform ~100 µm structures that expand up to twofold in Matrigel. Live/dead assays confirm high viability, supporting MoC integration. Fluorescent tracing enables cell tracking for up to 16 days. **Conclusions:** Overall, this MoC enables real-time analysis of GC

metastasis and supports translational and personalized applications by combining biologically relevant 3D tumor models with microfluidic control.

Keywords: Multi-Organ-On-A-Chip; Metastasis; Organoids; Microfluidics.

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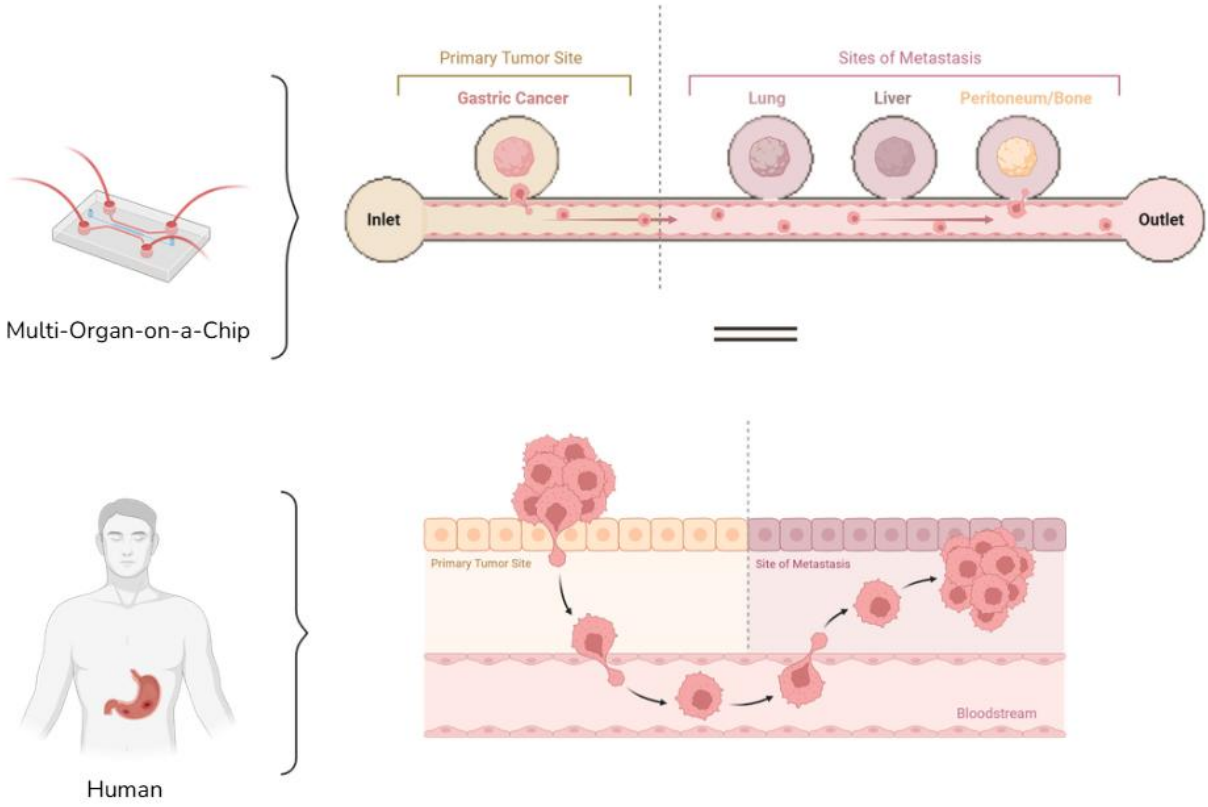

Figure 1. Schematic representation of the gastric cancer multi-organ-on-a-chip (MoC) platform.

24716 | Analyzing Obesity impact in Breast Cancer

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Background & Aim: Breast cancer (BC) is a heterogeneous disease classified into four molecular subtypes, Luminal A, Luminal B, HER2-enriched, and triple-negative (TNBC), with Luminal A showing the most favorable prognosis and TNBC the most aggressive behavior [1]. Obesity is a chronic disease associated with metabolic dysregulation and increased risk of several health issues, however its role in BC remains unknown. Some evidence suggests that obesity promotes tumor progression while others indicate a potential protective effect [2,3]. The aim of this work is to investigate how obesity affects breast cancer development, focusing on Luminal A (MCF-7) and TNBC (MDA-MB-231) molecular subtypes. **Methods:** In order to achieve this aim, MCF-7 and MDA-MB-231 cell lines were cultured and incubated with 20% concentration of serum from obese (Ob) and normoponderal (healthy)(H) individuals. Afterwards, cell viability (MTT), migration (injury), and proliferation (BrdU) assays were conducted. Two control groups were included for comparison: a negative control (0% FBS) and a positive control (20% FBS). **Results:** Preliminary results indicated that MCF7 cells viability was not affected by incubation with healthy and obese sera or FBS. MCF7 cells migration was slightly more decreased when incubated with Ob serum than with H serum, or with 20% FBS. Regarding MDA cells, healthy serum appeared to have reduced viability compared to obese individuals and negative control, while the positive control did not significantly differ. MDA cells' migration was not decreased by obese serum, as cells completed wound closure greater than healthy serum and both negative and positive control conditions. Cell proliferation results are still ongoing. **Conclusions:** In conclusion, despite preliminary, these findings suggest that obesity may affect BC progression differently depending on the molecular subtype, highlighting the need to integrate metabolic disease into therapeutic decision-making.

Keywords: Obesity, Breast Cancer, viability, proliferation and migration

Acknowledgments: The authors would like to thank the patients and the healthy volunteers for generously contributing samples to this study.

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24502 | Anthropogenic Sound Pollution an emerging stressor for animal Welfare: Insights and Possible Solution

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Background & Aim: Anthropogenic noise is a significant environmental stressor in natural and artificial habitats; however, mitigation strategies in aquariums remain limited. Acoustic sensitivity and behavioral responses to noise vary considerably across taxa. For example, seahorses exposed to low-frequency boat motor noise exhibit behavioral changes, an increased incidence of gas bladder disease, and higher susceptibility to stress-related disorders. In contrast, in penguins, exposure to crowds and noise has produced neutral or positive effects, including increased pool use without major behavioral disruption. This study evaluates the acoustic impacts on two species from distinct taxa and investigates whether immersive experiences can improve visitor behavior in a public aquarium. **Methods:** Two species (the penguin *Spheniscus humboldti* and the seahorse *Hippocampus abdominalis*) were assessed under three noise conditions: low (absence of visitors and background noise), medium (presence of visitors and background noise) and high (scheduled school visits combined with background noise). Acoustic data within enclosures were collected using a hydrophone, while external sound levels, visitor numbers, and student behavior were simultaneously recorded. Enclosure occupancy and animal behavior were video-recorded and analyzed. School groups voluntarily participated in an immersive activity involving exposure to a short underwater audio recording from the seahorse enclosure, after which visitor behavior was reassessed. **Results:** Preliminary findings indicate that low noise levels enhance behavioral diversity, activity, and enclosure dispersion in seahorses. In penguins, higher noise levels increased behavioral diversity and activity but reduced enclosure dispersion due to visitor attraction. Immersive experiences increased student awareness and behavioral control upon entering the exhibition. **Conclusions:**

Anthropogenic noise may act as either a stimulant or a stressor depending on species-specific acoustic sensitivities, highlighting the need for adaptive management strategies and visitor education in public aquariums.

Keywords: Anthropogenic noise; Animal welfare; Behavioral responses; Seahorses; Penguins.

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24537 | Assessing the environmental factors affecting detectability on transect counts of red-legged partridge in Southern Portugal

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Background & Aim: The red legged partridge (RLP, *Alectoris rufa*), is a highly prized gamebird species in the Iberian Peninsula. Since the 1980s, however, wild populations have undergone sharp declines, threatening the species' conservation status and its sustainable management as a hunting resource [1]. Thus, effective management relies on robust population dynamics models supported by reliable field data. Central to these models is the estimation of abundance and demographic parameters across key phenological phases, typically derived from standardized population counts. Such estimates, however, are influenced by variation in detectability. This study aims to identify the abiotic factors that significantly affect the RLP detectability across hunting grounds in the Alentejo region. **Methods:** Driven line transect surveys were conducted in 9 hunting grounds during the post-reproductive (July) and pre-hunting period (September). Surveys were carried out shortly after sunrise and before sunset, totalling 1422.9 km of transects (four replicates/transect/month). A total of 2817 RLP detections were recorded over 38 sampling days. Field observations were integrated with topographic variables (elevation, slope and aspect) [2], habitat productivity (NDVI) [4], and weather conditions (temperature and wind speed [3], and on-site cloud cover), using QGIS and R. A hierarchical Distance Sampling framework is being implemented, fitting various detection functions and covariate structures to identify the best-fitting models for detectability and abundance estimation. **Results:** Preliminary analyses indicate consistently higher counts in September than in July, suggesting temporal variation in detectability. Ongoing modelling of the detection function will refine abundance estimates and population size inference. Ultimately, this approach will enhance the capacity to model population dynamics and to implement

adaptive, evidence-based management that reconciles hunting with conservation of this emblematic species.

Keywords: Hierarchical Distance Sampling; Abiotic covariates; Driven line transects, Detectability function

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The authors of this study would like to thank the many colleagues that helped in the sampling effort.

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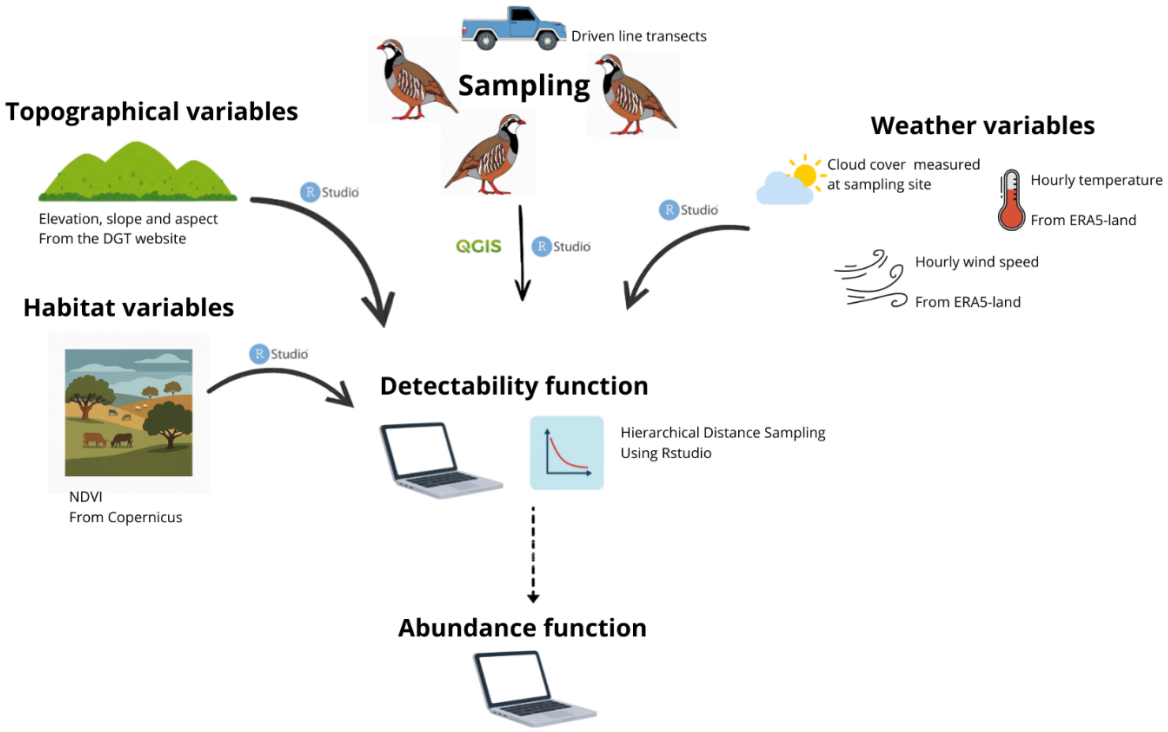

Figure 1: Representation of the workflow applied in this study.

24498 | Behavioural responses of the starfish *Marthasterias glacialis* in classical sociability paradigms

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Background & Aim: Little is known about the existence of structured social interactions in echinoderms. Despite the absence of a centralized brain, these organisms possess a decentralized nervous system and specialized sensory structures capable of integrating chemical, mechanical, and visual stimuli. The presence of these mechanisms suggests an ability to detect and respond not only to environmental stimuli but potentially also to signals from conspecifics. The starfish *Marthasterias glacialis* is described as a solitary species, with key ecological roles in benthic communities, acting as a regulator of populations, a nutrient recycling agent, and a potential bioindicator of environmental quality. Nevertheless, despite its ecological relevance, it remains unclear whether this species exhibits interaction patterns that may be interpreted as social behavior. In this context, the present study aims to investigate whether *Marthasterias glacialis* displays social-like behaviors. **Methods:** The experimental protocol, currently being conducted at the Estação Litoral da Aguda, integrates three complementary behavioral paradigms, allowing an integrated assessment of intraspecific interactions: aggregation assays with spatial and temporal monitoring; social preference tests using bilateral flow systems in which one flow contains social chemical cues; and simulated predation risk experiments using predator chemical signals to investigate whether the presence of conspecifics modulates anti-predator responses. **Results:** Data collection is ongoing, and spatial proximity patterns, physical contact, and behavioral responses to conspecific and predator stimuli are being analyzed. **Conclusions:** The expected results aim to clarify the existence and characteristics of social-like behaviors in starfish, broadening knowledge in the field of marine invertebrate ethology and deepening the understanding of their functional role in coastal ecosystems, with potential implications for conservation strategies and marine biodiversity management.

Keywords: *Marthasterias glacialis*, behavior, sociability

24657 | Beyond Cholesterol Lowering: How Simvastatin Shapes the Zebrafish Epigenome and Development

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Background & Aim: Pharmaceuticals are recognized as contaminants of emerging concern due to their persistence, bioactivity at low concentrations and incomplete removal in wastewater treatment plants (WWTPs). Statins such as simvastatin have been detected worldwide in WWTP influents, effluents, and surface waters at ng/L levels, raising concern over their continuous release into aquatic ecosystems. Here, we expect to evaluate if environmentally relevant concentrations of simvastatin, a lipid-lowering drug, induce measurable changes in the transcriptome and epigenome of zebrafish. By integrating gene expression profiling and epigenetic markers, this study will elucidate the molecular mechanisms underlying simvastatin induced toxicity and clarify how pharmaceuticals affect epigenetic regulation. **Methods:** Ecotoxicological assays with zebrafish embryos (FET test 236) will be used to evaluate the response to simvastatin during early development stages. In parallel, gain and loss of function experiments targeting HMGCR, the key enzyme inhibited by statins in the mevalonate pathway, will be implemented using a F0 CRISPR-Cas9 knockout method [4] and morpholinos, to elucidate molecular targets. RNA-seq will be conducted to identify differentially expressed genes and 5-mC assay to measure DNA methylation profiling. **Results:** Exposure to simvastatin (1,5 and 2,5 µg/L) lead to spinal deformities, pericardial and yolk edemas and a developmental delay. CRISPR-Cas9 assays targeting only one exon of *hmgcra* showed similar but slightly more pronounced phenotypes. **Conclusions:** The results obtained to date indicate that simvastatin induces alterations in zebrafish development. These changes might be directly associated with HMGCR inhibition or may result from other disruptions within the mevalonate pathway. Therefore, the remaining part of this work pretends to clarify the extent to which HMGCR

inhibition explains the observed phenotypic outcomes and if epigenetic modifications play a role in the registered changes.

Keywords: Statins, HMGCR, Zebrafish, Epigenetics

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24706 | Beyond Cholesterol Lowering: Impact of Simvastatin in Prenylation and their Developmental and Epigenetic Consequences

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Background & Aim: Contaminants of emerging concern (CECs) refer to newly identified synthetic chemicals or biological agents detected in the environment with potential or confirmed harm to the environment and humans, such as statins.[1, 2] Statins inhibit HMG-CoA reductase (HMGR), the mevalonate pathway's rate-limiting enzyme.[3] A downstream consequence of HMGR inhibition is loss of prenylation by reduced farnesyl diphosphate synthase (FDPS) substrate.[4,5] Protein prenylation refers to the addition of farnesyl or geranylgeranyl groups to proteins, enabling membrane localization and intracellular signaling, important for mitosis and embryonic development, for example.[6,7] HMGR conservation across metazoans suggests that statins can affect non-target organisms, an environmental concern.[2] The impact of statin-induced prenylation loss on the developmental epigenome is still unknown. We hypothesize that it alters embryonic development and epigenome in zebrafish (*Danio rerio*). **Methods:** A F0 *fdps* knockout (KO) in one-cell stage zebrafish embryos was generated following Kroll et al. (2021) and validated by Sanger sequencing.[8] The KO models, wild-type (negative control) and simvastatin-exposed embryos (positive control) are assessed for survival, hatching rate and morphometric data by Fish Embryo Toxicity (FET) test 236. Transcriptomic and DNA methylation studies assess gene expression and epigenetic changes. Altered prenylation is also confirmed by immunoblot through membrane-to-cytosol redistribution of prenylated proteins. **Results:** KO testing showed high zebrafish embryonic lethality by 24 hours post-fertilization (hpf) with abnormal, enlarged and disorganized blastomers detectable as early as 4 hpf, consistent with prenylation's role in mitosis and embryonic development.[6,7] **Conclusions:** These results highlight prenylation's critical role in development and guide future approaches, including

morpholinos and downstream KOs, to assess the environmental and epigenetic impact of statins.

Keywords: Contaminants of Emerging Concern; Statins; Prenylation; Knockout; Zebrafish

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24902 | Can embryo morphokinetics predict ploidy status?

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Background & Aim: Assisted Reproductive Technologies aim to identify human embryos with optimal implantation potential. Static morphology has limited predictive value. Time-lapse systems enable continuous embryo monitoring with dynamic data, yet chromosomal status remains decisive for viability [1]. Aneuploidy often causes implantation failure and miscarriage, rising with maternal age. Preimplantation Genetic Testing for Aneuploidy detects these abnormalities but requires invasive biopsy [2]. As human embryo development correlates with ploidy, this study evaluates human blastocyst ploidy effects on morphokinetics and explores non-invasive ploidy predictors [3]. **Methods:** This retrospective study was conducted at CEIE, including secondary data from routine clinical PGT cycles from January 2024 to August 2025. Oocytes were retrieved, enzymatically and mechanically denuded, and fertilized. Embryos were cultured and continuously monitored from fertilization (Day 1) to biopsy (Day 5-6) in a GERI time-lapse incubator (37 °C, 6.5% CO_2, 5% O_2). Grading followed ESHRE / ALPHA (Istanbul) Consensus Guidelines. Blastocyst biopsies underwent NGS-based chromosomal analysis [4]. Ethical approval was granted by the CEIE Institutional Ethics Committee. No additional procedures were performed on the embryos beyond the standard clinical workflow. Statistical analyses (SPSS) included descriptive and comparative tests (t-test or Mann–Whitney U) after Shapiro–Wilk normality assessment [5]. **Results:** Preliminary analysis (ongoing) show that euploid and aneuploid embryos showed significant differences in the timing of compaction, blastocyst formation, and expansion. Euploid embryos reach the blastocyst stage earlier than aneuploid embryos ($p<0.05$), likely due to more efficient cell division and reduced chromosomal segregation errors. **Conclusions:** These findings suggest that morphokinetic markers may help distinguish euploid from aneuploid embryos and support non-invasive embryo selection.

Keywords: Embryo morphokinetics; PGT-A; Time-lapse imaging; Aneuploid embryos

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24314 | Cardiac responses of Mytilus to natural thermal stress across the Iberian Peninsula

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Background & Aim: Intertidal mussels experience rapid thermal variation during emersion, creating strong physiological constraints that are expected to intensify in the actual warming scenario. Species with large latitudinal distribution, such as *Mytilus* spp. have physiological strategies to cope with these physical differences. We evaluated whether non-invasive heart-rate monitoring captures natural thermal stress in *Mytilus* and if these responses vary with latitude and shore exposure across the Iberian Peninsula. **Methods:** We sampled *Mytilus* spp. on 17 rocky shores from Galiza to south Portugal. During low tide, cardiac activity was recorded non-invasively for 60 s per individual using an infrared Pulse sensor connected to a smartphone app. Individuals were assigned to contrasting exposures (hot: sun-exposed, south-facing; cold: shaded, north-facing). Within each site, hot vs cold exposures were compared using two-sided Wilcoxon tests ($p<0.05$). Regional patterns were interpreted along a north–center–south gradient. **Results:** Cardiac activity differed between exposures in multiple sites, with cold exposures acting as thermal microrefugia and hot exposures concentrating stress. The frequency of significant hot–cold differences increased toward warmer regions (North: 6/9 sites; Center: 4/5 sites; South: all sites). Overall patterns supported a latitudinal stress gradient: North → stability; Center → controlled stress; South → high stress. **Conclusions:** Our conclusions are that Non-invasive field heart-rate monitoring detects natural thermal stress in *Mytilus* and reveals microhabitat buffering superimposed on a latitudinal gradient. This approach supports physiology-informed monitoring of rocky-shore vulnerability under climate warming.

Keywords: Cardiac activity; Intertidal; *Mytilus*; Thermal stress; Iberian Peninsula

24830 | Caring for the Originals: a conservation-restoration approach of nomenclatural types in the PO Herbarium (MHNC-UP)

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Background & Aim: The Museu de História Natural e da Ciência da Universidade do Porto (MHNC-UP) houses the PO Herbarium, preserving about 120,000 specimens of vascular plants, bryophytes, lichens, fungi and algae, including around 300 recognised nomenclatural type specimens. The collections span from the mid-nineteenth century to the present and are associated with Portugal, with additional material from Macaronesia, Spain, other European regions and significant collections from Angola, Mozambique, and São Tomé and Príncipe. These specimens are primary taxonomic references used in the formal description and identification of plant species. As unique scientific records, they have significant historical, scientific and documentary value, but their preservation is challenged by the fragility of plant material, ageing mounting supports and past storage conditions. MHNC-UP is undertaking a project to assess, conserve and restore approximately 120 herbarium type specimens collected in different countries, most gathered in Portugal by botanists such as Gonçalo Sampaio, Arnaldo Rozeira, Joaquim Tavares, Alphonse Luisier, J. Mendonça and J. Ormonde. **Methods:** The project follows a structured conservation workflow integrating condition assessment, minimal-intervention treatments, photographic documentation and preventive conservation. Each specimen is evaluated to record the condition of the herbarium sheet, mounting supports, plant fragments, surface dirt, mechanical damage and deterioration of adhesives or auxiliary materials. Treatments prioritise compatibility and reversibility, including controlled surface cleaning, stabilization and reattachment of fragments, replacement of degraded mounting elements and rehousing. High-resolution photographic documentation creates a detailed visual record and reduces unnecessary handling. **Results:** Treatments improve the physical and chemical stability of specimens. Reinforced mounting supports reduce handling risks, while archival storage materials prevent volatile organic compound (VOC) emissions and enhance physical stability. High-resolution images enable remote access and support taxonomic research while minimizing

manipulation. **Conclusions:** By integrating conservation treatments, preventive strategies and systematic photographic documentation, the project supports long-term preservation, accessibility and future botanical taxonomic research.

Keywords: Herbarium collections; Type specimens; Conservation and restoration; Preventive conservation; Botanical heritage; Collection management.

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24327 | Characterization of Oxygen-Consuming Devices for the cyanobacterium *Synechocystis sp.* PCC 6803

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Background & Aim: Cyanobacteria are photoautotrophic prokaryotes that are emerging as green cell factories. However, the O_2 produced during photosynthesis can impair the activity of O_2-sensitive enzymes, such as hydrogenases [1]. To tackle this issue, Oxygen-Consuming Devices (OCDs) based on O_2-binding proteins, such as the hemoglobin Vhb from *Vitreoscilla* C1 that was shown to increase respiration levels in *Escherichia coli* mutants lacking native terminal oxidases, were developed [2]. These OCDs were introduced in the model cyanobacterium *Synechocystis* sp. PCC 6803 (wt) and Δ*hoxYH* [3], a strain lacking a functional bidirectional hydrogenase and in which a Fe-hydrogenase is going to be introduced (Project PhotoSynH2). The main goal of this work was to evaluate the expression/conditions that favor the production of Vhb in *Synechocystis* strains. **Methods:** Synthetic devices designed/synthesized for the regulated expression of Vhb or Vhb fused with *Synechocystis* native hemoglobin – SynHb. Devices cloned into replicative plasmids and introduced into wt and Δ*hoxYH* by natural transformation. Vhb and Vhb_SynHb expression assessed by Western blot. **Results:** Vhb and Vhb_SynHb were successfully expressed in both strains. However, Vhb expression levels were significantly lower than those of Vhb_SynHb. The expression levels of Vhb_SynHb were stable for 7 days after the induction. Increasing the concentration of iron or the addition of the heme precursor (5-aminolevulinic acid) did not increase Vhb_SynHb expression levels. **Conclusions:** The fusion of Vhb with the *Synechocystis* native hemoglobin seems to increase expression/stability in both strains, as demonstrated previously for other proteins [4]. Supplementation with additional iron or heme precursor did not increase protein expression levels, but their impact on enzymatic activity still needs to be evaluated. The work presented here represents a step toward creating conditions suitable for O_2-sensitive processes, such as H_2 production.

Keywords: Cyanobacteria; Hemoglobin; Oxygen-consuming devices; *Synechocystis*; Vhb

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24817 | Characterizing the proteome of leaf vacuoles from the medicinal plant Catharanthus roseus

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Background & Aim: Vacuoles play a key role in plant environmental interactions by sequestering specialized metabolites, allowing them to exert their toxic defense without interfering with cell physiology. The medicinal plant *Catharanthus roseus* accumulates in leaf vacuoles, at low levels, the monoterpenoid indole alkaloids (MIAs) vinblastine and vincristine, used in cancer chemotherapy [1,2]. We previously isolated *C. roseus* leaf vacuoles and tonoplast fractions and sequenced the protein complement of both samples using Mass Spectrometry. Here, we aim to: i) integrate the best transcriptomic data sources available for *C. roseus* to generate an enhanced transcriptome, ii) map the peptide sequences to this reference, iii) identify candidate genes implicated in MIA metabolic fluxes and iv) obtain the functional portrait of *C. roseus* leaf vacuoles. **Methods:** To generate a non-redundant, consolidated transcriptome assembly, multiple high-quality transcriptomes were curated and aligned to the two gold-standard reference genomes. This optimized assembly is being used as the database for proteomic mapping via MaxQuant and Protein Discoverer. Label-free quantification will be employed for protein identification to derive relative abundances. Finally, the functional architecture of vacuoles will be reconstructed through Gene Ontology (GO) enrichment and protein-protein

interaction networks using PANTHER, STRING, and Cytoscape. **Results:** A consolidated transcriptome was assembled, providing a robust reference for the ongoing mapping of proteomic data. A prior preliminary analysis suggests *C. roseus* leaf vacuoles are involved in defence and specialised metabolism, and that candidate genes to the MIA missing biosynthetic steps and tonoplast transport should be identified. **Conclusions:** Proteomic analysis of *C. roseus* leaf vacuoles has the potential to indicate candidate genes key to the full understanding of the MIA pathway and the success of strategies leading to higher levels of the anticancer MIAs.

Keywords: Madagascar periwinkle; Monoterpenoid indole alkaloids; Vacuole; Proteome; Transcriptome.

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24362 | Comparative analysis of differential gene expression between sexes in Cnidaria

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Background & Aim: The Phylum *Cnidaria* comprises a wide diversity of organisms, from corals and anemones (Anthozoa) to hydrozoans (Hydrozoa). Despite all this diversity, little is known about the gene expression of sex-biased genes in this basal group of Metazoa. Therefore, this work aims to identify differentially expressed genes (DEGs) in males and females of 6 species of Cnidaria (*N. vectensis*; *H. symbiolongicarpus*; *F. ancora*; *F. vityazi*; *K. echinatum*; *P. compressa*) through bioinformatic analysis of transcriptomes. **Methods:** Male vs. Female transcriptome data (≥3 replicates/sex) were retrieved from NCBI. After quality control (FastQC) and filtering (Trimmomatic), *de novo assembly* was performed for species that lacked a reference genome using Trinity. Transcript abundance was quantified using Kallisto, followed by differential expression analysis in R (tidyverse, edgeR, biomaRt, limma, ggplot2). Annotation used Diamond and Homology search was done on Exonerate. **Results:** Many sex-specific genes, such as male spermatogenesis markers (e.g. CatSper and Testis specific) and female markers (e.g. Vitellogenin and ZP proteins) were identified. Furthermore, many sex-determination related genes, such as Dmrt and Sox were found. **Conclusions:** Characterizing these markers provides a molecular framework for understanding gametogenesis and sex reproduction origins in early-diverging metazoans.

Keywords: *Cnidaria*; Transcriptomics; Sex-determination; Differential gene expression.

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24578 | Cyanobacteria species from Parque Nacional Peneda-Gerês: new insights into their antioxidant and UV-protection potential

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Background & Aim: Cyanobacteria from Peneda-Gerês Nacional Park (PNPG) remain unexplored despite the park status as a biodiversity hotspot [1]. These microorganisms produce bioactive compounds, such as pigments, with significant antioxidant (AOX) and photoprotective potential [2]. This study aims to identify cyanobacterial strains from PNPG, characterize their pigments profile and evaluate their safety and potential for cosmetic applications. **Methods:** Strains Gerês 1A and Gerês 11 were isolated and taxonomically and morphologically characterized. Targeted fractions were prepared from the dry biomass using acetone and water. Chemical profiling of the fractions involved HPLC for carotenoids and spectrophotometry for phycobiliproteins and total phenolics content (TPC). AOX potential was assessed *via* nitric oxide ($^{\bullet}NO$) and superoxide anion radical ($O_2^{\bullet-}$) scavenging assays. Cellular biocompatibility was evaluated in human keratinocytes (HaCaT) using the MTT viability assay. This model will further be used to predict the UV-protection capacity of the fractions. **Results:** HPLC analysis identified chlorophyll *a* as the most abundant compound in both strains (**Figure 1**). TPC was significantly higher in acetonic fractions compared to aqueous ones. Regarding phycobiliproteins, Gerês 11 exhibited higher phycocyanin levels, while Gerês 1A was richer in phycoerythrin. In AOX assays, acetonic fractions were potent $^{\bullet}NO$ scavengers (EC_{25}=0.021µg/mL). Conversely, aqueous fractions were more effective in $O_2^{\bullet-}$ scavenging (EC_{25}=40.01µg/mL). Preliminary MTT assays showed that HaCaT cells remain viable when exposed to increasing concentrations of aqueous fractions; for acetonic fractions some cytotoxicity was observed for higher concentrations (50-200 µg/mL). **Conclusions:** The PNPG cyanobacteria are rich in high-value pigments with AOX potential. The high cell viability observed in HaCaT cells, coupled with AOX potential, validates these strains as safe and promising ingredients for innovative cosmetic products.

Keywords: Cyanobacteria, PNPG, carotenoids, UV radiation, antioxidants

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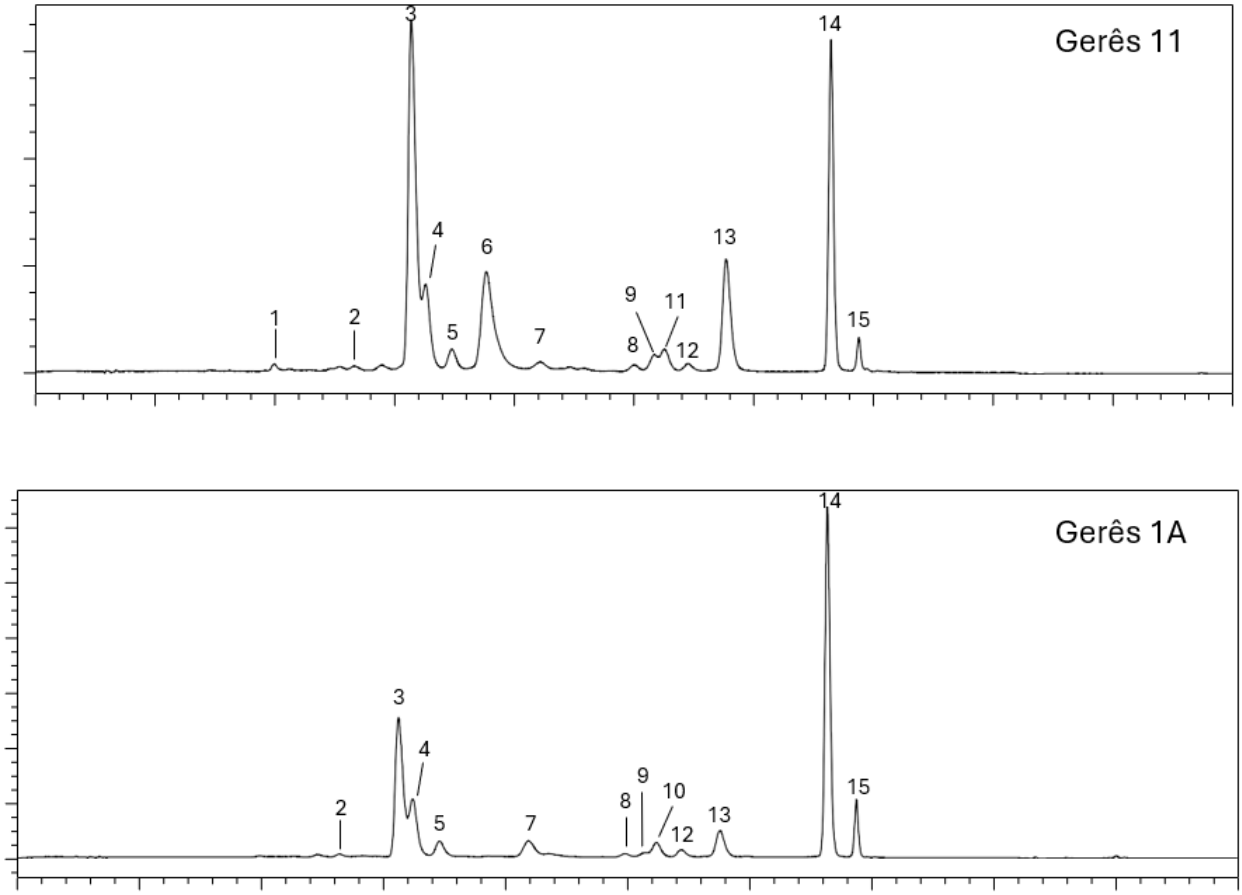

Figure 1: Carotenoid and chlorophylls profile of acetonic fractions of the cyanobacteria strains Gerês 11 and Gerês 1A. HPLC-PDA recorded at 450 nm. Chlorophyll-*a* derivatives (1 and 5), Lutein (2), Chlorophyll-*a* (3), Zeaxanthin (4), Unidentified xanthophyll (6 and 13), Myxoxanthophyll (7), β-cryptoxanthin (8), Unidentified carotenoid (9 and 11), Echinenone (10), α-carotene (12), β-carotene (14) and Unidentified carotene (15).

24436 | Cytotoxicity Evaluation of Dihydro-Methylone and Dihydro-Pentedrone in SH-SY5Y Cells

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Background & Aim: Synthetic cathinones are new psychoactive substances whose toxicological evaluation has traditionally focused on parent compounds; however, increasing evidence suggests that metabolites may substantially contribute to overall biological effects and toxicity [1]. Their metabolic pathways are well characterized and include reactions such as *N*-demethylation, *O*-demethylation, and β-keto reduction [2,3], yet the toxicological impact of these metabolites remains underexplored. Notably, carbonyl-reduced metabolites have been shown to exhibit higher cytotoxicity than their parent cathinones, underscoring their toxicological relevance [4]. This work aims to evaluate the cytotoxicity of β-hydroxy metabolites of methylone and pentedrone in SH-SY5Y cells. **Methods:** The cytotoxicity of dihydro-methylone and dihydro-pentedrone was evaluated using dopaminergic SH-SY5Y cells. The cells were exposed for 24 h to 10–6000 µM of both β-hydroxy metabolites. Cytotoxicity was assessed using a cell viability assay, and concentration–response curves were generated to determine toxicity parameters. These were fitted by nonlinear regression to estimate EC_{50} values. **Results:** Preliminary studies of cytotoxicity dihydro-pentedrone and dihydro-methylone showed neurotoxicity in a concentration-dependent manner, with an EC_{50} of 1.44 mM and 2.37 mM, respectively, indicating greater cytotoxic potency than the corresponding parent cathinones [5]. **Conclusions:** β-Hydroxy metabolites may be more cytotoxic than parent cathinones, reinforcing the need to include metabolites in toxicological profiling.

Keywords: Synthetic cathinones; SH-SY5Y cells; β-hydroxy metabolites; Cytotoxicity

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24825 | Development of Transethosome Nanocarriers for the Delivery of Anti-Ageing Bioactive Compounds

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Background & Aim: Skin ageing involves structural and functional changes driven by intrinsic and environmental factors, including oxidative stress, reduced fibroblast activity, and decreased collagen production. These processes contribute to dryness, wrinkle formation, and weakening of the skin barrier. The outermost skin layer, the *stratum corneum*, acts as a strong barrier that limits the penetration of many topically applied compounds, reducing their effectiveness. Nanotechnology-based delivery systems have emerged as promising strategies to improve the stability and dermal delivery of bioactive compounds. The aim of this study was to develop transethosomes for the encapsulation of an anti-ageing bioactive compound to enhance its stability and skin delivery. **Methods:** Transethosomes were prepared using the thin-film hydration method. The formulations were characterized by measuring particle size, polydispersity index and zeta potential using dynamic light scattering. Encapsulation efficiency and formulation stability were also evaluated. Cytocompatibility was assessed using HaCaT keratinocyte cells through cell viability assays. In addition, cellular uptake studies were performed to investigate the internalization of the transethosome formulations in skin cells. **Results:** Preliminary results indicate the successful formation of transethosomes with nanoscale size distribution, low polydispersity index, and suitable physicochemical properties for topical delivery. The formulations demonstrated efficient encapsulation and improved stability of the bioactive compound. In vitro studies with keratinocyte cells confirmed good cytocompatibility and suggested effective cellular interaction of the developed nanocarriers. **Conclusions:** These findings suggest that transethosomes represent a promising strategy to enhance the stability and dermal delivery of anti-ageing bioactive compounds, supporting their potential application in advanced skin care formulations.

Keywords: Transethosomes; skin ageing; topical delivery; keratinocytes

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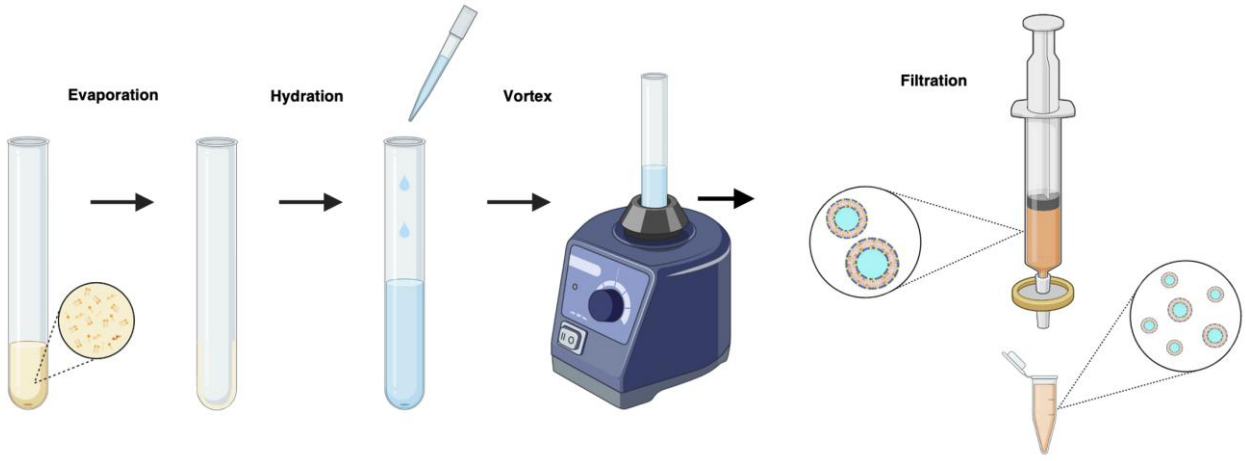

Figure 1: Transethosomes preparation

24888 | Digestibility of dietary zinc from different origins in European seabass (*Dicentrarchus labrax*)

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Background & Aim: Aquaculture helps meet the growing global demand for animal protein, requiring strategies that balance productivity, animal health, and environmental sustainability, with nutrition directly influencing growth performance, feed conversion, and stress resistance. Diet formulation research still focuses mainly on macronutrients, and mineral requirements remain poorly defined for several fish species, despite their potential to improve performance [1]. Zinc (Zn) has received limited attention despite acting as an enzymatic cofactor, regulating antioxidant and immune responses [2]. Its bioavailability depends on mineral interaction, diet composition and Zn source, complicating requirement estimates. European seabass (*Dicentrarchus labrax*) is a major aquaculture species with poorly defined zinc requirements [3] hindering diet optimization and risking deficiency or oversupplementation. The present study aims to compare the bioavailability of different Zn sources in juvenile European seabass. **Methods:** An 8-week digestibility trial will evaluate 5 diets (in triplicate): one control diet and four diets supplemented with 37.5 mg Zn/kg of diet two organic sources (Zn-methionine and Zn-glycinate) and two inorganic sources (Zn sulfate and Zn oxide). Apparent digestibility will be determined using chromium oxide as inert marker. Additionally, tissue Zn retention will be quantified in liver, skin and vertebrae along with oxidative status and the expression of biomarkers associated with Zn metabolism (e.g. metallothein). **Results:** Organic Zn sources are expected to show higher bioavailability reflected in greater digestibility, tissue retention and metallothionein expression with improved antioxidant modulation. **Conclusions:** These findings are expected to support the identification of suitable Zn sources for European seabass contributing to improved diet formulation and reduced zinc excretion into the aquatic environment, promoting more sustainable aquaculture practices.

Keywords: Zinc (Zn); European seabass (*Dicentrarchus labrax*); Bioavailability; Sustainability

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24423 | Dissecting IL-10 and T cell interactions for improved IL-10-based therapies

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Background & Aim: The anti-inflammatory cytokine interleukin-10 (IL-10) emerged as a promising therapeutic candidate for inflammatory diseases; however, severe side effects, including anemia and increased serum interferon (IFN)-γ, have limited its clinical use.[1] Paradoxically, IL-10-based immunotherapies showed beneficial effects in cancer by promoting $CD8^+$ T cell activation and enhancing IFN-γ and granzyme B production, highlighting the dual anti- and pro-inflammatory roles of IL-10.[2] The Immune Regulation group (i3S) developed *in vivo* models of IL-10 overexpression and identified mechanisms underlying therapy-associated toxicity. In pMT-10 mice, a transgenic system of increased IL-10 production as a response to zinc administration,[3] IL-10 exposure drives strong T cell activation and emergency myelopoiesis.[4] Current work aims to elucidate the mechanisms of IL-10-mediated T cell reprogramming. **Methods:** Systemic effects of elevated IL-10 in pMT-10 and pMT-10.IL-$10Ra^{fl/fl}$CD4-Cre mouse models, are assessed using flow cytometry, histological analysis, and molecular biology approaches. **Results:** In pMT-10 mice, IL-10-reprogrammed T cells are senescent, infiltrate several organs, and cause alterations compatible with accelerated aging, including a $CD4^+$ and $CD8^+$ T cell naïve/effector-memory subset imbalance and accumulation of $PD\text{-}1^+CD38^+$ cells. Tissue alterations are also observed in the pancreas and adipose tissue. In pMT-10.IL-$10Ra^{fl/fl}$CD4-Cre mice, T cells lack IL-10 receptor signaling despite high systemic IL-10, allowing discrimination between direct and indirect IL-10 effects. Preliminary results suggest reversal of the T cell subset imbalance and reduced tissue pathology. Ongoing efforts aim to further elucidate the molecular and cellular mechanisms by which IL-10 reprograms T cells. **Conclusions:** Overall, direct IL-10-mediated T cell reprogramming appears central to IL-10 therapeutic limitations, and targeting IL-10 T cell interactions may reduce adverse effects and enable improved therapies.

Keywords: Interleukin-10; Immunotherapies; Immunity; Senescence

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24303 | Dissecting the Effect of Astrocyte Condition Media on Neuronal Morphology and Functionality

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Astrocytes, the predominant glial cell type in the mammalian brain, regulate synapse formation and function partially through secreted proteins, contributing to the excitation-inhibition balance of neural circuits. Primary neuronal cultures are widely used to study the synaptogenic potential of astrocyte-secreted factors. Astrocyte-conditioned media (ACM) has been shown to promote both excitatory and inhibitory synapse formation and to alter neuronal morphology [1]. However, our knowledge of which proteins play a role in synaptogenesis is limited, with only a handful of proteins, such as thrombospondins (TSPs) and neurocan, proven to induce the formation of excitatory and inhibitory synapses, respectively [1] [2]. To address this knowledge gap, we have developed a glia-free primary neuronal culture system to evaluate the effect of specific astrocyte-secreted proteins on synaptogenesis. Immunofluorescence for cell-type-specific markers confirmed negligible astrocytes, microglia and OPC contamination. As a proof-of-concept, neurons were treated with either conditioned media (CM) or ACM, and synapse density was quantified using excitatory and inhibitory synaptic markers. Preliminary data indicates that ACM increases overall synapse number, as expected. To evaluate structural changes, neurons were transfected with a membrane-targeted fluorescent reporter and dendritic length and branching were quantified following ACM treatment. In the initial trial, ACM treatment was associated with an increase in dendritic length and branching compared to CM treatment. Overall, our data indicates we have implemented a suitable in vitro system to investigate the effect of astrocyte-secreted proteins on neuronal development and synaptogenesis.

Keywords: astrocytes, astrocyte-conditioned media, synaptogenesis, neuron.

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24764 | Do E-cadherin-dysfunctional cells exploit dormancy to survive in restrictive extracellular matrix environments?

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Background & Aim: E-cadherin coordinates intercellular junctions and signalling pathways essential for maintaining epithelial polarity and differentiation [1]. Alterations of E-cadherin, observed in approximately 70% of carcinomas, are strongly associated with invasion and metastasis [2]. However, the mechanisms by which loss of cell-cell adhesion promotes cancer cell survival and dissemination remain unclear. We propose that cancer cells exploit dormancy as a survival strategy, adapting to the extracellular matrix (ECM) conditions that disrupt differentiation programs [3-5]. Our main aim is to identify an ECM-induced dormancy signature that may uncover novel therapeutic vulnerabilities in dormant cancer cells. **Methods:** We established an *in vitro* model using a cadherin-negative cell line transfected with vectors encoding wild-type E-cadherin or the I326N and E758G variants identified in diffuse gastric cancer. E-cadherin expression and localization were assessed by Western blot and immunofluorescence. Transfected cells were used to form spheroids embedded in distinct ECMs, which were analyzed for structural organization and proteomic profiles. **Results:** The I326N and E758G variants resulted in a significant reduction in total protein levels and localization, when compared with the wild-type, consistent with functional impairment. In 3D culture, spheroids derived from wild-type and mutant cells displayed marked differences in morphological organization. Wild-type cells formed larger and compact aggregates, whereas mutant cells generated smaller spheroids that dispersed more diffusely throughout the ECM scaffold. Preliminary data indicate altered COUPTF1 and NDRG1 expression, suggesting their involvement in E-cadherin–mutant cell dormancy. **Conclusions:** This work provides evidence that E-cadherin dysfunction reshapes cellular architecture and molecular programs in response

to the ECM environment, possibly supporting adaptive dormancy. We envision that these ECM-associated readouts may serve as actionable targets to eliminate dormant cancer cells.

Keywords: E-cadherin; Cell dormancy; Extracellular matrix; Cancer invasion.

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24254 | Effect of salinity on ectoine biosynthetic gene cluster expression in Planctomycetota

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Background & Aim: *Planctomycetota* is a ubiquitous, ecologically versatile and metabolically diverse phylum, abundant in marine environments, where fluctuating salinity conditions impose osmotic stress and require efficient adaptive responses[1-4]. Ectoine, a widespread compatible solute involved in osmoprotection and of biotechnological relevance, is encoded in planctomycetal genomes, yet its regulation remains poorly characterised[5-6]. This study aims to assess the salinity tolerance of six marine planctomycetal strains and establish a basis for analysing the relationship between salt stress and ectoine gene cluster expression. **Methods:** The analysed strains belonged to the species *Gimesia benthica* (strains Pd1 and Ent1), *Gimesia maris* (strain 534-30), *Rubinisphaera brasiliensis* (strain Gr7), *Rubinisphaera margarita* (strain ICM_H10) and *Bremerella alba* (strain FF15). Cultures were grown in M14 medium[7] at 3.5% NaCl (control) and at 4.5%, 5.5%, 6.5%, 7.5%, and 8.5% NaCl (experimental conditions). Genomic DNA was extracted using the E.Z.N.A. DNA/RNA isolation kit[8]. The 16S rRNA gene was amplified by PCR and visualised on a 1% agarose gel. Strain-specific primers targeting the ectoine biosynthetic cluster were designed for subsequent quantitative PCR (qPCR) analysis. **Results:** Growth assays revealed strain-specific tolerance patterns. All strains grew at 3.5% and 4.5% NaCl. At 5.5%, ICM_H10 failed to grow, while the remaining strains persisted. At 6.5%, only FF15 and Gr7 maintained growth. At 7.5% and 8.5% NaCl, growth was observed solely for FF15. **Conclusions:** The strains exhibit distinct osmotic tolerance profiles, reflecting the metabolic diversity within *Planctomycetota*. Notably, only FF15 and Gr7 sustained growth at higher salinity levels, possibly reflecting enhanced activation of osmoprotective pathways, including ectoine biosynthesis, a hypothesis to be tested in future expression analyses. These findings provide a basis for understanding ectoine-mediated osmoadaptation in this phylum.

Keywords: *Planctomycetota*; Biosynthetic Gene Cluster; Ectoine; Abiotic Stress; Salinity

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24532 | Effects of Environmental Enrichment on Exploratory Behaviour and Puzzle Interaction in African Grey Parrots

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Background & Aim: Environmental enrichment is used by zoos to promote animal welfare and cognitive stimulation by encouraging natural behaviours in captive species [1], [2], [7], [8], [9]. The African grey parrot (*Psittacus erithacus*) is known for its high intelligence and complex behavioural patterns, resulting in greater sensitivity to environmental conditions [1], [3], [4], [6], [7]. The present study aimed to evaluate the interaction of 12 African grey parrots with feeding puzzles and analyse the impact of environmental enrichment on exploration and food-seeking behaviour [1], [3], [7], [9]. **Methods:** The study was conducted on 12 African grey parrots kept in captivity at a zoo. In an initial phase, the birds were exposed to two types of puzzle feeders over 5 sessions. The intervention phase consisted of varying stimuli over 10 sessions, associated with environmental enrichment and categorized by food search, destruction, and combination of cognitive and sensory elements [9]. In the last phase, puzzle feeders were reintroduced to assess behavioural changes during 5 sessions after exposure to various types of environmental enrichment. Throughout the study, the total duration of sessions and interaction times were recorded using a stopwatch, as behavioural responses were measured using an adapted ethogram [10]. **Results:** During the initial study phase, the birds displayed low interaction with the puzzles and were consistent with an initial neophobic response to new objects [5]. This parameter became a behavioural reference for the study, allowing for the evaluation of subsequent responses after environmental enrichment. **Conclusions:** The initial behaviour demonstrated by the African grey parrots, which displayed low interaction with the puzzles, established an initial behavioural reference, as it will be decisive in assessing whether environmental enrichment can modify the birds' behaviour and motivation in relation to exploration and interaction with cognitive challenges.

Keywords: environmental enrichment; exploratory behaviour; puzzle feeder; animal welfare; novel object response

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24580 | Effects of increasing precipitation on the breeding success of Adélie penguins (*Pygoscelis adeliae*) in Antarctica

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In Antarctica, climate change has regionally varying effects, including increasing snowfall, rising temperatures and consequently more rainfall. When increased precipitation coincides with the breeding season of Adélie penguins (*Pygoscelis adeliae*), flooding nests can lead to nest abandonment or chick hypothermia, affecting breeding success. Previous work showed significant declines in Adélie penguin populations in the Antarctic Peninsula affected by warming and increased precipitation. However, linking breeding success to specific precipitation events requires fine scale spatio-temporal data. Here, we compare the effects of precipitation and snowfall to rainfall transitions on critical breeding stages between two colonies of Adélie penguins in East Antarctica (Pétrel Island) and in the northern Antarctic Peninsula (Ardley Island) using two long-term monitoring programs. Regional and large-scale climate data are obtained from the ERA5 reanalysis of the European Centre for Medium-Range Weather Forecasts (ECMWF) and the regional climate model, Modèle Atmosphérique Régional (MAR). Moreover, we use *in situ* and ground-based remote sensing meteorological data. Precipitation data from vertically profiling radars at both sites are used to quantify how precipitation phase transitions and extreme events affect egg and chick survival. Regionally different population trends have already been identified, with declining populations in the Antarctic Peninsula and more stable to increasing populations in East Antarctica. Precipitation occurrence in early stages exhibits negative impacts on breeding success including even complete failure. Over the rapidly warming Antarctic Peninsula, higher breeding failures related to warm weather and precipitation events are expected. This work highlights the importance of regional long-term monitoring and the

collection of fine-scale spatio-temporal breeding and weather data synchronously, contributing to the priorities of the Antarctica InSync international initiative.

Keywords: Antarctica; climate change; breeding success; seabirds

24765 | EGF and GM-CSF promote trophoblast proliferation, migration and invasion, strengthening endometrial crosstalk

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Background and Aim: Embryo implantation is a dynamic process that requires tightly regulated trophoblast proliferation, migration, and invasion into the maternal decidua[1]. Growth factors and cytokines at the maternal–fetal interface play essential roles in this process[2]. Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Epidermal Growth Factor (EGF) activate pro-survival and pro-motility signaling pathways, and extracellular matrix remodeling, processes fundamental for implantation[3,4,5,6]. However, their functional effects on trophoblast behavior remain incompletely characterized. This study evaluated GM-CSF and EGF effects on trophoblast viability, migration, and implantation-related genes using *in vitro* models. **Methods:** BeWo and HTR8/SVneo trophoblast cell lines were treated with GM-CSF or EGF (0.1–10 ng/mL). Cell viability and cytotoxicity were assessed by MTT and LDH assays, migration by wound healing in HTR8/SVneo cells, morphology by Giemsa staining, and implantation-related gene expression (CLDN1, TJP1, MMP2, MMP9, MMP11) by RT–qPCR, followed by statistical analysis. **Results:** MTT and LDH confirmed treatment tolerability, with a dose-dependent metabolic reduction in BeWo but not HTR8/SVneo cells. Giemsa staining showed preserved cellular morphology, supporting the absence of structural damage. Scratch assays revealed increased wound closure following exposure to both GM-CSF and EGF, indicating enhanced migratory capacity. RT–qPCR analysis showed increased expression of the junctional markers CLDN1 and TJP1, indicating modulation of cell–cell adhesion, together with upregulation of MMP2, MMP9, and MMP11, consistent with enhanced extracellular matrix remodeling—particularly in response to GM-CSF at 5 ng/mL. **Conclusion:** GM-CSF and EGF promote trophoblast migratory behavior and implantation-related gene expression without cytotoxicity, supporting their role in regulating

trophoblast functional competence. IVF embryo culture media supplementation with those factors might contribute to ART outcome improvement.

Keywords: Embryo implantation; Trophoblast function; Embryo Transfer Medium; GM-CSF; EGF.

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24597 | Establishing Experimental Models to Investigate Regulation of Putative Target Genes by PRRXL1 Transcription Factor in the Developing Spinal Cord

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Background & Aim: Chronic pain is a debilitating condition lacking effective treatment. One way to understand how the pain nervous system operates is to investigate the molecular mechanisms that underlie its assembly. The dorsal spinal cord (DSC) is a major center for processing somatosensory information, composed of excitatory and inhibitory neuronal subtypes. The excitatory neurons specifically express the transcription factor PRRXL1, which is necessary for the assembly of the pain circuitry [1]. Our lab has identified a genome-wide list of PRRXL1 direct target genes. However, the direct regulatory impact of PRRXL1 on its target genes remains to be functionally characterized. This work aims to establish in our lab two model systems: an *in vitro* model that mimics DSC excitatory neurons [2, 3] and a developing chick DSC model. These models will be used to perform PRRXL1 gain-of-function (GOF) studies. **Methods:** We are implementing a reported model [2, 3] of retinoic acid (RA)-differentiated P19 cells by performing a timeline characterization of cell morphology and gene expression by immunocytochemistry and RT-PCR, respectively. In the chick DSC model, we are characterizing the expression pattern of PRRXL1 target genes by RNA *in situ* hybridization. Afterwards, we will perform PRRXL1 GOF assays in both model systems and analyze the effect on the expression levels of the target genes. **Results:** We already designed and validated the primers for gene expression analysis in RA-differentiating P19 cells. The characterization of the cellular model will be presented. In addition, we already generated and are testing RNA *in situ* probes for the expression pattern analysis of selected PRRXL1 target genes. Finally, we will present preliminary data of PRRXL1 GOF assays using both models. **Conclusions:** We hope to successfully establish both models to investigate PRRXL1-mediated transcriptional regulation and provide novel insights into the gene regulatory networks governing excitatory spinal neuron specification.

Keywords: PRRXL1, Spinal Cord Dorsal Horn, Neuronal cell line, Transcriptional regulation

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24458 | Establishing mussel primary cell lines as a cellular platform for sustainable antifouling research

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Background & Aim: Biofouling is the colonization of submerged surfaces, such as ships' hulls, by micro- and macroorganisms, leading to increased maintenance costs, hydrodynamic drag with higher fuel consumption and greenhouse gas emissions, and to the transport of invasive species, which disrupts local ecosystems. Antifouling coatings help prevent these issues. However, commercial coatings containing copper or booster biocides harm marine life[1].There is an urgent need for sustainable, nontoxic antifouling coatings and our group focuses on identifying new eco-friendly bioactive compounds[2].Antifouling research has mainly focused on phenotypic assays and antifouling compound development would benefit from the identification of molecular targets and the elucidation of mechanisms of action to comply with regulatory requirements[3],[4]. A robust cellular platform is essential to this end. To date, only one molluscan immortalized cell line has been described[5]. Progress is limited by contamination, low cellular proliferation index, cell senescence, and culture medium-related problems[5],[6]. This study aims to establish mussel primary cell lines and use them as cellular platforms to attempt immortalization and to assist future antifouling research. **Methods:** Adult mussels (*Mytilus galloprovincialis*) will be collected, and species will be identified by DNA extraction and 18S rRNA sequencing. Samples from different tissues will be dissected and decontaminated. Cells will be obtained by explant migration and enzymatic digestion. Various culture conditions will be attempted (% fetal bovine serum, additives, adhesion coatings, osmolarity). Cells morphology and proliferation will be assessed under bright field microscopy. **Results:** An optimized protocol for mussel primary cell line suitable for transfection and transgene expression will be obtained. **Conclusions:** Establishing mussel primary cell lines will advance antifouling research and support broader fields such as mussel biology and ecotoxicology.

Keywords: Primary cell line, Mussel, Antifouling

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24631 | Establishment of an in vitro Model for the L3-L4 Transition of Anisakis simplex and its Molecular Characterization

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Background & Aim: *Anisakis simplex* is a zoonotic marine nematode with a complex life cycle involving crustaceans, fish, molluscs and marine mammals [1]. Humans become accidental hosts through ingestion of raw or undercooked fish or molluscs containing infective third-stage larvae (L3), leading to anisakiasis and allergic reactions. The transition from L3 to the fourth larval stage (L4) represents a critical developmental step associated with morphological and molecular changes [2]. This study aimed to establish an *in vitro* model for the L3–L4 transition and to generate molecular material for characterization of stage-specific gene expression in this species. **Methods:** L3 larvae were isolated from the viscera of *Trachurus trachurus* and maintained in RPMI-1640 medium supplemented with antibiotics [3]. To induce moulting, larvae were incubated at 37°C under reduced oxygen conditions, simulating the definitive host environment. Morphological changes were monitored by optical microscopy. Total RNA was extracted from L3 and L4 stages, followed by cDNA synthesis and PCR amplification of selected targets. **Results:** The *in vitro* conditions induced the L3–L4 transition in approximately 20% of larvae after 10 days of incubation. Morphological analysis of moulted larvae confirmed the development of three prominent lips and the disappearance of the larval boring tooth, consistent with L4 characteristics. High-quality RNA was obtained from both stages, and successful cDNA amplification confirmed the suitability of the model for downstream molecular analyses. qRT-PCR of selected targets detected stage-dependent gene regulation, particularly relevant for known allergen expression. **Conclusions:** The established in vitro model enables controlled study of the L3–L4 transition in *A. simplex* and provides molecular templates for future transcriptomic and allergen characterization studies. This platform represents a valuable tool for advancing the understanding of anisakiasis and parasite-host interactions.

Keywords: *Anisakis simplex*; Larval moulting**;** zoonotic parasite; L3-L4 transition.

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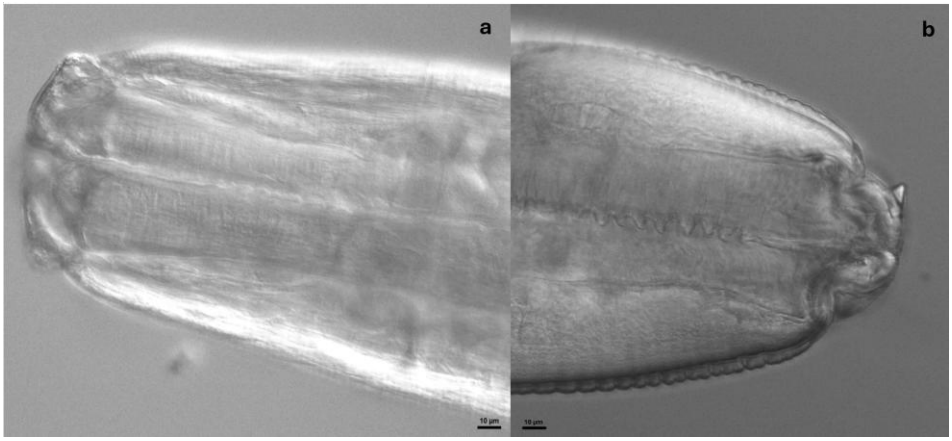

Figure 1: Comparative optical microscopy of *Anisakis simplex* cephalic regions: (a) L4 stage showing the development of three distinct lips and the absence of larval structures; (b) L3 stage characterized by the presence of the boring tooth (white arrow). Scale bars: 10 µm.

24643 | Evaluating the Predictive Role of Thrombosis-Related Genetic Polymorphisms in Cancer Cachexia: Insights from a Hospital-Based Study

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Background & Aim: Beyond the direct impact of cancer, malignant diseases are frequently associated with comorbid conditions, namely thrombosis and cachexia, which deteriorate patient prognosis [1]. Cancer-associated thrombosis (CAT) and cancer-associated cachexia (CAC) seem to share underlying biological mechanisms and risk factors, including genetic risk factors [1-3]. Among these, single-nucleotide polymorphisms (SNPs) are the most common ones [4]. This study was performed to explore the influence of four recognized CAT-related SNPs, namely *F3* rs1361600, *FGG* rs2066865, *PROCR* rs10747514 and *TFPI* rs10153820, on the susceptibility to CAC development and the disease severity in a cohort of cancer patients from the North region of Portugal [5, 6]. **Methods:** A cross-sectional observational study was carried out at the Portuguese Institute of Oncology of Porto (IPO Porto) with 141 cancer patients. The presence of CAC was evaluated based on the criteria of Fearon and the CASC-IN tool [7, 8]. The four SNPs were evaluated using real-time polymerase chain reaction (qRT-PCR) employing the TaqMan

allelic discrimination technology [9]. **Results:** Concerning the patients' characteristics, the SNPs *F3* rs1361600 and *FGG* rs2066865 were shown to be significantly associated with patients' body mass index (χ^2 test, p=0.006 and p=0.043, respectively). Regarding the impact on CAC status, *PROCR* rs10747514 was linked to the disease occurrence across different disease assessment tools (χ^2 test, p<0.05). Specifically, AA genotype carriers presented a three-to-four-fold risk increase of developing CAC compared to G allele carriers (AG and GG genotypes). As for the influence on patients' survival, carriers of the *TFPI* rs10153820 AA genotype presented lower overall survival (OS) compared to their counterparts (carriers of AG and GG genotypes), considering the different disease assessment tools (Log-rank test, p<0.05). **Conclusions:** This study suggests that CAT-related SNPs could play a role in the development and severity of CAC, with implications in inflammation and metabolic pathways. These SNPs could potentially inform improved prophylactic strategies for CAC, ultimately enhancing both the quality of life and survival outcomes of cancer patients.

Keywords: Neoplasms; Inflammation; Blood Coagulation; Single-Nucleotide Polymorphisms; Polymerase chain reaction.

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24397 | Evaluation of an essential oil-based bioinsecticide for the control of *Philaenus spumarius* and its phytotoxic effects on plant hosts

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Abstract: *Xylella fastidiosa* is a xylem-associated bacterium causing a destructive disease affecting numerous plant species, including crops and ornamental plants of economic importance in Europe. The bacterium is transmitted by insect vectors that feed on xylem sap. Among them, *Philaenus spumarius* (Hemiptera: Aphrophoridae) is considered the main vector in Europe, as it shares several host plants, is abundant in affected areas, and has repeatedly tested positive for *X. fastidiosa*. Since no effective curative treatment exists, control relies mainly on destroying infected and potentially infected plants within quarantine zones, causing significant economic and ecological costs. After *P. spumarius* was identified as the main vector, control strategies targeting it began, including habitat modification, push–pull strategies, biological control, and insecticides. Synthetic insecticides can be effective and, in some regions, are mandatory to control nymph populations. However, they may contaminate water, soil, and air, affect beneficial non-target organisms, and promote insecticide resistance. Essential oils have emerged as potential alternatives, generally more selective and less harmful to mammals and the environment. Some have already been tested on *P. spumarius*, though results were variable. It is also important to evaluate their effects on host plants due to possible phytotoxicity. In this project, selected essential oils will be tested on 4th and 5th instar nymphs of *P. spumarius*. In parallel, phytotoxicity will be assessed on host plants through symptom observation and biochemical analyses, including H_2O_2 levels, reduced glutathione, and lipid peroxidation after foliar application. This project aims to demonstrate the potential of essential oils as a sustainable alternative for controlling *P. spumarius* in agriculture.

Keywords: Biological control agents; Botanical insecticide; Integrated pest management; Meadow spittlebug;

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24211 | Evaluation of the impact of genetic polymorphisms in IFITM3 on susceptibility to SARS-CoV-2 infection in cancer patients

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SARS-CoV-2 infection was responsible for the COVID-19 pandemic. Due to its high transmissibility and infection rate, the implementation of preventive measures was necessary, particularly for more predisposed individuals, such as cancer patients. These individuals show increased susceptibility to COVID-19 infection due to their immunosuppressed state cause by both aggressiveness of the disease and the treatments required. Therefore, it is important to study new biomarkers, such as genetic polymorphisms, to allow the stratification of cancer patients according to their susceptibility to infection and response to vaccination. The interferon-induced transmembrane protein 3 (IFITM3) plays a key role in preventing the release of viral genetic material into the cytoplasm and, consequently, cell infection. Thus, this project focuses on the influence of two IFITM3 genetic polymorphisms (SNPs), *rs12252* and *rs34481144*, on susceptibility to COVID-19 infection. Genotyping of genomic DNA from diseased and healthy individuals was performed using real-time PCR, and the results were subsequently analysed using IBM® SPSS® Statistics software (version 29.0.0.0). None of the polymorphisms showed a direct increase in the risk of SARS-CoV-2 infection. However, carriers of the AG genotype for *IFITM3 rs12252* showed an increased risk of COVID-19 infection in men, while carriers of the TT+TC genotypes for *IFITM3 rs34481144* showed an increased risk of COVID-19 infection in men with haematological tumours, and carriers of the CC+CT genotypes showed an increased risk of infection in cancer patients with type 2 diabetes. Regarding survival rates, only cancer patients with the CC genotype of *IFITM3 rs34481144* had a shorter lifespan compared to those with the CT+TT genotypes. And increased the risk of mortality in cancer patients without dyslipidaemia and without diabetes. Therefore, these SNPs may be potential biomarkers for the stratification of cancer patients and for improving personalized therapy.

Keywords: COVID-19; Cancer; Genetic Polymorphisms; IFITM3; Biomarkers

24848 | Experimental approaches assessing why there are so many colours in Littorina marine snails

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Background & Aim: Shell colour polymorphism can have many functions including thermoregulation, predation avoidance (by crypsis) and communication [1,2,3]. Experimental approaches can be used to try to disentangle the main factors driving this variation in systems with prevalent colour diversity, as in *Littorina* marine snails[4]. The main goal of this study was to understand the role of diet in shell colour polymorphism in *L. obtusata* and the role of crypsis in *L. saxatilis*. **Methods:** To evaluate the effects of diet we implemented a pilot study in the lab after collecting 24 *L. obtusata* (12 yellow and 12 brown) from Praia Norte (Viana do Castelo, Portugal) together with macroalgae of *Ascophyllum nodosum* displaying yellow and brown colour sections. Snails were distributed into three aquaria with equal algae biomass (half brown, half yellow): 1) 6 brown snails; 2) 6 yellow snails; and 3) 6 brown and 6 yellow snails. Snails and algae were weighted periodically to assess consumption during 7 weeks and their stomachal content was later analysed to determine the proportion of each algal section they consumed. In parallel, photos of *L. saxatilis* in their background collected via citizen science projects from their natural habitats are being analyzed to test if their shell colour tends to be more similar to the background than expected by chance (as expected due to crypsis). **Results:** In all diet treatments, gut content analysis showed that snails ate a higher percentage of algae sections with a colour that is more similar to their shell colour(treatment 1, 70%; treatment 2, 80%; and 80% for brown and 58% for yellow snails in treatment 3). The analysis of the shell photos is still ongoing and will be presented at the meeting. **Conclusions:** Our preliminary results revealed a tendency for shell colour morphs to consume algae that match their own shell colour. This pattern suggests a possible association between diet and colour polymorphism, that needs to be further explored beyond this pilot study.

Keywords: Colour polymorphism ; diet ; predation

24299 | Exploring Metabolic Stress Conditions in Annulus Fibrosus Cells

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Background & Aim: Annulus fibrosus (AF) is the outer ring of the intervertebral disc, responsible for maintaining IVD integrity and preventing herniation. The AF cells are exposed to a tightly regulated, poorly vascularized microenvironment that sustains mechanical integrity and metabolic balance in the spine[1]. IVD physiological conditions include a pH of 7.1-7.4, glucose levels of 1-5 mM, lactate 2-16 mM and oxygen levels of 1-5%[2]. During IVD degeneration, impaired nutrient transport promote enhanced glycolysis, lactate accumulation, reduced ATP synthesis and progressive acidification (pH 6.8-6.2)[3], leading to unbalanced production of inflammatory markers and extracellular matrix (ECM) degradation. However, the response of AF cells to the degenerated IVD microenvironment is still under investigation. This study aims to investigate human AF cell response to metabolic stress by integrating pH and glucose variations in 2D cultures. **Methods:** Human AF cells were isolated with informed consent from patients undergoing microdiscectomy surgery, cultured (2D) in IVD media (as previously established[4]), and exposed for 24h to controlled variations in pH (6.25-6.90) and glucose concentration (0.5-2 mM). Metabolic activity, cytotoxicity, morphological changes (F-actin) and COX-2 expression were assessed. **Results:** Extracellular pH exerted the most pronounced effect on AF cell behaviour, with all tested conditions inducing actin constriction and increased COX-2 expression, compared to basal conditions. pH 6.25 led to reduced metabolic activity, cytoskeletal remodeling and cell detachment, despite low cytotoxicity. Glucose deprivation resulted in reduced metabolic activity without F-actin alterations, with 2 mM glucose group showing COX-2 expression. **Conclusions:** This work allowed for the establishment of in vitro experimental conditions to assess the role of glucose and pH on AF cell metabolism, highlighting pH 6.25 as the critical tolerance threshold.

Keywords: Intervertebral disc degeneration; Annulus fibrosus cells; Metabolic stress; Extracellular acidification; *In vitro* models

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24891 | Exploring the role of Heparan Sulfate 6-O-Sulfation in glycosaminoglycan remodeling and cell behavior in gastric cancer

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Background & Aim: Gastric cancer remains a leading cause of cancer-related mortality. Cumulative evidence supports the role of glycoconjugates in gastric cancer development [1], with aberrant proteoglycan expression being linked to cell malignancy features and poor prognosis [2,3]. Heparan sulfate (HS) proteoglycans (HSPGs), expressed at the cell surface and extracellular matrix, interact with multiple ligands and receptors, modulating signaling pathways, and their activity is strongly influenced by HS sulfation patterns, particularly 6-*O*-sulfation [4]. HS 6-*O*-sulfation is dynamically regulated during HS biosynthesis by HS 6-*O*-sulfotransferases (HS6STs) which add a sulfate group to the C6 position of GlcN residues and post-synthetically by 6-*O*-endosulfatases (HSulfs) that catalyze extracellular 6-*O*-desulfation (Figure 1). Although dysregulation of these enzymes has been associated with cancer [1], their specific role in gastric cancer progression remains unclear. This study aimed to elucidate the contribution of HS 6-*O*-sulfation in the regulation of cellular glycosaminoglycan (GAG) expression and tumor cell behavior. **Methods:** Glycoengineered gastric cancer cell models were generated by HS6ST1 knockout or HSulf-2 overexpression. Cellular and secreted GAG composition was assessed by disaccharide analysis, western blot, flow cytometry, and transcriptomic analysis. Functional assays are being performed, including wound healing assays to evaluate cell migratory capacity and the chick chorioallantoic membrane (CAM) assay to assess cell behavior in an in vivo context. **Results:** Abolition of HS 6-*O*-sulfation, via HS6ST1 knockout, led to increased total HS levels, both intracellularly and in the secretome, together with upregulation of syndecan-4, a cell surface HSPG associated with poor prognosis in gastric cancer. **Conclusions:** These findings demonstrate that HS 6-*O*-sulfation plays a critical role in regulating GAG composition and secretion, which may impact gastric cancer cell behavior,

highlighting HS sulfation imbalance as a contributor to tumor progression and a potential therapeutic target.

Keywords: Gastric cancer; Glycosaminoglycans; Heparan sulfate proteoglycans; HS 6-*O*-sulfotransferase 1 (HS6ST1); HS 6-*O*-endosulfatase 2 (HSulf-2).

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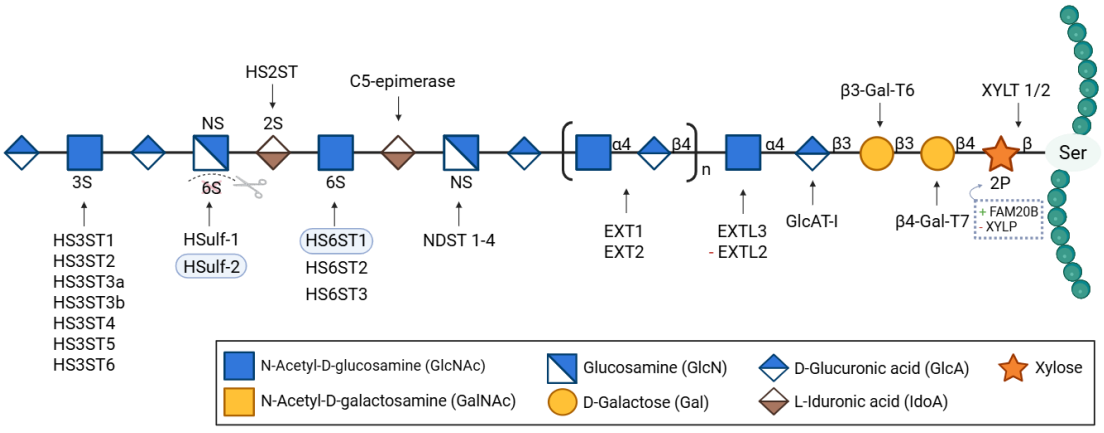

Figure 1: Overview of Heparan Sulfate biosynthesis and remodeling by HS6ST1 and HSULF-2. Created using BioRender (2026).

24402 | Exploring the skin protective effects of marine cyanobacteria from Cape Verde

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Background & Aim: Cyanobacteria are among the oldest living organisms on Earth and can survive under extreme conditions, including temperature, radiation, salinity, and pH variantions [1]. There adaptability is associated with the production of several secondary metabolites, some of which have potential applications in the cosmetic industry, particularly as natural bioactive ingredients [1,2]. This study, evaluated the chemical composition and biological activity of two marine cyanobacterial strains, *Leptothoe* sp. LEGE 181186 and *Salileptolyngbya* sp. LEGE 181191, to assess their potential as skin-protective agents. **Methods:** Two fractions with different polarities (acetonic and aqueous) were prepared from dry biomass. Their chemical profile was characterized by determining total phenolic content (TPC), carotenoid composition using high-performance liquid chromatography (HPLC), and phycobiliprotein content. Cytotoxicity was assessed using the MTT assay in human keratinocytes (HaCat) cell line. Antioxidant activity was evaluated by measuring the scavenging capacity against the physiological free radicals superoxide anion radical ($O^{\bullet -}$) and nitric oxide radical ($^{\bullet}NO$) generated *in vitro* (**Figure 1**). **Results:** The chemical analysis of the aqueous fractions showed a predominance in phycobiliproteins while the acetonic fractions had a larger concentration in phenolic compounds, carotenoids and chlorophylls. All fractions demonstrated antioxidant activity. Acetonic fractions showed better results in $^{\bullet}NO$ scavenging while aqueous fractions were more effective against superoxide anion. The preliminary results of the MTT cytotoxicity assay in the HaCat cell line suggest that aqueous fractions do not present toxicity, while the acetonic fractions are only toxic at higher concentrations. **Conclusions:** Both marine cyanobacterial strains have shown antioxidant potential and low cytotoxicity, supporting their potential as natural ingredients in cosmetic formulations.

Keywords: antioxidant activity; cytotoxicity; pigments; cosmetics; cyanobacteria.

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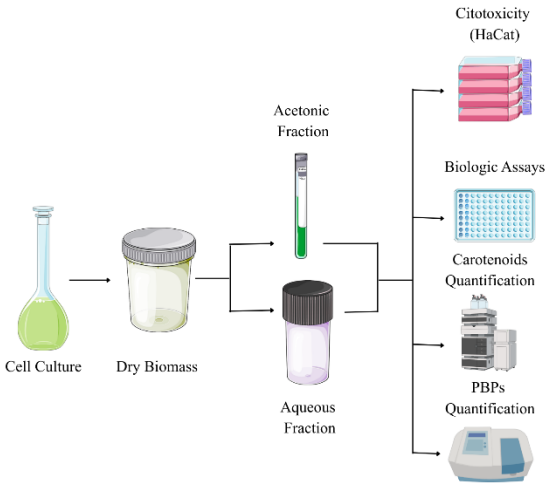

Figure 1: Graphic Methodology

24679 | Fermentation of *Ulva sp.* biomass for application as soil biofertilizer

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Background & Aim: Global soil degradation necessitates sustainable alternatives like seaweed-based biofertilizers. Although seaweeds are rich in nutrients and other biostimulant compounds, their direct use is limited by logistical issues, namely biomass preservation and storage. Bokashi technology offers a solution through anaerobic fermentation, suppressing pathogens and preserving biomass. However, seaweed fermentation is challenging due to their high moisture, salt, and low C/N ratios. This study aims to optimize *Ulva spp.* fermentation as a biofertilizer by testing various incorporation ratios with wheat bran and moisture levels.[1,2,3,4] **Methods:** A multifactorial study evaluated *Ulva sp.* and wheat bran mixtures at six seaweed incorporation ratios (0, 20, 40, 60, 80, 100%) and three moisture levels (35, 45, 60%). Samples were inoculated with effective microorganisms and fermented anaerobically for 21 days. Pre and post fermentation pH, electrical conductivity, and nutritional content (polysaccharides, minerals and lactic acid) were analyzed to evaluate the fermentation efficiency and characterize the resulting composts. **Results:** Moisture and seaweed proportions significantly influenced fermentation success. The 60% moisture level performed best across all ratios. Specifically, the required benchmark of pH below 4.5 was achieved with 20% and 40% seaweed incorporation at 60% moisture. No effective fermentation occurred at 35% moisture. Electrical conductivity increased with higher seaweed ratios. Ongoing analyses will further detail the nutritional profile of these biofertilizers. **Conclusions:** *Ulva spp.* biomass can be successfully stabilized through Bokashi fermentation under specific parameters. Ratios up to 40% seaweed with 60% moisture provide an optimal pathway for transforming marine biomass into stable organic products. These findings support the development of sustainable soil amendments using seaweed.

Keywords: Ulva sp., Bokashi fermentation, Biofertilizer, Marine biomass e Waste valorization.

Acknowledgments: We would like to acknowledge the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) for providing the necessary facilities and laboratory support to conduct this research. Special thanks are extended to the Fundação Amadeu Dias for its financial support through the Blue Young Talent (BYT) program which made this work possible. We also thank the research teams from the Blue Biotechnology, Environment and Health (BBEH) and Coastal Biodiversity (LBC) laboratories for their technical assistance and guidance during the experimental trials.

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24471 | Function of juvenile-like bill colour in adult common waxbills, *Estrilda astrild*

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Background & Aim: Carotenoid-based colour traits are widely regarded as honest signals of condition because their expression depends on dietary intake and physiological investment [1]. In the common waxbill (*Estrilda astrild*), adults normally show a red bill, whereas juveniles display a black bill. During the breeding season, some adult females develop black-mottled bills through melanin deposition, producing a juvenile-like appearance. The social significance of this reversible colour change is unknown [2]. This study tests whether juvenile-like bill coloration in adults alters social behaviour, particularly aggression and competitive interactions. **Methods:** We performed behavioural experiments on six captive groups of four adults (two males and two females; 24 birds). Bill colour was experimentally manipulated using non-toxic paint to create three treatments: control red bills, control blue bills and black bills. All individuals experienced the three conditions. Social behaviour was measured during standardized food competition tests following 90 minutes of food deprivation. A single feeder was introduced, and interactions were video recorded for 20 minutes. Time at the feeder, latency to feeder, locomotor activity, aggressive interactions and allopreening were quantified and analysed. **Results:** We predict that birds with black bills will receive fewer aggressive interactions. **Conclusions:** Juvenile-like bill coloration in adult waxbills might influence social interactions and may act as a signal reducing aggression. The study highlights the role of dynamic and context-dependent colour traits in regulating social behaviour in gregarious birds.

Keywords: Common waxbill; Social behaviour; Colour ornamentation; Carotenoids; Bill coloration.

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24481 | Genetic diversity in neutral and non-neutral genes in an Endangered shark species

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Background & Aim: Chondrichthyans (sharks, skates and chimaeras) diverged from other vertebrates ~450 million years ago (1), representing the earliest extant jawed vertebrates with fully functional innate and adaptive immune systems (2). The spiny dogfish (*Squalus acanthias*) is a highly migratory bathydemersal shark with a circumglobal distribution (3). Like other chondrichthyans, it has a long lifespan, late maturity, and low reproductive rate, increasing vulnerability to exploitation (3). Its populations have declined and become fragmentated due to overfishing and environmental change, leading to its classification as Endangered by the IUCN Red List (4,5). Unpublished NADH dehydrogenase 2 (ND2) and nuclear microsatellite data indicate reduced genetic diversity and significant differentiation in Adriatic and Black Sea populations relative to Irish Sea and Atlantic populations. This study aims to assess the genetic diversity and population structure in these four populations using functional immune markers: Toll-like receptors (TLR1 and TLR3) and Major Histocompatibility Complex (MHC) class I genes and compare with those retrieved for neutral markers. **Methods:** Genomic DNA was extracted from 40 individuals per population, PCR amplified with gene-specific primers and sequenced using next-generation platforms. The retrieved sequences will be used to estimate genetic diversity, population structure and differentiation using standard population genetic software. **Results:** Preliminary results indicate higher polymorphism in MHC class I than in TLR genes. **Conclusions:** By integrating neutral and immune-related markers, this work will help clarify the evolutionary processes shaping contemporary population structure in spiny dogfish, inform conservation units delimitation, and provide management strategies considering both demographic connectivity and adaptive potential undergoing environmental change.

Keywords: Chondrichthyans, *Squalus acanthias*, immune system, population structure

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24574 | GlyPhosMap: Mapping Glyco-Dependent Phosphoproteome Remodeling in Gastric Cancer

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Background & Aim: Aberrant *O*-glycosylation, particularly the expression of truncated *O*-glycans such as Tn and sialyl-Tn (STn), is strongly associated with gastric cancer (GC) progression (1). Using glycoengineered GC models, we previously showed that Tn/STn overexpression profoundly alters cell behavior, enhancing migration, invasion and chemoresistance despite reduced proliferation (2). However, the molecular mechanisms underlying this shift remain poorly understood (3). This project aims to map altered signaling landscapes in glycoengineered versus control models, identifying glycosylation-activated pathways to clarify drivers of tumor aggressiveness. **Methods:** Complementary glycoengineered cell lines will capture glyco-dependent phosphorylation across distinct GC phenotypes. Specifically, *C1GALT1* knockout AGS and MKN-45 cells (overexpressing Tn/STn antigens) model aggressive intestinal and diffuse-type GC. To resolve compartment-specific signaling, cells undergo subcellular fractionation by differential centrifugation to isolate nuclear, mitochondrial, membrane and cytoplasmic fractions. Following Lys-C/trypsin digestion, phosphopeptides are enriched using TiO_2 and Fe-NTA/IMAC, analyzed by LC–MS/MS and processed with dedicated bioinformatic pipelines to quantify phosphosites, assign subcellular localization and map glyco-dependent changes to signaling pathways. **Results:** Preliminary fractionation optimization yielded robust, highly pure fractions, confirming their suitability for downstream phosphopeptide enrichment. Building on this, we expect to generate comprehensive, compartment-specific phosphoproteomic maps comparing glycoengineered and wild-type GC cells. **Conclusions:** The successful isolation of high-purity subcellular fractions provides a solid foundation for mapping glyco-dependent phosphorylation signatures. Dissecting this glycosylation–phosphorylation axis will clarify how

truncated *O*-glycans rewire oncogenic signaling networks to drive aggressiveness and may reveal novel signaling hubs for targeted GC therapy.

Keywords: Gastric cancer; Aberrant glycosylation; Glyco-dependent phosphorylation; Subcellular phosphoproteomics.

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24701 | Habitat and abiotic drivers on the abundance and distribution patterns of amphibians in the Mindelo Ornithological Reserve

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Background & Aim: Amphibians are currently the most threatened vertebrate group, mostly due to habitat loss and fragmentation, intensive agriculture and invasive species expansion, climate change, diseases and pollution [1]. The Mindelo Ornithological Reserve (MOR) was the first protected area in Portugal and is recognised for its high amphibian diversity [2]. However, on-going habitat decline might impact amphibian diversity and abundance in the protected area [3]. Therefore, this work aims to assess the amphibian abundance in MOR, by habitat drivers and abiotic factors in shaping amphibian abundance and detectability. **Methods:** The study was based on repetitive sampling with regular inspection of 2 predefined transects in MOR. For each transect, the date, start and end time, daily precipitation (mm), temperature (ºC), relative humidity (%) and wind speed (km/h) were recorded. The location of each species was recorded in QField and processed in QGIS, and the data was stored in Excel and afterwards analysed in R software. Species abundance was estimated using N-Mixture models [4] at two different times of the year (autumn and spring) to assess seasonal differences in abundance and the influence of abiotic factors and habitat types on the species detectability and abundance. **Results:** During the last autumn survey, 9 amphibian species were recorded, with 1973 individuals. While the number of species observed the year before, in the same season, is equal, the total number of individuals was 2468. Nonetheless, due to heavy precipitation during the winter, it is expected that the amphibian detectability rates will increase and potentially elevate abundance compared to previous years. **Conclusions:** The analysis of amphibian abundance throughout the years enables the understanding of population trends and, thus, the long-term monitoring of amphibian communities within the MOR. This approach provides critical insight into the region's herpetofauna, supporting the development of conservation strategies.

Keywords: Conservation; Ecology; Long term monitoring; Population assessment.

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Figure 1: Satellite image of a transect in the Mindelo Ornithological Reserve (MOR). At each of the points marked with a blue cross in the transect, temperature (°C), relative humidity (%) and wind speed (Km/h) were recorded.

24478 | How does an epithelial tissue respond to mechanical challenges to mantain its organization?

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Background & Aim: Epithelia are subjected to mechanical forces during development, which contribute to their final shape [1]. However, adult epithelia can also experience mechanical challenges with the potential to deform and disrupt their normal organization [2,3]. Yet, we still understand poorly how adult epithelia respond to physiological mechanical challenges, such as contractions exerted by an enveloping muscle. Our aim is to understand how different epithelia respond to muscle contractions. **Methods:** We use *Drosophila melanogaster* as a model system, combining genetics, optogenetics and confocal microscopy to analyze epithelial cell responses to muscle contractions in the ovarian follicular epithelium and midgut epithelium. In particular, we are evaluating changes in cell morphology (e.g. cell height and apical cell area), cell adhesion and cytoskeleton organization in response to manual muscle removal. In parallel, we are we are also implementing optogenetic tools to acutely and reversibly modulate muscle contractions while preserving organ structure. **Results**: Our results show that light-induced activation of Channelrhodopsin in the ovarian muscle induces a tetanic contraction. Furthermore, our preliminary analysis of the follicular epithelium response to muscle removal indicates that the presence of muscle correlates with an increase in epithelial height and in actin accumulation at the lateral membrane. **Conclusions:** These results indicate that epithelia adjust their morphology and cytoskeleton organization in response to muscle activity. The ability to acutely trigger muscle contractions with optogenetics provides a controlled way to further test how these mechanical stimuli affect epithelial behavior. Together, this approach will help clarify how adult epithelia maintain their integrity when subjected to physiological mechanical forces.

Keywords: epithelia; muscle contractions; *Drosophila*; optogenetics; cytoskeleton

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24704 | Impact of human follicular fluid-relevant 17β-estradiol concentrations on capacitating spermatozoa

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Background & Aim: Polycystic Ovary Syndrome (PCOS) is a complex endocrinopathy and the leading cause of female infertility, characterized by hyperandrogenemia, oligo-ovulation, and polycystic ovaries. An altered hormonal and metabolic profile marks the pathophysiology of PCOS, which is extensively reflected in the follicular fluid (FF). During ovulation, the FF is released into the oviduct with the oocyte, altering the microenvironment where sperm capacitation and fertilization occur. Previous studies identified 17β-estradiol (E_2) as a significantly altered hormone in the FF from women with PCOS. Estrogen receptors are expressed in human sperm, nevertheless the impact of E_2 on its function is poorly understood. In this study, we aimed to disclose the impact of FF-relevant E_2 concentrations on human sperm capacitation. **Methods:** Human semen samples obtained from donors with normozoospermia (n=6) were pre-incubated in a modified capacitating BWW media at 37°C for 2h. Then, sperm was treated with E_2 based on the concentrations found in the FF of different phenotypes (non-PCOS CTRL: 55 ng/mL; PCOS group: 85 ng/mL; supra-PCOS: 200 ng/mL) for an additional 1h. Culture media without E_2 was used as negative control. After treatment, vitality (eosin-nigrosin staining) and motility (total and progressive) were assessed by optical microscopy. Acrosome reaction was assessed by Coomasie blue G250 staining. **Results:** Spermatozoa total and progressive motility is dose-dependently affected by E_2, showcasing a tendency towards a decrease in PCOS and supra-PCOS groups. Sperm vitality was not affected in any experimental conditions, suggesting that E_2 impacts motility without inducing cytotoxicity. **Conclusions:** Our results suggest that PCOS-relevant E_2 concentrations may reduce human sperm total and progressive motility. Although

further biological replicates are required to confirm this hypothesis, our work suggests that E_2 might play a role in PCOS-derived FF-induced dysfunction of human spermatozoa.

Keywords: 17β-Estradiol; fertility; follicular fluid; PCOS; spermatozoa

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Impacts of dietary antioxidants on the gut integrity of turbot postlarvae

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Background and Aim: Turbot (*Scophthalmus maximus*) larval production is a critical stage in aquaculture due to high mortality, small size and unknown nutritional requirements. Feed additives rich in polyphenols, such as grape seed and green tea extracts, may enhance intestinal integrity through antioxidant and immunomodulatory mechanisms. This study aimed to evaluate the effects of dietary supplementation with these extracts on intestinal morphology and larval performance[1,2]. **Methods:** Larvae were reared for 21 days (29–50 days after hatching, DAH) in a recirculating aquaculture system under controlled conditions. Larvae were distributed into fifteen tanks with three replicates per treatment. The control group received a commercial microdiet, while commercial-like experimental diets had the same formulation which was supplemented with grape seed and green tea extracts. At 50 DAH, larvae were sampled for standard length and histological analysis. Anterior intestine sections were collected using the first gill arch as reference. Alcian Blue Periodic Acid Schiff staining was applied for histomorphometry and mucin differentiation. Villus length and width, lamina propria and submucosa thickness, muscular layer thickness, absorptive area, and goblet cell number and type (mix goblet cells, acid goblet cells, neutral goblet cells) were quantified[3]. **Results:** Supplemented diets are expected to increase villus height and width, expand absorptive area, and enhance goblet cell density and mucus production. A reduction in lamina propria and submucosa thickness and lower inflammatory cell infiltration are also anticipated, indicating improved intestinal integrity[3]. **Conclusions:** Supplementation with polyphenols from grape seed and green tea is expected to promote an increase and improvement of intestinal villi, strengthen the intestinal mucosa through enhanced mucus production and promote a higher number of

goblet cells, that will increase the absorptive surface area, resulting in improved digestive efficiency.

Keywords: Mucins, Villus, Histology, Flatfish, Nutrition

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24670 | Marine Seaweeds as bioregulators of inflammation-related mediators and enzymes

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Background & Aim: Seaweeds constitute a ubiquitous group of photosynthetic organisms, recognized as one of the most promising marine sources of bioactive metabolites. With a wide range of applications, marine-derived metabolites have been explored in the field of pharmaceutics, in search for new and innovative drug scaffolds [1]. Inflammation is one of the areas where new drugs are most needed. This complex biological response, triggered by chemical or physical injury and tightly regulated by the immune system, can become chronic, and contribute to the development of multiple pathological conditions [2]. In this regard, several marine-derived compounds have been explored in the management of inflammatory processes, emerging as promising modulators of inflammatory pathways[3]. Therefore the present study aimed to extract, characterize, and evaluate the anti-inflammatory potential of bioactive compounds of underexplored marine seaweeds from Cape Verde (Figure 1). **Methods:** *Cladophoropsis membranacea* (Bang ex C. Agardh) Bogesen), *Digenea simplex* (Wulfen C. Agardh) and *Hypnea musciformis* (Wulfen) J. V. Lamouroux were collected in Calhau (São Vicente, Cabo Verde), lyophilized, and extracted using 70% acetone and 90% ethanol, combined with agitation or ultrasound-assisted extraction. Extracts were screened for antioxidant activity (DPPH and NO scavenging) and total phenolic content (Folin–Ciocalteu), followed by comparative statistical analysis to determine the optimal extraction conditions for each species. Selected extracts were further characterized by HPLC-DAD and LC-MS/MS and evaluated for their ability to inhibit lipoxygenase (LOX), a key enzyme involved in inflammatory processes. Ongoing studies will assess the anti-inflammatory effects of the most promising extracts in LPS-stimulated RAW 264.7 macrophages. **Results:** Preliminary results suggest the acetone extract, obtained by agitation, of the species *C. membranacea* as the most promising, associated with a more comprehensive carotenoid profile. **Conclusions**: Our preliminary

findings point to a correlation between carotenoid composition and anti-inflammatory capacity, suggesting that these species warrant more comprehensive future research.

Keywords: Anti-inflammatory; Carotenoids; Nitric Oxide; Oxidative Stress;

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Figure 1: Seaweeds biodiversity at Calhau, São Vicente, Cape Verde.

24616 | Mitigating the Impact of Marine Heatwaves on Gilthead Seabream through Dietary Vitamin C and E Supplementation

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Background & Aim: With the continual rise of the global population, the growing demand for sustainable aquatic food increasingly falls on aquaculture [1]. In the Mediterranean, the gilthead seabream (*Sparus aurata*) is one of the key species that fill this demand, but its sustainability is threatened by climate change, and the rising water temperatures that come with it [2, 3]. The temperature increase not only causes oxidative stress but also compromises the immune system, heightening disease susceptibility [4]. Vitamins C and E are critical vitamins in fish antioxidant defense and immune regulation, and are therefore promising dietary supplements to mitigate heat stress impact [5, 6]. This project aims to evaluate the roles of these vitamins, individually and in combination, in modulating stress and immune responses of *S. aurata* during exposure to an acute heat stress condition. **Methods:** Gilthead seabream (~100 g) are being fed experimental diets for 6 weeks under optimal rearing conditions. The diets' supplementation includes different concentrations of vitamins C and E, individually and in combination. Afterwards, the fish will be subjected to a short-term thermal stress challenge. The fish response to the heat-induced stress will then be evaluated through analyzing morphological differences in the gills' integrity, oxidative status (by assessing the activity of antioxidant enzymes), and immune parameters (by quantifying the expression of genes involved in the immune response). Plasma biochemical parameters will also be assessed to quantify the levels of known thermal stress markers. **Results:** We hypothesize that a specific supplementation level will prove the most efficient at mitigating the impact of the heat-induced stress, expecting to see in this group a higher integrity of the gills, an enhanced oxidative status, higher expression of immune response genes, and lower levels of thermal stress markers. **Conclusions:** These results will provide important data to further define the optimal dietary specifications for the growth of *S. aurata*, allowing for a more efficient and sustainable aquaculture of this species.

Keywords: *Sparus aurata*; Heat stress, Vitamin C; Vitamin E

Acknowledgments:

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24900 | Nanotechnology for Skin Protection: Lipid-Based Nanoparticles for Lutein Delivery

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Background & Aim: Skin ageing is a complex biological process characterized by the accumulation of cellular damage and the loss of physiological skin functions, with cellular senescence acting as one of its primary driving mechanisms [1]. Treating and preventing this process is essential to mitigate the impact of oxidative stress and UV radiation, which degrade the extracellular matrix and compromise the skin barrier [2]. Lutein is a lipophilic antioxidant with ROS-scavenging properties, yet its topical application is limited by poor aqueous solubility and high instability. The aim is to develop Lipid-based Nanoparticles to encapsulate and protect lutein, enhance its skin delivery, and evaluate their antioxidant capacity. **Methods:** This project develops Lipid-based Nanoparticles using hot high-pressure homogenization (HPH). The formulations will be optimized based on size, PDI, and encapsulation efficiency. *In vitro* assays using HaCaT keratinocytes and L929 fibroblasts cells will evaluate formulation safety and its capacity to reduce oxidative stress compared to free lutein. **Results:** The developed lipid-based nanoparticles are expected to exhibit suitable physicochemical properties, high lutein encapsulation efficiency, and good stability. Furthermore, the formulations are anticipated to demonstrate cytocompatibility and improved antioxidant activity in skin cell models compared to free lutein. **Conclusions:** These nanoparticles represent a promising strategy for improving the stability, delivery, and antioxidant performance of lutein in topical formulations, supporting their potential application in advanced anti-ageing dermatological therapies.

Keywords: Skin ageing, Lutein, Lipid-based nanoparticles, Antioxidant activity

Acknowledgments:

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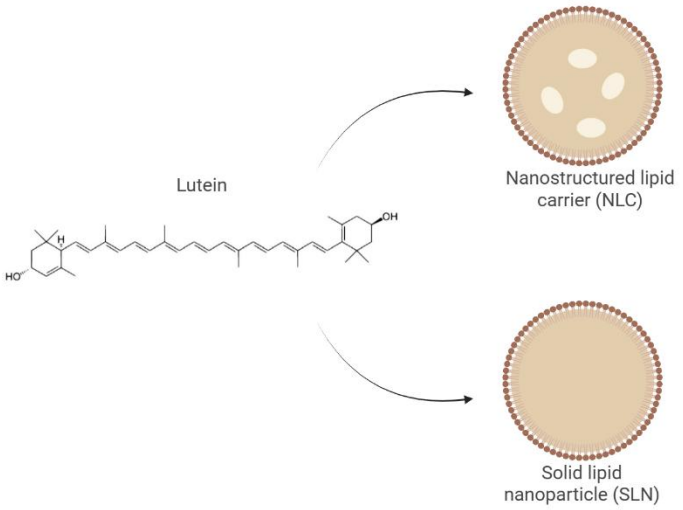

- Protection of lutein from degradation
- Overcomes poor aqueous solubility
- Enhanced skin delivery and antioxidante activity

Figure 1: Encapsulation of lutein in lipid-based nanoparticles.

24407 | New insights into the anti-inflammatory potential of new Cyanobacteria species from Peneda-Gerês National Park

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Background & Aim: Created in May 1971, Peneda-Gerês National Park (PNPG) is the oldest protected area and the only national park in Portugal. Alongside this macrobiome live cyanobacteria, widespread in Gerês rivers, cascades and thermal waters, with a promising biotechnological potential not explored so far [1]. In this study we aim to explore new strains from the PNPG, isolate and characterize them by assessing their biological identity, chemical profile, and antioxidant and anti-inflammatory potential. **Methods:** Strains Gerês 8 and Gerês 9A (Figure 1) were isolated from field samples, cultivated in Z8 culture medium and scaled-up until 4L. The produced biomass was extracted with targeted solvents, and chemical characterized using High Performance Liquid Chromatography (HPLC-PDA) and spectrophotometrically. The ability of the fractions to scavenge the physiological free radicals nitric oxide (•NO) and superoxide anion radical (O2•-) generated in vitro were determined spectrophotometrically. Cytotoxicity of the fractions was evaluated in human keratinocytes (HaCaT) using the MTT viability assay. Non-toxic fractions will be explored for their ability to inhibit enzymes involved in the inflammatory process and to inhibit the production of inflammatory mediators in a mammalian cell model of macrophages (RAW 264.7). **Results:** HPLC analysis identified chlorophyll-a as the most abundant compound in both strains, varying from 197.39 to 103.08 mg/g dry fraction, followed by β-Carotene varying from 25.85 to 26.13 mg/g dry fraction. Regarding phycobiliproteins, Gerês 9A was richer in phycoerythrin while Gerês 8 exhibited higher phycocyanin content. In antioxidant assays, acetonic fractions were more effective in •NO scavenging while aqueous fractions presented best results for O2•-. Preliminary MTT viability assays showed no toxicity of the fractions. **Conclusions:** Overall, both fractions from the cyanobacteria strains explored in this study presented promising potential to be further explored for the development of new natural anti-inflammatory ingredients.

Keywords: Inflammation, PNPG, Cyanobacteria, Free radicals, Carotenoids

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Funding: This research was funded by national funds through FCT, I.P., and by the European Commission's Recovery and Resilience Facility, within the scope of UID/04423/2025 (https://doi.org/10.54499/UID/04423/2025), UID/PRR/04423/2025 (https://doi.org/10.54499/UID/PRR/04423/2025), and LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020). Additional financial support was obtained from NORTE2030-FEDER-01796500, project co-funded by the European Union through the NORTE 2030 Regional Program.

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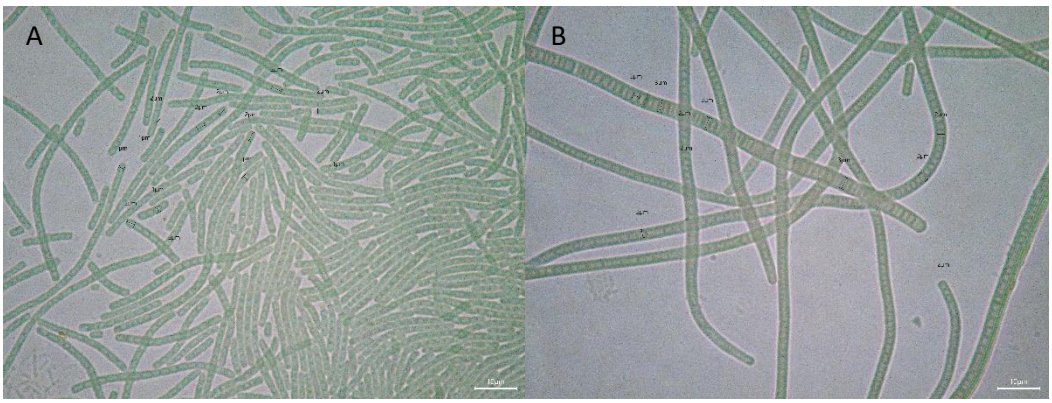

Figure 1. Microphotographs of Gerês 8 (A) and Gerês 9A (B).

24408 | Novel zebrafish model for pathogenicity validation of variants of unknown significance identified in a mitochondrial gene

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Background & Aim: Mitochondrial diseases (MD) affect 1 in 5.000 live births [1]. ACAD9 is a mitochondrial protein involved in fatty acid β-oxidation and Complex I assembly. Mutations in *ACAD9* are associated with MD of variable severity [2]. Zebrafish *(Danio rerio*), with conserved mitochondrial physiology and genetic similarity to humans is a valuable model to assess the functional impact of variants of unknown significance (VUS) in MD-related genes [3, 4]. This project aims to establish a new zebrafish *acad9* model through morpholino oligonucleotides (MO) mediated knockdown and to functionally characterize two *ACAD9* VUS, c.1258C>T (p.Arg420Cys) and c.170C>T (p.Pro57Leu). **Methods:** A splice-blocking MO targeting zebrafish *acad9* was titrated from 2-6 ng/µL and injected at the 1 cell stage. Phenotypic evaluations included tail-coil (30 hours post-fertilization - hpf), touch evoke response assays (48 hpf), and locomotor assays and morphological analysis (120 hpf). Human wild-type (WT) and mutant sequences were cloned into pCS2+ vectors, followed by mRNA *in vitro* transcription. Rescue assays involved co-injecting MO with WT or mutant mRNA at 1-cell stage. Behavioural evaluation will be performed until 48 hpf. Protein analysis will be performed by Western blot (Fig.1). **Results:** acad9 knockdown increased burst activity impaired touch evoke response and reduced movement and swim speed. Morphants also showed delayed development and increased pericardial area. Further experimentations will include rescue assays. In zebrafish, WT mRNA is expected to rescue the morphant phenotype, confirming model specificity. Successful rescue by mutant mRNA would support a benign classification, while failure to rescue marked by impaired

reflexes and diminished protein expression, denotes pathogenicity. **Conclusions:** This project will support functional validation to aid the clinical interpretation of VUS in human ACAD9-related deficiencies facilitating genetic counselling and pre-natal diagnosis for affected families.

Funding: This work is supported by FCT (2023.12019.PEX).

Keywords: *ACAD9*; *Danio rerio*; Mitochondrial Diseases; VUS; Phenotypic Rescue

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24841 | Parasites checklist of the common two banded seabream, *Diplodus vulgaris*

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Background & Aim: *Diplodus vulgaris* is a species of seabream that belongs to the family Sparidae. It is highly valued gastronomically, making it a valuable fishing resource, which makes it a promising species for aquaculture. The occurrence of parasites represent one of the bootle necks of fish production.Therefore our aims were to reviewed the parasites that have already been reported in this fish species, and later on we will do a survey of parasites present in a Portuguese populations of this species. **Methods:** We used the Google Scholar and Web of Science databases, and use the key words: "Diplodus vulgaris" and "Parasites", to select papers that reported the presence of parasites in *D. vulgaris*. **Results:** We were able to collect only six papers [1-6], that included our key words, and identified six taxonomic groups: Myxosporea, Monogenea, Digenea, Nematoda, Acanthocephala, and Copepoda. So far, no zoonotic parasites were reported in this fish species, however a great diversity of Monogenean were described. **Conclusions:** A wide diversity of parasites was recorded in common two banded seabream and the Monogenean seams to be the most well represented group pf parasites, according to a review of the literature.

Keywords: *Diplodus vulgaris*; Metazoan parasites; literature review.

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24600 | Peanut Peel Extracts to Boost Antioxidant and Immune Responses in carnivorous Fish: An Ex Vivo approach

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Background & Aim: Peanut peel (PP) is an agro-industrial by-product rich in bioactive compounds with strong antioxidant activity [1]. Its valorization through extraction with organic solvents or sustainable alternatives, such as natural deep eutectic solvents (NADES) [2,3], is particularly relevant given the need for natural antioxidants to improve fish welfare in aquaculture. Ex vivo models enable controlled physiological conditions while reducing animal use. This study aims to evaluate the effects of PP extracts on the immune and antioxidant responses of carnivorous aquaculture fish using intestinal explants before and after hydrogen peroxide challenge. **Methods:** PP bioactive compounds were extracted by microwave-assisted 50% ethanol or betaine–urea (1:2) NADES. An ex vivo assay was conducted using anterior intestine explants from *Dicentrarchus labrax* and *Sparus aurata*. Tissues were incubated with increasing extract concentrations for 2 h, followed by 0.04% hydrogen peroxide for 2 h. Viability was assessed by MTT assay, with 10% DMSO as negative control. Explants were preserved in Trizol for gene expression analysis. **Results:** Antioxidant activity was higher in the NADES extract, while total phenolic content was greater in the ethanolic extract. Intestinal explants treated with NADES showed reduced viability at 4%, 8%, and 10% in both species, while the ethanolic extract maintained viability at all tested concentrations. Mortality was observed only in the DMSO group. Analysis of antioxidant, anti-, and pro-inflammatory gene expression in challenged and non-challenged explants is ongoing. **Conclusion:** Bioactive compounds extracted from PP were higher in the NADES extract, which, consequently, increased explant toxicity in the higher concentration groups in the *ex vivo* trial. Further analyses are required to explore the potential of these extracts in modulating antioxidant, anti and pro-inflammatory gene markers expression in intestinal explants.

Keywords: By- product; Peanut peel; Ex vivo; immunity

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24769 | Polymorphism in *Discoglossus galganoi*: Pattern, Behaviour, and Physiology

Polymorphism in *Discoglossus galganoi*: Pattern, Behaviour, and Physiology

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Background & Aim: Colour pattern polymorphisms raise a central evolutionary paradox about the persisting alternative phenotypes within the same population, despite natural selection [1]. Although studied in some vertebrate groups, amphibian polymorphisms remain comparatively understudied. The Iberian Painted Frog, *Discoglossus galganoi*, exhibits two dorsal morphotypes, striped and spotted, providing an excellent model to investigate whether colour polymorphism reflects functionally relevant ecological divergence. This study aims to test whether morphotypes correspond to behavioural personalities, undergoing different stress levels and shaping skin microbiota composition, potentially contributing to the maintenance of polymorphism. **Methods:** Adult individuals were captured in Mindelo, through night searches. For each specimen, morphological data and environmental variables were recorded. Skin microbiota and corticosterone measures were sampled in the field to evaluate microbial diversity and baseline stress levels. In the laboratory, individuals undergo two repeated Open Field Tests to assess differences in exploration in both morphotypes [2]. Corticosterone was sampled before and after each trial to quantify stress responsiveness. **Results:** We expect striped individuals to display higher exploratory behaviour, reflecting a bolder personality type which is predicted to be associated with lower corticosterone levels and higher microbial diversity. Conversely, spotted individuals are expected to exhibit reduced exploration, higher stress responsiveness and lower microbiota diversity, due to less environmental exposure. **Conclusions:** This project provides an integrative understanding of intraspecific variation by linking polymorphic coloration to several functional traits, thereby contributing to deeper understanding on balanced selection and their functional background in polymorphic

amphibians, and guiding future research integrating fitness, genetic, and demographic data in this species.

Keywords: Anura; personality traits; intraspecific variation

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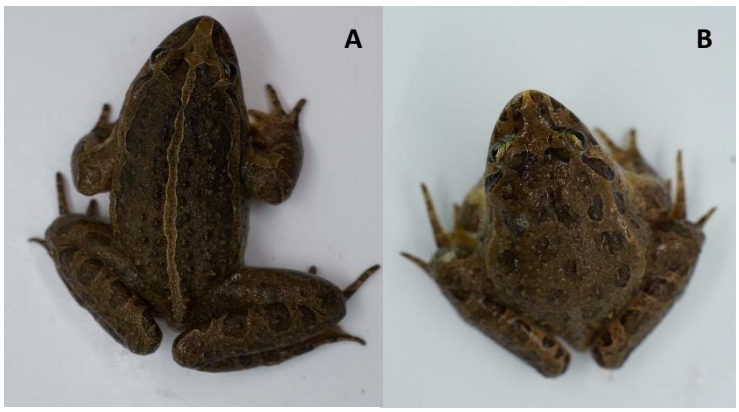

Figure 1: Stripped (A) and spotted (B) morphotypes of *Discoglossus galganoi*.

24911 | Carlos Teixeira Collection: fossil plants collected from boreholes from São Pedro da Cova and Midões - Archive and Database

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Background & Aim: The Carlos Teixeira Collection is a historic paleobotany collection, mainly from the Carboniferous of São Pedro da Cova, which was initially studied by Carlos Teixeira, in 1946. This work consists of the study and archive of this Collection, that integrates the Portuguese Mineral Deposits Museum of the National Laboratory of Energy and Geology of Portugal (LNEG) in S. Mamede de Infesta. **Methods:** For the preparation of the Collection database, three cabinets were studied and the specimens characterized according to 17 characteristics: catalogue number, cabinet, drawer/shelf, shelf identification, number of fossil specimens and rocks, presence of an original label, information in that label, fauna or flora, specimen identification, weight, length, height, location, museological interest, photo and notes. **Results:** An exhibition was created at the Portuguese Mineral Deposits Museum by the selection of well-preserved specimens to represent the Collection and arranging them within a display cabinet. **Conclusions:** This project improved the documentation and accessibility of the Carlos Teixeira Collection, contributing to a better understanding of the Collection and of paleontology in general.

Keywords: Paleobotany; Museology; Archive; Science Communication; Carboniferous

24853 | Polyphenol-rich extracts from invasive species: exploring metal chelation for neuroprotective strategies

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Background & Aim: Age-related neurodegenerative diseases associated with aging are a consequence of excessive levels of ROS[1,2,3] and free metal accumulation in the brain[4,5], which can trigger neuroinflammation[1,4] and protein aggregation[3,4]. This work focuses on the neuroprotective potential of polyphenols extracted from the flowers of Mimosa and the fruits of Sumac[6], Portuguese invasive species whose valorization contributes to environmental sustainability. The primary aim of this work is to extract and characterize these bioactive compounds[7] and evaluate their capacity to form stable polyphenol-metal complexes[8] as a potential neuroprotective strategy[1,2,4]. **Methods:** Polyphenols were obtained through sequential extraction to ensure the recovery of both extractable and macromolecular fractions[7]. Qualitative and quantitative analysis were performed using UV-Vis Spectrophotometry and HPLC-DAD/ESI-MS to identify specific anthocyanins, flavones, and tannins[9,7]. Biological efficacy was assessed through metal complexation competition assays with transition metals (Fe, Cu, Zn)[8,10] and the measurement of antioxidant capacity in cell-free assays[7]. **Results:** Profiling revealed diverse chemical profiles, including several anthocyanins[6], luteolin, and various ellagitannins, confirming that non-extractable (macromolecular) polyphenols represent approximately 80% of the total phenolic content in these matrices[7]. Complexation assays demonstrated that the flower extracts possess a high binding affinity for ferrous and cupric ions, forming stable complexes[2,4,8] that could effectively prevent free metals from participating in uncontrolled Fenton reactions[2,3,4]. **Conclusions:** Extracts from Mimosa and Sumac constitute rich and sustainable sources of complex polyphenols[6,9] capable of modulating metal homeostasis[2,4]. Their ability to sequester transition metals and neutralize ROS[2,3,8] highlights the potential of these invasive species as high-value matrices for developing new neuroprotective therapeutic agents[1,4].

Keywords: Neurodegeneration; Transition Metals; Oxidative Stress; Polyphenols; Metal Chelation

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24870 | Regulation of somatotropic signaling by FOXM1 in Progeria

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Background & Aim: Hutchinson–Gilford Progeria Syndrome (HGPS) is a fatal genetic disorder characterized by accelerated aging and severe clinical manifestations, including impaired growth, lipodystrophy, dermal and skeletal abnormalities, alopecia and cardiovascular complications. The transcription factor FOXM1 is progressively repressed in both accelerated and physiological aging. Induction of a FOXM1 transgene extends lifespan by approximately 25% in the progeroid mouse model *Lmna*$^{G609G/G609G}$ (LAKI) and is associated with increased body size. This suggests a potential effect on the somatotropic axis (Growth Hormone (GH)/Insulin-like Growth Factor-1 (IGF-1)), the principal endocrine pathway regulating growth and metabolic homeostasis. Here we explore the role of FOXM1 overexpression in the modulation of the somatotropic axis counteracting the growth repression in progeria using LAKI mouse overexpressing FOXM1 (LAKI-FOXM1). **Methods:** First, we measured IGF-1 and GH in the blood of WT, LAKI, and LAKI-FOXM1 mice by ELISA to determine somatotropic axis unbalance in LAKI and its restoration in LAKI-FOXM1. To assess in vivo metabolic alterations in LAKI and their improvement in LAKI-FOXM1, we evaluated blood glucose levels, glucose tolerance, and liver histology (Masson's Trichrome for fibrosis and CD45 Immunohistochemistry for immune cell infiltration). **Results:** Progeroid LAKI mice present liver fibrosis and immune cell infiltration, both of which are mitigated by FOXM1 overexpression. Assessment of glucose levels indicated hypoglycemia in the LAKI mice, which is corrected by FOXM1 overexpression. **Conclusion:** FOXM1 overexpression is capable of regulating the somatotropic signaling axis, reducing liver inflammation and fibrotic progression and normalizing glucose levels of LAKI mice. Collectively, these results point to a positive effect of FOXM1 in restoring liver function in progeroid LAKI mice.

Keywords: progeria, aging, somatotropic axis, FOXM1, IGF-1.

24289 | Replacing Fish Oil with animal by-products in Aquafeeds: Implications for European Seabass (*Dicentrarchus labrax*) Growth Performance, feed utilization, and fillet fatty acid profile

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Background & Aim: Aquaculture growth increases the need for sustainable diets, with reducing fish oil (FO) dependence being a major challenge due to its EPA and DHA content. As vegetable oils lack these essential fatty acids for marine fish species, alternative lipid sources are required. Fatty acid ethyl ester oil (FAEE), a by-product of omega-3 production with an EPA and DHA content between 5 and 8%, and *Hermetia illucens* larvae oil (HIO), a by-product from insect meal production, are promising FO alternatives due to their fatty acid profile, cholesterol content, and lipid-soluble vitamins [1, 2]. This study evaluated their effects on European seabass juveniles' growth, feed utilization, and plasma immune parameters. **Methods:** Four isoproteic and isolipidic diets (48% protein; 15% lipids) were formulated with FO, FAEE, and HIO. D1 was the positive control (71.7% FO, 20 % HIO), D2 the negative control (20% FO, 71.7% HIO), and D3 and D4 contained no FO, with HIO/FAEE at 51.7/40% and 20/71.7%. Diets were tested in triplicate. Fish (25 g) were stocked in 12 tanks (15 fish; 200 L) and fed for 84 days. At the end, the fish were euthanized, weighed, and plasma samples were collected from three fish. **Results:** No differences in growth, feed efficiency, or plasma immune parameters are expected. **Conclusions:** FAEE and HIO support sustainable feeds and circular economy strategies without compromising growth, feed efficiency, or fillet quality.

Keywords: Aquaculture; Aquafeeds; alternative ingredients; ethyl esters oil; insect oil.

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24879 | Revealing Joaquim Sampaio's botanical legacy: survey and cataloguing scientific illustrations preserved in the University of Porto Herbarium

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Background & Aim: The documentary collection of Joaquim António Ferreira Sampaio, preserved at the PO Herbarium, Museum of Natural History and Science of the University of Porto, includes numerous original scientific illustrations related to his botanical publications. However, these materials had not previously been systematically surveyed or analyzed. This study aims to identify, document, and categorize the scientific illustrations present in Sampaio's archival collection and establish links between the original drawings and their corresponding published works. **Methods:** A sequential methodological approach was followed. First, a survey of Joaquim Sampaio's archival materials preserved at the PO Herbarium was conducted, with detailed notes taken on the documents and their relation to known publications. Sampaio's published articles were then examined through online sources and physical collections in the libraries of the University of Porto. Identified scientific illustrations were digitalized and compiled, and reference tables were created to organize bibliographic information. Relevant archival documents were also digitalized to support preservation and access. Finally, an Excel database was created compiling all collected information, including bibliographic references, species names, and a unique code linking each illustration to its publication. **Results:** The survey enabled the identification and digitalization of several original illustrations related to Sampaio's botanical works. These were organized in a structured database connecting archival materials with their respective publications. Preliminary results reveal previously unreferenced or dispersed illustrations, highlighting the historical and scientific value of the collection. **Conclusions:** This work constitutes the first systematic survey of Joaquim Sampaio's scientific illustrations preserved at the PO Herbarium. The cataloguing and digitalization process improves preservation, accessibility, and documentation of this historical botanical material, supporting future research in botanical history, taxonomy, and scientific illustration.

Keywords: Botanical illustration; Herbarium archives; Joaquim Sampaio; Digital cataloguing; History of botany

24535 | Role of IQGAP proteins in regulating the diameter of the actin rings in neuronal axons

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Background & Aim: The axonal membrane periodic skeleton (MPS) is a cytoskeletal structure composed of actin rings interconnected by spectrin, first identified by super-resolution microscopy (1). It is essential for neuronal stability and function (2), yet the mechanisms regulating its circular organization and maintenance remain unclear. IQGAP scaffold proteins regulate actin filament circularization at the plasma membrane (3) and may therefore contribute to MPS organization. This study investigates the role of IQGAP proteins in the nanoscale organization of the axonal MPS. **Methods:** CRISPR-Cas9–mediated knockout models of IQGAP proteins were generated in primary hippocampal neurons and neuronal cell lines. Gene-editing efficiency was validated in neuronal cultures and Rat-2 cells. In parallel, a CRISPR-based IQGAP knockout model was established in differentiated SH-SY5Y cells. Super-resolution STED microscopy was used to analyse IQGAP distribution and MPS structural parameters. **Results:** IQGAP isoforms are endogenously expressed in neurons, with higher expression in mature neurons than at early developmental stages. Super-resolution imaging revealed that IQGAP3 exhibits a periodic axonal distribution with spacing consistent with the characteristic ~190 nm periodicity of the MPS (1). **Conclusions:** These results provide initial insights into the role of IQGAP proteins in axonal MPS organization and contribute to a better understanding of the molecular mechanisms underlying axonal cytoskeletal architecture.

Keywords: Actin rings, IQGAP proteins, super-resolution microscopy, primary hippocampal neurons, SH-SY5Y cell line.

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24559 | Rotating Enrichment Devices as a Welfare Tool for a Green Sea Turtle in a Public Aquarium

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Background & Aim: Environmental enrichment is strongly encouraged in zoos and aquariums, but despite growing demands regarding animal welfare, evidence-based enrichment protocols for sea turtles remain scarce. Therefore, this case study evaluated the effects of a rotating enrichment plan on behavioral diversity and spatial distribution in a resident green sea turtle (*Chelonia mydas*) in a public aquarium. **Methods:** An adult male green sea turtle at SEA LIFE Porto was studied in a within-subject design, with observations conducted under standard conditions (no enrichment) and during enrichment sessions, both lasting 30 minutes. The turtle was exposed either to no device (standard) or to a single enrichment device (vegetable tube, grazing mat, yoga ball, fixed scrub brush, or diver with scrub brush) three times per week. Each session combined 20 minutes of instantaneous sampling every 30 seconds with 10 minutes of space-use recording across 13 predefined areas. Behavioral categories, enrichment-specific interactions, diversity indices, and spatial data were analyzed using non-parametric tests and two-way ANOVAs. **Results:** Enrichment sessions increased behavioral diversity, with the Hill number rising from 2.77 under standard conditions to 3.55 (~28% increase), indicating a richer behavioral repertoire. Resting and hiding behaviors decreased, while total activity and exploration interactions (e.g. grazing, object and diver interaction) increased, without a corresponding increase in locomotion. Spatially, the turtle reduced use of a heavily utilized surface area and increased use of deeper, previously underused zones. Among devices, the vegetable-filled tube elicited the highest exploratory response, significantly exceeding the fixed scrub and yoga ball. **Conclusions:** The enrichment plan increased behavioral diversity and balanced habitat use in a resident green sea turtle, highlighting the value of systematic monitoring to optimize enrichment and welfare management in aquariums.

Keywords: Animal Welfare, Environmental Enrichment, Green Sea Turtle, Behavioral analysis

24595 | Seaweeds from the Cape Verde Archipelago: Antioxidant Potential and Biotechnological applications

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Background & Aim: Seaweeds represent a diverse group of organisms with different morphologies and colors that inhabit coastal regions all over the world [1]. To cope with harsh and changing conditions of their habitats, seaweeds have evolved the ability to synthesize a diverse array of specialized metabolites, with a large spectrum of biological activities [2, 3]. Although seaweeds are abundant along the coast of Cape Verde, they remain poorly explored [4]. Therefore, this study aims to investigate the biotechnological potential of seaweeds collected in São Vicente, with particular focus on antioxidant properties. **Methods**: Three brown seaweed species, *Dictyota dichotoma* (Hudson) J. V. Lamouroux, *Hydroclathrus clathratus* (C. Agardh) M. Howe and *Chnoospora minima* (Hering) Papenfuss, were collected in Calhau (São Vicente, Cape Verde), lyophilized, and extracted combining different solvent mixtures (acetone 90% *vs* ethanol 70%) and extraction methods (agitation *vs* sonication). The optimal condition for each species was selected based on extraction yields, total phenolic content (TPC), and DPPH and nitric oxide ($^{\bullet}$NO) scavenging capacity, after a comparative statistical analysis. The selected extracts were chemically characterized by HPLC-PDA and LC-MS/MS, to identify bioactive compounds present in the samples. **Results:** Extraction yields varied from 2.54% to 10.57% for the acetone extract of *C. minima* and the ethanolic extract of *H. clathratus,* respectively, both of which obtained using agitation. Statistical analysis revealed that the extraction method did not affect the evaluated bioactivities, whereas the contrary was observed for solvent composition (p <0.05 in ANOSIM test). *D. dichotoma* acetone extract displayed the best $^{\bullet}$NO scavenging ability (IC_{50}=0.5 mg/ml). In contrast, a low ability to inhibit tyrosinase was observed for all extracts. The preliminary chemical characterization indicated the presence of fucoxanthin, pheophytin *a* and chlorophyll *a* in all extracts. **Conclusions:** All selected extracts stood out for

their similar chemical composition and good antioxidant activity, underscoring the biotechnological potential of brown seaweeds from the Cape Verde Archipelago as promising sources of natural antioxidants for both cosmeceutical and pharmaceutical applications.

Keywords: Brown seaweeds; Cape Verde Archipelago; Carotenoids; Marine Biotechnology; Nitric Oxide.

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24942 | Surface-modified PLGA nanoparticles for the targeted delivery of vancomycin for the treatment of bacterial meningitis

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Background & Aim: Central nervous system (CNS) infections, particularly bacterial meningitis, remain associated with high mortality and neurological sequelae [1]. The therapeutic efficacy of the antibiotic vancomycin is limited by restricted penetration across the blood–brain barrier (BBB) and potential systemic toxicity at high doses. Nanotechnology-based drug delivery systems may improve antibiotic bioavailability. This work aims to develop and characterize surface-modified PLGA nanoparticles (NPs) loaded with vancomycin as a proof-of-concept strategy for targeted BBB delivery. **Methods:** Vancomycin-loaded PLGA NPs were produced using a double emulsion solvent evaporation method. Physicochemical characterization included hydrodynamic diameter, polydispersity index, and zeta potential determination by dynamic and electrophoretic light scattering. Encapsulation efficiency was assessed using UV-Vis spectrophotometry. Colloidal stability was evaluated over time during refrigerated storage at 4°C. A Factorial Design was implemented to optimize size, polydispersity index (PDI), zeta potential (ZP) and Encapsulation Efficiency (EE). **Results:** The developed NPs successfully encapsulated vancomycin and exhibited physicochemical properties consistent with nanometric drug delivery systems [2]. The formulations presented uniform size distribution, appropriate surface charge, and maintained colloidal stability for at least a period of 30 days. The implementation of a structured experimental design supported the identification of critical formulation parameters influencing NP characteristics. **Conclusions:** This work establishes a reproducible PLGA NP platform capable of efficiently incorporating vancomycin while maintaining physicochemical stability. These findings contribute to the rational design of

nanocarriers aimed at overcoming key challenges in BBB drug delivery and improving therapeutic strategies for bacterial meningitis.

Keywords: drug-delivery systems; brain delivery; antibiotics; blood-brain barrier; infectious diseases

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24321 | The potential of bio-inspired surfaces for mitigating marine biofilms

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Background & Aim: Marine biofouling is a dynamic process involving the settlement and growth of microorganisms, algae, plants, and invertebrates on submerged surfaces, with biofilm formation representing one of its earliest stages [1]. This phenomenon poses significant technical, economic, and environmental challenges in the marine industries [2]. Bioinspired strategies have emerged as sustainable antifouling solutions, with plant leaf-inspired surfaces showing potential to disrupt bacterial attachment and limit biofilm development [3, 4]. Therefore, this work aims to evaluate the effectiveness of such biomimetic topographies in mitigating marine biofilm formation. **Methods:** The replication of leaf topography was achieved through a moulding technique using dual-system liquid silicone. Long-term (up to 6 weeks) marine biofilms were developed on different biomimetic silicone leaf-inspired surfaces using *Cobetia marina,* a model biofilm-forming bacterium widely employed in marine biofouling studies, under *in vitro* conditions that simulate marine hydrodynamic environments. Perspex, a polymer commonly used in marine applications, and flat silicone surfaces were used as controls. Long-term field immersion assays were also conducted to assess the performance of biomimetic surfaces under real environmental conditions. Biofilm development was assessed using several approaches, including quantification of biofilm cells and structural analysis by Optical Coherence Tomography. **Results**: Long-term field immersion assays showed reductions of up to 50% in biofilm cells on biomimetic surfaces relative to flat silicone controls, highlighting the antifouling potential of biomimetic surfaces. **Conclusions:** These preliminary findings indicate that plant leaf-inspired topographies can effectively limit microbial colonization under natural environmental conditions. If sustained over time, this approach may represent a promising and environmentally responsible strategy for long-term marine antifouling applications.

Keywords: Biomimetic surfaces; Plant leaves; Biofouling; Marine biofilms; Antifouling surfaces

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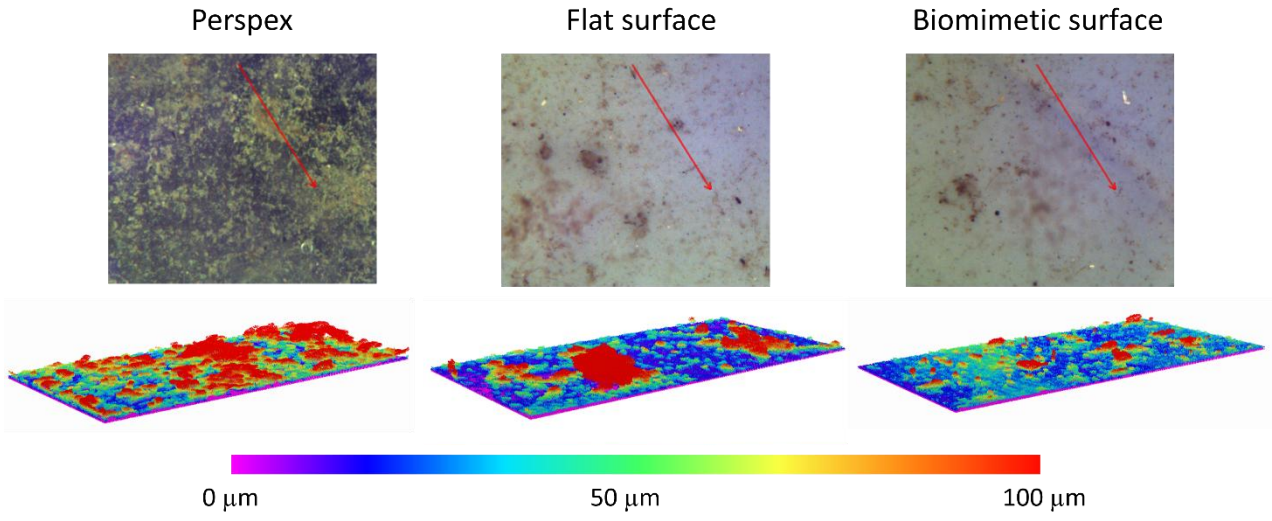

Figure 1: *In situ* biological colonization (first line) and representative 3D cross-sectional Optical Coherence Tomography images (second line) of biofilms developed on perspex, flat silicone, and on a biomimetic surface (White Cabbage) after 3 weeks of immersion. The colour scale represents the biofilm thickness (up to 100 µm).

24783 | The role of IL-10 in the aged brain

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Background & Aim: The anti-inflammatory cytokine interleukin-10 (IL-10) emerged as a promising therapeutic candidate for inflammatory diseases; however, severe side effects, including anemia and increased serum interferon (IFN)-γ, have limited its clinical use. [1] The Immune Regulation group (i3S) developed *in vivo* models of IL-10 overexpression and identified mechanisms underlying therapy-associated toxicity. In pMT-10 mice, a transgenic system of increased IL-10 production as a response to zinc administration, [2] IL-10 exposure drives strong T cell activation and emergency myelopoiesis. [3] Novel data show that IL-10-reprogrammed T cells are senescent, infiltrate several organs, and cause alterations compatible with accelerated aging. Currently, this project aims to dissect the systemic damaged caused by IL-10 elevation and the mechanisms of IL-10-mediated T cell reprogramming. **Methods:** The induced pMT-10 and pMT-10.IL-10Ra$^{-/-}$ mouse models are assessed using flow cytometry, histological analysis, and transcriptomic approaches, together with behaviour studies. C57BL/6 mice are used as controls. **Results:** Preliminary data suggest that, compared to control groups, induced pMT-10 mice show decreased motivation and impacted motor strength in behaviour studies, compatible with an aging phenotype. Transcriptomics data showed a strong correlation between IL-10 expression with age in the brain. Therefore, current efforts are focused on determining the types of alterations present in the brain of induced pMT-10 mice. In the near future, analysis of brain tissue (using flow cytometry and histological analysis) from the described mouse models will allow to assess the extent of brain alterations. **Conclusions:** This work reveals a novel role for IL-10 in aging. Beyond immunoregulation, increased IL-10 expression drives T cell senescence, systemic alterations, and behavioral decline compatible with accelerated aging, positioning IL-10 as a key player in age-associated processes.

Keywords: Interleukin-10; Immunity; Senescence; Behaviour; Brain

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24928 | The role of IL-10 overexpression in the neuroimmune system under chronic Methamphetamine exposure

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Background & Aim: Chronic Methamphetamine (Meth) exposure induces alterations in microglial morphology, altering microglia numbers and other immune cells. CD47, a synaptic "don´t eat me" signal and its receptor SIRP-α regulates neuron-microglia communication and synaptic pruning. Preliminary data from our laboratory show that CD47-SIRP-α signaling is dysregulated after chronic Meth exposure. Moreover, we also showed in an acute Meth administration model that IL-10 prevents Meth-induced neuroinflammation in a mouse model that overexpresses IL-10 (pMT-10) [1]. Within this framework, the central purpose of this investigation is to determine the role of IL-10 overexpression in the neuroimmune system in a chronic model of Meth administration. We have observed that IL-10 overexpression prevents/reverses Meth-induced microglia homeostasis dysregulation in male mice. Here, we aim to determine whether IL-10 overexpression is also able to modulate microglia homeostasis dysregulation in female mice. **Methods:** A mouse model that overexpresses IL-10 (pMT-10) under a zinc promoter was used. Female mice were administered with escalating doses of Meth (1-6 mg/kg/day, IP, 10 days), and IL-10 overexpression was promoted in the last four days of Meth administration. Brains were sliced in the cryostat, and immunostaining to evaluate microglial cells by confocal microscopy were performed. Microglia morphology analysis was performed using IMARIS 3D reconstruction and microglia cell density was also analyzed. **Results:** Preliminary results suggest that IL-10 overexpression effectively abrogates or prevents Meth-induced microglial homeostasis dysregulation in females, consistent with our previous findings in male mice. **Conclusions**: This study will provide deeper insights into the role of IL-10 overexpression in the modulation of Meth-induced neuroinflammation. Moreover, this research

will contribute to clarifying the distinct responses of males and females to psychostimulant exposure.

Keywords: Addiction; Microglia; Neuroinflammation; Interleukin-10; Sex-differences

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24472 | Thermal Heterogeneity and Intertidal Biodiversity Patterns Along the North-Western Iberian Coast

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The intertidal is one of the most dynamic ecosystems on the planet, experiencing strong environmental variability in temperature, salinity, moisture and wave exposure across latitudinal gradients and fine-scale microhabitats. Organisms inhabiting these environments are subjected to daily fluctuating physical stress. The northern Portuguese and Galicia coast represents a biogeographic transition zone where boreal and warm-temperate species coexist under a seasonal upwelling regime. Here, foundation species such as mussels, rockweeds, red turf algae, serve as one of the most important biological mechanisms to alleviate the pressure, playing a crucial role in the structure and maintenance of these systems. This region relies heavily on the ocean and is especially vulnerable to change driven by climate variability. Therefore, tracking biodiversity patterns here is crucial to identify possible shifts in community structure and ecosystem composition. In this study, we surveyed intertidal biodiversity across 18 rocky shores along northern Portugal and Galicia. At each shore, we conducted standardized photographic surveys to document community composition. Species were identified and quantified using AI-assisted image analysis tools. We integrated high-resolution temperature from field loggers with the standardized biodiversity surveys to assess how fine-scale thermal heterogeneity shapes intertidal community structure. We aim to characterize community patterns and quantify the influence of local thermal variability on species distribution. This approach helps clarify how fluctuations in temperature shape intertidal communities within a zone that's sensitive to the climate, while also creating a reference point to identify future ecological changes.

24655 | Toxicological Assessment of NDEA using Danio rerio as experimental model

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Background & Aim: Nitrosamines are toxic disinfection by-products (DBPs), considered emerging contaminants, formed during drinking water treatment through reactions between disinfectants and natural or anthropogenic precursors. They pose significant environmental and public health risks because of their carcinogenic and mutagenic effects, being already proven that long-term exposure to these chemicals can lead to different types of cancer. Although their occurrence has been known for decades, their toxicological mechanisms are still not fully understood. The aim of this project is to support the establishment of regulatory guideline values to protect public and human health. **Methods:** Toxicological assays with zebrafish (*Danio rerio*) embryos were conducted to evaluate morphological consequences of exposure to N-Nitrosodiethylamine (NDEA), using the Fish Embryo Acute Toxicity (FET) test (OECD 236), monitored through 96 hours post-fertilization. To study behavioral alterations, it was used the Locomotor Activity Monitoring System. At the end of the assay, it was also measured the length of the larvae, alongside with mortality, anomalies and behavior alterations. **Results:** Preliminary assays following 96h of NDEA exposure revealed significant physical abnormalities, including pericardial edema, scoliosis, yolk sac edema and tail malformation, also some were completely abnormal and were smaller than usual. Related to cognitive functions it was possible to see that some of these animals would swim a short distance with difficulty and then would stop, and others didn´t respond to the touch. **Conclusions:** These preliminary results show that the exposure to NDEA can affect the normal development of zebrafish, affecting both physical morphology and behavioral aspects. These data allowed us to establish the No Observed Effect Concentration (NOEC) and, subsequently, multi-omics (metabolomics and transcriptomics) studies will be conducted to elucidate the molecular pathways underlying these alterations.

Keywords: Disinfection by-products (DBPs), Nitrosamines, Human Health, Emerging Contaminants, Toxicity

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24959 | Transcriptomic signatures to pathogenic and non-pathogenic lagovirus infection in the European rabbit

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Background & Aim: Understanding why closely related viruses can cause markedly different disease outcomes in the same host species remains a central question in host-pathogen interaction. The European rabbit and rabbit haemorrhagic disease (RHD) represent a model to address this problem. RHD is caused by rabbit haemorrhagic disease virus (RHDV), a lagovirus of the family *Caliciviridae* that induces acute fatal hepatitis in European rabbits. Rabbit calicivirus (RCV) is a closely related lagovirus that causes asymptomatic infection. The circulation of a highly pathogenic virus and a non-pathogenic form in the same host provides a valuable system to investigate host molecular responses associated with different infection outcomes. **Methods:** European rabbits (n=13) were experimentally infected with either RHDV or RCV and euthanized at 4 (RHDV n= 2; RCV n=2) and 14 (RHDV n= 3; RCV n=3) days post-infection (dpi). For all individuals, RNA was extracted from the liver, duodenum, spleen and thymus, and analyzed using RNA-seq. Differential gene expression analyses were performed to identify genes likely associated with viral infection and tissue-specific responses. **Results:** RHDV infection induced a stronger transcriptional response than RCV. The number of differentially expressed genes was higher in RHDV-infected animals, with a predominance of upregulated genes that are associated with host immune response to infection. Our results show that the duodenum shows the highest number of differential expressed genes, while the liver, spleen and thymus exhibited more moderate responses. **Conclusions:** These results support that RHDV infection is associated with broader transcriptional changes in the host compared with RCV infection. The strongest

responses were observed in the duodenum and liver, consistent with the tissues primarily targeted during the infection.

Keywords: European rabbit; rabbit calicivirus; rabbit haemorrhagic disease virus; RNA-seq

24871 | Trehalose-Glycerol Natural Deep Eutectic Solvent: Cytotoxic Impact on Canine Epididymal Sperm

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Background & Aim: Cryopreservation of dog epididymal spermatozoa is crucial for preserving germ cells from purebred and endangered canids after sudden death or orchiectomy. However, its efficacy is limited by cold shock and ice crystal formation, which compromise sperm quality. Cryoprotectants such as glycerol mitigate these effects, although high concentrations can be cytotoxic [1,2]. Natural Deep Eutectic Solvents (NADESs) have emerged as promising, less-toxic alternatives for cryopreserving various cell types [3]. This study evaluated the cytotoxicity of Trehalose-Glycerol (Treha-Gly) (1:30) NADES at different concentrations on dog epididymal sperm prior to freezing, to identify the most cytocompatible concentrations for subsequent freeze-thaw assessment. **Methods:** Cytotoxicity was assessed after 1 and 2 h of incubation at 37°C in spermatozoa collected from the cauda epididymis of seven dogs undergoing orchiectomy. The experimental groups included Tris-Citrate-Fructose (TCF, control) and Treha-Gly NADES at 5%, 10%, and 20% (v/v). Metabolic activity was measured using the MTT assay, a colorimetric test for cellular metabolic activity. Sperm motility parameters, including total motility (TM), progressive motility (PM), curvilinear velocity (VCL), straight-line velocity (VSL), and average path velocity (VAP), were evaluated with a Computer-Assisted Sperm Analysis system. Statistical analysis was performed using two-factor ANOVA followed by Tukey's post hoc test. **Results:** MTT results show no significant differences between Treha-Gly NADES concentrations and TCF at either time point. Preliminary motility analysis indicates that 20% Treha-Gly NADES markedly decreases TM, PM, VCL, VSL and VAP, while TCF, 5% and 10% Treha-Gly NADES maintain similar motility values. **Conclusions:** This study reveals that Treha-Gly NADES are non-cytotoxic to epididymal canine spermatozoa at 5-20%. Preliminary motility data

suggest that 5% and 10% are the most compatible concentrations for potential cryopreservation use.

Keywords: Canine Epididymal Spermatozoa; Cytotoxicity; Sperm motility; Natural Deep Eutectic Solvents; Cryopreservation.

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24184 | Uncovering the inter-tissue coupling that drive epithelial rotation

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Background & Aim: Collective cell migration is crucial for animal development, tissue repair and cancer dissemination. Unlike the leader-follower organization in most migrating groups, epithelial tissues display a distinct form of collective migration through circumferential rotation, but the mechanisms underlying this process remain poorly understood. The *Drosophila* egg chamber is an ideal model to study how epithelial rotation is mechanically coupled between tissues. Egg chamber rotation is driven by collective movement of the follicular epithelium (FE), relying on the actin cytoskeleton at the basal level. However, the FE also adheres apically to the germline, which also rotates. Recent analyses of tissue boundaries reveal robust inter-tissue adhesion enabling coordinated movement. Furthermore, optogenetic manipulation of contractility at the germline cortex disrupts FE migration, suggesting that the inter-tissue adhesion at the germline is critical for coordinated rotation. To this end, we aimed to determine when the interaction between these tissues was established during egg chamber development. **Methods:** We used *Drosophila melanogaster* as a model organism, focusing on ovarian egg chambers. We performed live imaging and immunofluorescence to analyse egg chamber dynamics. Images were acquired by confocal and spinning disk microscopy, and quantitative analyses were performed using ImageJ, combined with kymograph. **Results:** Analysis revealed that the hexagonal morphology of the FE could be visualised indirectly through the underlying germline which could be observed from stage 6 of oogenesis onwards. We also observed higher colocalisation values and increased migration speeds during stages 6 and 7. **Conclusions:** We were able to visualise the mechanical coupling between the germline and the FE, as evidenced by the germline reproducing the characteristic hexagonal shape of the FE. This coupling appears to correlate with epithelial cell migration speed, both reaching a maximum at stage 7.

Keywords: *Drosophila*; epithelium; cell polarity; cell migration

24568 | Unravelling pseudo-unipolarization and axon asymmetry of sensory dorsal root ganglia

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Background & Aim: Dorsal root ganglia (DRG) sensory neurons (SNs) have a peculiar morphology where a single axon bifurcates into two axons (central and peripheral) with different properties and functions (1;2). The cell biology of DRG neuron pseudo-unipolarization (PU) is still not fully understood. In this project, using a rat DRG co-culture system where SNs recapitulate PU, we will elucidate the mechanisms behind DRG neuron PU and central/peripheral axon-specific protein localization. **Methods:** We optimized the culture protocol based on (3) and quantified the percentage of DRG neurons presenting PU at 3 different time points (7, 14, and 21 days *in vitro* – DIV). To understand the mechanisms behind PU (cell body bulging and/or membrane fusion), we are monitoring by live imaging the maturation of rat primary SNs, where nuclear positioning and positioning of other cell components (membrane, Golgi, actin) will be followed. We tested alternative methods for efficiently transferring the reporter genes to SNs, including transfection and transduction. Using drug-mediated modulation, we are also assessing the importance of cytoskeleton dynamics and organization for PU to occur. **Results:** At DIV21, 42% of DRG neurons were pseudo-unipolarized, which is within the expected range. In the presence of drugs, there was a decrease in the percentage of pseudo-unipolarized DRG neurons. We observed that transfection with Lipofectamine 3000 resulted in a large number of cells expressing the reporters. However, the transduction was the best choice because the AAV-PHP.S used has a greater tropism for sensory neurons. **Conclusions:** Overall, at DIV21, there was a higher percentage of pseudo-unipolarization. In the presence of drugs, this percentage suffered a decrease. AAV-PHP.S was a better strategy for transferring the reporter gene to the DRG

neurons. Both microtubules and myosins participate in PU. However, the full characterization of how pseudo-unipolarization occurs in these neurons is still under study.

Keywords: DRG neurons; pseudo-unipolarization; cell body bulging; membrane fusion; axonal cytoskeleton

Acknowledgments:

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24552 | Unravelling the Domestication History of the Indian Peafowl: Integrating Genomic Insights of Modern, Historical and Ancient DNA

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Background & Aim: The Indian peafowl (*Pavo cristatus*) is an iconic bird species. Although it is native to India and Sri Lanka [1], this pheasant sparked the interest of many cultures, which propelled its dispersal and domestication as early as 1 000 B.C, according to historical records [2]. Nowadays, the Indian peafowl is bred in captivity worldwide [3] but its domestication history remains greatly understudied. This project aims at understanding the domestication and evolutionary history of the Indian peafowl by integrating genomic, phylogenetic and demographic approaches on modern, historical and ancient DNA samples. **Methods:** We used contemporary Indian peafowl whole-genomes (*P. cristatus*, n=392) to understand genomic diversity and population structure of wild and domesticated populations and to evaluate introgression with the green peafowl (*P. muticus*, n=46). By employing next-generation sequencing on museum *(P. cristatus*, n=74; *P. muticus*, n=27) and archaeological peafowl samples, we will evaluate changes in genetic variation over the course of domestication in Europe. Furthermore, by combining modern and historical data with phylogenetic and demographic modelling we will evaluate the process of domestication of the Indian peafowl. **Results:** Preliminary results show high interspecific divergence between the blue and green peafowl, with very low intraspecific genetic diversity in modern blue. The low divergence within domestic blue in comparison to domestic green peafowl is consistent with a single origin of the blue peafowl and a very intensive captive breeding during the past. Both nuclear and mitochondrial genomes indicate evidence on introgression between species, and the absence of geographic structure due to continued exchange of individuals at a continental scale. **Conclusions:** Early results show a distinction between domesticated and wild specimens and the historical portion of this study will reveal further insights on the timeline and demography regarding peafowl domestication.

Keywords: Peafowl; Avian Domestication; Evolutionary History

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24565 | UV Radiation Repair in Pigmented Bacteria: Implications for Planetary Surface Habitability

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Background & Aim: Ultraviolet (UV) radiation is a major constraint on surface habitability in planetary environments lacking atmospheric shielding. High UV fluxes induce severe DNA damage and limit microbial survival. Understanding DNA damage and repair under UV exposure is therefore essential to constrain biological limits under planetary radiation stress. This study aims to quantify genomic damage and evaluate DNA repair capacity in pigmented environmental bacterial isolates under controlled UV exposure. **Methods:** Environmental microorganisms were isolated from Arctic environmental samples using multiple culture media (PYGV, PYGVb and PYGV + NAG) under selective and non-selective conditions. Pigmented colonies were selected based on visible coloration, with a focus on dark brown to black isolates potentially producing melanins. A UV-Vis dataset from the literature was compiled to guide pigment identification. Pigment absorption profiles will be analyzed using UV-visible spectrophotometry. DNA will be extracted before irradiation, immediately after exposure, and after incubation under optimal growth conditions, and DNA integrity will be assessed by agarose gel electrophoresis. **Results:** A dataset of UV-Vis absorption spectra from 12 literature studies on melanin has been compiled, revealing two main spectral behaviors among melanins: either a broad-band monotonic absorption across the UV range with no distinct peak, or defined UV maxima between 210 and 330 nm. In parallel, pigmented isolates from Arctic samples and 7 dark-pigmented strains from CIIMAR culture collections and laboratory stocks have been selected. These preliminary results provide the basis for comparing pigment spectral properties with DNA damage and recovery after high UV exposure. **Conclusions:** By linking pigment characteristics with molecular damage and repair dynamics, this study seeks to better constrain microbial resilience under radiation stress and support astrobiological models of life under UV radiation.

Keywords: Ultraviolet radiation; DNA damage; DNA repair; Pigmented bacteria; Astrobiology

24453 | When Females Sing: Island-Mainland Differences in Territorial Behaviour

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Background & Aim: Bird song is a complex behaviour whose study has advanced neurobiology, behavioural, evolutionary ecology, and conservation. Although ancestral in songbirds, female song remains understudied [1]. Because it is often linked to territorial behaviour, studying populations where both sexes defend territories allows testing hypotheses about its evolution and function. Oceanic islands provide ideal natural laboratories for such questions: their isolation, reduced species diversity, milder climates, and limited resources promote behavioural divergence from mainland ancestors. Higher densities, limited area and resources increase territorial pressure, shaping behaviour [2]. We aim to investigate how island-driven territoriality influences the presence and function of female song within the *Fringilla* complex. **Methods:** We simulated territorial intrusions in the mainland Common Chaffinch (*F. coelebs*), which has lost female song, and in two insular species, the Canary Island Chaffinch (*F. canariensis*) and the Blue Chaffinch (*F. teydea*), where it has re-emerged. Trials were conducted in Portugal and Tenerife. We measured the territorial and acoustic responses of individuals to conspecific song playbacks and 3D-printed models. Spectral analysis was performed with Avisoft, and the statistical in R. **Results:** We expect island populations to show stronger and more aggressive territorial responses in both sexes, with *F. teydea* exhibiting the strongest responses, reflecting high resource defensibility and specialization. Female song is predicted to be more frequent and complex on islands, and male and female roles more similar due to increased cooperative territorial defence. **Conclusions:** This study tests whether elevated island territoriality is associated with the maintenance and functional relevance of female song, clarifying the role of competition in its persistence and re-expression. This work highlights female song as an adaptive trait shaped by ecological context and its importance for understanding behavioural evolution in island birds.

Keywords: Acoustic; Behaviour; Insularity; Song;

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Figure 1 Canary Island Chaffinch, by Ana V. Leitão

24780 | Bridging In Vitro and In Vivo Models with an Organotypic Bone Infection Model

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Background & Aim: Reliable experimental models are essential to investigate bone-bacteria interactions and evaluate novel therapeutic strategies. Conventional *in vitro* systems lack the native extracellular matrix and three-dimensional complexity of the bone tissue, whereas *in vivo* models raise ethical and logistical concerns. *Ex vivo* organotypic systems bridge this gap by preserving tissue architecture and cell-matrix interactions while providing a controlled, reproducible, and physiologically relevant alternative. This study aims to establish and validate an *ex vivo* bone infection model using an embryonic chick femur organotypic culture system inoculated with *Staphylococcus aureus*, modeling key features of osteomyelitis in a non-animal platform. **Methods:** Fertilized chicken eggs will be incubated for 13 days, after which femurs will be harvested and maintained in organotypic culture for up to six days. Infection will be induced by intramedullary inoculation of S. *aureus* (ATCC 25923). At defined time points, bacterial burden will be quantified by colony-forming units (CFUs) enumeration following bone homogenization. Histological and histochemical analyses, including Gram and Alcian Blue/Sirius Red staining, will assess bacterial localization, biofilm formation, and bone matrix alterations. Quantitative PCR will evaluate bacterial virulence-associated genes and host osteogenic and inflammatory markers. **Results:** Infected femurs are expected to demonstrate reproducible and quantifiable bacterial loads, structural disruption of the bone matrix, impaired osteogenic gene expression, increased inflammatory mediators and adaptive bacterial transcriptional responses associated with biofilm formation and persistence. **Conclusions:** This *ex vivo* chick femur model represents a reproducible and ethically responsible platform bridging simplified *in vitro* assays and complex *in vivo* studies, supporting investigation of bone infection and the testing of innovative antimicrobial and bone-regenerative therapeutic approaches.

Keywords: *Ex vivo* model; Organotypic culture; *Staphylococcus aureus*; Bone infection.

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2. DOI: 10.1016/j.bone.2022.116355
3. DOI: 10.1016/j.actbio.2014.06.014
4. DOI: 10.1007/s11914-020-00649-5
5. DOI: 10.1016/ j.joen.2021.06.006

CHEMISTRY

24290 | Carbon Dot Nanozymes with SOD-Like Activity: ROS Detection Using a Sequential Injection Lab-on-Valve System

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Background & Aim: Reactive Oxygen Species (ROS), such as superoxide anion radical ($O_2^{\bullet -}$), are critical drivers of oxidative stress and tissue damage, namely in chronic wounds. Although Superoxide Dismutase (SOD) regulates these levels, its therapeutic application is limited by high costs and instability [1]. Therefore, more stable and cost-effective alternatives are needed, and Carbon Dots (CDs) are promising nanozymes if they exhibit SOD-like activity. In this context, the present study aims to optimize a ROS detection method in a lab-on-valve integrated microsequential analysis system (µSIA-LOV), and to assess the ROS scavenging activity of synthesized Carbon Dots. **Methods:** Superoxide radicals were generated via pyrogallol autoxidation in an alkaline medium (pH 9.3) and detected through reduction of the tetrazolium salt WST-1 into a soluble formazan, measured spectrophotometrically at 450 nm [2]. This reaction was automated using a µSIA-LOV system, and key parameters, including reagent concentrations, volumes, and reaction time were carefully optimized. The scavenging activity of commercial SOD and synthesized CDs was evaluated at different concentrations, enabling the comparison between them. **Results:** Optimized µSIA-LOV parameters ensured a stable and reliable analytical signal. The scavenging activity of commercial SOD was validated in a dose-dependent manner. Following this, the synthesized Carbon Dots SOD-like activity was found to be even higher than the commercial SOD. **Conclusions:** The automatic µSIA-LOV system proved to be a cost-effective, rapid alternative to conventional methods and the CDs exhibited ROS scavenging potential, demonstrating their potential as effective SOD-mimicking nanozymes. Future studies will involve determining the kinetic parameters of these nanozymes and evaluating their performance in wound fluid to mimic physiological conditions.

Keywords: Lab-on-valve; ROS scavenging; Superoxide dismutase; Nanozymes; Carbon dots.

Acknowledgments:

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24336 | Click chemistry mediated conjugation of an ionic liquid to a peptaibol to improve water solubility and bioactivity

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Background & Aim: Peptaibols are a large family of natural, non-ribosomal linear peptides primarily produced by fungi of the genus *Trichoderma*. These peptides are structurally unique due to the presence of rare amino acids throughout their sequence, such as α-aminoisobutyric acid (Aib) and isovaline (Iva), as well as an acetylated *N*-terminus and a *C*-terminal alcohol. These structural characteristics confer high resistance to proteolytic degradation, granting broad bioactivity to peptaibols. However, their low water solubility hinders their use in pest control formulations for agricultural applications [1]. Longibramide E is an 11-residue peptaibol produced by the fungus *T. longibrachiatum*, widely used as an ideal model for the synthesis of new molecules for crop protection [2, 3]. Recently, ionic liquids (ILs) have become increasingly attractive as low-cost highly customizable and "green" organic salts for diverse utilities, including enhancement of antimicrobial potency and water solubility [4]. This study aims to improve these two features in longibramide E, by conjugating it to an IL with a broad spectrum of antibacterial activity. **Methods:** The IL was synthesized *via* the reaction of 1-hexadecylimidazole (C16Im) with propargyl bromide. In parallel, longibramide E was produced by solid-phase peptide synthesis (SPPS), and next modified at the *C*-terminus by coupling to azidoacetic acid. The alkyne-modified IL and the azide-modified peptaibol were finally coupled to each other *via* a copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) "click" reaction. **Results:** The synthesis of the target IL-peptaibol conjugate was successful. Ongoing studies focus on the conjugate's *in vitro* bioactivity and water solubility, both expected to be enhanced compared to the parent peptaibol, based on literature reports [5]. **Conclusions:** Conjugation to ILs could be a sustainable and low-cost solution to overcome the drawbacks of peptaibols, enabling their use in crop protection and beyond.

Keywords: Click-chemistry; Ionic liquids; Peptaibols; Water solubility.

Acknowledgments: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 -Laboratório Associado para a Química Verde – Tecnologias e Processos Limpos.

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24542 | Development of Antidotes for Envenomation by Snakes of the Elapidae Family

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Background & Aim: Three-finger toxins (3FTx) are abundant in elapid venoms. Although they share a conserved scaffold of three β-stranded loops projecting outward from a central hydrophobic core, they consist of multiple subfamilies with diverse biological activities. This research focuses on the α-Neurotoxin subfamily, typically the group with highest lethality, divided into short-chain and long-chain subgroups, the former having four disulfide bonds and the latter an additional bond located in loop II (fig. 1). These toxins inhibit nicotinic acetylcholine receptors (nAChRs), leading to paralysis and to respiratory failure. Despite their clinical relevance, inhibitor development remains limited, mainly due to their structural rigidity and sequence variability between different species [1-3]. Hence, this project aims to contribute to the discovery of effective inhibition strategies. **Methods:** Multiple sequence alignments of both short-chain and long-chain α-neurotoxins were performed, conserved regions were identified and analyzed through visual comparison on AlphaFold3 predicted toxin structures, incorporating consensus sequences derived from literature sources [4, 5]. Molecular dynamics simulations were performed to assess structural stability. Target residues were then defined for non-covalent virtual screening, to identify a reduced set of promising ligands. **Results:** Target residues identified for long-chain 3FTX were K24, C31 and C57, and for short-chain were K25, K26 and C53 (consensus sequences numbering). These sites remained stable and somewhat accessible during simulations. Non-covalent virtual screening is currently ongoing. **Conclusions:** This research will contribute to α-neurotoxin inhibitor discovery. Targeting conserved regions may enable the development of universally effective inhibitors, overcoming the limits of structural rigidity and interspecies variability.

Keywords: 3FTx; α-neurotoxin; Multiple sequence alignment; Molecular dynamics; Virtual screening.

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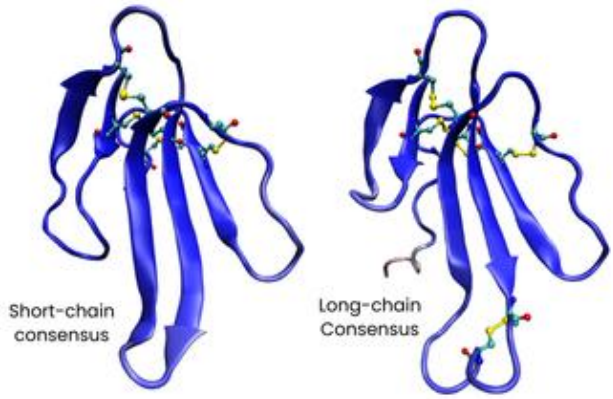

Figure 1: Comparison between AlphaFold3 predicted protein structure and cysteine bonds in short-chain and long-chain α-neurotoxins (consensus sequences from literature). Beta coloring on the toxin (deepness of the blue depending on the confidence of the algorithm that that region of the structure is positioned correctly).

24922 | *Dunaliella salina* as a lipid source for functional feed

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Background & Aim: *Dunaliella salina* is a microalga recognized as valuable source of bioactive compounds. It has been studied for its high content of ß-carotene and polar lipids, both of which possess antioxidant and health-promoting properties. In aquaculture, two major challenges persist: the excessive use of antibiotics and the need for optimized nutrient delivery. Nanoparticles have emerged as tools for targeted and efficient delivery of bioactive molecules. This work aims to extract and characterize *Dunaliella salina* lipids and explore their incorporation into nanoparticle formulations to produce functional feed for fish aquaculture. **Methods:** A total of 100 mg of *Dunaliella salina* powder was homogenized using a vortex mixer with a solution of methanol/chloroform (1:2, v/v). The mixture was then centrifuged, and the supernatant was subjected to evaporation under a nitrogen stream. The dried lipid extracts were weighted and resuspended in methanol for further analysis. The antioxidant capacity of the extracts was determined using the 2,2-diphenyl-1-picryhydrazyl (DPPH) assay, the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and the ferric reducing antioxidant power (FRAP) assay, while the lipid profile was obtained through Gas Chromatography with flame ionization detection. **Results:** The lipid yield for *Dunaliella salina* extract was 5.7 ± 0.7 (% of dry weight). For the DPPH, ABTS and FRAP assays, the antioxidant power was 37.0 ± 0.5, 105.3 ± 0.9 and 94.3 ± 3.5 µM Trolox equivalents, respectively. The lipid extract displayed a diversified fatty acid profile, dominated by palmitic and oleic acids and including other nutritionally relevant saturated, monounsaturated and long-chain polyunsaturated fatty acids. **Conclusions:** It is possible to infer that *Dunaliella salina* lipid extract shows promising antioxidant activity and contain specific fatty acids crucial to support optimal growth, stress resilience and immune function, showing potential as a lipid source for functional feed.

Keywords: antioxidant activity, conventional extraction, lipid profile

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24554 | Employing Computational Techniques to Study hASIC1a Selective Permeability

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Background & Aim: Acid-sensing ion channels (ASICs) are proton-gated channels involved in the perception of pain. Within this family, ASIC1a is a homotrimer of particular therapeutic interest as it modulates synaptic transmission, memory, and fear conditioning. [1] In addition to Na^+ permeability upon channel activation, ASIC1a is the only subtype permeable to small amounts of Ca^{2+}, which play a critical role in neurodegeneration during acidosis and ischemia.[2,3] Little is known about this slight permeability, particularly when it comes to why the channel favors the passage of Ca^{2+} over K^+, despite the significantly higher extracellular concentration of the latter. Thus, we intend to computationally study this phenomenon by simulating a system comprising the open conformation of an open human ASIC1a (hASIC1a) embedded in a realistic plasma mammalian membrane in a KCl and $CaCl_2$ solution. **Methods:** The structure of the hASIC1a was obtained via homology modeling using an open chicken ASIC1a monomer (PDB 4NTX[4]) as the template. After the biological assembly and refinement of the hASIC1a homotrimer, the system was built in CHARMM-GUI[5,6]. Following this step, we performed molecular dynamics (MD) simulations to observe the passage of the different ionic species through the channel's pore. The resulting path from these trajectories is being used in Umbrella Sampling simulations, and the Weighted Histogram Analysis Method will then be applied to its results to calculate the potential of mean force (PMF) for each ion. **Results:** Ultimately, this will allow the estimate of the Gibbs free energy variations associated with the passage of Ca^{2+}, K^+ and Cl^- and the subsequent identification of specific ion-binding sites and energy barriers that function as selectivity filters. **Conclusions:** By combining MD simulations with a realistic membrane and free energy calculations, this study aims to fill an existing knowledge gap regarding the unique permeability of ASIC1a towards Ca^{2+}, which may be proven useful for future therapeutic approaches.

Keywords: human acid-sensing ionic channel 1a; realistic asymmetric plasma mammalian membrane; homology modeling; molecular dynamics simulations; free energy calculations

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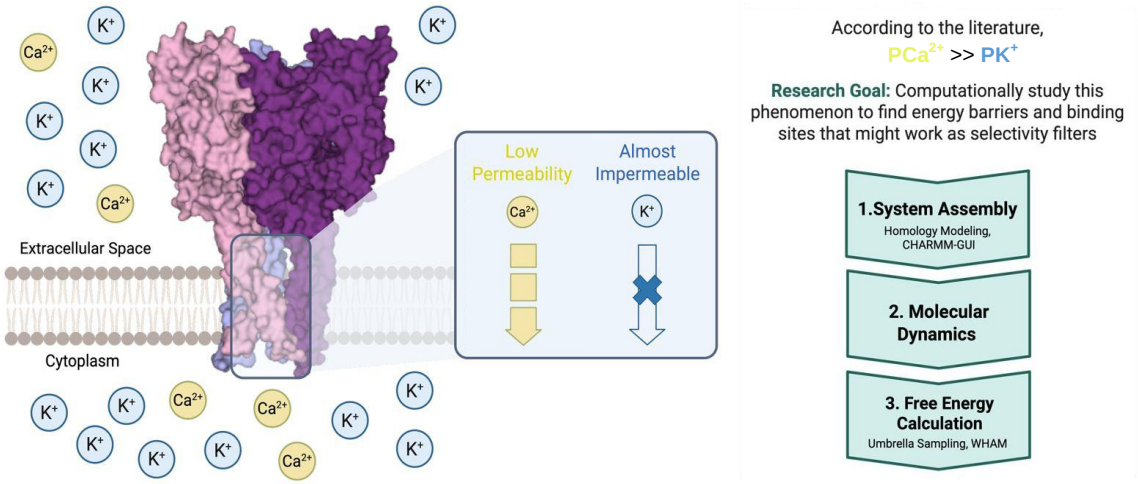

Figure 1: Accompanying Graphical Abstract for the project, showcasing the hASIC1 and its respective permeability level towards calcium and potassium cations according to the literature, as well as the necessary procedures employed in order to computationally study its selective permeability.

24530 | Evaluation of π-electron correlation in anthracene derivatives

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Background & Aim: Electron correlation in π-conjugated aromatic systems plays a key role in the thermodynamic stability and functional performance of organic semiconductors yet remains difficult to quantify experimentally.[1] In this work, we present an integrated experimental-computational study on several phenylanthracene derivatives designed to study the effect of substitution pattern and molecular structure on phase equilibria and electronic properties. **Methods:** The target compounds were synthesized by the Suzuki-Miyaura cross-coupling reaction and purified by vacuum sublimation. The standard molar enthalpies and entropies of sublimation were determined using the Knudsen effusion method, and the solid-solid and solid-liquid phase equilibria were evaluated by differential scanning calorimetry (DSC). A comparative study using UV-Vis and NMR spectroscopy was carried out to assess the effect of structural modifications. **Results:** Significant differences were observed between several diphenylanthracene isomers by UV-Vis and NMR spectroscopy. These differences suggest that the stabilization in 1,8-diphenylanthracene involves the H9 hydrogen atom, possibly forming an electronic bridge between the two substituents. On the other hand, multiconfigurational theoretical calculations support the presence of significant static electronic correlation in the 2,6-diphenylnaphthalene and 2,6-diphenylanthracene derivatives, showing that the origin of the energetic stabilization lies in very extensive π-conjugation. Sublimation equilibrium studies were extended to several naphthalene and anthracene derivatives, revealing that volatility is mainly related to the molecular surface area. DSC experiments revealed the existence of readily accessible polymorphs for the 9,10- and 1,8-diphenylanthracene compounds. **Conclusions:** The overall results establish a consistent link between molecular architecture, electronic correlation and phase behavior in functionalized acenes, and provide a quantitative thermodynamic basis for future determination of gas-phase formation enthalpies and for the rational design of larger acene-based materials with tailored stability and electronic properties.

Keywords: Anthracene derivatives; π-conjugation; Electron correlation.

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24522 | From Sea to Solution: Green Extraction of Algal Polysaccharides with Antioxidant Properties

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Background & Aim: Algal polysaccharides, particularly those derived from seaweeds, are increasingly explored in aquaculture as natural immunomodulators capable of enhancing host immune responses and improving resistance to infections [1]. The aim of this work was to explore the use of a green extraction method to obtain and characterize polysaccharide-enriched algal extracts from different species. **Methods:** Extracts from freeze-dried biomass of macroalgae (*Saccharina latissima*, *Laminaria ochroleuca*) and microalgae (*Chlorella vulgaris*, *Dunaliella salina*) were obtained using a sonication probe in water (5 g L^{-1}), and performed in triplicate. The extracts were characterized for (i) carbohydrate content (sulfuric acid/UV spectrophotometry method [2]), (ii) antioxidant activity through 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Ferric Reducing Antioxidant Power (FRAP) assays, and (iii) functional groups using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR). Biocompatibility was tested in primary European seabass leukocytes using the Presto Blue assay at 50–5000 µg mL^{-1}. **Results:** Extraction yields exceed 50% for all the algae species studied, except for *Chlorella vulgaris* (25%), and the carbohydrate content of the macroalgae extracts was around 50 mg mL^{-1}, whereas for the *Dunaliella salina* and *Chlorella vulgaris* was around 100 and 200 mg mL^{-1}, respectively. Macroalgae displayed higher antioxidant capacity with ABTS assay, whereas microalgae had better results with FRAP assay. ATR-FTIR spectra revealed characteristic glycosidic linkage peaks in all samples. **Conclusions:** The use of a

sonication probe provided an easy, energy-efficient, fast, and environmentally friendly extraction method using water as solvent, while still achieving high extraction yields. The obtained extracts exhibited promising antioxidant potential; however, further *in vitro* and *in vivo* studies will be conducted to confirm and clarify their potential biological effects.

Keywords: algae; sonication probe; algal polysaccharides; biocompatibility

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24318 | Magnetic Electrochemical Immunoassay for Crustacean Allergen Analysis

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Background & Aim: Accurate allergen labeling on packaged foods is critically important as undeclared allergens may pose serious health risks. Electrochemical biosensing platforms are promising approaches for the rapid and sensitive analysis of allergenic proteins in complex food matrices, providing an efficient tool for food safety monitoring [1–3]. **Methods:** A sandwich-type electrochemical immunoassay was developed through the combination of magnetic beads and screen-printed carbon electrodes for the chronoamperometric determination of tropomyosin, a major crustacean allergen. Scanning electron microscopy (SEM) was employed to evaluate the morphology and surface characteristics of the magnetic particles and to confirm their functional suitability. **Results:** Tropomyosin was quantified within 30 min using a 40-µL sample aliquot. The assay displayed a linear response over the range of 1.0–200 µg/g, with a limit of detection of 1.0 µg/g. High precision (CV<10%) was achieved across model food matrices. **Conclusions:** The immunoassay provides reliable quantitative assessment of tropomyosin in processed food samples, since the use of magnetic particles facilitates rapid and efficient separation and washing steps, enhancing both assay sensitivity and selectivity. This approach effectively minimizes matrix interference, resulting in improved analytical performance, which is essential for safeguarding sensitized individuals and ensuring compliance with food safety regulations.

Keywords: Crustacean; Food allergy; Immunosensor; Electrochemical biosensor; Magnetic Beads.

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24652 | MIP-Based Biosensors for Food Allergen Detection: A Comprehensive Review

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Background & Aim: The need for rapid, reliable, and selective detection of food allergens is increasing due to their impact on public health and food safety. Biosensors, composed of a recognition element and a transducer, provide fast and user-friendly analytical tools. However, natural bioreceptors such as antibodies and enzymes often suffer from limited stability, short lifetime, and high production costs. Molecularly imprinted polymers (MIPs) have emerged as synthetic receptors capable of mimicking antibody-like recognition while offering enhanced robustness and easier integration into sensor platforms. This work presents an updated bibliometric review of MIP-based biosensors focused on food allergen detection. **Methods:** A systematic bibliometric analysis was conducted using Scopus [1] and PubMed [2] databases. The keywords “MIP biosensor” and “allergens” were applied to publications between 2016 and 2026. The term “point-of-care” (POC) was additionally included to evaluate the development of portable applications. Articles were screened based on relevance to food allergen detection. **Results:** The search identified 381 articles in Scopus and 8 in PubMed specifically addressing MIP-based biosensors for allergens. Broader searches retrieved 1,001 related records in Scopus and 27 in PubMed, indicating sustained growth in this research area. The analysis reveals increasing scientific output and expanding interdisciplinarity, involving chemistry, materials science, engineering, and biotechnology. A clear trend toward portable and mobile-integrated biosensing systems was observed, highlighting the transition toward POC technologies. **Conclusions:** MIP-based biosensors show strong potential as stable, cost-effective alternatives to natural bioreceptors in food allergen detection. Their integration into portable platforms may contribute to more accessible and reliable monitoring systems, supporting advances in next-generation analytical technologies.

Keywords: Molecularly imprinted polymers; Food safety; Synthetic bioreceptors

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24634 | Multigram Scale Synthesis of Aza-Proline for Application in Medicinal Chemistry

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Background & Aim: Azapeptides, a notable class of peptide derivatives, are formed through the replacement of one or more α-carbon atoms of a peptide by nitrogen atoms.[1] This subtle, yet effective modification provides peptides with a diverse range of pharmacological properties.[1] The incorporation of aza-amino acid residues into biologically active peptides is known to enhance proteolytic resistance,[2] thereby increasing the stability and bioavailability of peptide drugs.[1,2] In some cases, azapeptides also exhibit improved activity and selectivity toward biological targets.[3] Therefore, the development of aza-peptides represents a highly promising strategy in medicinal chemistry for the design of peptide-based therapeutics. **Methods:** In this work, an efficient multigram-scale synthesis of aza-proline, a proline surrogate with relevant applications in peptide synthesis and design, is described. The synthesis relies on *N*-alkylation and *N*-carbonylation of hydrazine derivatives using inexpensive reagents and standard laboratory techniques to afford the desired aza-amino acid. **Results:** A robust and straightforward methodology was developed for the preparation of aza-proline in multigram quantities, achieving an overall yield of 67% over five reaction steps. Additionally, crystalline samples suitable for X-ray diffraction analysis were obtained, enabling, for the first time, the structural characterization of aza-proline in the solid state. **Conclusions:** Overall, this work contributes to the state of the art by providing practical and scalable access to a relevant proline surrogate, thereby facilitating its broader application in peptide design and medicinal chemistry. The availability of multigram quantities of structurally characterized aza-proline is expected to accelerate the development of azapeptide-based therapeutics with improved stability and biological performance.

Keywords: Aza-proline; Azapeptides; Medicinal chemistry; Peptide synthesis; Proline surrogates.

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24224 | Nanomaterial-based analytical methods for monitoring antibiotics in honey

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Background & Aim: Honey is a complex food matrix that can accumulate trace levels of antibiotic (ATB) residues, raising concerns about antimicrobial resistance and the European Union (EU) zero-tolerance policy for prohibited substances [1]. Conventional ATB detection methods, including immunoassays and chromatographic techniques, are often limited by time-consuming pretreatment, high costs, and the use of organic solvents [1, 2]. In this context, nanomaterial-based fluorescent sensors (NMFS), particularly semiconductor quantum dots (QDs) and carbon dots (CDs), have emerged as promising alternatives due to their tunable optical properties and surface functionalization [2]. This work reviews recent advances in NMFS for ATB detection in honey. **Methods:** PubMed and Web of Science were systematically searched for studies reporting NMFS methods for antibiotic detection in honey. **Results:** These sensors show linear detection ranges from the nM level up to hundreds of µM, with good recoveries (91–116%). Limits of detection range from low nanomolar values in optimized systems (e.g., NH_2-SQDs, LOD ≈3 nM) [3] to higher values in portable paper-based formats (e.g., CdZnTe QDs with MoS2 nanosheets, LOD ≈56 µM) [2]. Most approaches rely on fluorescence modulation mechanisms, including quenching [4], ratiometric responses [5] or dual fluorescence-colorimetric readouts [6]. In QDs and CDs-based systems (e.g., MoS_2 QDs@UiF-8 and NH_2-SQDs), ATB binding induces fluorescence changes via inner-filter effects [7] or charge-transfer-related quenching [8]. Selectivity is further improved by incorporating recognition elements such as monoclonal antibodies or aptamers [2, 9]. **Conclusions:** NMFS represent a promising analytical approach for ATB monitoring in honey, offering high sensitivity, good recoveries, and the potential for rapid, on-site analysis. Future research should focus on improving robustness and reproducibility while maintaining sustainability, ensuring compliance with EU regulatory requirements.

Keywords: antibiotics; honey; fluorescent sensors; quantum dots; carbon dots

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24801 | Plas2Nano: Transforming plastic waste into smart colorimetric sensing technologies

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Background & Aim: The accumulation of non-recyclable plastic waste is a critical environmental challenge that affects water quality. Poly(ethylene terephthalate) (PET) residues contribute to coastal pollution and microplastic formation [1]. Within the Plas2Nano project and as an endorser of the EU Mission "Restore our Ocean and Waters", this work aims to revalue PET as a chemical precursor for the design and synthesis of new colorimetric sensors for hazardous metal ion detection [2]. This strategy follows Green Chemistry principles and promotes circular economy and low-cost environmental and biomedical monitoring [3]. **Methods**: During European Ocean Days clean-up initiatives, PET waste was collected from beaches in Gaia and Aveiro. Then, PET was chemically depolymerized, under microwave irradiation, via aminolysis with hydrazine hydrate to produce terephthalate dihydrazide (*TPD*). *TPD* served as intermediary for condensation reactions with vanillin derivatives, enabling the creation of the sensors. Structural characterization was performed by NMR, FTIR, and MS, while UV–Vis spectroscopy evaluated metal-binding sensitivity and selectivity. **Results**: Aminolysis reaction conditions were optimized, providing an efficient route to obtain the *TPD* monomer from PET waste, which was then functionalized with vanillin derivatives to produce the desired sensors (Figure 1). The new sensors showed distinct visual responses to metal ions, including Cu^{2+} and Fe^{3+}. Calibration models enabled quantitative metal determination. **Conclusions**: This work demonstrates that coastal PET waste can be transformed into new sensing materials through a process already protected by a national patent [4]. Beyond laboratory validation, these chemical devices have been demonstrated in many schools, presented at European Ocean Days (Brussels), and will integrate future activities at the ICBAS BYS project, reinforcing the connection between sustainable chemistry, innovation, education, and social impact.

Keywords: PET upcycling; Colorimetric and fluorescent sensors; Chemical probes; Metal ion detection; Waste valorization

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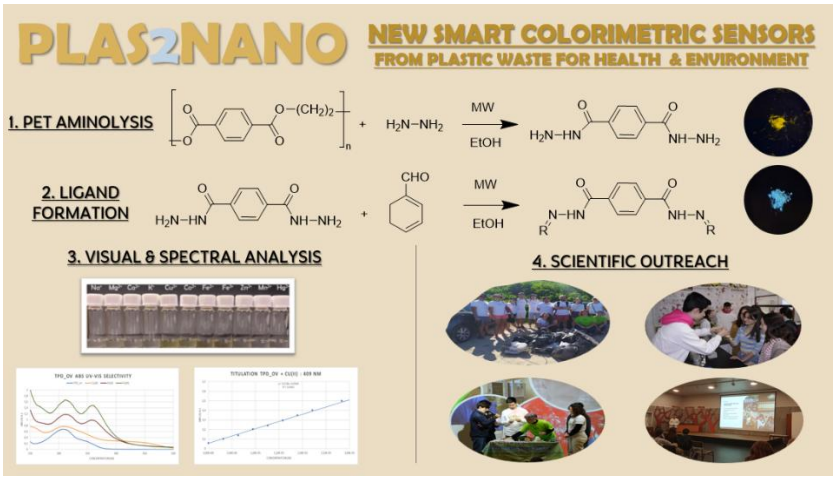

Figure 1: Plas2Nano: Colorimetric and Fluorescent Sensors. Image drawn in ChemDraw Professional 18.0

24452 | Study of Electronic Correlation in Aromatic Organic Compounds: Experimental Quantification of its Magnitude and Evaluation of its Electronic Nature

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Background & Aim: This work is part of a wider study on π-electronic correlation phenomena in aromatic organic compounds[1], seeking to understand how such interactions affect the physical and chemical behavior of conjugated systems. The present study focuses on aromatic molecules with a four-ring acene core, whose thermodynamic properties are analyzed to clarify the role of π-electronic correlation in their optical responses and structural organization. A comparative analysis with structurally related compounds containing different acenes and phenyl substituents was performed to identify systematic trends and assess how gradual extension of the π-conjugated framework affects their overall electronic behavior. **Methods:** The compounds (figure 1) were synthesized via Suzuki-Miyaura cross-coupling reaction. The thermodynamic properties were examined using Knudsen effusion combined with a quartz crystal microbalance (KNQ) and differential scanning calorimetry (DSC). The optical properties were studied by UV-Vis spectroscopy. Density functional theory (DFT) calculations, including geometry optimization and vibrational frequencies, complemented the experimental results. **Results:** All compounds were successfully synthesized and characterized. Enthalpies and entropies of fusion and sublimation were obtained. Solid-solid phase transitions were identified, offering further insight into structural stability and intermolecular interactions. **Conclusions:** The study provided valuable insight into the relationship between π-electronic correlation and the thermodynamic, structural, and optical behavior of extended aromatic systems. Through the combination of experimental thermodynamic measurements with complementary optical and structural analyses, it was possible to evaluate how molecular architecture influenced energetic stability and electronic delocalization. Comparative assessment of compounds with different acenes revealed consistent trends that contributed to a broader understanding of π-electron interactions in conjugated organic materials.

Keywords: Molecular energetics; Polyaromatic hydrocarbons; Electron correlation

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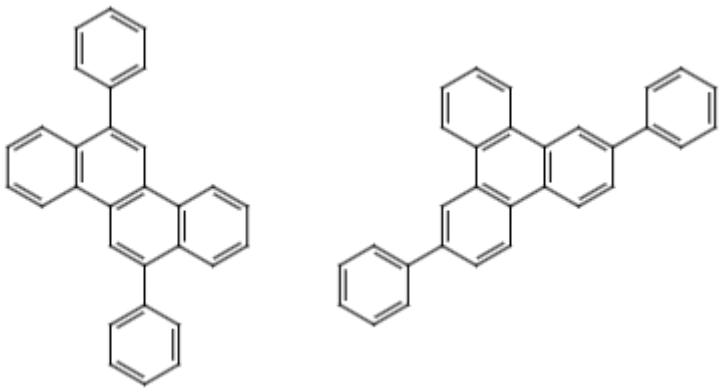

Figure 1: Structure of the compounds studied: 6,12-diphenylchrysene (left) and 2,7-diphenyltriphenylene (right).

24305 | Synthesis and molecular modification of chalcone derivatives with potential biological activity

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Background & Aim: Chalcones (1,3-diphenylpropan-1-ones) are bioactive open-chain flavonoids constituted by two aromatic rings (A and B) linked through a three-carbon α,β-unsaturated carbonyl system. The presence of this carbonyl system makes these derivatives promising building blocks for the synthesis of bioactive compounds, such as other classes of flavonoids as dihydrochalcones, as well as diarylpropanes. Our laboratory has extensive experience in this field and has identified some chalcone derivatives with significant biological potential, particularly as antifouling agents and as antimicrobial adjuvants [1,2]. To expand the library of chalcone derivatives and explore their potential biological activities, a series of chalcones, and structure related dihydrochalcones as well as diarylpropanes, with different substitution patterns in A and B rings, was planned. **Methods:** The synthesis of the chalcones was successfully achieved by a base-catalyzed aldol condensation reaction between appropriately substituted acetophenones and benzaldehydes. Dihydrochalcones and diarylpropanes were obtained by catalytic hydrogenation of chalcones using Pd/C or $Pd(OH)_2$/C as catalysts under H_2 atmosphere at room temperature. **Results:** Three chalcone derivatives were successfully synthesized and characterized by ^1H and ^{13}C nuclear magnetic resonance techniques. **Conclusions:** In the future, the library of compounds will be enlarged, and the potential biological activities will be explored to perform structure-activity relationship studies.

Keywords: Chalcones; Dihydrochalcones; Diarylpropanes; Synthesis; Structure elucidation

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24389 | Synthesis of a Xanthone Derivative via Benzophenone Intermediate and Microwave-Assisted Acylation

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Background & Aim: Xanthone derivatives stand out due to their chemical versatility and wide range of biological activities [1]. Developing xanthone derivatives offers promising opportunities to discover new bioactive agents and to create valuable tools for chiral separation [2]. Herein, the total synthesis and structure elucidation of a xanthone derivative are described to obtain a promising starting material for the preparation of bioactive derivatives and chromatographic selectors for liquid chromatography. **Methods:** Total synthesis of a xanthone derivative by the synthesis of a benzophenone intermediate, followed by acylation via microwave-assisted reaction, and an ether hydrolysis to obtain the final compound. The structures of the xanthone derivative and intermediates were established by spectroscopic methods (NMR and IR). **Results:** The synthesis, purification, and structure elucidation were successfully accomplished with yields ranging from 50% to 98%. **Conclusions:** The synthetic strategy selected to obtain the xanthone derivative demonstrated to be efficient. Future studies will focus on the synthesis of derivatives and the evaluation of their antitumor and antimicrobial activities. Another application to be explored will be as tools for chiral separation.

Keywords: Xanthone derivative; Benzophenone intermediate; Synthesis; Structure elucidation

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24700 | Synthesis of C-1 and C-4 Modified Thioxanthone Derivatives as Precursors for Metal-Based Antifungal Agents

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Background & Aim: The development of new antifungal agents has become an urgent challenge due to rising resistance, limited drug classes, and high toxicity associated with current therapies[1]. Metal coordination of bioactive ligands has emerged as a promising strategy, often introducing new modes of action and improving activity against multidrug-resistant strains[2]. This project aims to design and synthesize a library of thioxanthone derivatives selectively modified at the C-1 and C-4 positions. Thioxanthones are considered a privileged scaffold due to their intrinsic pharmacological properties and previously reported antifungal activity[3,4]. In this work, a chelating moiety will be introduced to this scaffold and subsequently be complexed with four metals (Zn, Cu, Fe, and Ga) to evaluate their antifungal potential. **Methods:** Two different synthetic routes were planned for both C-1 and C-4 modifications. For C-1 functionalization, 1-chloro-4-propoxy-9*H*-thioxanthen-9-one (**TX**) was subjected to a copper-catalyzed Ullmann-type C-N cross-coupling with a hydroxyurea derivative to afford **TX1**. For C-4 modification, TX first underwent ether cleavage, followed by a Williamson ether synthesis and subsequent basic ester hydrolysis to afford **TX2**. A copper-catalyzed Ullmann-type C–N coupling was chosen to generate an aminated thioxanthone derivatives, which will later be conjugated with the chelating moiety. All synthesized compounds were purified either by column or preparative chromatography, and their structures were confirmed by NMR. **Results:** The target **TX1** derivative was synthesized as a red solid (11.3% yield), while intermediate **TX2** was obtained in three steps with 79% yield. Further C-1 and C-4 functionalizations are currently ongoing. **Conclusions:** Preliminary data

validate the synthetic strategy. Future work will consist of completing the functionalized libraries, performing metal complexation, and evaluating in vitro antifungal activity.

Keywords: Thioxanthone, Antifungals, Multidrug resistance, Chelating ligands, Ullmann coupling.

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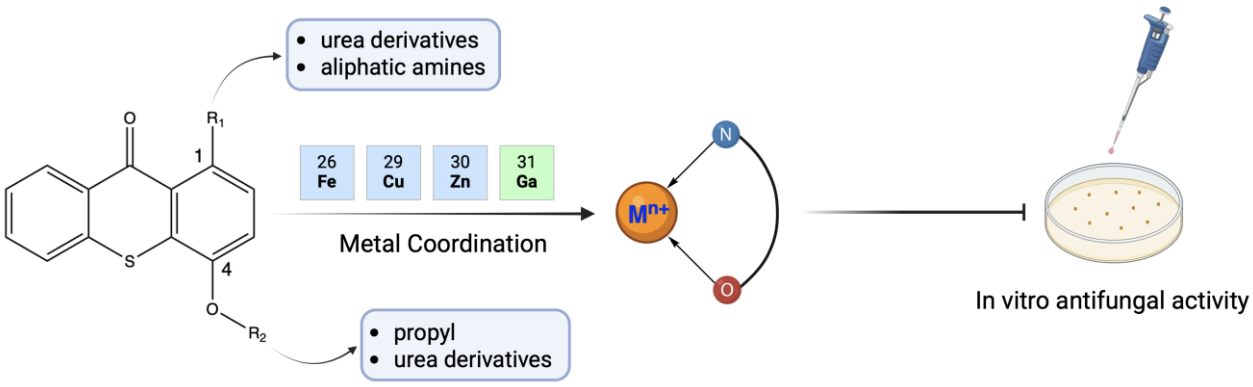

Figure 1: C-1 and C-4 Modified Thioxanthone Derivatives as Precursors for Metal-Based Antifungal Agents

24688 | Synthesis of kojic acid–chalcone hybrids as multifunctional agents for skin hyperpigmentation control

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Background & Aim: Excessive melanin production is associated with several hyperpigmentation disorders, driving the continuous search for safe and effective tyrosinase inhibitors. Kojic acid is a well-established depigmenting agent widely used in cosmetic formulations due to its ability to inhibit tyrosinase via copper chelation at the enzyme's active site.[1] However, its limited stability and moderate potency encourage structural optimization.[2] Chalcones are open-chain flavonoids well known for their antioxidant and anti-inflammatory properties, and their tyrosinase inhibitory effect has also been reported.[3] Taking this into account, the combination of these two bioactive scaffolds through molecular hybridization represents a promising strategy to develop multifunctional depigmenting agents. **Methods:** New kojic acid–chalcone hybrids were rationally designed and synthesized using a copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) approach to generate a 1,2,3-triazole linker. A propargylated chalcone intermediate was obtained via Claisen–Schmidt condensation in high yield and subsequently conjugated with an azide-functionalized kojic acid derivative. Structurally related hybrids were also prepared to enable structure–activity relationship studies. **Results:** The synthetic strategy successfully afforded a small library of triazole-linked kojic acid–chalcone hybrids. **Conclusions:** This study synthesized new chalcone-kojic acid hybrids with potential as tyrosinase inhibitors and for skin health benefits. Further research to assess antityrosinase activity will be performed. This research will contribute to developing safer and more effective skin-lightening ingredients, addressing kojic acid limitations.

Keywords: Chalcones; Kojic Acid; Click Chemistry; Synthesis; Skin hyperpigmentation

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24473 | Synthesis of phase I metabolites of a non-nitrocatechol catechol-O-methyltransferase inhibitor targeting dopaminergic metabolism and iron-driven ferroptosis

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Background & Aim: Dopaminergic degeneration, combined with increased dopamine metabolism, can lead to dopamine deficiency observed in neurodegenerative diseases such as Parkinson's disease [1]. The main therapeutic strategy to restore dopaminergic neurotransmission is the co-administration of the dopamine precursor L-DOPA and catechol-O-methyltransferase (COMT) inhibitors [2]. Although these approaches offer symptomatic relief, they do not halt the progression of neurodegeneration and raise concerns regarding long-term efficacy and toxicity [3]. This work aims to contribute to the study of the pharmacokinetics of a novel neuroprotective and selective COMT inhibitor (Compound 9), developed by our team [4], by synthesizing its predicted phase I metabolites for the characterization of its metabolic profile and safety. **Methods:** Development of new chemical entities; Structural elucidation by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). **Results:** The synthesis of the Compound 9 metabolites, currently in progress, involves a Michael addition between 3-hydroxy-4H-pyran-4-one and appropriately substituted primary arylamines under mild acidic conditions. Reaction conditions are being optimized to reduce the formation of byproducts and improve the reactions' yields. The structures of the final products have been confirmed by NMR spectroscopy and MS. Following chemical characterization, the compounds will be evaluated for COMT inhibition and drug-like properties. The most promising candidates will be assessed for cytotoxicity in differentiated SH-SY5Y and HepG2 cells and for their protective effects against ferroptosis induced by RSL-3 and erastin. **Conclusions:** Altogether, this work will contribute to validating the safety profile of Compound 9.

Keywords: Dopamine metabolism, COMT inhibitor, neurodegenerative diseases, Parkinson's Disease

Acknowledgments: This work was funded by FEDER funds through the Operational Program Competitiveness Factors COMPETE and national funds by the FCT-Foundation for Science and Technology under the research grants for RISE-Health (UID/06397/2025, https://doi.org/10.54499/UID/06397/2025), RESTORE (COMPETE2030-FEDER-00776500 and 2023.17955.ICDT; https://doi.org/10.54499/2023.17955.ICDT). This work was also funded by project IMPULSE and services of EU-OPENSCREEN (Horizon Europe: 101132028; DOI: 10.3030/101132028). D.C. (2024.07926.CEECIND) and S.B. (2023.06106.CEECIND) contracts were also supported by FCT and FEDER/COMPETE funds.

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24705 | Turning Waste into Resource: Optimized Protocol for High-Purity Cellulose Extraction from Rice Husk

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Background & Aim: Rice is the most consumed food in the world, with great yet still unexplored potential. Its consumption generates about 20% of rice husk (RH) per grain produced, producing a significant amount of agrowaste [1,2]. Although it represents only a fraction of total rice residues, RH is particularly valuable due to its lignocellulosic composition, mainly cellulose, and lignin, which are abundant and versatile materials [3]. RH contains silica, which adds to its research value and complexity. Although cellulose extraction from forest biomass is a well-studied topic, obtaining it from residual sources like RH remains challenging. Many methods exist for extracting cellulose such as Kraft (the most widely used), Sulfite, Soda, mechanical, and biological pulping. Biomass composition differs from source, and region of occurrence. Bleaching is a key process that gives cellulosic biomass its characteristic white color and higher purity level. The selected method affects both cellulose quality and the environmental impact of the process [4]. The scope of this work is to establish a protocol that efficiently extracts cellulose from RH, with the highest purity and yield. **Methods:** The RH composition was elucidated according to NREL and TAPPI standard procedures. Cellulose was extracted via soda pulping and characterized through physicochemical and spectroscopic techniques. A central composite design was used to optimize the alkaline extraction and bleaching phases, evaluating the effects of temperature, concentration, and reaction time at three levels. **Results:** RH characterization established the lignocellulosic baseline, allowing optimized soda and bleaching conditions to yield high-purity cellulose efficiently. **Conclusions:** RH is a renewable resource of high-value products. The cellulose obtained from RH exhibit characteristics with potential to be used in the production of sustainable materials.

Keywords: Rice husk; Cellulose extraction; Soda pulping; Process optimization; Circular Economy.

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24601 | Hydroxyapatite-Biochar Renewable as Promising Catalysts for (Electro)chemical Processes

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Background & Aim: This research focuses on Goal 9 & Goal 13 of the UN Sustainable Development Goals[1], promoting a circular economy. Using renewable biomass materials, fishbones (FB) and shrimp shells (SS) cutoffs, by-products from sea food distributors, were employed to create a combined material. This is being explored in electrocatalytic carbon dioxide reduction, to remove CO_2 from the atmosphere, and catalytic hydrogen transfer of levulinic acid (LA) and furfural (FF) to biofuel additives. Hydroxyapatite (HA) was prepared from FB utilized for its Ca and K rich composition, ion-exchange capacity & tunable acid-base ability. It was combined with shrimp shell biochar (BioC), improving electron transfer capabilities. **Methods:** HA was prepared by alkaline hydrolysis[2] and calcination[3]. Shrimp shells were converted using pyrolysis at 900°C. The BioC & HA were combined at various ratios: 1:0.5, 1:1 & 0.5:1[4,5]. Material was characterised using FTIR, SEM, elemental analysis, ICP, BET. Initial electrochemical tests have followed the oxygen reduction reaction (ORR), to understand the electrochemical potential of the raw materials. Shown Fig 1. **Results:** BET showed BioC to have the highest surface area (~275 m^2/g) and with a slight decrease for materials with more HA. Electrocatalytic ORR with BioC showed the most promising results with jL of -4.07 j/mA cm^{-2} and nO_2 of 3.35 electron on average Fig 2. Catalytic hydrogen transfer has been briefly evaluated for LA and FF conversion with conversions up to 97% and a selectivity of 98% towards Isopropyl 4-oxovalerate Fig.3. **Conclusions:** The preliminary results for BioC:HA catalysts showed they are promising materials compared with state of the art. Replacing catalysts in industry with more sustainable ones brings us one step closer to creating a zero-waste circular economy.

Keywords: Catalysts; Biochar; Sustainability; Shrimp shells; Renewable.

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We would like to thank Nueva Pescanova group and Mar Ibérica company for the collaboration.

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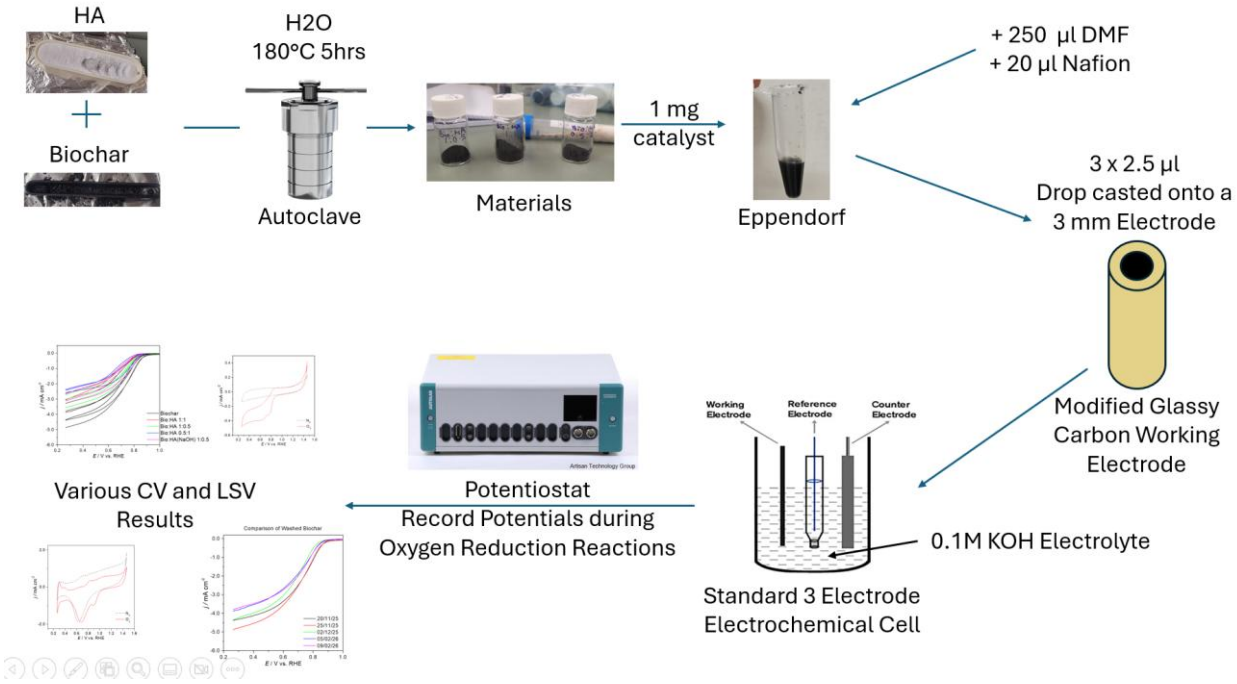


Figure 1: basic diagram of the method and procedure.

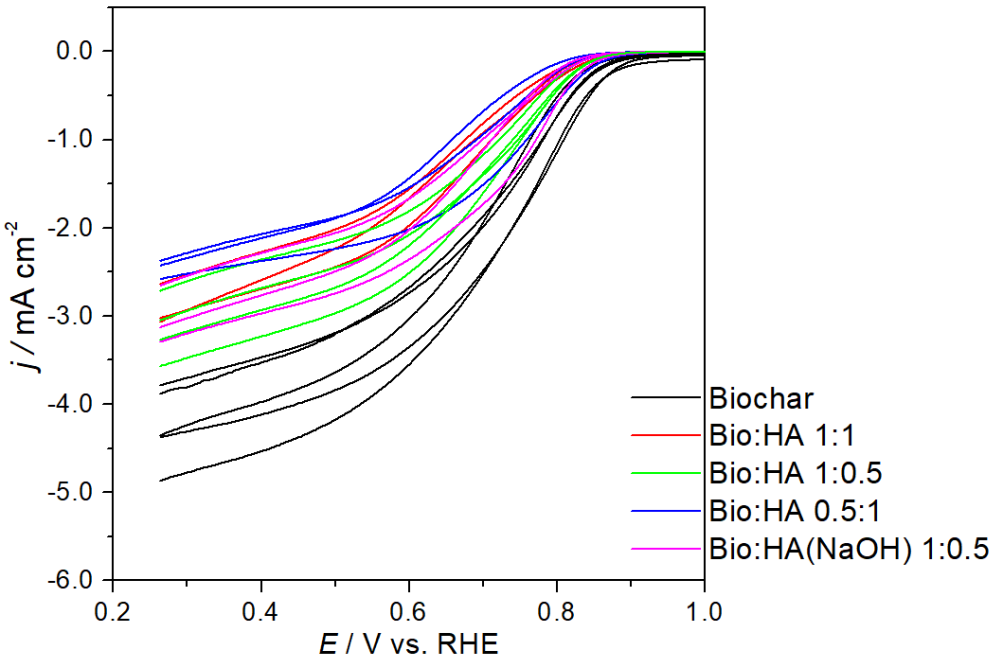
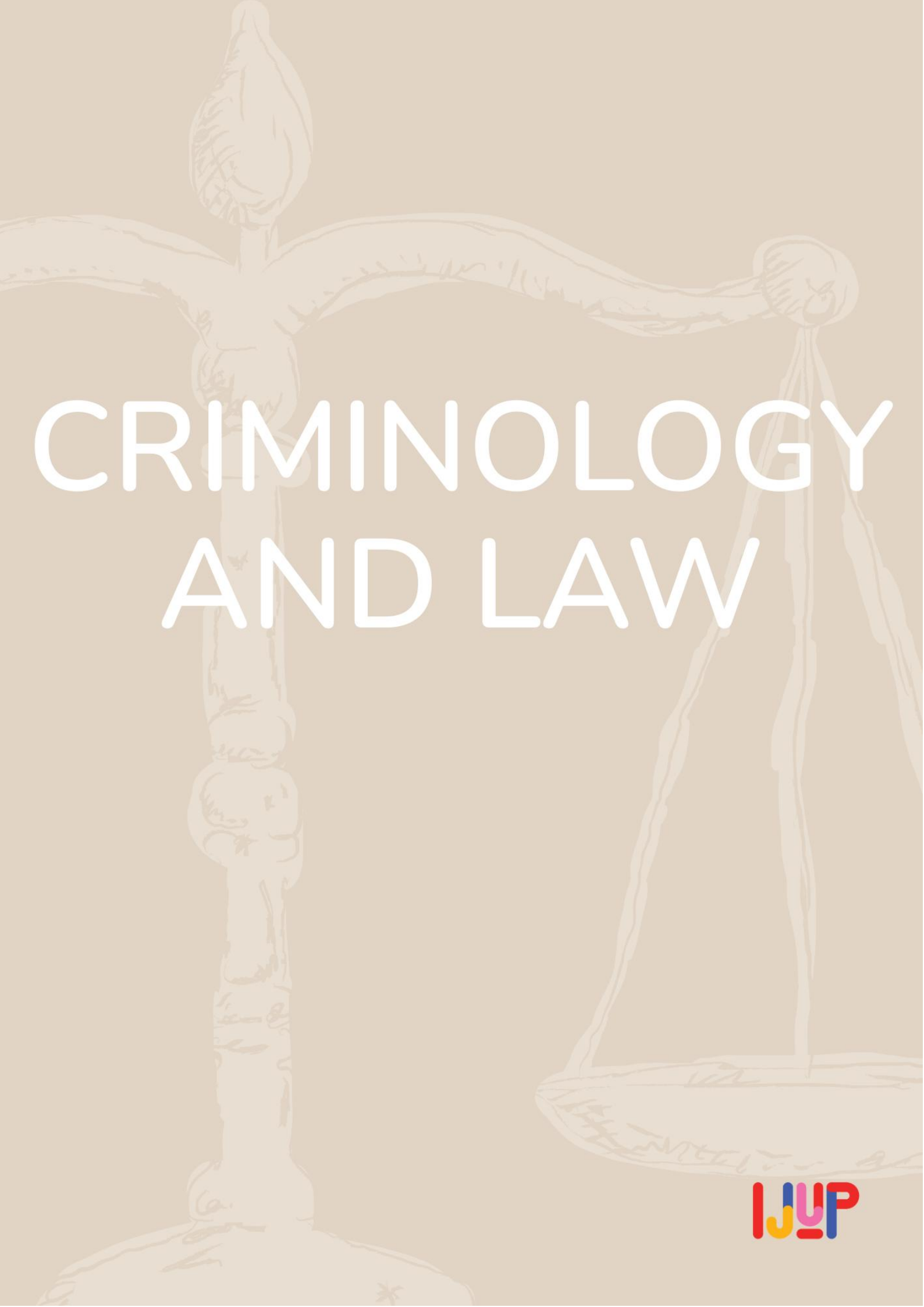


Figure 2: Electrochemical ORR following all materials prepared. BioC shown in black.

Catalyst Name	Conversion %	Selectivity for (Isopropyl 4-oxovalerate) %	Selectivity for (Y-Valerolactone) %
HA	69.9	100	0
Bio:HA 0.5:1	97.9	98	2

Figure 3: Table showing the conversion and selectivity of HA and BioC:HA 0.5:1 towards different biofuel additives. Conditions: Isopropanol, Levulinic acid, tetradecane in a high-pressure reactor, 10 hrs at 160°C.



CRIMINOLOGY AND LAW

24266 | Doxing as an expression of Vigilantism: Bystanders perceptions

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Background & Aim: Doxing, defined as the non-consensual disclosure of personally identifiable information online, has emerged as a manifestation of digital vigilantism [1]. Often driven by distrust in formal institutions, it may function as an informal mechanism of social regulation and punishment [3]. Despite its potential to cause psychological, reputational and physical harm, doxing is sometimes publicly legitimized when framed as "social justice" [2]. This study aims to analyse public perceptions of the moral legitimation of doxing and the predisposition to engage in such behaviour when targets are alleged criminal offenders. **Methods:** A quantitative experimental vignette design will be employed. Participants will be exposed to hypothetical scenarios involving alleged offenders, systematically varying offence severity, social reactions and contextual legitimacy cues. Data will be collected through an online questionnaire administered to a non-probabilistic sample. Measures will assess moral evaluation, perceived legitimacy of doxing and predisposition to engage in such behaviour, while also examining the influence of individual factors (e.g., gender, political orientation, moral beliefs and justice perceptions) and collective factors (e.g., perceived online social norms, social approval or disapproval dynamics). A pilot pre-test will assess clarity and reliability. **Results:** As the study is ongoing, the presentation focuses on the theoretical framework. It is hypothesized that moral acceptability and intention to dox will be higher when the alleged offence is perceived as severe, evidence is perceived as strong, and when perceived social norms indicate approval. **Conclusions:** The expected findings may clarify psychological and social mechanisms underlying informal digital justice. By identifying conditions under which doxing becomes socially legitimized, this research contributes to understanding tensions between formal legal systems and informal social control in digital environments.

Keywords: Doxing; Digital vigilantism; Informal social control; Legitimacy; Vignettes

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24909 | Visual Behaviors and Discursive Strategies in the Attribution of Trust to Online Commerce

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Background & Aim: Currently, technology is transforming everyday life and patterns of criminality. The limitations of the traditional marketplace have accelerated the expansion of e-commerce, fostering not only legitimate consumer practices but also the growth of online consumer fraud [1]. E-commerce fraud generates significant economic and emotional harm to victims and society, contributing to a progressive erosion of trust in digital commercial environments [2]. Recently, fraudulent strategies have become increasingly sophisticated, notably the creation and dissemination of fraudulent e-commerce websites that closely replicate the appearance and functionality of legitimate websites [3]. Within this framework, Visual Criminology becomes relevant to interpreting the power of visual representations in shaping public opinion and social responses to crime [4]. Thus, this investigation aims to understand visual behaviors and perceptions in relation to images from e-commerce websites. **Methods:** The data will come from mixed methods, complementing eye-tracking techniques, which involved viewing images from e-commerce websites, with semi-structured interviews using photo-elicitation techniques. Expected number of participants for interviews is 12 and for the eye-tracking database is 50. **Results:** This investigation is ongoing and data is being collected and analyzed. Preliminary results indicate variations in attention and behavioral patterns. Most eye-tracking results show a higher number of fixations on areas of commercial products. Furthermore, interviews reveal that participants use discursive strategies guided by previous experiences to attribute trust to online commerce. **Conclusions:** Results from this study will contribute to an understanding of the influence of digital visual stimuli on selective attention and individual behavior on e-commerce websites. Additionally, this study contributes to the development of specific interventions aimed at mitigating harm to online consumers.

Keywords: E-commerce; Fraudulent E-commerce Websites; Perceptions; Online Consumer Fraud; Visual Behaviors

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24331 | Human Trafficking and Financial Crimes: Perceptions and Experiences of Portuguese Authorities regarding the Importance of Jointly Investigations

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Background & Aim: Trafficking in Human Beings is a serious violation of human rights[1,2], constituting a transnational crime frequently committed within the context of organized crime[3]. It is financially motivated, constituting the second most widespread illicit economy worldwide, perceived by perpetrators as a high-profit, low-risk activity[4,5]. These profits are then reinvested in legitimate activities to be laundered through sophisticated schemes and circuits[6,7]. Investigating the flow of these profits and using financial data in trafficking investigations could allow authorities to identify victims, uncover networks and lead to more arrests[8,9]; removing these assets is central to dismantling the networks and preventing money laundering[10]. Despite widespread international recommendations regarding the join criminal investigation of both phenomena, little is known about the experiences of law enforcement concerning these practices in Portugal. Therefore, this on-going study aims to explore the perceptions and experiences of Portuguese authorities regarding the importance of jointly investigating trafficking of human beings and financial crimes such as money laundering. **Methods:** Data were collected through semi-structured interviews with experts in the field and analysed through a thematic analysis framework. **Results:** Preliminary findings suggest that conducting financial investigations alongside trafficking investigations not only yields material evidence to complement victim testimony in court, but also cuts off funding to traffickers or networks, enabling authorities to dismantle them. Additionally, the financial data analysed and shared by financial institutions could enable the identification of suspects or victims through money flows. **Conclusions:** These findings highlight the importance of jointly investigating human trafficking and money laundering, following the flow of profits to identify and dismantle networks, as well as to identify and support victims.

Keywords: Human Trafficking; Money Laundering; Financial Investigation; Joint Investigation

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24950 | Immigration and Fear of Crime: the role of Police Proximity in this relationship

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Background & Aim: Fear of crime is considered a major concern for populations, the scientific community and public policy [4]. Individual's fear of crime can be affected by different factors including perceptions of immigration [1] and police proximity, especially in terms of police visibility [5]. The main aim of this study is to explore the relationship between perceptions of immigration and fear of crime, as well as the role of perceptions of the police in this relationship, which may exert a moderating effect. **Methods:** This ongoing study adopts a quantitative methodology, using a survey as a data collection instrument. Sociodemographic characteristics, political orientation, levels of fear of crime and perceived insecurity, perceptions of immigration in the country, and perceptions of the police, particularly in terms of visibility and satisfaction will be measured. Participants will be recruited through universities and dissemination on social media platforms. A sample of no fewer than 500 respondents is expected. **Results:** It is expected that people who present more unfavorable perceptions of immigration will express higher levels of fear of crime and that police visibility will at least mitigate this relationship. **Conclusions:** Finally, this study is considered particularly relevant because immigration has long been, and continues to be, a widely debated issue across the world, while the existing evidence remains contradictory. Previous research suggests that citizens tend to associate immigration with crime [2]. However, empirical studies indicate that this relationship is largely socially constructed rather than supported by evidence [3]. This study is also relevant because, to the best of our knowledge, there is no existing literature examining these three elements together. In this sense, it may offer an important contribution to academic and scientific knowledge, while also informing policies aimed at enhancing citizens' sense of security, as well as immigration and policing policies.

Keywords: Fear of crime; Immigration perceptions; Policing; Police Visibility.

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24877 | Media Influence on the Construction of the "Ideal" and "Non-Ideal" Rape Victim in Portugal

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Background & Aim: Over the past decades, crime has become a central topic in news coverage, with a significant portion of the population experiencing crime primarily through media representations [1]. Consequently, the media plays a significant role in shaping social perceptions of crime and its victims, influencing how cases are understood and evaluated by the public [2]. In this context, the concept of the "ideal victim" [3] highlights that the social recognition of victimization often depends on specific characteristics attributed to the victim and the context in which the crime occurs. Therefore, this ongoing study aims to analyze how Portuguese media represents rape victims considering the concepts of "ideal victim" and "non-ideal victim", with particular focus on two groups frequently marginalized in this process: male victims of rape and individuals engaged in prostitution. Specifically, it seeks to understand how victims' characteristics influence the selection, prominence, and description of cases in news reporting, as well as to compare how these representations differ between sensationalist and non-sensationalist newspapers. **Methods:** To achieve these goals, a qualitative approach will be conducted, based on the content analysis of news articles published in Portuguese media outlets, comparing coverage from sensationalist and non-sensationalist newspapers. The analysis will focus on language used, narrative framing, and discursive elements associated with the attribution of credibility, empathy, or blame to victims. **Results:** Given the results obtained in previous studies, it is expected to find differences in the media representation of rape victims, indicating a tendency for greater visibility and empathy when victims conform to the stereotype of the ideal victim, whereas victims who do not fit these patterns may receive less prominence or be more prone to victim-blaming narratives. Differences may also emerge between sensationalist and non-sensationalist newspapers in terms of framing, emphasis, and narrative tone. **Conclusions:** This study aims to contribute to understanding how media discourse may reproduce or challenge stereotypes surrounding sexual victimization.

Keywords: Rape; Ideal Victim; Non-ideal Victim; Media Representations; Victim-blaming

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24326 | Pets as a coping strategy for children exposed to domestic violence

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Background & Aim: Children exposed to domestic violence (DV) represent a global social concern, being considered indirect victims of the abuse as they may suffer impacts like psychological trauma and behaviour changes, which will create consequences in their relationships and their natural development [1]. Therefore,victims need to be heard and helped to overcome the trauma and reduce its consequences. In this scope, the literature has been emphasizing that children's pets may play an important role in helping them cope with the DV, once they can reduce stress and anxiety, while helping children overcome the experience and improve their wellbeing [2]. However, children's perceptions of their pet's role in DV, have been neglected as objects of research. Thus, this ongoing study aims to explore children's perceptions of their pet's role as a coping strategy while examining their views of the DV they witnessed. Precisely, it seeks to examine how children perceive their pets' emotional support and how this relationship relates to their coping processes. **Methods:** To reach these goals, a qualitative study, using semi-structured interviews with children exposed to DV will be conducted. Data will be analysed by a thematic analysis to identify patterns and key themes in children's narratives regarding their pets' support and role. **Results:** Results are expected to highlight the ways in which children perceive their pets as sources of comfort, emotional support, and companionship during episodes of DV, which will help contextualize children's perspectives and the potential protective role of pets in mitigating the negative impact of the exposure. **Conclusions**: This study aims to contribute to understanding the positive role pets can play in supporting children exposed to DV. By emphasizing children's perspectives, it aims to inform interventions and therapeutic practices that integrate pets into victim support, ways to improve their duty and the needs of the victims.

Keywords: Children; Pets; Domestic Violence; Perceptions; Qualitative study.

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24264 | Political Corruption: Portuguese Public Perceptions

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Background & Aim: Corruption is a global phenomenon with significant social and political consequences. Despite its impact, political corruption in Portugal continues to be marked by high unreported figures, exceeding those observed in other types of crime [1]. Unlike violent crimes, corruption does not generate immediate threat perception, contributing to normalization and invisibility. Literature suggests that public perceptions influence how political corruption is recognized and reported, particularly through fear of crime, institutional distrust, and procedural justice perceived as flawed [2]. Deficits in these domains may weaken social capital and institutional legitimacy, facilitating the persistence of political corruption. Therefore, this study aims to analyse how social perceptions perpetuate political corruption crimes among Portuguese university students, examining predictors such as fear of crime, trust in justice institutions, procedural justice, social capital, populist attitudes, and political orientation. **Methods:** A cross-sectional quantitative study will be conducted using an online questionnaire to be administered to Portuguese university students. Validated scales will measure the variables of interest. A pilot pre-test will assess clarity and reliability. **Results:** The presentation focuses on the theoretical framework. Based on prior literature, lower institutional trust and weaker perceptions of procedural justice are expected to predict greater normalization and lower reporting intentions [3]. **Conclusions:** The expected findings may clarify how public perceptions underly the persistence of political corruption in Portugal. By identifying perceptual predictors, it may inform strategies to strengthen institutional legitimacy and civic engagement, contributing to the literature on corruption perception.

Keywords: Political corruption; Social Perceptions; Dark figures of crime; Perceived Insecurity

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24251 | The incel phenomenon as a contemporary manifestation of terrorism – an exploratory study

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Background & Aim: The incel community, a subculture within the broader manosphere, is characterised by misogyny, fatalism and a sense of self-perceived victimhood [1]. Grounded in a rigid view of social hierarchy based on attractiveness and the nihilistic black pill worldview, incels perceive their condition as both permanent and unjust [2]. In response to this, some members have committed or attempted acts of mass violence, which research suggests are primarily driven by a desire to punish a society perceived as responsible for their exclusion [3]. Nevertheless, mass misogynistic violence is rarely classified as terrorism, often being reduced to gender-based violence, even if its features align more closely with those of the latter. Therefore, the present study seeks to explore whether the incel phenomenon can be understood as a contemporary form of terrorism. **Methods:** To achieve this goal, a documentary analysis of news media reports on mass violence linked to the incel community will be employed. Concretely, the study seeks to analyse how the media portrays incels, and whether their discourses frame such acts as terrorism, adopting Schmid's revised academic consensus definition of terrorism [4] as the analytical framework to identify both convergences and divergences between the characteristics of incel related violence and those of terrorism. **Results:** A substantial overlap is expected between incel mass violence and several core elements of terrorism, including the instrumentalisation of fear, the desire for retribution, the pursuit of public visibility, and the framing of violence as a means of societal change. Findings are also expected to reflect the growing academic and legal tendency to classify these acts as terrorism, despite ongoing debates about the ethical and social implications of such labelling. **Conclusions:** This study highlights the complexity of discourse surrounding incels and violence, emphasising the need for further research and a deeper understanding of this phenomenon.

Keywords: Incels; Mass violence; Media; Terrorism.

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24884 | The Relationship Between Prostitution and Substance Use: Analysis of Life Trajectories Using the Biogram Method

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Background & Aim: Prostitution and psychoactive substance use are frequently associated in the literature. However, despite extensive international research, this relationship remains largely unexplored in the Portuguese context, limiting the understanding of its dynamics and contributing to the invisibility of this population. Engagement in street prostitution is often linked to social exclusion, marginalization, and persistent stigma experienced by sex workers [1]. In this context, prostitution may facilitate the adoption of risky behaviors, particularly drug use [2], which often emerges as a mechanism to sustain this use within a context of survival [3]. This study aims to analyze the life trajectories of individuals who engage, or have engaged, in street prostitution and who use, or have used, drugs**. Methods:** A qualitative design will be employed using the biogram method. Data will be collected through semi-structured interviews with 20 participants. The biogram approach reconstructs life histories by integrating heterobiographical and autobiographical data, enabling the chronological mapping of significant life events and turning points [4]. This method allows the identification of patterns and changes across participants' trajectories. **Results:** As the study is ongoing, the presentation focuses on the theoretical framework and methodological approach. It is expected that the findings will provide insights into how trajectories of prostitution and drug use intersect and evolve. **Conclusions:** The expected findings may contribute to a deeper understanding of the social and behavioral dynamics underlying the intersection between prostitution and substance use. By identifying patterns within life trajectories, this study seeks to inform both scientific knowledge and intervention strategies targeting vulnerable populations.

Keywords: Life Trajectories; Prostitution; Drug Use; Biogram.

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24953 | Victimization and Participation in Restorative Justice: Consequences, Needs and Expectations

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Background and Aim: Previous research consistently shows that victims wish to be included in the criminal justice process [3]. As a result, since the 1980s, policies promoting victim participation, particularly restorative justice (RJ), have been implemented. RJ encourages the involvement and participation of those directly affected by crime, enhancing their role within the criminal justice process [4]. However, despite the generally positive perceptions, limited research has examined victims' attitudes and views towards RJ. **Methods:** Using a qualitative design and semi-structured interviews, this study seeks to explore the participants' perceptions on the aspects (e.g., consequences of victimization, perceived needs, and expectations regarding the process and outcome) that may contribute to a victims' willingness to participate in a RJ program. An interview guide was developed around four dimensions: Victimization, RJ, Victims' Needs, and Victims' Expectations, as well as a sociodemographic questionnaire to characterize the sample. Eligible participants are adults (aged over 18), of both sexes, who have been victims of one of the following crimes: Property crimes (theft, vehicle theft, vandalism), Violent crimes (robbery, assault), and Cybercrime (online fraud, online identity theft). **Results:** Most participants are expected to have limited prior knowledge of RJ, though many may show interest in the approach. A key motivation for participation is curiosity about the offender, particularly understanding the reasons behind the crime. Victims also express a need for involvement in the justice process, as well as a desire for reparation, closure, and emotional healing through RJ interventions [1]. **Conclusions:** The question is no longer whether RJ should be implemented, but how it can be effectively implemented [2]. By examining victims' perspectives in the Portuguese context, this study contributes to existing literature and enhances the understanding of what motivates participation in RJ programs.

Keywords: Restorative Justice; Victimization; Participation; Victims' willingness.

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24886 | VicTory: Restorative and Victim-Centred Approach to Mitigate Hate and (Violent) Extremism

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Background & Aim: Hate crimes and violent extremism pose a growing global challenge with profound and lasting impacts on victims[1]. Restorative Justice (RJ) remains underexplored in this field[2], despite evidence of its potential to reduce reoffending and enhance victim empowerment[3]. Developed within the EU-funded VicTory project[4], this study aims to gather insights from professionals working directly with victims of hate and violent extremism, to better understand existing practices and the potential relevance of restorative approaches. **Methods:** An online survey was conducted on the EU Survey platform, reaching 55 professionals from the project's partner countries (Portugal, Italy, Spain, Finland, and Hungary) who work with those victims, including criminal justice practitioners, victim support workers and other relevant stakeholders. The first part of the survey focuses on assessing victims' needs and the perceived risks and safeguards of RJ practices. The second examines organisational capacity, training requirements, systemic barriers, community involvement, and professionals' views on the applicability of restorative practices. **Results:** The results revealed that victims are focused on emotional and psychological support, prioritising procedural justice over punitive retribution. Moreover, several systemic challenges were pointed out by professionals, such as training deficits and lack of proper institutional frameworks and funding. **Conclusions:** The study contributes to ongoing debates on the potential of restorative practices as an important tool for victim support, specifically for victims of hate crimes and extremist-related incidents, identifying opportunities to strengthen social, institutional and governmental responses, targeted training and policy initiatives, in this context. Ultimately, it seeks to highlight the importance of developing structured, well-regulated restorative frameworks and specialised training programmes to ensure a safe and victim-centred implementation.

Keywords: Victim Support; Hate Crime; Extremism; Restorative Justice

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ENGINEERING

24693 | A Participatory Design Approach to Serious Games for Adolescent Digital Well-being

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Background & Aim: Social media is central to adolescent life and identity, yet problematic use correlates with depression, anxiety, loneliness, and body dissatisfaction. Despite these risks, few serious games target this area. Part of a LIACC project, this study combines technology, psychology, and digital education to create an interactive platform. The goal is to develop a serious game - informed by adolescents and healthcare/judicial professionals - to facilitate learning and self-reflection regarding social media's adverse effects. **Methods:** Following a Participatory Design approach involving adolescents (ages 10-19) and health/justice experts, the study spans three phases: recruitment and assessment, co-design and iterative development, and final evaluation. Data collection includes semi-structured interviews, focus groups, and pre/post-questionnaires using the Social Media Addiction Scale and System Usability Scale. **Results:** This work expects to produce an evidence-based prototype featuring realistic social media simulations and consequence-based branching narratives. By bridging clinical evidence with lived digital experiences, the project aims for high engagement and usability. Expected outcomes include increased awareness of risks like cyberbullying and addiction. Results will provide a validated framework of game mechanics to foster healthier digital habits and behavioral intent. **Conclusions:** Serious games leverage adolescent familiarity with digital environments to support mental health education. By involving stakeholders, this project ensures the game is developmentally appropriate. Ultimately, the work seeks to demonstrate that collaborative design improves the usability and impact of digital interventions for adolescent well-being.

Keywords: Serious Games; Social Media Use; Mental Health; Adolescents; Participatory Design

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24252 | All-Solid-State Energy Harvesting, Storage and Sensing Devices

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Background & Aim: Networks aim to increase velocity and reliability to meet the rising demands of the Internet of Things (IoT), while inserting disruptive technological displacements such as Artificial Intelligence (AI) [1]. Regarding the upcoming demands, next-generation solid-state devices should convey energy autonomy by harvesting power from their environment, while linking wireless communication, edge processing, and long-term operational reliability [2]. We have developed a new harvesting and storage wireless cell. The edges of the cell are metal electrodes with a $Li_{2.99}Ba_{0.005}OCl$ layer deposited on top of the electrodes **Methods:** The experimental setup employed copper foil and aluminum or zinc foil. A 1 × 1 cm² area on each foil was coated with the electrolyte powder, which was then sealed with commercial lacquer to prevent moisture absorption. The copper and aluminum electrodes were arranged facing each other in one dimension, with a maximum separation distance of 12 cm. The open-circuit voltage (OCV) was measured using a digital multimeter. The electrodes were placed on different insulating polymers such as PLA polymer, or Polylactic acid, and directly on lab's ceramic board. **Results:** The gap-dependent electrochemical behavior of the Cu/ferroionic//air//ferroionic/Al (or Zn) devices was further analyzed through CV (I-V) measurements (Fig. 1). I-V curves recorded across varying air-gap distances (Fig. 1a) show that as the gap increases, the overall current decreases, and the electrochemical response becomes increasingly non-linear. **Conclusions:** Ferroionic $Li_{2.99}Ba_{0.005}OCl$ layers enable the formation of structured, centimeter-scale air-gap electrostatic potentials through the interplay of mobile ion relaxation and polaron pinning effects. Ferroionic edge-gated architectures thus provide a versatile platform for programmable electrostatic control, with potential applications in solid-state transistors and batteries, energy harvesting, and high-sensitivity long-range sensing.

Keywords: batteries; sensors; energy harvesters; ferroionics; wireless

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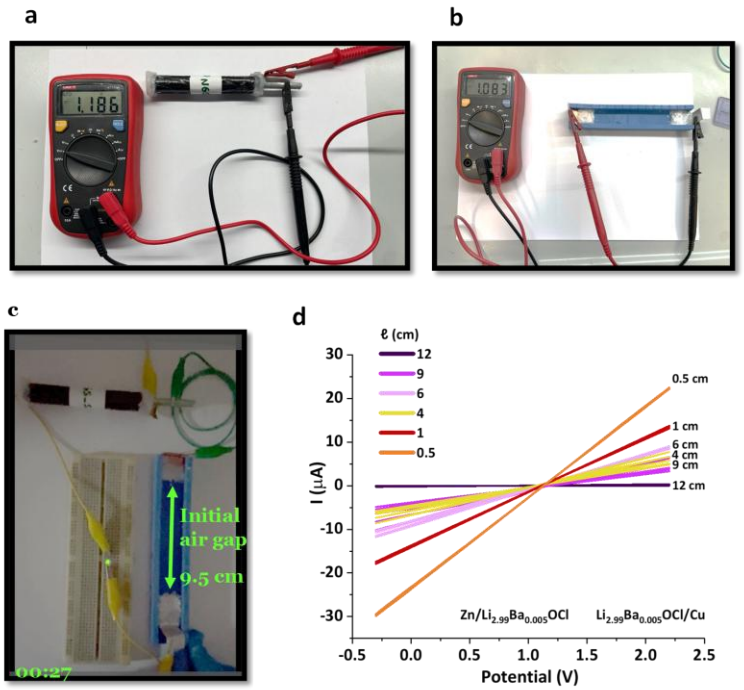

Figure 1: **a,** Open circuit voltage (OCV) of a Cu//$Na_{2.99}Ba_{0.005}OCl$//Al coaxial cell; **b,** OCV for a Cu/$Li_{2.99}Ba_{0.005}OCl$//Air//$Li_{2.99}Ba_{0.005}OCl$/Zn cell where the gap is 9.5 cm; **c,** cell in **b,** connected in series with the coaxial in **a,** and lighting a green LED (requiring 1.8 V to light) when the air gap varies dynamically from 9.5 to 0.5 cm after 1 min and 09 s. **d**, I-V cycles for different air-gap lengths in a Cu/$Li_{2.99}Ba_{0.005}OCl$//air//$Li_{2.99}Ba_{0.005}OCl$/Zn cell.

24929 | An electrochemical biosensor for stroke diagnosis and differentiation

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Background & Aim: Stroke represents the second leading cause of death worldwide, characterized by the interruption of blood flow in brain tissue [1]. A quick differentiation between hemorrhagic (ICH) and ischemic (AIS) stroke is essential for prehospital management, choice of reperfusion strategies and bleeding control. Biomarkers such as glial fibrillary acidic protein (GFAP) and S100β have emerged as promising tools for stroke classification [2]. Therefore, the objective of this study is to establish a link between existing studies and clinical application, with the development of an electrochemical biosensor that allows GFAP to be quantified accurately and quickly, thereby enabling a faster diagnosis and differentiation of stroke events. **Methods:** The electrochemical sensor was developed from a modified screen-printed carbon electrode with graphene oxide to promote the immobilization of capture antibodies via covalent bonding. Bovine serum albumin was used to prevent non-specific binding, thereby reducing background noise. Quantification of the antigen in the sample was performed using a sandwich immunoassay, employing a detection antibody and PLGA nanoparticles coated with gold nanoparticles for signal amplification. The gold nanoparticles were synthesized using an optimized Turkevich method to control particle size and ensure uniform distribution. Both nanoparticles were characterized by dynamic light scattering, electrophoretic light scattering and UV-vis spectroscopy. The developed biosensor was characterized using chronoamperometry (CA), cyclic voltammetry (CV) and scanning electron microscopy (TEM). **Results:** Under the optimal conditions, an analytical curve was attained using the CA signal (μA) after exposure of the immunosensor to different concentrations of GFAP (pg mL^{-1} - ng mL^{-1}). **Conclusions** The developed immunosensor enables a successful and highly sensitive detection of GFAP with electrochemical amplification achieved through the combination of PLGA and AuNPs.

Keywords: Stroke, Electrochemical biosensor, Glial fibrillary acidic protein (GFAP), Signal amplification

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24593 | Analysis and Optimisation of a Photo-Induced Hydrogen Production Process

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Photocatalytic hydrogen (H_2) generation under visible light irradiation represents a promising strategy for sustainable fuel production, particularly when integrated with wastewater treatment processes, where organic contaminants can serve as sacrificial agents and enhance H_2 evolution [1,2]. This dual-function approach offers a pathway to simultaneously address renewable energy generation and environmental remediation challenges, contributing to circular and resource-efficient systems. Nevertheless, the overall photocatalytic efficiency remains constrained by intrinsic catalyst properties, photoreactor configuration and operating conditions. In this context, the present work investigates a visible-light-driven photocatalytic system aimed at optimising reactor configurations for the coupled production of H_2 and wastewater treatment. Emphasis is placed on the synthesis and comprehensive characterisation of visible-light-responsive photocatalysts, targeting enhanced light absorption, improved charge carrier mobility, and increased physicochemical stability. Structural, morphological, optical, and surface properties are evaluated to establish correlations between material characteristics and photocatalytic performance. Photocatalytic experiments are conducted under systematically varied operating conditions, including catalyst loading, light intensity and pollutant concentration, in order to assess their influence on H_2 production rates and overall process efficiency. The experimental results are analysed to identify performance-limiting factors, evaluate reaction behaviour, and determine optimal operating windows. The findings are expected to provide insights into the interplay between catalyst design and reactor operation, supporting the development of efficient and sustainable photo-induced H_2 production technologies.

Keywords: Photocatalytic hydrogen production; Process optimisation; Sustainable energy

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24923 | Ballast Technical-Financial Study

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Background & Aim: Ballast consists of crushed, angular rock fragments typically derived from hard and durable rocks such as granite or basalt. Several types of ballast exist. The grain size and composition may vary, including materials such as sand, gravel, and crushed stone. It is mainly used in railway infrastructure to support rails, ensuring track stability and proper drainage conditions. Ballast also contributes to reducing vibrations caused by train movement and limits vegetation growth along the track. In public infrastructure projects, it is important to evaluate the financial aspects related to the supply of ballast, including the identification of suitable extraction places. **Methods:** Based on the identification of potential extraction places, a financial analysis is essential to estimate the costs associated with rock extraction, material processing, and transportation. When the required quantity of natural ballast can't be obtained from available sources, it is relevant to explore alternatives solutions to the supply of "geological" ballast using other materials. **Results:** Considering the large quantities of ballast required for railway construction and the existence of multiple potential supply locations, a comparative economic assessment becomes necessary. Such analysis allows the identification of the most cost-effective and logistically best solution for supplying the required material. Transportation costs, from exploitation to building sites, can be a critical item in the decision making; thus, locations and processing must be effective within the cost structure. **Conclusions:** This study aims to evaluate the costs related to the supply of ballast for railway infrastructure, such as extraction, processing, and transportation stages, as well as possible alternative materials.

Keywords: Ballast; Extraction; Cost

24934 | Biocompatibility and Uptake Assessment of Functionalized PLGA Nanoparticles in Brain Endothelial Cells for Alzheimer's Therapy

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Background & Aim: Alzheimer's disease (AD) is a common form of neurodegenerative disease. Patients diagnosed with this condition reveal progressive cognitive decline, accentuated memory loss, and behavioural changes [1]. Presently, there is no cure for this disease, and most treatments just relieve some of the neurological symptoms. The main difficulty when developing therapies for AD consists of penetrating the blood-brain barrier (BBB) [2]. The use of functionalized nanoparticles (NPs) allows for the transport of drugs and targeting of the brain [3, 4]. In this study, polymeric NPs with the surface functionalized with proteins were evaluated regarding their biocompatibility and uptake capacity in brain endothelial cells. **Methods:** The poly(lactic-co-glycolic) acid (PLGA) NPs were produced, the surface was modified, and their capacity to transport an AD drug model (donepezil) was assessed through differential scanning calorimetry. The *in vitro* tests were conducted using the endothelial cell line hCMEC/D3, and the NPs uptake and cytotoxicity were assessed. **Results:** The PLGA NPs synthesized were successfully functionalized, conjugated, and proved to be efficient in transporting the AD's drug. Furthermore, the NPs are non-cytotoxic for the hCMEC/D3 cells at the tested concentrations. **Conclusions:** Protein-functionalized PLGA NPs were successfully synthesized and thoroughly analysed, exhibiting physicochemical characteristics capable of penetrating the BBB and transporting drugs for the treatment of AD. The *in vitro* assays demonstrated that the NPs exhibited low levels of cytotoxicity, indicating that the NPs are safe for drug transport into the brain.

Keywords: Nanomedicines, Surface-functionalization, Blood-Brain Barrier, Neurodegenerative Diseases

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24651 | Biomass based materials for the electrocatalytic degradation of pharmaceutical pollutant

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Background & Aim: The global water crisis is intensifying and, so, conventional water and wastewater treatment technologies must be developed to efficiently remove contaminants of emerging concern (CECs) [1]. Among these, venlafaxine (VFX), an antidepressant frequently detected in surface waters is mandated for monitoring in quaternary treatment under EU Directive 2024/3019 [2,3]. **Methods:** Electrochemical advanced oxidation processes (EAOPs) were explored under mild conditions to produce reactive oxidants (e.g., $HO^{\bullet}$) for the oxidation and mineralization of VFX into less harmful species. Moreover, carbon-based catalysts were synthesised via one-pot technique and assessed as suspension electrodes in batch slurry reactors. Grape pomace was used as biomass for the biochar (BC), using thiourea, melamine and dicyandiamide as functionalisation agents leading to BC-T, BC-M and BC-D. These were also explored for self-immobilisation onto three-dimensional inert macrospheres for continuous operation. **Results:** By pairing boron-doped diamond (BDD) and nickel sheet electrodes in a 6 mM NaCl aqueous solution, suspended BC-T under galvanostatic 10 mA reached >80% VFX removal within 5 minutes of reaction, whereas electrolysis alone removed <20%. Further studies were performed to investigate the best synthesis conditions and by using other electrodes and electrolytes. The supported catalysts were added to an integrated system in order to obtain continuous removal of VFX from water. **Conclusions:** EAOPs coupled with carbon-based catalysts show strong potential as an efficient and sustainable water treatment technology, enabling both pollutant degradation (100% removal in 30 min) and value recovery from solid waste-derived materials from winery industry.

Keywords: Biochar, Venlafaxine, Electrocatalysis, Electrochemical advanced oxidation processes

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24382 | Characterisation and Valorisation of Sands Resulting from Water Treatment Processes

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Background & Aim: Water treatment processes produce residual materials, which are commonly disposed of in landfills or storage ponds. The composition of these residues is influenced by the source of the raw water, the chemicals used during treatment, and the specific unit operations applied. Within the framework of the circular economy, effective waste management is essential, and the 'zero waste' strategy seeks to minimize environmental impact by encouraging the reuse and valorization of by-products. One such approach is the utilization of sands resulting from water treatment in construction or land reclamation projects. Using sand recovered from treatment facilities as a substitute for natural sand aligns with circular economy principles. Therefore, evaluating its potential for reuse—whether in construction, land reclamation, or environmental engineering—requires thorough characterization specific to its origin. **Methods:** Three sludge samples (~3 kg each) were collected from two water treatment plants in northern Portugal. Water content was determined per PN-EN 15934, and sand was dried at 105°C to constant weight. Granulometric analysis followed PN-EN 933-1:2012. Fractions smaller than 0.074 mm were analyzed via laser diffraction (Malvern MASTERSIZER 2000). Chemical composition was assessed using X-ray fluorescence spectrometry (Oxford Instruments X-MET7000). Ongoing work includes further tests to evaluate properties for specific applications. **Results:** The circular economy is increasingly relevant in water management. Reusing waste generated during treatment helps reduce both the consumption of natural resources and the amount of waste directed to landfills. The findings to date suggest that sand recovered from water treatment sludge may be suitable for use in the construction industry as a replacement for natural sand. However, additional testing is necessary to ensure environmental safety—such as leachability studies—and to assess mechanical strength.

Keywords: Water treatment plant; Sludge management; Circular economy; Recycling; Filter sand; Reuse

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24474 | Co-design and Development of a Serious Game to Promote Well-Being in Older Adults

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Background & Aim: Global population ageing is associated with higher prevalence of loneliness, depression, and reduced general well-being among older adults. Serious games have shown positive effects in mental health contexts, including depression reduction [1], however, very few have been designed to promote well-being in older adults, and even less involve co-design with the older adults [2]. This project aims to design, develop and evaluate a co-designed digital serious game focused on enhancing the well-being of adults aged 65 and older. **Methods:** The project will start by defining requirements from currently available studies and developing them into initial prototypes developed for mobile devices. Older adults will participate in iterative co-design sessions with the main objective of refining the game in terms of accessibility, interactions and core gameplay. Each session should also include some measurements related to engagement, usability and well-being before and after the sessions. **Results:** The expected outcome is a fully functional and tested mobile serious game that aims to improve the general well-being of older adults by making use of previously tested elements like reminiscence-based themes [3]. It is also expected that the inclusion of co-design will improve the usability and the engagement of the serious game, further expanding the knowledge in the area. This is expected to then benefit the well-being of the older adults, as well as decreasing the feeling of loneliness and depression. **Conclusions:** This work addresses a gap in the serious game area toward the elderly and their well-being. Due to the addition of co-design, it is expected to deliver a final product as well as recommendations for future games created in this field.

Keywords: Serious games; Older adults; Well-being; Co-design; Accessibility

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24988 | Data Intercomparison as a Tool for Quality Assurance in Mining Waste Valorisation Studies

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Background & Aim: The extractive industry generates vast amounts of waste, particularly tailings, which pose significant environmental, safety, and economic risks, including dam failures and pollution. Despite these challenges, mining remains essential, as recycling alone cannot meet growing material demand driven by digital and energy transitions. Within a circular economy framework, extractive waste can be reprocessed and valorised, transforming an environmental liability into a secondary source of raw materials. The present work is part of the ValorWaste project and focuses on data analysis from the characterisation of four mining waste samples conducted by four project partners. The aim is to proceed to an intercomparison and validation of the results to ensure their quality and comparability. **Methods:** The study included characterisation data generated at FEUP, SECIL, INOE, and CUT, compiled and processed on an online platform developed by ASE. The data include chemical elemental composition, mineralogical analysis, SEM analysis, and radionuclide concentrations. The data analysis is ongoing, with the data being processed and uploaded to the platform. The forthcoming phase concludes with the intercomparison of the results. **Results:** The intercomparison supports the quality assurance of the produced data. Based on the characteristics of mining waste samples, a valorisation pathway for each material will be defined. In general, the preliminary analysis indicates a good match between the characterisation data provided from the four partners, except for radionuclide activity concentrations. **Conclusions:** The intercomparison of characterisation data shows good overall consistency among project partners, supporting data quality and comparability. Minor discrepancies in radionuclide activity highlight the need for further methodological harmonisation. Overall, the results provide a solid basis for defining safe

and effective valorisation pathways for the studied material within a circular economy framework.

Keywords: Mining waste; Circular economy; ValorWaste project; Analytical comparability; Decision support

Acknowledgements: This work is part of the ValorWaste project “Valorisation and Integration of Extractive Waste Towards the Sustainability of Raw Materials Industry” with the reference ERA-MIN3/0002/2023 and with DOI 10.54499/ERA-MIN3/0002/2023 funded by the Fundação para a Ciência e a Tecnologia, I.P., through national funds within the framework of the M-ERA-NET3 network “ERA-NET Cofund in raw materials. This work was developed at R&D Unit CERENA, supported through FCT Project UID/04028/2025 (https://doi.org/10.54499/UID/04028/2025).

24304 | Deep brain stimulation outcome prediction in obsessive-compulsive disorder through intracranial electrodes' localization

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Background & Aim: Obsessive-compulsive disorder (OCD) is a debilitating psychiatric disorder characterized by intrusive thoughts and repetitive behaviors, and linked to dysfunctions within the cortico-striato-thalamo-cortical (CSTC) brain circuits [1]. Deep brain stimulation (DBS) has emerged as a therapeutic option for refractory cases, but its clinical efficacy is heterogeneous and appears to depend on electrode location, which determines the volume of tissue activated (VTA) and the modulation of specific white matter tracts within the CSTC circuits [2], [3]. This study aims to identify associations between electrode location and clinical outcomes, developing predictive models to optimize stimulation targets. **Methods:** A cohort of 11 OCD patients treated with DBS at ULS São João was analyzed, with electrodes targeting different brain regions including the bed nucleus of the stria terminalis, the anterior limb of the internal capsule, and the ventral capsule/ventral striatum. A multimodal imaging pipeline was applied for fusion of preoperative magnetic resonance imaging (MRI) and postoperative computed tomography (CT) for 3D electrode reconstruction. Subsequently, VTA modeling and subject-specific diffusion MRI tractography will be used to identify intersecting fibers, and spatial and connectivity features will be extracted for exploratory and predictive analyses of clinical response. **Results:** Preliminary results show improvement or stability in Y-BOCS scores, the clinical scale that rates OCD patient symptoms, after DBS. Electrode reconstructions align with CT artifacts, and tractography demonstrates consistent spatial organization relative to adjacent structures. **Conclusions:** This work establishes an integrated framework for electrode localization, stimulation modeling, and structural connectivity analysis in DBS for OCD. Final results are

expected to identify anatomical and stimulation features predictive of response, enabling refined targeting and personalized neurostimulation strategies.

Keywords: Obsessive-compulsive disorder; deep brain stimulation; tractography; predictive modeling.

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24482 | Detection of Ofloxacin and Cephalexin in Water Samples using Biofilm-modified Electrodes

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Background & Aim: The presence of antibiotics (AB) in aquatic environments poses a high risk to ecosystem health and contributes to the spread of antimicrobial resistance [1]. Monitoring these contaminants is challenging because they are often present at low concentrations in complex water matrices. Conventional analytical techniques are time-consuming and require trained personnel and specialized laboratories. This study explores the potential of biofilm-based electrochemical sensors using *Shewanella oneidensis* for the detection of ofloxacin (OFX) and cephalexin (CEX), two AB frequently detected in water systems [2]. **Methods:** *S. oneidensis* biofilms were developed on commercial screen-printed carbon electrodes, exposed to water samples containing different AB concentrations (0–4 µg/mL), and analyzed by cyclic voltammetry using a potentiostat. Several experimental conditions were initially optimized, including the culture medium, water exposure time, and electrochemical analysis parameters. After optimization, a total of 21 samples were analyzed to validate the system for AB detection. Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic accuracy of *S. oneidensis*-modified electrodes and to determine optimal cut-off values for distinguishing between samples with and without OFX or CEX. **Results:** The data indicated that a 30-min exposure period, bacterial growth in Tryptic Soy Broth medium, and electrochemical measurements conducted at a scan rate of 0.01 V/s represented the most suitable conditions among those tested. The presence of AB appeared to decrease the measured current at +0.7 V. Sensitivity values of 0.93, 1.00 and 0.86 were obtained for overall antibiotic detection, and ofloxacin and cephalexin detection, respectively. **Conclusions:** The developed method enables rapid detection of the tested contaminants and shows potential as a portable screening tool for on-site water monitoring. Further optimization and validation are required.

Keywords: Antibiotic contaminants; Electrochemical biosensors; Cyclic voltammetry; Water monitoring; Antimicrobial resistance.

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24235 | Development & Topological Optimization of a Hydraulic Block

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Background & Aim: In order to improve performance in accordance with the Tsiolkovsky equation, the weight of the INVICTUS II rocket's hydraulic system must be reduced. While conventional manifolds are reliable, they are also heavy. This work aims to develop and compare a conventional manifold and two topologically optimised hydraulic blocks (one for CNC machining and one for AM) to find the lightest, safest and most cost-effective solution. **Methods:** Based on N_2/N_2O compatibility, the material options were narrowed down to SS 304, SS 316L, Al 6061-T6 and Al 6082-T6. The mechanical and fatigue properties of these materials were then compared, and Al 6061-T6 was selected. Topology optimisation (density-based methods) was then applied, taking into account 3-axis CNC and SLM constraints. FEA with pressure loads was used to evaluate Von Mises stress and fatigue performance. **Results:** Preliminary results show aluminum 6061-T6 enables weight reduction without sacrificing safety. Topology-optimised designs reduced mass compared to the manifold, with the greatest reduction achieved by the additive manufacturing solution due to its geometric freedom. Initial FEA shows all designs remain within the elastic domain under pressure, with improved stress distribution. **Conclusions:** This study demonstrates the effectiveness of topology optimisation in reducing the weight of hydraulic manifolds while maintaining their structural integrity. Aluminium 6061-T6 was found to be the most suitable material due to its favourable strength-to-weight ratio and fatigue performance. Although additive manufacturing enables greater weight reduction thanks to increased geometric freedom, three-axis CNC machining remains a viable, cost-effective alternative. Ultimately, the optimised designs contribute to improved rocket performance and support more efficient and sustainable aerospace engineering solutions.

Keywords: Topology Optimization; Additive Manufacturing; Hydraulic Manifolds

24450 | Development and Optimization of Composite Electrolytes for Solid-State Sodium-Ion Batteries

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Background & Aim: Solid-state batteries offer a safer and more stable alternative to conventional liquid-based systems, with the potential for improved thermal stability and extended lifespan[1], while the solid-state electrolytes based on poly(ethylene oxide) (PEO) have garnered interest due to their viscoelastic properties, ease of processing, and compatibility with sodium ions[2]. **Methods:** This work investigates the electrochemical performance of a composite solid electrolyte[3] for sodium-ion batteries incorporating PEO[4], focusing on optimizing both composition and manufacturing of Cu/$Na_{2.99}Ba_{0.005}OCl$/Zn pouch cells, in a simplified configuration without traditional electrodes, with Cu as the positive current collector and Zn as the negative. To determine the ideal balance between ionic conductivity and mechanical stability, various binder/electrolyte ratios were tested through different processing methods. Each method is being evaluated for its influence on the electrolyte's structural integrity and homogeneity[5,6], across different temperatures to assess performance stability under variable thermal conditions. **Results:** Electrochemical characterization (PEIS, CV, Galvanostatic Intermittent Titration Technique and charge/discharge tests) revealed a strong dependence on processing method, with certain combinations significantly enhancing ionic transport and overall performance. After assembly, the dry-cell demonstrated an open circuit voltage of approximately $1{,}07\ \mathrm{V}$. When set to discharge, while operating with a current value varying from $6.29\ \mu\mathrm{A}\cdot\mathrm{cm}^{-2}$ to $6.51\ \mu\mathrm{A}\cdot\mathrm{cm}^{-2}$, the cell exhibited a potential difference between $0{,}98\ \mathrm{V}$ and $1{,}12\ \mathrm{V}$ after 50 days. **Conclusions:** These findings offer a foundation for a significant step toward the development of next-generation sustainable batteries through an original composite polymer-ceramic electrolyte design, laying the groundwork for energy storage solutions capable of meeting the rigorous demands in both high-performance applications and portable electronics.

Keywords: Polyethylene Oxide; Composite Solid Polymer-ceramic electrolyte; Sodium-ion; Solid-State; Sustainable Battery

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24674 | Development of Cognitive and Socioemotional Skills in School-Age Children: Co-Designing a Serious Game

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Background & Aim: Cognitive and socioemotional skill development during early school years greatly affects children's present and future academic achievements and psychological and overall well-being [1] [2]. Educational neuroscience highlights play as a powerful mechanism to support these domains, fostering motivation, active engagement, attention and self-regulation, and even the development of more skills like imagination [2]. Digital Serious Games (SGs) integrate learning mechanics into engaging, interactive, feedback-driven environments, enabling personalized and immersive experiences that enhance knowledge acquisition and the practice of cognitive and socioemotional skills [3]. However, there is a lack of SGs that intentionally integrate the development of both skill domains and many of the SGs designed to develop socioemotional skills target only specific clinical populations [4]. Thus, this project aims to design and develop an engaging, evidence-based and developmentally appropriate SG to foster both cognitive and socioemotional skills in school-age children from the general population. **Methods:** To support the SG's design and development, an exploratory participatory approach will follow three phases: 1) co-design with children, their guardians, teachers and healthcare professionals; 2) prototype testing; and 3) final usability and preliminary impact evaluation. Interviews, questionnaires and creative activities with children will be used. **Results:** The SG is expected to improve both cognitive and socioemotional skills in children aged 6–9, while being engaging and based on evidence from the literature and insights from children and professionals. **Conclusions:** This work will contribute to research on SGs for children by addressing the lack of SGs supporting both cognitive and socioemotional skill development and providing evidence for holistic play-based learning in childhood.

Keywords: Serious Games; Cognitive Skills; Socioemotional Skills; School-Aged Children; Co-Design.

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24556 | Development of Intelligent Planner for Serious Games in Cognitive Rehabilitation

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Background & Aim: Acquired Brain Injury (ABI) often leads to persistent cognitive deficits affecting autonomy and quality of life. Although Cognitive Rehabilitation (CR) is essential, traditional computerized tools frequently lack personalization. Serious games (SG) combined with artificial intelligence (AI) offer adaptive and motivating alternatives [1]. This project aims to develop a serious games platform integrating an intelligent planner to personalize cognitive rehabilitation for adults with ABI under clinical supervision. **Methods:** A multidisciplinary team of engineers, neuropsychologists, and legal experts will adopt a user-centered, co-design methodology structured in three iterative phases: (1) requirements elicitation through semi-structured interviews with patients and rehabilitation professionals (≥18 years); (2) prototype development and usability workshop; and (3) refinement and re-evaluation. The platform will integrate short cognitive mini-games and an intelligent planning agent that recommends task sequences and adjusts difficulty based on performance metrics. Usability and user experience will be assessed using GEQ [2], SUS [3], and USEQ [4] questionnaires, complemented by qualitative feedback. Data will be anonymized in accordance with national data protection legislation. **Results:** The expected outcomes include the identification of user-centered functional requirements, the development of a validated prototype, and preliminary evidence of usability, engagement, and acceptability among patients and professionals. It is anticipated that adaptive planning mechanisms will improve task personalization and optimize rehabilitation pathways. **Conclusions:** This project is expected to demonstrate the feasibility of integrating an intelligent planner into a serious games platform for cognitive rehabilitation. By promoting personalization, motivation, and clinical alignment, the system may contribute to advancing patient-centered digital neurorehabilitation solutions.

Keywords: Serious Games; Cognitive Rehabilitation; Artificial Intelligence; Intelligent Planning; Acquired Brain Injury

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24687 | Emotion Regulation in the Family: A Co-Designed Serious Game Approach

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Background & Aim: Serious Games (SGs) are games whose purpose lies beyond entertainment, aiming to educate and change patterns of experience or behavior [1]. Recent studies highlight the promise of using SGs in promotion and improvement of mental health among the youth [2]. The development of Emotion Regulation (ER) skills begins in early childhood, mostly influenced by socialization and caregivers' own ER processes, with adolescence representing a critical stage that presents both risks and opportunities for ER [3]. This work presents *Emotion Odyssey: Unlock the Secrets 2.0*, a multiplayer SG designed to promote ER skills in adolescents and their parents. The initial project focused on the preliminary design and development of the SG. Building on this foundation, the present study advances to a second phase emphasizing participatory refinement, iterative development, and the evaluation of both usability and clinical effectiveness. **Methods:** The development of this SG will follow a co-creation methodology with the stakeholders (adolescent-parent dyads and mental health professionals) to ensure the final product aligns with the preferences and requirements of its intended users while supporting evidence-based ER strategies. The first stage will assess the usability of the previous work and collect insights to inform game design decisions. The intermediate stage will focus on integrating new ideas and feedback on the previously identified requirements. The final stage will focus on the evaluation of the SG usability and clinical effectiveness. **Results:** The expected results include a user-centered version of *Emotion Odyssey 2.0* that promotes adolescents' and parents' ER skills, collaborative engagement, and provides a socially interactive gaming experience for the family. **Conclusions:** The co-design and development of this SG aims to address the challenge of developing adaptive ER strategies in the family through a user-centric approach informed by evidence-based knowledge.

Keywords: Emotion Regulation, Co-Design, Serious Game, Adolescents, Parents.

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24435 | Evaluating the Technical and Economic Feasibility of Reusing Mining Waste in Circular Economy Applications

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Background & Aim: Mining activities generate vast quantities of waste, posing substantial environmental and safety concerns. These wastes are typically stored in tailings storage facilities, creating ongoing risks to the environment and public safety. Circular economy principles in mining waste management involve re-mining and reusing tailings to limit environmental harm and improve resource efficiency. The present work is focused on evaluating two types of mining waste to determine their technical and economic feasibility for reuse: i) granite sludge produced during block sawing and ii) tailings from rare earth beneficiation processes. **Methods:** The study included the characterization of the material: moisture content, particle size distribution, pH, chemical and mineralogical composition, water absorption, slump, density, compressive strength, and environmental safety tests. The research remains ongoing, with the forthcoming phase focusing on the comprehensive compilation and analysis of all experimental results obtained thus far. **Results:** The characterization results will indicate the potential for these materials to be reused, either incorporated into construction materials or ceramics (granite sludge and tailings), used to produce geopolymers, or used as backfill (tailings). The results should demonstrate the technical and economic viability of reusing mining waste, thereby reducing landfill volumes and creating new economic opportunities within a circular economy framework. Further analysis is planned to confirm the long-term durability and environmental safety of these potential applications. **Conclusions:** Preliminary findings indicate that both studied materials exhibit physical and chemical characteristics suitable for reuse in construction products and backfill applications. Continued research and comprehensive analysis will be essential to confirm the technical and economic viability of these strategies, ensuring both environmental protection and sustainable resource management.

Keywords: Granite sludge; Rare earth tailings, Resource efficiency, Construction materials, sustainability

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24614 | Evaluation of interactions between inorganic nanoparticles and biomolecules: implications for biomedical applications and nanotechnological safety

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Background & Aim: Nanotechnology emerged as a prominent field for bioaplications, such as the development of diagnostic systems, targeted therapies, and biomedical devices. Understanding how nanoparticles behave in contact with biological environments becomes essential to predict their interactions and toxicological outcomes [1]. This study aims for an improved knowledge of the physicochemical and media-dependent properties of silver nanoparticles (AgNPs), silica nanoparticles (SiO_2), and titanium nanoparticles (TiO_2) dispersed in commonly used cell culture media. Furthermore, *in vitro* cytotoxicity assays are performed to assess how the observed properties modulate nanoparticle-cell interactions. **Methods:** Commercially available nanoparticles are dispersed in DMEM, supplemented with increasing concentrations (0-10%) of fetal bovine serum (FBS). Dynamic Light Scattering (DLS), electrophoretic light scattering (ELS), and nanoparticle tracking analysis (NTA) are employed to quantify nanoparticle's hydrodynamic size and colloidal stability. UV-Vis spectroscopy monitors their optical properties, aggregation, or surface modifications induced by serum proteins. Electron microscopy techniques allow the evaluation of nanoparticle's morphologic properties, size, and their integrity before and after incubation. In vitro cytotoxicity assays are conducted, leading to the assessment of cell viability and morphological modifications. **Results:** FBS concentration influences the nanoparticles behavior, highlighting the influence of protein content on nanoparticle dispersion and corona formation, resulting in changes in their hydrodynamic size and stability. **Conclusions:** This project evaluates the critical role of the biological media in modulating nanoparticle's physicochemical properties and biological responses, contributing to a more reliable interpretation of nano-bio interactions, thus supporting the development of safer and more predictable nanomaterials for biomedical applications.

Keywords: inorganic nanoparticles; cell culture media; fetal bovine serum; cytotoxicity.

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24612 | Evaluation of the Antibiofilm Performance of Bioinspired Surfaces for Application in Urinary Catheters

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Background & Aim: Urinary catheters (UC) are widely used in clinical practice. However, their use frequently results in catheter-associated urinary tract infections (CAUTIs), which can compromise device performance and reduce patients' quality of life [1]. CAUTIs are primarily driven by the ability of pathogens to adhere to UC surfaces and form biofilms. Topographical surface modification, inspired by the non-fouling properties found in nature, is a promising, chemical-free strategy to control biofilm development without promoting selective pressure on pathogens. This study aimed to produce and evaluate the antibiofilm potential of leaf-inspired biomimetic topographies for application in UC and to assess whether they act synergistically with antimicrobial therapies. **Methods:** Leaf microtopographies were replicated using a two-step silicone casting technique [2]. Flat silicone surfaces were used as control. The antibiofilm potential of the biomimetic surfaces was evaluated against 24 h *Escherichia coli* biofilms formed in artificial urine medium at 37 °C under static conditions. Subsequently, the 24 h-old biofilms were treated with ciprofloxacin (CIP; 200 µg/mL) for 6 h under the same conditions. After biofilm formation and CIP treatment, the biofilm cell suspensions were analysed to determine the number of colony-forming units (CFU) per cm^2. **Results:** All biomimetic surfaces reduced the number of biofilm cells compared to the control surface, confirming their antibiofilm performance (up to 0.46 Log CFU/cm^2 reduction). Antibiotic treatment was highly effective, significantly reducing bacterial density compared to the pre-treated biofilms (≈ 2 Log CFU/cm^2 reduction). After CIP treatment, all biomimetic surfaces outperformed the control surface, with the Holly topography being the most effective, achieving a median reduction of 0.91 Log CFU/cm^2. **Conclusions:** These findings highlight the potential of biomimetic surfaces for application in urinary catheters to inhibit uropathogen biofilm formation.

Keywords: Biomimetic surfaces; Biofilms; Urinary catheters; Antibiotic tolerance; *Escherichia coli*.

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24889 | Evaluation of Using Bio-based solvents in the Extraction of Bioactive Compounds from Chestnut Burs

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Background & Aim: Portugal is one of Europe's largest producers of *Castenea Sativa*, a chestnut species found in southern Europe and Asia and extensively used in the food industry [1]. However, chestnut harvesting generates significant amounts of agricultural residues such as shells, burs and leaves, which are usually discarded or burned, leading to biomass waste and environmental impacts [1,2]. In particular, the bur is a by-product of chestnuts rich in phenolic compounds, whose production in Portugal varies between 10 and 15 thousand tons per year [3]. These compounds have antioxidant, antimicrobial, and anti-inflammatory properties, making them valuable for food, pharmaceutical, and cosmetic industries [1,4]. The conventional approach for polyphenols extraction relies on the use of organic solvents such as benzene, hexane, and acetone. However, these solvents have several significant disadvantages such as toxicity and environmental concerns. In this sense, bio-based solvents emerge as promising alternatives [5]. Thus, this work evaluates the performance of bio-based solvents in solid-liquid extraction of bioactive compounds from chestnut burs. **Methods:** In the experimental procedure followed, a pre-treatment of the sample was carried out in which the bur was reduced to powder. Next, solid-liquid extractions were performed using ethanol and selected bio-based solvents suitable for the cosmetics industry. Each extract was analyzed using TPC, FRAP and ABTS assays to determine total phenolic content and antioxidant activity. The most promising solvent was selected for process optimization. **Results:** Preliminary results indicate that bio-based solvents enable the extraction of phenolic compounds from chestnut burs, producing extracts with measurable antioxidant activity. **Conclusions:** These findings highlight the potential of bio-based solvents as sustainable alternatives for the recovery of bioactive compounds from chestnut industry residues.

Keywords: Chestnut burs; Bio-based solvents; Phenolic compounds; Solid–liquid extraction; Antioxidant activity

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24493 | Exploring the Potential of Pd/Cu-MWCNT Bimetallic Catalyst for Water Decontamination

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Background & Aim: Water pollution is a major environmental and public health concern worldwide, due to biological and chemical contaminants. Because of their unique properties, including high surface area, chemical stability, and antimicrobial potential, carbon-based nanomaterials, such as multi-walled carbon nanotubes (MWCNTs), have gained increasing interest for applications in water treatment processes. This project aimed to study the antimicrobial activity of the palladium and copper-MWCNTs (Pd/Cu-MWCNT) bimetallic catalyst against two water contaminant bacteria, *Pseudomonas fluorescens* and *Enterococcus faecalis*, in the presence and absence of nitrate (target chemical pollutant) [1]. **Methods:** Antimicrobial assays were performed by exposing the bacteria to Pd/Cu-MWCNTs (0.05% and 0.5%) for 5 h at 25 ºC and 160 rpm, followed by colony-forming units (CFU) enumeration at 0, 15 min, 30 min, 1 h, 2 h, 3 h, 4 h and 5 h. The activity of these materials was then tested against both bacteria in the presence of nitrate for 1 h under the same conditions. **Results:** The cell culturability data demonstrated the antimicrobial activity of Pd/Cu-MWCNTs against the studied bacteria. Culturable *E. faecalis* cells were reduced by 1.5 log and 2.8 log after 1 h and 5 h of contact, respectively, with 0.5% Pd/Cu-MWCNTs. In turn, the culturability of *P. fluorescens* was completely inhibited after 1 h of contact with 0.5% Pd/Cu-MWCNTs. Additionally, the presence of nitrate did not compromise the antimicrobial activity of the bimetallic catalyst. **Conclusions:** Overall, this work demonstrated the antimicrobial activity of Pd/Cu-MWCNTs, against two water contaminant bacteria, both in the absence and in the presence of their target pollutant, nitrate. These findings highlight the potential of these materials for water biodecontamination.

Keywords: Water decontamination; Metal-functionalized multi-walled carbon nanotubes; Antimicrobial activity; Water contaminant bacteria

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24987 | Fluoroquinolones removal from water by a biobased adsorbent

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Background & Aim: This study aims to apply a *Spirulina*-based adsorbent for the removal of fluoroquinolones (FQs) from water. This material was previously prepared by the research group [1] and has shown great potential in the removal of several micropollutants from drinking water [2,3] and wastewater [1]. Moreover, it did not reveal genotoxic/cytotoxic activity [3]. **Methods:** A liquid chromatography with fluorescence detection method is currently being optimized to separate a group of target FQs with a Kinetex F5 column under reverse phase. Batch adsorption of FQs will be tested in ultra-pure water before testing real water samples (e.g., surface water and wastewater). For this, the selected FQs will be spiked at 1 mg/L and put into contact with the biobased material. The simultaneous adsorption of the target FQs will be followed through the optimized chromatographic method. **Results:** Adsorption kinetic models will be fitted to experimental data obtained for each FQ, to confirm the material's ability to remove these compounds from water, since previous studies do not focus specifically on this class of compounds. **Conclusions:** Based on previous works [1-3], the *Spirulina*-based material has promising applications as an adsorbent, even in (waste)water treatment plants. Therefore, the study of the ability of this material to remove these antibiotics from water can generate important knowledge to mitigate their presence and, consequently, the development of antimicrobial resistance.

Keywords: Water treatment; Fluoroquinolones; Biosorbent; Liquid-chromatography.

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24335 | Green Adsorbents for Uranium Removal from REE Mining Wastewater

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Background & Aim: Rare earth element (REE) beneficiation generates wastewater containing hazardous contaminants, including naturally occurring radioactive materials such as uranium. Sustainable and low-cost treatment strategies are required to support water reuse and reduce environmental risks. This work evaluates natural mineral adsorbents for uranium removal from aqueous solutions, aiming to identify efficient and "green" eco-friendly alternatives for mine wastewater treatment. **Methods:** Diatomaceous earth (DE), bentonite and kaolin were characterized in terms of cation exchange capacity (CEC), elemental composition and particle size. Diatomaceous earth was organically modified with HDTMABr to enhance adsorption performance. Batch adsorption experiments were conducted under controlled conditions using uranium-spiked synthetic waters (10–500 ppb), varying pH, contact time, temperature, and adsorbent dosage. Uranium concentrations were quantified by ICP-MS. **Results:** Bentonite and DE showed relatively high CEC values (68.8 and 44.2 mmol/100 g), whereas kaolin presented low exchange capacity (7.79 mmol/100 g) and was excluded from adsorption tests. Bentonite was also discarded due to excessive swelling and gelation behaviour. The modified DE demonstrated high removal efficiency, achieving up to 92% uranium removal. Adsorption increased with uranium concentration and was favoured at pH 6–8. Effective removal was obtained using low adsorbent doses, indicating cost-efficient performance. **Conclusions:** Organically modified diatomaceous earth proved to be a promising natural adsorbent for uranium removal, combining high efficiency with low material requirements. These results support its potential application in sustainable mine wastewater treatment and contribute to circular economy and zero-pollution strategies.

Keywords: Uranium adsorption, Green adsorbents, Diatomaceous earth, REE beneficiation, Mine wastewater

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24446 | Green Extraction Methodologies to Valorise Chestnut Shells

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Background & Aim: Food waste is an increasing challenge, particularly from agro-industrial processes. Chestnuts are widely processed, and industrial freezing involves brulage, generating significant residues [1]. In Trás-os-Montes (Portugal), a major European chestnut-producing region, large quantities of shells, burrs, and leaves are produced. Within this framework, the CyChest project aims to valorise brulage-treated chestnut shells by extracting bioactive compounds for incorporation into high-value-added products. **Methods:** The work involves optimising supercritical fluid extraction, SFE-CO_2, a green extraction methodology, and comparing the results with a simple Soxhlet extraction using polar and non-polar solvents. The optimisation of SFE-CO_2 parameters will be performed according to Kessler, *et all* [2], while Soxhlet extraction will follow the procedure previously described by Vázquez, *et all* [3] with minor modifications. Then, the extracts will be assessed for mass recovery, total phenolic (TPC) and flavonoid content (TFC), antioxidant capacity (DPPH), and chemical profile by GC-MS. TPC will be determined using the Folin–Ciocalteu method [4], while TFC and DPPH assays will follow previously reported spectrophotometric methods [5,6]. **Results:** Preliminary results from chestnut shells collected in 2022 showed that both extraction techniques yielded low recoveries (<1%) and comparable results. Chromatographic profiles were highly similar between extracts obtained with the same extraction time. The compositional analysis showed that oleic acid was the predominant compound in both extracts, followed by L-ascorbic acid and linoleic acid. In addition, γ-sitosterol was detected in significant amounts, together with several long-chain alkanes. **Conclusions:** Although further analyses using more recent biomass (2025 shells) are required, preliminary results indicate that industrial chestnut shell residues are rich in carboxylic acids and steroidal alcohols. These findings highlight chestnut shells as a valuable, low-cost biomass with strong potential for industrial applications. Their valorisation supports sustainable

practices and the development of functional, high-value-added products, in line with circular economy principles.

Keywords: Chestnut Shells; Waste Valorisation; Supercritical Fluid Extraction; Soxhlet Extraction; Industrial Waste

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24401 | Hydrodeoxygenation of a Furfural-Levulinic Acid Condensation Product for the Synthesis of Aviation Fuel Precursors

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Background & Aim: Lignocellulosic biomass is a renewable carbon source for producing advanced fuels that can reduce dependence on fossil resources and lower greenhouse gas emissions [1]. This work aimed to produce aviation fuel precursors from levulinic acid and furfural, key molecules derived from biomass. **Methods:** A sodium hydroxide catalyzed condensation reaction was carried out between levulinic acid and furfural, according to Amarasekara et al. [2], to obtain the solid reaction substrate. The solid product was purified by Soxhlet extraction using cyclohexane as the extraction solvent for 4 h. Purified and crude product were analyzed by gas chromatography-mass spectrometry (GC-MS). Bi- and monometallic catalysts composed of 2.5% Co-10.5% Mo and 5% Ru supported on carbon nanotubes (CNT) and alumina were synthetized and denoted as Co-Mo/CNT and Ru/Al_2O_3, respectively. The hydrodeoxygenation (HDO) reaction was conducted in a batch reactor using 0.5 g of substrate, 50 mL of n-decane, and 0.25 g of Co-Mo/CNT at 350 °C, 30 bar of H_2, 300 rpm for 6 h [3]. This reaction was also performed using 1.0 g of substrate and 0.2 g of Ru/Al_2O_3 in 30 mL of water at 260 °C and 40 bar of H_2 [4]. The resultant liquid phase was analyzed by GC–MS. **Results:** The crude product mass was 0.9426 g (48.5 % yield). GC-MS showed similar spectra for crude and purified samples, indicating cyclohexane purification is unnecessary. Peaks observed at 11.1, 12.2 and 12.7 min correspond to compounds with molecular formulas $C_{15}H_{24}O$, $C_9H_{10}O_2$ and $C_{10}H_{10}O_4$, respectively. In n-decane, the main product was a lactone, $C_{15}H_{22}O_2$, whereas in water, products included alkanes, furanones, lactones and aromatic derivatives among C_8-C_{15} hydrocarbons. **Conclusions:** A purification step is not necessary, as the crude product exhibited comparable composition to the purified material. This condensation reaction produces a key precursor that, upon HDO, yields products suitable for aviation fuel, demonstrating the potential of this approach.

Keywords: condensation; hydrodeoxygenation; aviation fuel; levulinic acid; furfural

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24589 | Improving healthcare capacity by predicting urgent 30-day readmissions after stroke: a machine learning approach

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Background & Aim: Stroke is a leading cause of death and disability worldwide and a substantial burden for healthcare systems, namely due to frequent hospital readmissions, which cost on average €9120 per event in Canada [1]. Although factors such as age and stroke severity are associated with higher readmission risk, there is little quantifiable correlation among these [2]. Therefore, identifying these relationships can support timely prevention of readmissions. As such, we present a machine learning approach to predict the risk of unplanned 30-day readmission risk after stroke discharge, aiming to ensure a timelier follow-up and to allow and prevent costly and avoidable readmissions. **Methods:** Data were extracted from the MIMIC-IV database, restricting the cohort to patients with an International Classification of Diseases (ICD) stroke code that survived the 30-day period following discharge. We implemented a Gradient Boosting Machine, optimized with grid search paired with cross-evaluation and a feature importance test (SHAP) was performed to assess model interpretability. **Results:** Despite modest discrimination (AUC=0.58), the model focused on risk stratification, achieving a recall of 0.62 and identifying 1 readmitted patient for every 6 patients flagged as high risk. Although follow-up costs are hard to estimate, they are likely lower than emergency readmissions, especially as telemedicine reduces monitoring costs. Regarding the top predictors, SHAP analysis identified discharge home (0.161) and non-white ethnicity (0.049) as associated with lower readmission risk, and age 65–84 (0.047) as associated with higher risk. This information is relevant to support clinical decisions and help validate predictions. **Conclusions:** This approach provides a first version of an interpretable risk stratification model for 30-day stroke readmission that can support proactive care after discharge and has clear potential to reduce avoidable readmissions by guiding targeted follow-up and resource allocation.

Keywords: Stroke care, healthtech, machine learning, AI for health

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24895 | in vitro evaluation of double surface-modified PLGA nanoparticles for controlled drug release and enhanced neuronal uptake in a neurodegenerative disease model

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Background & Aim: Alzheimer's disease (AD) is a prevalent neurodegenerative disorder characterized by progressive cognitive decline [1]. Due to the presence of the blood-brain barrier (BBB), a highly selective membrane that restricts the passage of drugs into the central nervous system (CNS) [2], current therapeutic strategies show limited efficacy, highlighting the urgent need for innovative treatment approaches. Engineered nanoparticles (NPs) have been shown to enhance passage across the BBB, offering a promising strategy for targeted drug delivery to the central nervous system [3, 4]. This explores the *in vitro* potential of double-surface-modified NPs for the controlled release of an acetylcholinesterase inhibitor and their capacity to reach the brain cells. **Methods:** Poly(lactic-co-glycolic acid) (PLGA) NPs, with surface functionalization with selected proteins to target overexpressed receptors of the BBB cells and molecules able to increase the cells' permeability, were characterized in terms of size, polydispersity index (PdI), and zeta potential by dynamic light scattering (DLS). Drug release studies were performed in simulated biological conditions. System stability was characterized under simulated gastrointestinal conditions. Cytotoxicity assays were conducted using the SH-SY5Y cell line to assess the biocompatibility of the developed NPs, followed by evaluation of cellular uptake. **Results:** The developed NPs exhibited appropriate physicochemical properties and a controlled drug-release profile. The system also maintained stability under simulated gastrointestinal conditions. Biocompatibility was confirmed in SH-SY5Y cells, and protein functionalization enhanced cellular uptake. **Conclusions:** The findings demonstrate that double surface modification is an effective strategy to enhance cellular internalization while preserving

controlled drug release and biocompatibility. This platform may represent a promising approach for improving targeted nanotherapeutics in AD treatment.

Keywords: Neurological disorders, Blood-Brain Barrier, Polymeric Nanoparticles, Brain targeting

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24864 | Multilingual Language Models for Span-Level Detection of Persuasion Techniques in Disinformation Discourse

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Background & Aim: The automatic detection of manipulative and persuasive discourse is increasingly relevant in political communication and online media, where large volumes of content make manual monitoring impractical. In addition, disinformation often leverages these techniques to persuade readers and influence public opinion, increasing the need for automatic methods in persuasive discourse. One approach to address this challenge is through Natural Language Processing, where Transformer-based models represent the current state-of-the-art for span-level detection tasks. Although these models have significantly advanced text classification tasks, cross-lingual persuasion detection at the span level remains underexplored for mid to low resource languages such as European Portuguese. This study aims to evaluate the effectiveness of multilingual transformer models for span-level persuasion technique detection under a cross-lingual transfer setting. **Methods:** Supervised training was first performed on English dataset from the CheckThat! 2024 Task3, followed by fine-tuning on the Portuguese one. Two architectures were evaluated: BERT multilingual and XLM-RoBERTa. Additionally, a generative large language model (Gemini) was tested in a few-shot setting. Performance was measured using micro and macro F1. **Results:** BERT multilingual outperformed XLM-RoBERTa in both micro-F1 and macro-F1, indicating stronger cross-lingual generalization in fine-grained persuasion detection. The generative model achieved lower quantitative performance but demonstrated potential as a complementary qualitative analysis tool. **Conclusions:** The results show some potential on using multilingual transformer models for identifying persuasion techniques in Portuguese through cross-lingual transfer. These findings highlight the potential of using cross-language approaches for supporting the automatic analysis of manipulative discourse in online media in less representative languages.

Keywords: Disinformation; Persuasion Detection; Multilingual NLP; Cross-Lingual Transfer; Transformer Models

24675 | New LED systems combined with MoS2/5-Aminolevulinic Acid for Cancer Photothermal and Photodynamic Therapy

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Background & Aim: Breast cancer is the most frequently diagnosed malignancy in women and a leading cause of cancer-related mortality [1]. Despite multimodal treatments, local recurrence and toxicity remain major challenges. Light-based therapies such as photothermal therapy (PTT) and photodynamic therapy (PDT) have emerged as promising localized approaches for tumor ablation. PTT converts near-infrared light into heat, while PDT activates photosensitizers such as 5-aminolevulinic acid (5-ALA) to generate cytotoxic reactive oxygen species via protoporphyrin IX (PpIX), both ultimately leading to cancer cell death [2]. This work aims to develop a selective dual phototherapeutic platform combining MoS_2 nanomaterials and 5-ALA, activated by new custom-built LED systems, using three-dimensional (3D) co-culture breast cancer models composed of malignant and normal epithelial cells to induce localized cytotoxic effects while preserving healthy tissue after breast-conserving surgery. **Methods:** MDA-MB-231 breast cancer and MCF-10A non-tumorigenic breast epithelial cells were cultured as two-dimensional (2D) monolayers and three-dimensional (3D) spheroids, including co-culture spheroids composed of both tumor and non-tumor cells. MoS_2-treated models were irradiated with near-infrared LEDs (≈810 nm) to induce hyperthermia (≈42–50 °C), while 5-ALA-treated cells were exposed to blue LEDs (≈400 nm) to promote PpIX activation. Cell viability and proliferation were assessed using fluorescence-based assays. **Results:** In 2D models, 5-ALA-mediated PDT reduced metabolic activity and proliferation of MDA-MB-231 cells, whereas MCF-10A cells exhibited comparatively higher metabolic activity, supporting treatment selectivity. In addition, MoS_2-mediated photothermal therapy following 20 minutes of near-infrared irradiation significantly reduced the viability of MDA-MB-231 cells and increased tumor cell death. To evaluate these effects in a more physiologically relevant context, 3D breast cancer spheroid models were generated. Ongoing experiments are assessing dual PTT/PDT effects in 3D co-culture spheroids combining

breast cancer and normal breast epithelial cells. **Conclusions:** Both 5-ALA-mediated PDT and MoS_2-mediated PTT demonstrated selective cytotoxic effects in breast cancer cells in 2D models. Their combined application will be further investigated in 3D co-culture spheroid models.

Keywords: Breast cancer; Photothermal therapy; Photodynamic therapy; MoS_2 nanomaterials; 3D spheroids.

Acknowledgments:

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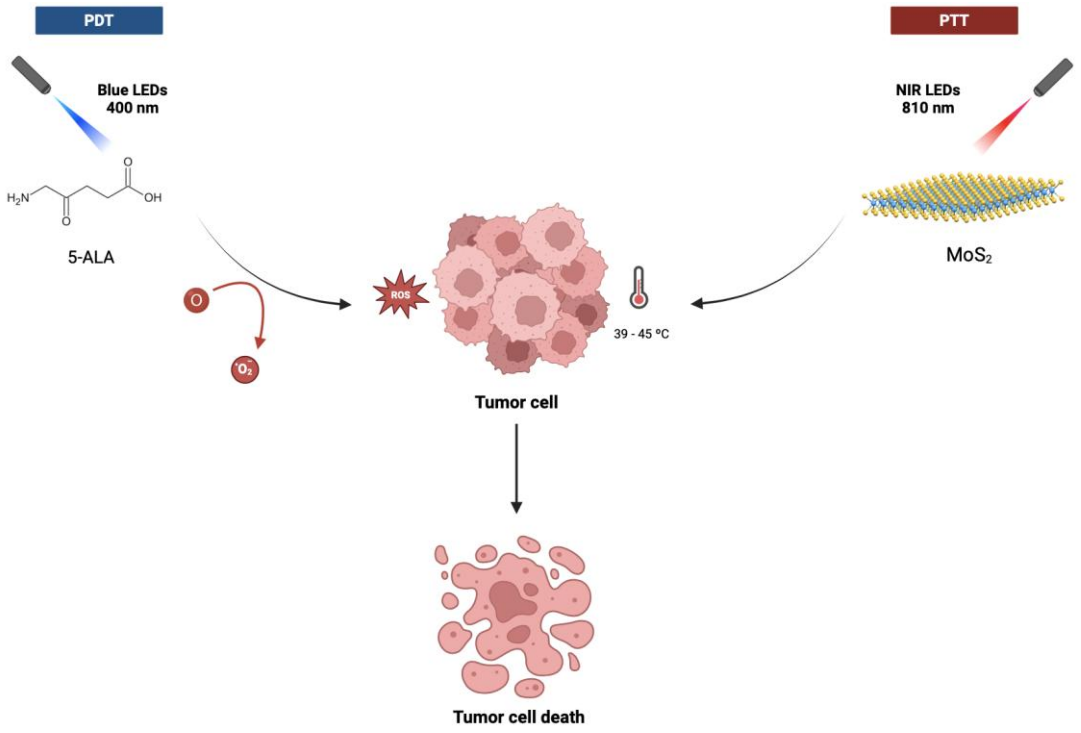

Figure 1: Schematic representation of the dual phototherapeutic strategy. Photodynamic therapy (PDT) is mediated by 5-aminolevulinic acid (5-ALA) activated by blue LEDs (400 nm), leading to intracellular protoporphyrin IX (PpIX) activation and reactive oxygen species (ROS) generation. Photothermal therapy (PTT) is achieved using MoS_2 nanomaterials activated by new portable near-infrared (NIR) LED (810 nm) devices developed by us, inducing localized hyperthermia (≈39–45 °C). Both approaches promote tumor cell death. Created in BioRender.com.

24427 | Optimization of Composite Cathodes for Sodium-Ion Batteries

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Background & Aim: Challenges with lithium-ion batteries (LIBs), particularly resource availability and safety, have accelerated the development of more sustainable alternatives [1]. Sodium-ion batteries (SIBs) have re-emerged as promising candidates due to sodium abundance and improved sustainability [2]. In solid-state configurations in particular, performance is limited by interfacial resistance [3]. This work aims to develop and optimize composite cathodes to improve the electrochemical performance of sodium-based systems. **Methods:** Composite cathodes were fabricated via a wet method and optimized to a controlled composition of $Na_3V_2(PO_4)_3$ (NVP), conductive carbon, binders, and sodium-based electrolytes. Morphology and composition were analyzed by SEM/EDX, while surface topography and potential distribution were investigated by AFM/SKPM. Electrical transport properties of the cathodes were evaluated through sheet resistance measurements and Hall effect characterization. Electrochemical performance is being assessed using PEIS to deconvolute resistance contributions, cyclic voltammetry (CV), and electrochemical cycling at various C-rates within voltage windows established from CV analysis. **Results:** Preliminary results indicate that composite formulation and electrolyte selection influence the structural integrity and electrochemical response of the cathodes. Morphological analysis showed homogeneous particle distribution. Electrochemical measurements indicate distinct impedance evolution and satisfactory cycling responses among the tested systems. Ongoing analysis aims to further correlate structural, electrical, and electrochemical trends. **Conclusions:** The preliminary findings suggest that composite cathode design plays a critical role in governing interfacial resistance and overall electrochemical behavior in sodium-based battery systems.

Keywords: Sodium-ion batteries, solid-state, composite cathodes.

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24451 | Photosensitive Cell for Hybrid Harvesting & Storage

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Background & Aim: The increasing demand for sustainable energy and the urgency of climate change accelerate the shift to renewables. Although LIBs dominate, safety risks, flammable electrolytes and critical raw materials limit sustainability [1]. Solid-state and sodium-based alternatives improve safety and material availability [2]. Ferroelectric solid electrolytes, with spontaneous polarization and high dielectric constants, enable enhanced electrostatic storage, interfacial charge amplification, negative capacitance, and self-charging effects [3]. Hybrid systems integrating photovoltaic harvesting and electrochemical storage in one solid-state device emerge as promising solutions [4]. **Methods:** This work adopts an experimental solid-state architecture employing $Na_{2.99}Ba_{0.05}OCl$ as a ferroelectric solid electrolyte integrated with photoactive shell materials. Devices are fabricated in coaxial and pouch-cell configurations to evaluate structural effects. Copper and Zinc are used as the current collectors, resulting in a Zn/$Na_{2.99}Ba_{0.05}OCl$/Cu configuration covered by the photosensitive shell. Optical properties of the electrolyte and shell materials are analyzed by UV-Vis spectroscopy to determine their absorbance spectrum. To assess cell electrochemical characterization, potentiostatic electrochemical impedance spectroscopy and cyclic voltammetry are conducted. Photoelectrochemical assessment is performed through current-voltage (I-V) characteristics under illumination, allowing analysis of the device's overall operational stability. **Results:** UV-Vis analysis confirmed the light-absorption properties of both shell materials and the electrolyte, electrochemical tests revealed ionic transport and interfacial behavior and illuminated I-V measurements demonstrated measurable photoactivity in the integrated devices. **Conclusions:** These findings confirm the potential of integrating ferroelectric solid electrolytes with photoactive shells for safer hybrid photo-storage systems.

Keywords: Hybrid Device; Ferroelectric Solid-State; Photoactive Materials

Acknowledgments: This work was supported by the Department of Chemical and Biological Engineering at the Engineering Faculty of University of Porto. A special acknowledgment to the Agenda “NGS – New Generation Storage”, ref. no. C644936001-00000045, investment project no. 58, under the Incentive System “Agendas for Busi-ness Innovation”, funded by the PRR - Recovery and Resilience Plan and the European Union’s NextGeneration EU funds, for providing the funding for this study. This study was funded by the European Union – NextGeneration EU. However, the views and opinions expressed are solely those of the author(s) and do not necessarily reflect the views and opinions of the European Union or the European Commission. Nei-ther the European Union nor the European Commission can be held responsible for them.

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24852 | PLGA Nanoparticles Loaded with Meclofenamic Acid: A Novel Drug Delivery Strategy for Glioblastoma Therapy

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Background & Aim: Glioblastoma Multiforme (GBM) is the most aggressive primary malignant brain cancer associated with poor prognosis and a median survival of 15 months, despite existing treatments. A significant challenge in GBM therapy is the inability of conventional drugs to achieve therapeutic concentrations in the tumor due to their inability to cross the blood-brain barrier and due to the tumor heterogeneity [1]. Meclofenamic acid (MFA) is a nonsteroidal anti-inflammatory drug (NSAID) that has shown potential in GBM therapy by disrupting tumor cell communication and enhancing sensitivity to conventional chemotherapy [2]. Encapsulation of MFA into biodegradable and biocompatible poly(lactic-co-glycolic) (PLGA) nanoparticles (NPs) may enable controlled release and stability [3], while surface coating with chitosan promotes selective release in the acidic tumor environment [4]. **Methods:** Chitosan-coated PLGA NPs loaded with MFA were prepared by a single oil-in-water (O/W) emulsion solvent evaporation method. Four formulations were produced by modifying key parameters to evaluate their influence on NPs physicochemical properties, including size, polydispersity index (PDI), zeta potential, and MFA encapsulation efficiency (EE). A base formulation employing 10 mg of PLGA, 1% (w/v) of polyvinyl alcohol (PVA), and one sonication cycle of 10 s (on/off) was first prepared. Then, in the other formulations, one parameter was varied at a time: (i) 3 cycles of sonication, (ii) 20 mg of PLGA, and (iii) and 3% (w/v) of PVA. **Results:** The size and zeta potential of the MFA-loaded NPs formulations were evaluated using dynamic light scattering. Data suggest that modifications between formulations lead to differences in particle size, PDI, and zeta potential. The EE obtained for the NPs ranged within acceptable values. **Conclusions:** Chitosan-coated

MFA-loaded PLGA NPs with suitable characteristics were successfully produced. Further optimization will be carried out to enhance EE and formulation performance.

Keywords: Brain cancer; Poly(lactic-co-glycolic acid); brain delivery; nonsteroidal anti-inflammatory drug; drug repurposing

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24598 | Predictive Models for the Prevention of Blooms and Spread of the Invasive Macroalgae Rugulopteryx okamurae in Portuguese Coastal Ecosystems

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Background & Aim: In Portugal, coastal enrichment remains a key issue, as nutrient input and favorable climate promote macroalgal growth as we can see in Figure 1. Portaria nº 270-A/2025 reinforces environmental monitoring, risk mitigation, and coastal management through data-based approaches, highlighting the need for predictive tools [1]. Macroalgal dynamics depend on complex, nonlinear interactions involving temperature, radiation, nutrient inputs, river discharge, and sea hydrodynamics [2,3]. Traditional statistical techniques struggle to capture these dependences, whereas machine learning (ML) offers strong predictive capabilities for complex ecological systems [4]. This study tests whether ML models can accurately predict macroalgal concentrations in Portuguese coastal waters using environmental and climatic variables, aiming to develop a data-driven basis for ecosystem monitoring. **Methods:** Environmental variables (temperature, precipitation, wind) will be obtained from national meteorological services, and hydrological data will include river discharge and nutrient loads. Macroalgae concentration will serve as the response variable. Data preprocessing will involve temporal alignment, imputation, outlier removal, and creation of lagged variables. The dataset will be split chronologically into training and test sets (70/30). Multiple Linear Regression will serve as a baseline, compared with Random Forest and Gradient Boosting models. Performance will be assessed using RMSE, MAE, and R^2. Explainable AI (feature importance, SHAP) will identify key environmental drivers of macroalgae, aiding management. **Results:** Ensemble ML models outperformed linear regression, with lower RMSE and greater predictive stability during high-concentration periods. Key predictors included temperature, nutrient levels, and lagged

precipitation. **Conclusions:** ML improves prediction of macroalgae in Portuguese coastal waters, advancing ecological forecasting and early-warning for eutrophication.

Keywords: Algal bloom, Invasive macroalgae, *Rugulopteryx okamurae*, Machine Learning, Predictive Modeling

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Project NUTRIBRAIN - Coastal Flora for Mental Well-being Promotion: Development of food supplements based on dune plant compounds with impact on BDNF-TrkB signalling, with reference COMPETE2030-FEDER-00686600, operation nº 15759, supported by FEDER (COMPETE 2030) and national funds by FCT / MECI

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Figure 1 [5]:

24815 | Production of engineered polymeric nanoparticles loaded with tarenflurbil to cross the blood brain barrier and treat Alzheimer's disease

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Background & Aim: Alzheimer's disease (AD) is one of the growing neurodegenerative disorders, expected to triple in 30 years, characterized by cognitive and memory impairments. The current treatment options are unable to address AD, since they cannot prevent neuronal loss, but only slow down the disease progression. The blood-brain barrier (BBB) hinders the entry of most therapeutic agents into the brain, while small molecules may transit, but are suboptimal for therapeutic purposes. The mechanisms that lead to AD can be linked to the senile plaques present in the brain of patients, in which amyloid-beta (Aβ) peptides self-assemble into fibrils and induce neurotoxicity. Tarenflurbil presents the ability to modulate the Aβ production, however, it presents low capacity to cross the BBB. Nanoparticle-based drug delivery systems can enable receptor-mediated transcytosis (RTM), thereby enhancing the transport mechanism across the BBB [1, 2]. One of the promising ligands to target the brain is transferrin [3]. Poly (lactic-co-glycolic acid) (PLGA) nanoparticles exhibit biocompatibility, reduced toxicity, and high drug-loading capacity [4]. **Methods:** Tarenflurbil-loaded PLGA nanoparticles were produced by the single-emulsion solvent evaporation method, subsequently functionalized with transferrin after activation of polymer carboxylic groups with 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The nanoparticles' size, polydispersity index, and zeta potential were analyzed by dynamic light scattering. The efficacy of the surface modification was assessed by UV-Vis spectroscopy using the BCA kit and verified by Fourier transform infrared spectroscopy. **Results:** The nanoparticles exhibited optimal sizes between 100-200 nm, a low polydispersity index, and negative zeta potentials. The nanoparticles' surface was efficiently conjugated. **Conclusions:** Functionalized PLGA nanoparticles were successfully synthesized. Their

characteristics have proven ideal for drug delivery applications, with promising properties for BBB penetration. Future research will focus on evaluating *in vitro* cytotoxicity.

Keywords: Nanomedicines; Drug delivery systems; Poly (lactic-co-glycolic acid); Transferrin.

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24759 | Quantification and Qualification of Microglia Movements

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Background & Aim: At i3S, researchers often manually analyze microscopy videos to track immune cells of the central nervous system (called microglia) and understand how they move and change shape, while simultaneously exploring factors that influence the integrity and function of the cells [1]. Considering that each video has dozens of microglia and their movements must be accounted for across its entire duration. Consequently, manually tracking and quantifying microglial dynamics across the entire video is a highly time-intensive process. This project aims to develop an automated system capable of analyzing microscopy videos, detecting microglial cells, and measuring the cell elongation and retraction processes over time. **Methods:** We first manually label microglia in the videos and use these annotations to fine-tune a foundation model (a large artificial intelligence model trained on vast amounts of data) for microglia segmentation. Subsequently, microglial movements and morphological alterations will be quantified using a similar model, as exemplified in Figure 1. Currently, five videos (with eleven frames each) featuring dozens of microglia are being utilized to fine-tune the model. **Results:** Early results show that large pre-trained foundation models, such as micro-SAM [2] and Cellpose-SAM [3], combined with human guidance, can accurately identify microglia in microscopy videos in a fraction of the time required for manual analysis. **Conclusions:** The proposed framework is capable of accurately identifying microglial cells and their respective morphological changes over a period, effectively making the segmentation process more efficient.

Keywords: Biology; Artificial Intelligence; Computer Vision; Cell Segmentation

Acknowledgments: This work is being developed in the context of a thesis for the Master in Informatics and Computing Engineering at FEUP.

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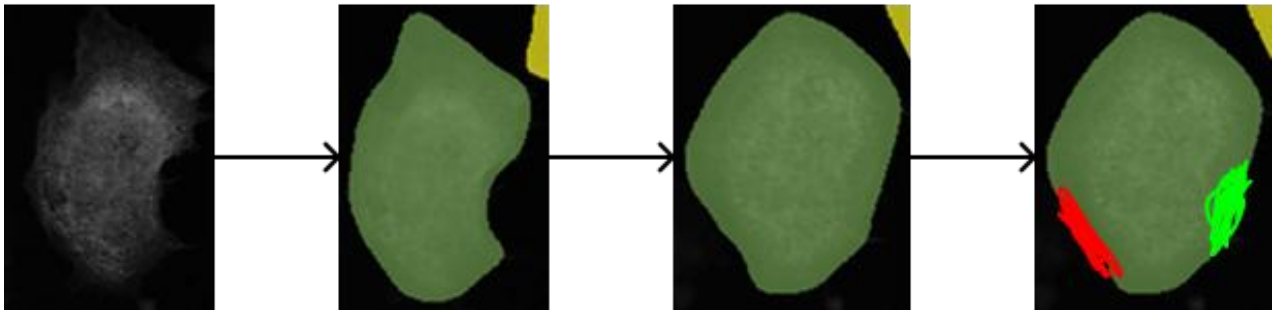

Figure 1: Proposed output of the framework being developed

24440 | Selective extraction of ammonia from landfill leachate by direct contact membrane distillation

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Background & Aim: Landfill leachates can contain high concentrations of ammonia [1–3], representing both an environmental problem and a potential resource for recovery. Ammonia recovery aligns with the circular economy principles [4]. Ammonia has different applications, the main one being the production of fertilizers [3]. Membrane distillation (MD) is a promising process for ammonia removal and recovery [1,5]. By optimizing the operating conditions (such as pH and temperature), it is possible to transfer volatile ammonia through a hydrophobic membrane. This work aims to recover ammonia from a nanofiltration permeate of a landfill leachate, using an MD process. **Methods:** A laboratory-scale MD is being used to test polytetrafluoroethylene membranes, and the possible occurrence of fouling and scaling is under study by membrane characterization. **Results:** Optimization of the MD process should enable the selective transfer of ammonia from the alkaline feed to the acidified permeate, driven by controlled temperature gradients and pH differences across the membrane. Optimizing these conditions increases ammonia volatilization while maintaining effective rejection of non-volatile organic compounds and dissolved salts. Fouling phenomena can occur on the feed side, primarily due to the precipitation of organic and inorganic matter, potentially impacting membrane permeability over time. **Conclusions:** The development of this optimized direct contact membrane distillation process can provide an efficient alternative, both in terms of energy and ammonia recovery from landfill effluents.

Keywords: Landfill leachate; Membrane distillation; Ammonia recovery; Resource valorization.

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24406 | ValorWaste Project: Gamifying Circular Economy Learning for Youth

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Background & Aim: The transition to sustainable energy systems depends on access to critical raw materials, yet the mining industry faces significant environmental challenges and low public acceptance. Addressing these issues begins with education, particularly for younger generations, to promote awareness and understanding of the circular economy. Within the ValorWaste project, two serious games were developed: an introductory version for primary school students and an advanced version, available in both physical and digital formats, targeted at high school students. **Methods:** Implementing a Game-Based Learning approach, the primary school game introduces key ideas such as responsible mining, environmental protection, and circular economy principles through scenario-based challenges. The high school version is structured as a one-versus-one resource management game that simulates real-world processes, including extraction, environmental liabilities such as sulfidic tailings, and technological solutions to these problems. A digital multiplayer prototype was developed using AI-assisted coding and a real-time database, facilitating remote play across multiple devices. **Results:** A functional suite of educational games was successfully developed. The primary school game fosters an understanding of resource recovery and environmental restoration, while the high school game accurately incorporates concepts such as *Life Cycle Assessment* and the *End-of-Waste framework*. Playtesting showed that requiring players to address environmental liabilities to earn *Sustainability Tokens* helps internalize the costs of environmental impact. The digital prototype provides reliable, real-time synchronization, enabling remote testing and gameplay. **Conclusions:** Gamification is highly effective for science communication. The ValorWaste games translate complex sustainability goals into engaging experiences, showing strong potential to improve environmental literacy and support societal awareness in the raw materials sector.

Keywords: ValorWaste project; Game-Based Learning; Circular Economy; Mining Waste; Science Communication

Acknowledgements: This work was developed under the Project ValorWaste- “Valorisation and Integration of Extractive Waste Towards the Sustainability of Raw Materials Industry” with the reference ERA-MIN3/0002/2023 and with DOI 10.54499/ERA-MIN3/0002/2023 funded by the Fundação para a Ciência e a Tecnologia, I.P., through national funds within the framework of the M-ERA-NET3 network “ERA-NET Cofund in raw materials. This work was developed at R&D Unit CERENA, supported through FCT Project UID/04028/2025 (https://doi.org/10.54499/UID/04028/2025).

ENVIRONMENT

24533 | Transport Accessibility to Education and Spatial Inequality: A Multimodal GIS-Based Analysis of Vila Real (Portugal)

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Background & Aim: Mobility both reflects and shapes environmental and social challenges. Although transport systems can promote inclusion, they also reproduce accessibility inequality and accessibility poverty (Karner et al., 2025). In low-density areas, children's access to education often depends on household car availability, and driving-time metrics may overestimate territorial equity, masking structural dependence on a private vehicle (Pizzol et al., 2021). Students in rural or transitional areas perceive inaccessibility mainly when transport constraints intersect with social disadvantage (Moleman et al., 2025). This study tests whether car-based assessments overestimate equity in access to education and whether modal disparities intensify at higher educational levels due to school centralization. **Methods:** A GIS-based comparative analysis was conducted using walking and car-based scenarios. Schools from primary to secondary level were georeferenced, and 5-, 10-, and 15-minute service areas were calculated. Youth population (0–19 years) was analyzed at parish level. Given data aggregation limits, the study prioritizes spatial differentiation between mobility regimes. **Results:** A clear center–periphery gradient emerges. The urban core concentrates both schools and youth population, while rural parishes show fragmented pedestrian accessibility. Car-based accessibility covers most of the territory within 15 minutes, but this reflects potential rather than effective access. As educational level increases, centralization intensifies, reducing accessibility by non-motorized modes and reinforcing car dependence. These disparities increase time burdens and unevenly shape participation in educational and extracurricular activities. **Conclusions:** Car-centric metrics mask inequality Including public transport would likely reveal additional disparities. This study contributes to a spatial justice perspective to support more equitable educational planning in low-density territories.

Keywords: Transport equity; Educational accessibility; Spatial Justice; GIS Analysis

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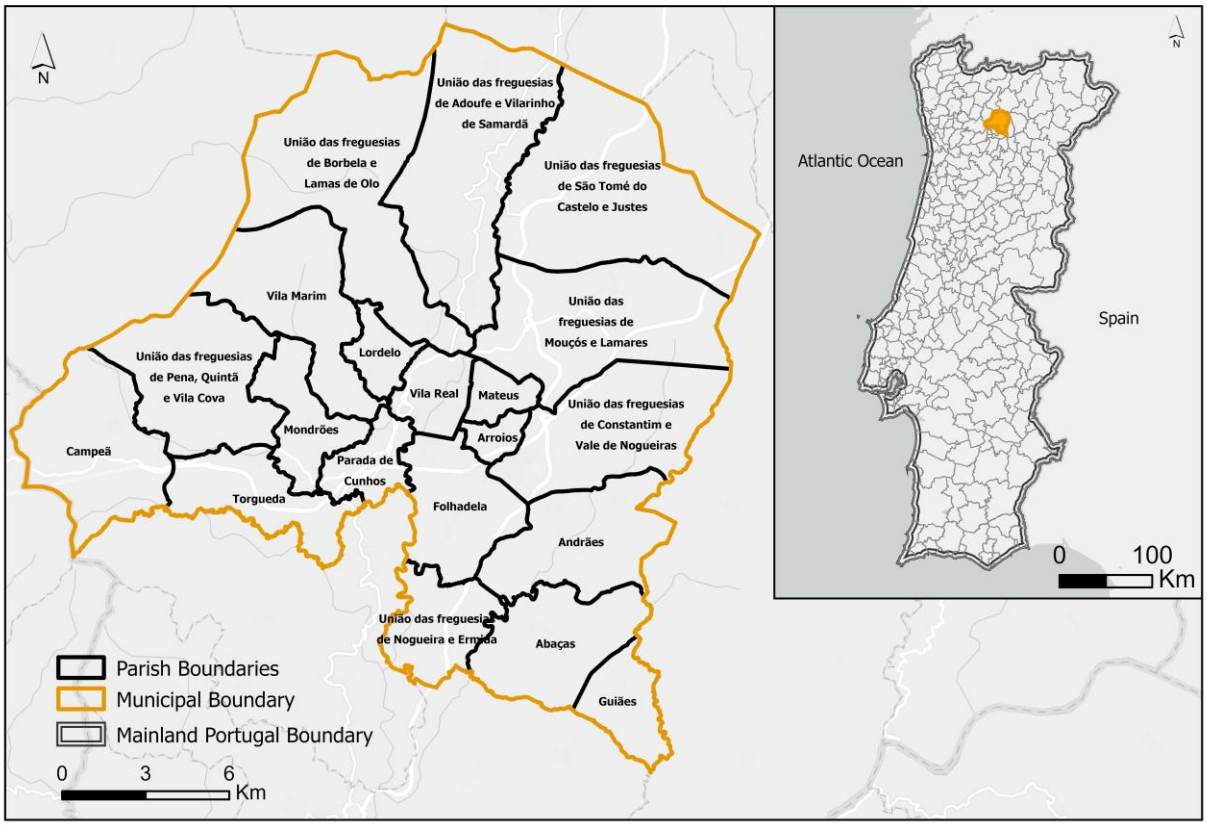
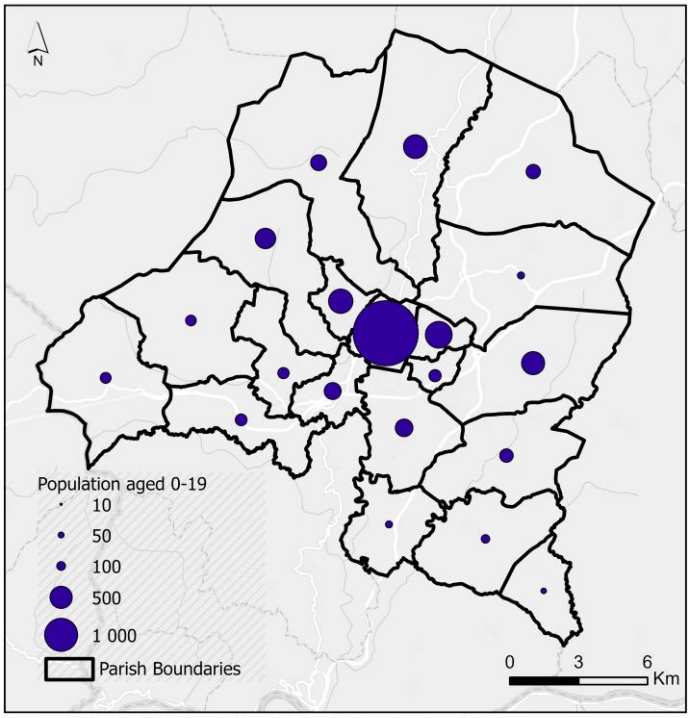
Figure 1: Study Area Setting: Vila Real, Portugal



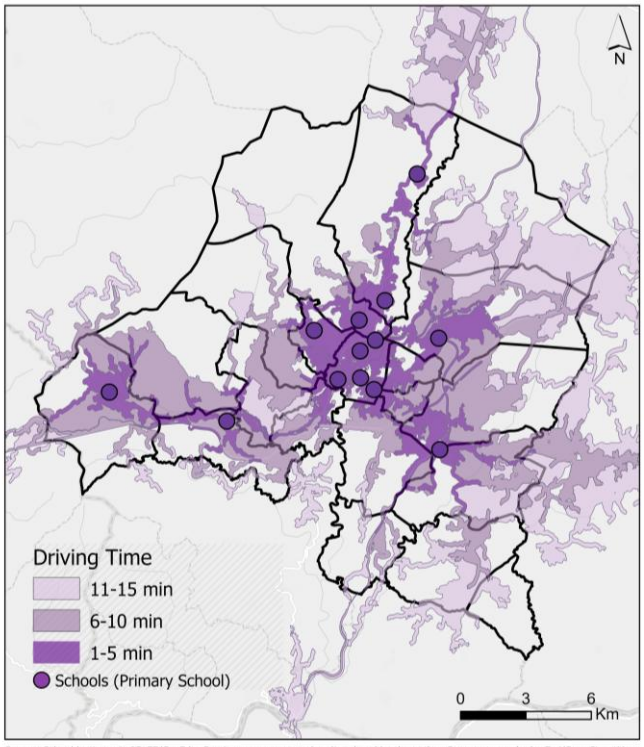
Source: General Directorate of Territory, CAOP2025; own elaboration

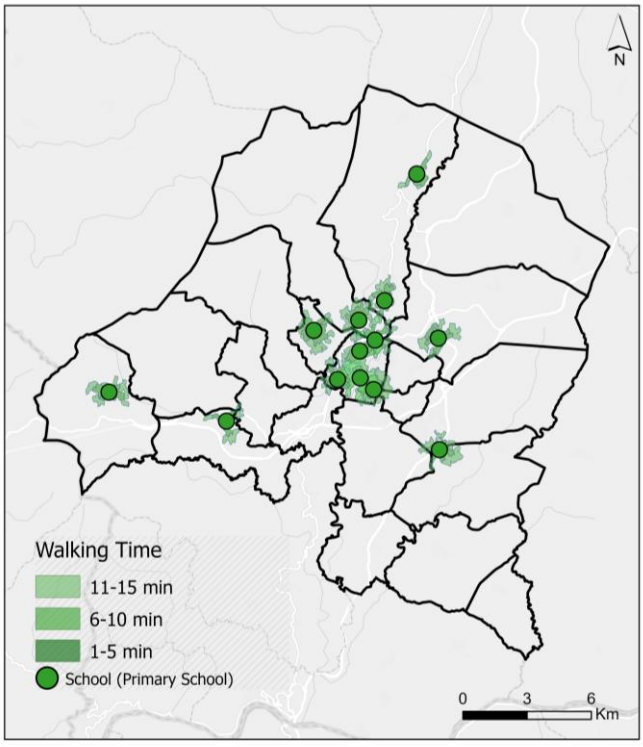
Figure 2: Population of the Municipality of Vila Real aged 0-19



Source: Resident Population (Nº) by place of residence at the tima of the Census [2021]; own Elaboration

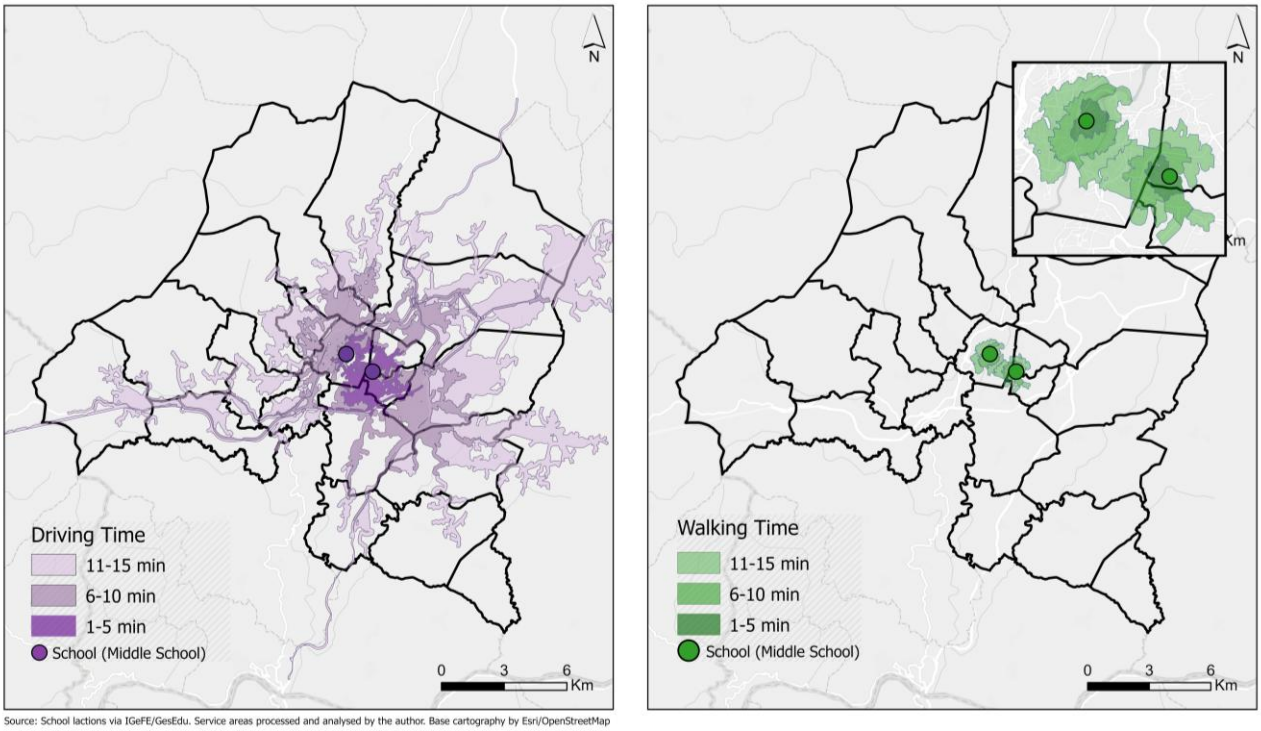
Figure 3: Areas of influence of Primary School, by driving time and walking time





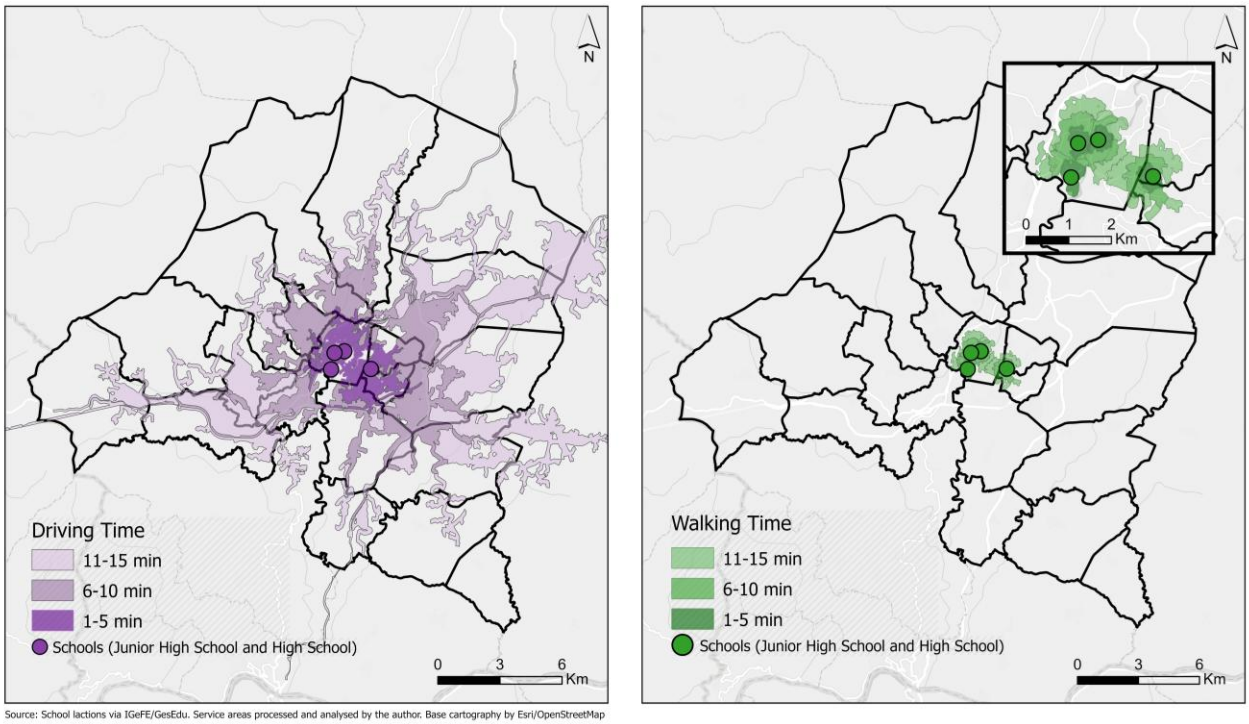
Source: School lactions via IGeFE/GesEdu. Service areas processed and analysed by the author. Base cartography by Esri/OpenStreetMap

Figure 4: Areas of influence of Middle School, by driving time and walking time



Source: School lactions via IGeFE/GesEdu. Service areas processed and analysed by the author. Base cartography by Esri/OpenStreetMap

Figure 5: Areas of influence of Junior High School and High School, by driving time and walking



Source: School lactions via IGeFE/GesEdu. Service areas processed and analysed by the author. Base cartography by Esri/OpenStreetMap

time

24894 | From Point Clouds to Policy: 3D Modeling as a Decision-Support Tool

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Background & Aim: Urban Digital Twins (UDTs) are emerging as strategic urban planning tools that transcend traditional cartography by integrating geospatial data and 3D simulations. The objective of this study is to develop a 3D model of Vila Nova de Gaia and compare Level of Detail (LOD) methodologies (LOD1 and LOD2) to inform territorial planning decisions. **Methods:** The study used point clouds, photogrammetry, cadastral footprints, and land use maps. ArcGIS Pro and ArcGIS Online were used for processing. The methodologies included using spatial joints to enrich building attributes, 3D extrusion for Level of Detail (LOD) 1, and geometric extraction from point clouds for LOD 2. A comparative analysis was conducted to assess computational efficiency versus geometric fidelity. **Results:** Two 3D modeling approaches were implemented: LOD1 allowed for quick deployment of regional analyses, and LOD2 provided the superior geometric realism necessary for shading and visibility simulations, such as roofs and chimneys. High-density point clouds (≥8 points/m²) were critical for accurate feature extraction. **Conclusions:** UDTs are essential to evidence-based urban planning. The choice between LOD1 and LOD2 strikes a balance between operational efficiency and simulation accuracy. High-quality base data is essential for the utility of models. These tools promote transparency and citizen participation, supporting sustainable city management.

Keywords: Urban Digital Twin, 3D modeling, GIS, urban planning, ArcGIS.

24913 | Preparation of Plateosaurus sp. for a Future Exhibition

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Background & Aim: *Plateosaurus* is a sauropodomorph dinosaur and their fossils have provided great insight into early sauropodomorph anatomy, evolution and paleobiology. This specimen from Frick, discovered in 2017, was transported to Dinosaurier-Park Münchehagen and afterwards to Dino Parque Lourinhã. This work aimed to prepare and properly store several of the bones of this specimen, including cervical vertebrae, a pubis and a claw, ensuring their preservation for future study and exhibition. **Methods:** For this work, mainly mechanical preparation techniques were employed to carefully remove the sedimentary matrix and consolidate the bones. Tools included an eletric microhammer, scalpel, tungsten pointer, toothbrush, cotton, and water. Each bone was carefully cleaned, consolidated, catalogued, and stored, following the paleontological protocols for this fossil's conservation and storage established by Dinosaurier-Park Münchehagen. **Results:** It resulted in fully cleaned and stabilized bones that are now properly stored in boxes for long-term preservation. This specimen is ready for exhibition at Dino Parque Lourinhã, with all bones secured and accessible for future scientific work. **Conclusions:** This work demonstrates the successful preparation and conservation of key *Plateosaurus* skeletal elements, which are going to be object of research and exhibition. The preparation not only preserves the specimen but also enhances its scientific and educational value, contributing to the understanding of Late Triassic sauropodomorph anatomy.

Keywords: Plateosaurus; fossil preparation; mechanical preparation; Late Triassic; Frick

Acknowledgments:

We thank the Sauriermuseum Frick, Dinosaurier-Park Münchehagen, and Dino Parque Lourinhã, for providing access to the specimen and supporting this project. We are especially grateful to all the volunteers who contributed their time and effort. A special thanks to João Marinheiro and Cátia Ribeiro for their work in preparing the specimen.

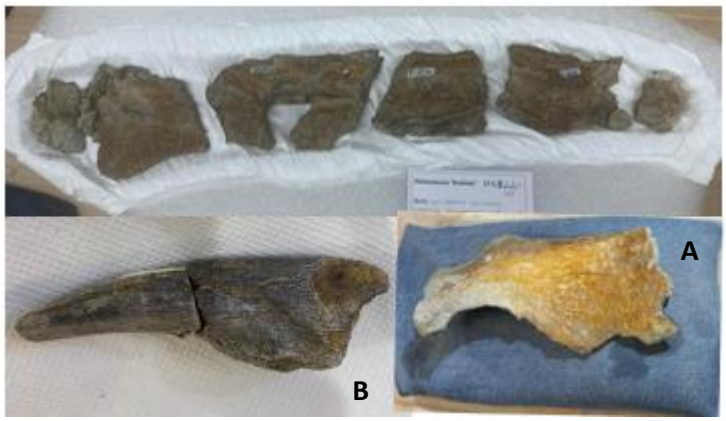

Figure 1: *Plateosaurus* specimen. A. Cervical vertebrae; B. Claw; C. Pubis.

24550 | Hazard assessment of remediation technologies to eliminate contaminants of emerging concern from secondary municipal wastewaters

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Background & Aim: Municipal wastewater is a major pathway for the release of complex contaminants into aquatic ecosystems, and conventional treatment does not effectively remove contaminants of emerging concern (CECs). In line with the Directive (EU) 2024/3019, which requires large WWTPs to remove at least 80% of selected CECs during treatment, advanced technologies such as ultrafiltration (UF) and UV radiation are increasingly implemented. However, reductions in target CECs may not translate into lower ecological risk due to potential transformation products. This study aimed to evaluate how advanced treatment affects the ecotoxicity of secondary municipal wastewater by applying the Fish Embryo Toxicity (FET) test with zebrafish (*Danio rerio)* embryos and integrating morphological, behavioral and molecular endpoints. **Methods:** Wastewater samples, collected before (secondary effluent) and after advanced treatments (UF and UV), were assessed by FET test (OECD 236). Morphological abnormalities were monitored for 96 hours. Behavioral alterations were evaluated using a Locomotor Activity Monitoring System, while transcriptomic analyses were performed to identify changes in gene expression related to exposure. Statistical analyses were then conducted to compare the treatments and determine any significant differences in toxicity and molecular response profiles. **Results:** No visible morphological abnormalities were observed in treatment groups. Behavioral changes and transcriptomic/genetic analyses have not yet been analyzed because the trial is still ongoing. Overall, developmental alterations were minimal, with no consistent pattern of acute toxicity detected. **Conclusions:** Combining advanced remediation technologies with high throughput ecotoxicological, behavioral and transcriptomic approaches enable a more comprehensive evaluation of treated wastewater safety than chemical analysis alone. As this work is ongoing, the results obtained so far support improved environmental risk assessment and the development of more sustainable wastewater management strategies.

Keywords: Contaminants of Emerging Concern, Wastewater Treatment, Advanced Remediation Technologies, Ecotoxicological Assessment, Zebrafish

Acknowledgments:

This work is part of the international Interreg BlueWater project, which aims to improve the water quality of rivers and estuaries in the Iberian Peninsula. The project is being developed in collaboration with CIIMAR, FCUP, and FEUP.

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24414 | Integrating Macro-litter and Microbial Colonization into River Monitoring: Evidence from the Leça River

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Background & Aim: In Portugal, monitoring programs for river ecological assessments follow the Water Framework Directive [1], but macro-litter and its associated microbiota remain largely overlooked, despite their growing relevance for ecosystem health [2]. This study aimed to evaluate the ecological status of the Leça River, combining conventional water quality parameters [3] with analysis of macro-litter and associated microbiota. **Methods:** Seven sampling sites (P1-P7) were selected along the river. Summer and autumn 2025 field campaigns were conducted, and in situ physico-chemical parameters and inorganic nutrients were evaluated. Macro-litter was classified using litter classification frameworks [e.g., 4]. Microbiological colonization was assessed on plastic, textile, and ceramic items collected at P1, P3, and P6 sites, using non-selective (Nutrient Agar) and selective (MacConkey Agar) culture media. **Results:** Physico-chemical parameters revealed water degradation along the river in both seasons, resulting in a moderate ecological status, due to reduced O_2 levels, high biochemical oxygen demand, and nutrient concentrations. Plastic was the dominant macro-litter category, followed by pottery/ceramics and cloth. P6 exhibited the highest microbial colonization on non-selective media, on textile items, while P3 showed greater colonization by Gram-negative bacteria, on plastic items. This higher abundance at P3 supports the hypothesis that macro-litter may act as a vector for potentially pathogenic microorganisms, including members of the *Enterobacteriaceae* family [2]. **Conclusions:** Leça River degradation can reflect the combined effects of chemical contamination and solid waste accumulation. The association between litter typology, microbial colonization, and downstream water quality reinforces macro-litter as a meaningful indicator of pollution sources and potential health risks. Integrating waste-

associated microbiota into monitoring programs can strengthen the ecological assessment of rivers.

Keywords: Leça River; Macro-litter; Microbiota; Environmental Monitoring; Water Quality.

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24859 | Nanoencapsulation of *Trichoderma atroviride* for safer biological crop protection in vineyards

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Background & Aim: Biological control agents based on *Trichoderma atroviride* are sustainable alternatives to copper-based fungicides in viticulture, particularly against grapevine trunk diseases caused by *Neofusicoccum parvum* and grey mould induced by *Botrytis cinerea* [1]. However, conidial viability declines under field conditions, limiting biocontrol reliability [2]. Encapsulation in lipid nanoparticles (LN) may overcome these limitations by protecting biological materials, improving plant surface adhesion, and enabling controlled release [3]. This study aims to develop a biocompatible LN formulation for encapsulation of *T. atroviride* conidia, assess conidial viability after encapsulation, and evaluate the safety of the nanoformulation towards non-target fungi. **Methods:** LN will be designed using a solvent-free methodology and ecofriendly compounds. Formulations will be characterized by mean particle size, homogeneity, surface charge, and stability. Conidial viability will be assessed by colony-forming unit enumeration. As a preliminary safety assessment, non-target effects of the LN will be evaluated on *Lentinus sajor-caju*, a lignocellulose-degrading basidiomycete representative of beneficial fungal communities. Radial mycelial growth of *L. sajor-caju* will be measured on Malt Extract Agar treated with LN. In parallel, efficacy assays against grapevine pathogens are ongoing. **Results:** Nanoencapsulation is expected to maintain conidial viability while producing stable LN with suitable properties for agricultural use. LN are not expected to inhibit growth of *L. sajor-caju*, supporting biological compatibility. **Conclusions:** These preliminary results provide an initial assessment of the environmental safety and biological compatibility of LN-based nanoformulations for *T. atroviride* delivery. Ongoing work will evaluate efficacy against *B.*

cinerea and *N. parvum*, contributing to sustainable alternatives to copper-based fungicides in viticulture [4].

Keywords: Lipid nanoparticles; Grapevine pathogens; Sustainable viticulture; Environmental safety

Acknowledgments: This work was supported by - Fundação para a Ciência e Tecnologia, I.P. through the Strategic Project "GreenUPorto - Centro de investigação em Produção Agroalimentar Sustentável", with references UID/05748/2025, DOI https://doi.org/10.54499/UID/05748/2025, UID/PRR/05748/2025, with DOI https://doi.org/10.54499/UID/PRR/05748/2025 and through individual research PhD grant attributed to CG by project reference 2023.01531.BDANA, DOI https://doi.org/10.54499/2023.01531.BDANA. This work was also supported by FCT under the scope of the R&D Project Nano-RNABiocide (2023.14644.PEX). TA also thanks FCT for the contract under the CEEC-Individual—6th Edition with the reference 2023.08988.CEECIND.

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24711 | RESTAURA: Study of Soil Health and Rehabilitation of Mining Areas – Tapada Mine Case Study

Eduardo Amorim[1]; Maria de Lurdes Dinis[1]; Maria Cristina Vila[1]; Gonçalo Pereira[1]; Beatriz Bento[1]; António Fiúza; Daniel Ruivo[2]; Patrícia Santos[2]; Alexandre Lima[2]

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Background & Aim: Northern Portugal has abundant metallic and non-metallic mineral resources, with significant past mining activity focused on Au, Sb, Pb, Zn, Ag, Sn and W. Mining waste from past extraction and processing activities in this region remains near the mines, with no recovery or mitigation measures in place. The main objective of this work is to assess environmental contamination in these areas and to contribute to the definition of strategies to mitigate and restore soil health. The work is being developed within the RESTAURA project. The former Au-Sb Tapada mine, located in the municipality of Gondomar, Porto district, was selected as a case study. **Methods:** The methodology includes i) identifying and quantifying hazardous elements in the area surrounding the waste dumps through an integrated study supported by petrographic, geochemical, physical and environmental data; and ii) promoting soil treatment and the intelligent reuse of mining waste. This is an ongoing work, and the characterisation of the samples is underway. **Results:** Based on existing information, Sb is the primary mining contaminant/tracer and provides strong evidence of mining impacts and leaching/runoff; As and Pb align with antimony (spatial overlap) and are likely derived from a common source (tailings/mineralised zones). The pH and conductivity will indicate metal mobility; the distribution of soil organic carbon and cation exchange capacity will be crucial for studying soil fertility and properties; and natural leaching tests and potential for acid generation will assess the chemical stability of the sampled material. **Conclusions:** Although this is an ongoing study, the results to date underscore the environmental risks posed by abandoned mining areas lacking remediation measures and emphasise the need for targeted management strategies. The data generated constitute a necessary baseline for the definition of mitigation, stabilization and soil restoration solutions, including the intelligent reuse of mining waste.

Keywords: Environmental geochemistry; Mining contamination; Antimony; Abandoned mines; Reuse of mining waste

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24381 | Sustainable removal of uranium from wastewater generated during rare earth ore processing

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Background & Aim: Owing to global freshwater scarcity and increasingly stringent environmental regulations, water recycling in the mining industry has become essential, particularly in flotation processes, which are among the most water-intensive stages of mineral beneficiation. However, recirculation of flotation water leads to the accumulation of dissolved ions, residual reagents, and metal species that can impair process performance, increase operational costs, and pose environmental risks. Therefore, the development of sustainable and efficient technologies for the removal of contaminants such as uranium from flotation water is crucial to enable safe water reuse and reduce environmental discharge. In this context, this study aimed to investigate the applicability of 8-hydroxyquinoline–modified diatomaceous as a sustainable adsorbent for uranium removal from flotation water. **Methods:** Diatomaceous earth was organically modified with 8-hydroxyquinoline to enhance its uranium removal efficiency. The effects of various experimental conditions including adsorbent dosage, initial uranium concentration, contact time, pH, temperature, and the presence of flotation reagents were evaluated through batch adsorption experiments. **Results:** Modification with 8-hydroxyquinoline significantly enhanced the uranium removal efficiency compared to raw diatomaceous earth. Preliminary batch experiments indicate that uranium removal is strongly affected by pH and the initial uranium concentration, whereas adsorbent dosage and contact time have minimal impact, suggesting that the adsorption process is rapid. **Conclusions:** Organically modified diatomaceous earth functionalized with 8-hydroxyquinoline demonstrated a strong potential as an effective natural adsorbent for uranium removal. These findings support its application in sustainable mine wastewater treatment.

Keywords: Uranium adsorption; Sustainable adsorbents; Diatomaceous earth; REE beneficiation; Mine wastewater

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HEALTH SCIENCES

24876 | Antibiotic resistance in environmental isolates from a One Health perspective

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Background & Aim: Antimicrobial resistance is a growing global health concern and environmental waters exposed to anthropogenic contamination can contribute to the spread of resistant bacteria. This study aimed to investigate the presence of antibiotic-resistant bacteria in seawater impacted by a potentially contaminated urban water stream. **Methods:** Three water samples, pluvial water and urban water streams reaching the beach and seawater, were analyzed to detect potential seawater contamination origins, using the membrane filtration method. Membranes were incubated on MacConkey agar with and without antibiotics (cefotaxime and meropenem) to select resistant bacterial isolates. *E. coli* ATCC 25922 was used as a control strain. Lactose-fermenting colony forming units (CFU) were selected as representative coliforms (fecal contaminant indicators) for susceptibility testing by disk diffusion method. Identification tests included chromogenic agar, oxidase and SIM tests as an exploratory approach, to further characterization. Two carbapenem-resistant isolates were randomly selected from CFU growing in carbapenem added media, for further investigation using the Carbapenem Inactivation Method (CIM). **Results:** Several CFU were obtained in selective media containing antibiotics, indicating the presence of resistant microorganisms in environmental samples. Five representative isolates were randomly selected for further characterization. Two CIM positive isolates showed carbapenem degradative mechanism. Preliminary identification suggested isolate A as *Klebsiella* spp. and isolate B as *Pseudomonas* spp.. Although few analyzed samples, these were enough to detect this alarming contamination. **Conclusions:** These findings highlight the presence of carbapenem-resistant bacteria in the water stream reaching the beach, creating a hotspot of contamination of bathing seawater with these resistant threats. We will need a wide view, including a One Health approach, to understand the origin of this dangerous contamination.

Keywords: Enterobacterobacterales, β-lactam antibiotics, Extended-spectrum beta-lactamases, Carbapenemases, Multidrug resistance.

24967 | From insect to table: What Bacteriological Hazards are on the Menu?

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Background & Aim: Alternative proteins, including plant-based and insect-derived sources, are increasingly demanded due to environmental concerns, ethical issues related to animal protein consumption, and the need to sustainably meet the nutritional requirements of a growing global population (1). Edible insects have emerged as a promising solution, offering high protein content, essential vitamins and minerals, and lower greenhouse gas emissions compared to traditional livestock production (1). This study aims to identify and characterize the microbiological hazards associated with the production, processing and commercialization of insects for human consumption. **Methods:** A literature review was conducted using PubMed, Google Scholar, Scopus, Web of Science and guidelines from EFSA, FAO and the European Commission, focusing on the search terms “edible insects” and “microbial contamination”. **Results:** Analysis indicated that while the chitinous exoskeleton of insects may retain spores of *Bacillus* and *Clostridium*, or bacteria such as *Listeria monocytogenes* and *Escherichia coli*, as well as small debris, sieving substantially reduced their presence (2). Nevertheless, blanching and drying steps effectively decreased water activity to levels incompatible with the survival of pathogenic microorganisms (3). No specific pathogens were detected that could compromise the safety of insect-based food products, assuming feed complied with EFSA standards and hygiene practices are adequately applied throughout the production chain. **Conclusions:** This means that when produced under controlled hygienic conditions and following EFSA feed and processing standards, insect-based foods present a low microbiological risk to consumers. These findings reinforce the importance of strict safety and quality control and highlight the potential of edible insects as a sustainable, safe alternative protein source for human nutrition. However, further investigation is needed to clarify the currently available information.

Keywords: insects, alternative protein, microbiological risks

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24439 | Ichthyoplankton and Microplastics in Portuguese Estuaries

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The early stages of fishes represent a critical phase for the survival and recruitment of fish populations, being highly vulnerable to (a)biotic factors and anthropogenic pressures. To increase their chances of survival, several marine species use estuaries as nurseries to maximize the survivorship of these stages. However, estuaries are increasingly subject to anthropogenic pressures, given their proximity to urban areas, with inputs of several contaminants such as microplastics. Microplastics (MPs - plastic particles < 5 millimeters) can cause various direct or indirect complex effects on ecosystems, namely compromising the development of early life stages of fishes and even their survival. This study aims to investigate the spatial-temporal distribution patterns of larval fish assemblages, and MP pollution of two Portuguese estuaries, the Douro and the Lima estuary. To achieve this aim, seasonal samplings have been carried out, with four sampling stations along each estuarine-coastal gradient. At each sampling station, planktonic tows were performed sub-superficially with a planktonic net of 500um mesh-size. In the laboratory, after sorting, fish larvae were quantified and identified to the lowest possible taxon. The remaining sample was subjected to a protocol [1] to isolate and quantify the MPs. Retrieved MPs were afterwards characterized by type, size and color. Preliminary results of two seasons, summer and autumn 2025, showed that families Gobiidae, Labridae and Atherinidae were the most abundant, representing 93,6 % of the total larval fish assemblage. MPs collected were mainly fibers and fragments of blue color and sizes up to 2 mm. Future results will allow to assess potential temporal and/or spatial overlap of MPs and fish larvae, in order to understand the level of MP contamination in each estuary and assess potential ecological impacts of this contaminant on the early life of fishes.

Keywords: Ichthyoplankton; Fish larvae; Microplastics; Estuaries.

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24399 | Impact of neonatal infection by Group B Streptococcus on the host´s immune system

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Neonatal infections remain a major global health problem and account for a substantial number of life-threatening conditions, including sepsis. Commensal bacteria such as *Streptococcus agalactiae* (Group B streptococcus, GBS) and *Escherichia coli* become pathogenic due to the immaturity of the neonatal immune system and are the main causative agents of these infections. Individuals who survive neonatal infection often exhibit impaired immune responses later in life, although the underlying mechanisms remain poorly understood. The thymus, the primary organ for T-cell development, is highly active in the neonatal period and plays a crucial role in immune development and self-tolerance. Although previously considered immune-privileged, it is now known to be susceptible to infection. However, the long-term effects of neonatal infection on thymic function and immune maturation remain largely unexplored. To investigate the impact of neonatal GBS infection on thymic function and T-cell responses, we used a previously established mouse model that recapitulates human neonatal GBS infection. In this model, mice were naturally infected at delivery, and thymic and T-cell populations were analyzed at defined time points. Thymus and spleen were collected for microbiological and immunological analyses, and immune cell populations were characterized by flow cytometry. Our results show that GBS colonizes the thymus shortly after birth, inducing severe thymic atrophy with a marked reduction in thymic and T-cell populations. These alterations persisted two weeks after infection despite bacterial clearance. Furthermore, regulatory T-cell differentiation and Aire$^+$ thymic epithelial cells were significantly reduced, and infected mice exhibited splenic T-cell lymphopenia and decreased regulatory T-cell frequencies. In conclusion, neonatal GBS infection induces sustained thymic dysfunction and long-lasting immune alterations, providing mechanistic insight into reduced immune fitness following early-life infection.

Keywords: Bacterial infection, Immunological response, Mouse model, Thymus, T cell regulation

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24677 | Integrating Culture-Dependent and Independent Profiling to Uncover Fecal Microbiota and *Campylobacter* in Broiler Chickens

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Background & Aim: Poultry production is a major reservoir of zoonotic pathogens, responsible for nearly 60% of human infectious diseases (1). Broilers often carry *Campylobacter jejuni*, *Campylobacter coli*, and non-typhoidal *Salmonella spp.* without clinical signs, facilitating contamination of the food chain (1). In Portugal, high levels of antimicrobial resistance (AMR) have been reported in zoonotic isolates from poultry systems (2,3), reinforcing concerns within the One Health framework (4). Although surveillance programs are established, the link between farm performance and zoonotic burden remains unclear. This study aims to compare microbial load and the presence of *C. jejuni* and *C. coli* in poultry farms with contrasting performance metrics from the same origin. **Methods:** Three high-performing and three low-performing broiler houses will be selected. Fecal samples will be collected over one production cycle, encompassing three representative time points (0, 14 and 35 days). Microbial detection and quantification will follow ISO culture-based methods, complemented by conventional PCR and quantitative real-time PCR targeting *Campylobacter spp., C. jejuni*, and *C. coli*. **Results:** Preliminary results from 18 samples show that 38.9% (n=7) were positive for Campylobacter spp. via culture-based methods. In high-performing farms (A, B, C), 66.7% of the samples showed no Campylobacter growth, with the highest load reaching $7.7x10^4$ CFU/g. Conversely, low-performing farms (X, Y, Z) showed a higher frequency of positive samples (44.4%), although the maximum microbial load recorded in this group was $4.1x10^4$ CFU/g (4.61 log10 CFU/g). These initial findings demonstrate that Campylobacter is present in both production systems, but suggest a slightly higher prevalence in lower-performing units. **Conclusions:** These preliminary

data indicate that Campylobacter colonization is a persistent challenge regardless of farm performance, though a trend toward higher prevalence in lower-performing broiler houses is emerging. As the study progresses, the integration of molecular methods (PCR/qPCR) is expected to provide higher sensitivity, further clarifying the link between productive efficiency and zoonotic burden within the One Health framework in Portuguese poultry production.

Keywords: *Campylobacter* spp.; Zoonotic pathogens; Broiler production; One Health; Farm performance

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24684 | MALDIBANK: Optimization of Sample Preparation Protocols for MALDI-TOF MS Identification of Cyanobacteria

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Background & Aim: MALDIBANK aims to develop a standardized, high-quality MALDI-TOF MS spectral database for microbial identification. Spectral reliability strongly depends on sample preparation, particularly for environmental microorganisms such as cyanobacteria, which often present resistant cell structures. This work aimed to determine the most suitable extraction protocol for MALDI-TOF analysis of cyanobacteria by comparing two methods: (i) freeze-thaw combined with bead-based mechanical cell lysis (Precellys) and (ii) a solvent-based protocol using ethanol 70% (ET) or acetone (AC) washes followed by protein extraction with formic acid 70% and acetonitrile. **Methods:** Prior to extraction, cyanobacterial cultures were centrifuged and washed. In the first protocol (FT), cells underwent two freeze-thaw cycles and bead-based disruption, followed by centrifugation and supernatant recovery. In the second protocol, biomass was washed twice with ET or AC, dried, and extracted with formic acid 70% and acetonitrile. Samples were applied to MALDI plates with matrix and analyzed in the 2,000-20,000 m/z range. Spectral quality was evaluated based on peak intensity, peak number, signal-to-noise ratio and reproducibility. **Results:** Preliminary observations show differences in spectral profiles between protocols. FT produced higher peak richness and signal intensity in the region between 3,000 and 8,000 m/z, suggesting enhanced protein release but increased baseline noise. AC and ET generated cleaner baselines and well-defined dominant peaks. No relevant differences were observed between acetone and ethanol washes, as similar reference peaks were detected. **Conclusions:** All protocols are suitable for MALDI-TOF MS analysis but produce different spectra characteristics: mechanical disruption improves extraction, whereas the solvent-based methods enhance spectral clarity. Ongoing work aims to identify the most robust and reproducible approach to producing standardized reference spectra within MALDIBANK.

Keywords: MALDI-TOF MS; Cyanobacteria; Sample preparation; Spectral optimization

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24484 | Microbial colonization of marine tunicates – opportunities for bioactive compound discovery

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Background & Aim: Ascidians, a well-presented class of Tunicates, known for their complex host-microbiome relationships, usually harbor different cell types in their tunic, together with different microbial symbionts, which can produce various bioactive compounds [1,2,3,4,5,6]. Interestingly, some ascidian species show lesser susceptibility to epibiosis on their tunic than others, raising the question of how differences in microbial community composition, and/or their by-products, influence epibiosis. This study therefore aims to explore the differences in the tunic-associated microbiome and metabolites of two ascidian species with different epibiosis susceptibility. **Methods:** Seawater samples and five replicates of two ascidian species (*Styela clava* and *Clavelina lepadiformis*) were collected, COI-verified and their tunics were dissected. A methanolic crude extract of the tunic tissue was prepared and analyzed by LC/MS-based untargeted metabolomics [7]. Both the tunic and the seawater samples were analyzed using metatranscriptomic analysis of biosynthetic gene cluster (BGC) expression and metagenomic reconstruction of microbial genomes [8,9]. **Results**: Preliminary results show distinct metabolite-cluster patterns between the two ascidian species, indicating potential for the discovery of compounds associated with epibiosis prevention/promotion. Regarding metagenomics, the expected differences in tunic-associated microbiomes will be linked to their metabolomic profiles. **Conclusion:** This study provides insights into species-specific microbiomes and secondary metabolites, providing the groundwork for future investigations of their roles in epibiosis.

Keywords: Ascidians; Epibiosis; Microbiome; Untargeted metabolomics

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24874 | Parasites of seabass (*Dicentrarchus* labrax) of Peniche and Algarve

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Background & Aim: Parasites are widely recognized as effective biological tags for distinguishing fish stocks, as fish acquire specific parasites within defined endemic areas, enabling researchers to track population movements or determine stock origin. In this preliminary study, we investigated the utility of parasites for differentiating stocks of *Dicentrarchus labrax* from Portugal. **Methods:** Seabass (*Dicentrarchus labrax*), from two sampling locations Algarve and Peniche, were fished between January ad March 2025. A parasitological survey was done in its major organs, including gills, digestive tract, liver, heart and spleen, were examined and the recovered parasites were preserved in 70% ethanol for later identification [1]. Prevalence and mean abundance were calculated for each parasite taxon (Algarve and Peniche together, as preliminary data), and comparative statistical analyses will be conducted for taxa meeting the minimum criteria required for a reliable biological tag. **Results:** For the n=32 fish analysed, so far, we recorded 6 parasite taxa. The highest prevalence was observed for *Anisakis* sp. (50%) and *Diplectanum aequans* (46.9%), but the latter showing the highest mean ± SD abundance (8 ± 14). Digenea, *Caligus* sp. *Lernanthropus kroyeri,* and *Serranicotyle labracis* presented lower prevalence values (15.6-18.8%) and low abundances. Preliminary patterns analysis suggest heterogeneous parasite distribution among hosts from different localities, but more data will be analysed soon and more robust data will be presented in the final poster. **Conclusions:** The relatively high prevalence and infection levels observed for some taxa, particularly *Diplectanum aequans* and *Anisakis* sp., indicate that these parasites meet key baseline criteria for potential tools of biological tags. Future statistical analyses will determine their discriminatory ability.

Keywords: seabass; parasites; infection levels; stock discrimination; biological tags

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24394 | Quality Assessment of Untreated Water Sources in Rural Parishes of Chaves, Portugal

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Background & Aim: Safe drinking water is essential for nutrition and health[1]. Although Portugal has high public water coverage, rural populations such as those in Chaves often rely on untreated sources (wells, springs, fountains) due to infrastructural gaps, cost, or perceived quality[2]. These non-regulated sources are rarely monitored and may contain microbiological or chemical contaminants, affecting vulnerable groups. The current study extends previous research of our group[3] by enlarging the sample and integrating physicochemical parameters with microbiological analysis to strengthen the overall assessment of water quality. **Methods:** Water samples were collected from private properties (21 groundwater sources - wells, boreholes, mines) and natural sources (19 streams/fountains) across four parishes from Chaves. Analyses followed Decreto-Lei 69/2023 and ISO standards for microbiological and physicochemical parameters. **Results:** The analysis revealed widespread microbial contamination, with 87% of the samples testing positive for fecal indicators. Coliforms were most common (85% of samples; wells 86%, fountains 84%), followed by *Enterococcus* spp. (57%; wells 48%, fountains 68%), *E. coli* (45%; wells 52%, fountains 37%), and *C. perfringens* (15%; wells 14%, fountains 5%). Physicochemical parameters varied widely: pH ranged 5.30-7.56 (57.5% of water samples were acidic), nitrates <2,2-212 mg/L (exceeding legal limits in 10% of samples, indicating possible agricultural or wastewater contamination), nitrites 0.030-0.377 mg/L, conductivity 25.9-504 µS/cm, TDS 29–504 mg/L, and dissolved oxygen 5.92-10.66 mg/L. Results suggest variable mineralization and local contamination sources, with overall good oxygenation, especially in fountain samples. **Conclusions:** Untreated rural water sources showed high

microbiological contamination and occasional nitrate exceedances. Their continued domestic use may represent an under-recognized health risk, highlighting the need for improved monitoring and community awareness.

Keywords: microbiological contamination; chemical contamination; rural water sources; drinking water quality

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24630 | The microbiological risk of companion animals in veterinary hospital environments

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Background& Aim: Antimicrobial resistance (AMR) represents a growing global concern in both human and veterinary medicine. Companion animals may act as reservoirs and disseminators of antimicrobial-resistant bacteria within veterinary hospital environments. This study aims to assess the microbiological risk associated with companion animals admitted to veterinary healthcare facilities, contributing to improved biosecurity strategies. **Methods:** Perianal, axillary and fecal samples were collected from companion animals to detect and isolate clinically relevant bacteria (*Escherichia coli*, *Klebsiella spp.*, coagulase-positive *Staphylococcus*, *Enterococcus* spp. and *Pseudomonas* spp.). Isolates were identified and subjected to antimicrobial susceptibility testing. **Results:** A total of 19 samples (10 dogs and 9 cats) were analyzed. Fourteen *Klebsiella* spp. Isolates were obtained, five (36%) of which were extended-spectrum β-lactamase (ESBL) producers. Twenty *E. coli* were recovered, including three (14%) ESBL-producing strains. Additionally, 34 *Enterococcus* spp. isolates were detected, with no vancomycin-resistant *Enterococcus* spp. (VRE) identified. Six coagulase-positive staphylococci were isolated, with no detection of methicillin-resistant *Staphylococcus aureus* (MRSA) or methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). High antimicrobial resistance rates (≥50%) were observed for penicillins in *E. coli* (57%), first-generation cephalosporins in *Klebsiella* spp. (64%), fluoroquinolones in *Klebsiella* spp. (50%), and tetracyclines in *Enterococcus* spp. (51%). **Conclusions:** The detection of resistance to multiple antimicrobial classes highlights the role of companion animals as potential reservoirs of antimicrobial-resistant bacteria. These results reinforce the importance of implementing effective infection control and biosecurity measures to limit the dissemination of AMR within veterinary healthcare environments.

Keywords: Multidrug-resistant bacteria; antimicrobial resistance; companion animals; veterinary hospitals; One health

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24205 | Neurofilament light chain in plasma: diagnostic and prognostic value in schizophrenia - a systematic review

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Background & Aim: Schizophrenia is a chronic mental illness, with functional/structural brain repercussions and serious impact on those affected. The use of biologic markers in Neurology has been established, but its utility in the psychiatric field remains uncertain. This systematic review aimed to determine whether the measurement of neurofilament light chain (NfL), a marker of axonal damage, in the plasma of schizophrenic patients could have diagnostic and prognostic value in schizophrenia. **Methods:** Literature review was conducted using the electronic databases Pubmed, Scopus and Web Of Science (last searched on December 8th, 2025). The inclusion criteria were: cross-sectional studies, case-control studies, randomized controlled trials and case reports, which determined NfL plasma values in individuals with diagnosed schizophrenia, schizophrenia spectrum disorders or first psychotic episode and were *in vivo* human studies. Newcastle-Ottawa Quality Assessment Scale (adapted for cross-sectional studies) was used to assess the quality of the included studies. **Results:** A total of 11 articles were included in this review, with a total of 3026 participants. Marked heterogeneity between studies was noted. Significant positive correlations were found between higher NfL plasma values and treatment resistance/more severe schizophrenia cases. Higher serum NfL levels were shown to predict lower cortical thickness in the left and right insula, and left inferior temporal regions, in treatment resistant patients, translating more severe neurodegeneration. Significant differences were also found between NfL plasma levels in schizophrenic patients *versus* patients with neurodegenerative conditions, but not when compared to healthy individuals, denoting a potential role in differential diagnosis. **Conclusions:** Evidence found in this review solidifies NfL as a possible future marker in psychiatric clinical practice for the diagnosis, prognosis and therapeutic approach in schizophrenia.

Keywords: Schizophrenia, Neurofilament light chain (NfL), Diagnosis, Prognosis.

24221 | Impact of Non-Nutritive Sweeteners on Placental Function: An In Vitro Study Using

HTR-8/SVneo Cells

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Background & Aim: Consumption of non-nutritive sweeteners (NNS) has been increasing over the years, yet clear safety guidelines for their use during pregnancy are lacking. [1,2] Proper placentation involves extravillous trophoblasts (EVTs) invasion of the maternal uterus, and any disfunction in this process can compromise fetal growth and pregnancy outcomes. [3] NNS are generally considered safe to consume during pregnancy, although recent studies suggest that these compounds can cross the placental barrier and may correlate with adverse pregnancy outcomes. [4] Therefore, this research aims to determine whether these compounds can effectively compromise the behavior of invasive EVTs. **Methods:** The effect of a 24h-exposure of the HTR-8/SVneo cell line (a model of **human first-trimester EVTs)** to three NNS (acesulfame-K, saccharin and sucralose) on cell viability (MTT and LDH assays), proliferation (^{3}H-thymidine incorporation), migration (wound healing), oxidative stress (TBARS), and glucose (^{3}H-deoxy-D-glucose) and methionine (^{14}C-methionine) uptake was evaluated. **Results:** While metabolic activity (MTT assay) was not affected by the NNS, all the NNS, and particularly sucralose, induced membrane damage, as shown by increased LDH release. Specific effects of the NNS on lipid peroxidation were observed (TBARS assay), with sucralose increasing and acesulfame K and saccharin reducing MDA levels. Cell migration was significantly reduced by saccharin and sucralose at the highest concentrations, whereas acesulfame K had no significant effect. Cell proliferation was differentially affected, with sucralose enhancing and saccharin reducing DNA synthesis at higher concentrations. Finally, these NNS slightly reduced ^{3}H-deoxy-D-glucose uptake, and increased ^{4}C-methionine uptake. **Conclusions**: Our findings suggest that NNS interfere with multiple aspects of extravillous trophoblast functions and homeostasis, which may be of relevance for placentation.

Keywords: Non-nutritive sweeteners; Extravillous trophoblasts; Placentation.

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24241 | Neuromodulation of orbitostriatal circuitry during contextual time-reward information processing in chronic pain

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Background & aim: Impaired delay gratification has been reported in chronic pain patients, leading to a preference for smaller immediate rewards over delayed ones. Alterations in the orbitofrontal cortex (OFC) and nucleus accumbens shell (NAcSh) have been described in chronic pain. However, the contribution of the OFC-NAcSh circuit to time-reward associations remains unclear. Here, we investigated the role of glutamatergic OFC-NAc projections in decision-making using optogenetics. **Methods:** To address this, male rats were trained in a two-lever operant task: one lever delivered a smaller reward (1 pellet) after a 3-second delay, while the other delivered a larger reward (3 pellets) after a 6-second delay. Rats underwent 5-day training period under immediate reinforcement contingencies. Rats with 80% or higher completed trials were selected for stereotaxic surgery for viral vector delivery and optic fiber implantation. Subsequently, neuropathic pain was inducted using the spared nerve injury (SNI) model. Control rats underwent identical procedures without nerve injury. Performance was evaluated during days 21 and 28 post-surgery, with optogenetic stimulation. Finally, brain tissue was later collected to verify viral transfection and optic fiber placement. **Results:** Results indicate that SNI rats showed a preference for the smaller immediate reward compared to control rats. Optogenetic stimulation of OFC-NAc projections did not significantly alter lever preference in SNI rats, but control rats increased preference for immediate rewards during stimulation. **Conclusion:** The findings indicate that optogenetic activation of OFC-NAcSh projections modulates reward-based decision-making, particularly in control rats. Furthermore, the differential effects observed in neuropathic pain conditions suggest that chronic pain alters the functional contribution of this circuit to time-reward associations and highlight its potential as a target for therapeutic strategies aimed at maladaptive decision-making.

Keywords: Delayed gratification; Orbitofrontal cortex; Nucleus accumbens shell; Neuropathic pain

24263 | Development and Characterisation of PLGA Nanoparticles Encapsulating Rivastigmine for Alzheimer's Disease Therapy

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Background & Aim: Alzheimer's disease (AD) is an irreversible and progressive neurological disorder characterised by neuronal degeneration, cognitive decline, amyloid plaques, and neurofibrillary tangles, accounting for 60–80% of dementia cases worldwide and projected to reach 131.5 million by 2050 (1). A major limitation in AD therapy is the blood–brain barrier (BBB), which prevents more than 98% of small-molecule and nearly all large-molecule drugs from entering the brain (2). Rivastigmine, a clinically approved acetylcholinesterase inhibitor, presents low bioavailability due to poor solubility and rapid metabolism (3). To overcome these limitations, this study aims to develop and optimise rivastigmine-loaded PLGA nanoparticles (NPs) as a non-invasive brain targeting strategy. **Methods:** PLGA NPs were prepared by solvent displacement and electrospraying. Solvent-displaced NPs were characterised by DLS (size, PDI, zeta potential), DSC, and TEM. Electrosprayed NPs were analysed by SEM and FTIR. Encapsulation efficiency (EE) was estimated by UV–Vis spectroscopy. **Results:** Solvent-displaced NPs exhibited mean diameters around 100 nm, low PDI (<0.25), and negative zeta potential (approximately –27 mV), confirmed by TEM. DSC showed a typical amorphous PLGA profile with a glass transition within the expected temperature range and no distinct melting peak. Electrosprayed blank NPs measured 198 nm, while rivastigmine-loaded NPs averaged 220 nm. FTIR of rivastigmine and PLGA was analysed. FTIR spectra of loaded NPs closely resembled PLGA, showing no drug-specific bands, indicating successful encapsulation. EE is expected to exceed 50%. **Conclusions:** These PLGA NPs exhibited physicochemical properties suitable for effective brain delivery and good physical stability, highlighting their potential as a brain-targeted drug delivery system.

Keywords: Alzheimer's disease, Nanoparticles, Blood-brain barrier, Treatment

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24268 | Acute Pancreatitis Associated with PD-1 Inhibitors: A Disproportionality Analysis of Global Pharmacovigilance Data

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Background & Aim: PD-1 inhibitors are immune checkpoint inhibitors (ICIs) used to treat various advanced cancers. Although effective, concerns have been raised about rare but serious immune-mediated adverse events, particularly pancreatitis. This study aims to assess the potential association between PD-1 inhibitors and acute pancreatitis using real-world post-marketing data from *VigiBase*. **Methods:** A disproportionality analysis was conducted using Individual Case Safety Reports (ICSRs) from VigiBase up to 16 June 2025, accessed via VigiLyze. ICSRs were included if a PD-1 inhibitor (nivolumab, pembrolizumab, cemiplimab, or dostarlimab) was listed as suspected or interacting. Reporting Odds Ratios (ROR) and Information Components (IC) were calculated with 95% confidence (CI) and credibility intervals (CrI), respectively. Additional stratified and sensitivity analyses were performed to assess signal robustness across drugs and Preferred Terms. **Results:** We retrieved 1,578 ICSRs involving PD-1 inhibitors and pancreatic adverse events, primarily associated with nivolumab (53.6%) and pembrolizumab (46.3%). Most cases involved male patients (55.2%) aged 45–74 years and were classified as serious (97.8%), including fatal (7.0%), life-threatening (6.1%) and hospitalisation-requiring events (54.8%). Pancreatitis (61.1%), immune-mediated pancreatitis (21.1%) and acute pancreatitis (17.0%) were the most frequently reported terms. Statistically significant disproportionality signals for acute pancreatitis were observed for all four PD-1 inhibitors, with stronger associations for nivolumab (ROR: 3.70; IC: 1.87) and pembrolizumab (ROR: 3.57; IC: 1.82), and emerging but significant signals for cemiplimab and dostarlimab. **Conclusions:** The study identified potential safety signals linking PD-1 inhibitors to pancreatitis, particularly nivolumab and pembrolizumab. Findings underscore the importance of continued pharmacovigilance and further studies to confirm and quantify these associations.

Keywords: Pharmacovigilance; Spontaneous Adverse Drug Reaction Reporting Systems; Immune Checkpoint Inhibitors; Drug-Related Side Effects and Adverse Reactions; Programmed Cell Death 1 Receptor (PD-1)

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24269 | Characterization of N88S Seipin-containing IB formation and chaperone interaction network in the yeast model of human N88S seipinopathy

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Background & Aim: Seipinopathies are autosomal dominant motor neuron diseases caused by toxic gain-of-function mutations (N88S and S90L) in the endoplasmic reticulum (ER) protein Seipin (1). Misfolded Seipin accumulates in cytosolic inclusion bodies (IBs), disrupts ER proteostasis, and chronically activates the unfolded protein response (UPR), ER-associated degradation (ERAD), and molecular chaperone systems. In a humanized yeast model of N88S seipinopathy, Seipin IBs colocalize with Kar2p and the disaggregase Hsp104p, indicating coordinated protein quality control to manage misfolded Seipin. However, IB composition and associated chaperone interaction networks remain undefined. We aimed to characterize N88S Seipin IB formation, ultrastructure, and the corresponding proteostasis networks. **Methods:** Stress signaling was quantified using stress responsive PACE- and CCAAT-driven mCherry gene reporters. Quantitative proteomics compared global protein abundance in WT and N88S strains, with or without proteasome impairment (*rpn4*Δ), to identify differentially enriched chaperones and stress-response factors. Hsp104-myc nanotrap pull-downs coupled with mass spectrometry identified IB-associated proteins. Transmission electron microscopy with Hsp104 immunogold labeling assessed ER and lipid droplet (LD) morphology and IB ultrastructure in WT and N88S mutant cells. **Results:** N88S seipin expression significantly increased stress reporter activity, indicating elevated proteotoxic stress. N88S seipin-expressing cells exhibited enlarged LDs, increased ER stacking, and altered IB subcellular distribution. Proteomic and pull-down analyses identified key chaperones, co-chaperones, and proteostasis regulators associated with N88S IBs, supporting coordinated disaggregation, refolding, and degradation mechanisms. **Conclusions:** This work defines conserved chaperone networks involved in managing the N88S-induced loss of proteostasis, paving the way for identifying human orthologs as potential therapeutic targets.

Keywords: Seipin, N88S seipinopathy, Endoplasmic Reticulum (ER) stress, Protein Quality Control (PQC), Chaperones.

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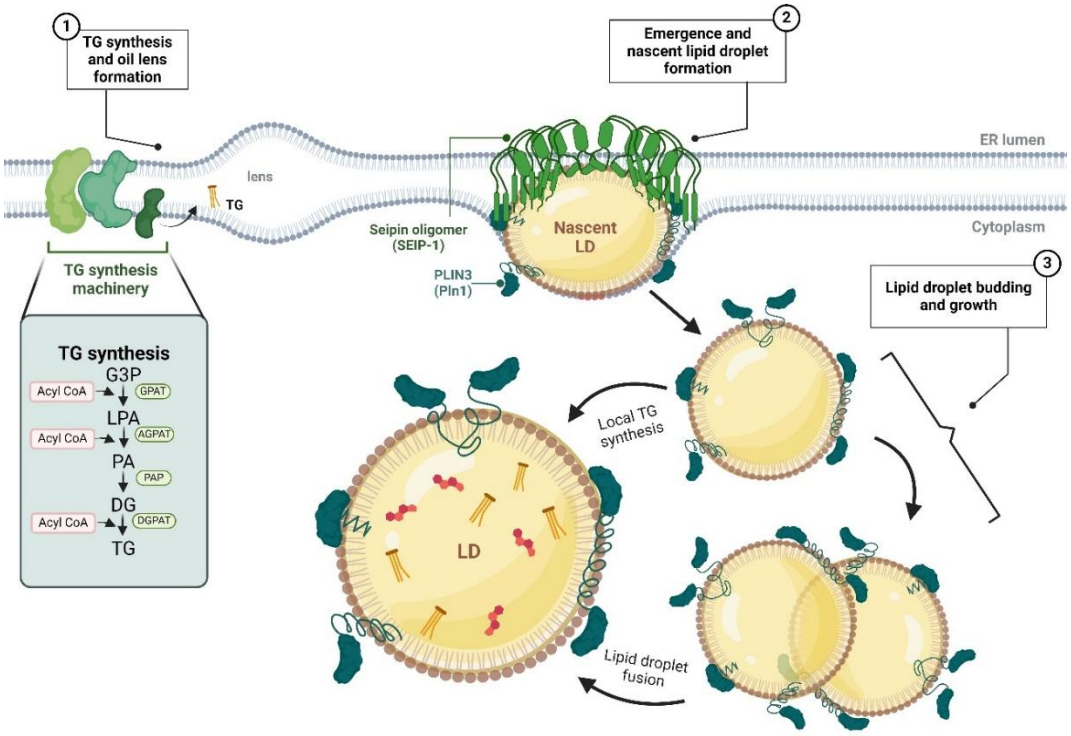

Figure 1. Schematic representation of major stages in lipid droplet (LD) biogenesis. Lipid droplets are derived from the ER through three key stages. The first stage involves TG synthesis (shown in the figure) and cholesterol esters by specific enzymes, which concentrate NL between the layers of the ER membrane. Once NL concentrations increase, they merge into a highly condensed lens structure. The second stage involves the recruitment of several proteins, including **seipin,** to the forming lens, thus promoting the emergence of the nascent LD. Finally, the third stage involves LD bud off from the ER by merging with other LDs/local TG synthesis. AGPAT, acylglycerolphosphate acyltransferase; DG, diacylglycerol; DGAT, acyl-CoA: diacylglycerol acyltransferase; G3P, glycerol-3-phosphate; GPAT, glycerol-3-phosphate acyltransferase; LPS, lysophosphatidic acid; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PLIN, perilipin.

24272 | Priming and metaplasticity in Transcranial Magnetic Stimulation across neuropsychiatric disorders – a review

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Background & Aim: Transcranial Magnetic Stimulation (TMS) has been considered a promising non-invasive treatment for multiple neuropsychiatric disorders. Several techniques have been developed in recent years to improve its therapeutic efficacy. An example of this approach is priming, a procedure administered immediately before TMS to potentiate the neural response through neuromodulatory effects. Although very promising, priming is a paradigm that requires further investigation. **Methods:** A systematic review was performed using PubMed, Web of Science, Scopus, and LILACS, in accordance with PRISMA 2020 guidelines. Electronic databases were systematically searched through January 2026 to identify studies assessing the effects of priming and metaplasticity in transcranial magnetic stimulation across neuropsychiatric disorders. Inclusion and exclusion criteria were established a priori according to the PICOS framework. **Results:** The search found 132 records, and after eliminating duplicates, 69 articles remained, of which 21 reports were selected for full-text review. Considering the inclusion and exclusion criteria, 19 studies were included in this systematic review. Among these, 12 were randomized controlled trials, 2 were retrospective observational studies, 1 was an open-label pilot study, 1 was a pseudo-randomized trial, 1 was a preliminary report, and 1 was a case study. Overall, priming interventions across magnetic, electrical, and pharmacological strategies showed the capacity to modulate cortical excitability and enhance rTMS-induced neuroplasticity. **Conclusions:** Priming neuromodulation appears to be an encouraging strategy to enhance the neurophysiological and clinical effects of rTMS across neuropsychiatric disorders, supporting the idea of neuromodulation guided by metaplasticity. Although promising, differences in stimulation protocols and study designs require further investigation using standard protocols and larger controlled trials to identify the best priming methods and their specific therapeutic benefits for each disorder.

Keywords: Transcranial Magnetic Stimulation (TMS); priming; metaplasticity; neuropsychiatric disorders.

24286 | Fecal Microbiota as a Potential Determinant of Productive Performance in Broiler Production

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Background & Aim: Broiler production systems have complex bacterial communities that influence productivity and food safety. Gut microbiota composition has been linked to feed efficiency and growth performance. (1). Indicator microorganisms such as total aerobic bacteria, spore-forming bacteria, *Escherichia coli*, Enterobacteriaceae, and *Enterococcus spp.* are widely used to assess hygiene status and microbial pressure within production systems (2). Moreover, *E. coli* and *Enterococcus spp.* are recognized reservoirs of antimicrobial resistance (AMR) genes in poultry production (3), with significant resistance levels reported in Portuguese systems (4). This study aims to detect and quantify specific bacterial groups in broiler farms with different levels of productivity. **Methods:** The best and worst performing broiler houses (sanitary and statistical units) will be selected, according to historical productivity data. Fecal and surface samples will be collected on 6 farms during one production cycle (replicates), encompassing three representative time-points: 0, 14 and 35 days. Detection and quantification of total aerobic counts, spore-forming bacteria*, E. coli*, Enterobacteriaceae and *Enterococcus* will follow ISO culture methods complemented by conventional PCR and quantitative real-time PCR. Statistical analyses will assess associations between bacterial counts and productive parameters. **Results:** Preliminary results showed no clear differences in fecal microbial loads between performance groups, with high variability observed across samples. These findings suggest that microbial abundance alone may not explain performance differences, highlighting the need for further analysis of microbiota composition. **Conclusions:** This study is expected to clarify the link between microbial burden and productive efficiency in broiler systems. The

findings are expected to improved biosecurity practices, and more targeted interventions within a One Health perspective in Portuguese poultry production.

Keywords: Broiler production; Indicator Bacteria; Antimicrobial resistance; One Health

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24295 | Cogni Party: Enhancing ABI Rehabilitation through Adaptive AI and Multiplayer Serious Games

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Background & Aim: Acquired Brain Injury (ABI) causes severe cognitive and psychosocial deficits, with spontaneous recovery and pharmacological treatments showing limitations [1]. Traditional rehabilitation struggles with adherence due to monotonous exercises lacking ecological validity [2]. While serious games (SGs) encourage the intensive practice required for neuroplasticity [3], current solutions often lack real-time difficulty adaptation and and frequently require patients to train alone. This study aims to develop a multiplayer SG with adaptive Artificial Intelligence (AI) to support ABI cognitive and psychosocial rehabilitation. **Methods:** *Cogni Party* will be a local multiplayer PC game emphasizing social interaction through party-style mini-games. It will be developed using Godot and Blender to ensure lightweight performance and accessibility across a wide range of hardware. The core innovation will integrate AI agents trained through Reinforcement Learning using the PPO algorithm and Godot RL Agents, enabling adaptive and intelligent in-game interactions. A co-design approach will be adopted, involving patients, families, and healthcare professionals through interviews, workshops, and usability testing. This process will ensure that the platform strictly meets clinical standards and accessibility requirements. **Results:** Expected outcomes include a platform that promotes social interaction through multiplayer games. In the absence of human peers, AI bots simulate opponents with similar cognitive skills. This dynamic adaptation prevents frustration, sustaining the high engagement needed to drive tangible clinical improvements. **Conclusions:** This project provides an innovative ABI rehabilitation tool combining evidence-based therapeutic principles with engaging social party game dynamics. By transitioning therapy into a shared, adaptive digital environment, it actively reduces social isolation, boosts motivation, and promotes accessible home-based training, serving as a robust complement to traditional therapies.

Keywords: Acquired Brain Injury, Cognitive Rehabilitation, Serious Games, Artificial Intelligence, Reinforcement Learning, Co-design.

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24296 | Development of a magnetically responsive Nano-Tenosome system for targeted tendon inflammation

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Background & Aim: Tendinopathy is a prevalent musculoskeletal disorder characterized by chronic inflammation and impaired tissue regeneration that lacks efficient therapies. The NF-kB pathway plays a central role in tendinopathy, making it a potential target in the development of innovative therapeutic strategies[1]. Exosomes are small vesicles secreted by cells, which are emerging as effective delivery systems for therapeutic molecules[2]. Recent evidence suggests that hypoxia enhances exosome release and alters their molecular cargo, which is involved in regulation of inflammation. Moreover, superparamagnetic iron oxide nanoparticles (SPIONs) have shown potential for remote control of inflammatory processes under external magnetic fields (MF), representing a promising strategy for tendon regeneration. Therefore, we have designed a magnetically responsive Nano-Tenosome system, integrating exosomes with SPIONs functionalized with an NF-kB inhibitor peptide to modulate inflammatory responses. **Methods**: Exosomes were isolated from human tendon-derived stem cells (hTDCs) cultured under hypoxic and normoxic conditions and analysed for exosomal markers (CD63, CD81, CD9) and associated molecules (VLA-4, Syntenin-1, Cytochrome C) by Western Blot and ELISA. SPIONs-peptide were incorporated into exosomes via magnetofection and characterized by SEM and zetasizer. Their therapeutic potential was evaluated in an inflammatory co-culture model of hTDCs and macrophages under MF, and cellular responses assessed by RT-qPCR, Western Blot, and cytokine profile for inflammatory and macrophage phenotype markers. **Results:** Preliminary results confirmed the successful isolation of exosomes, evidenced by the presence of CD63, CD81, CD9, VLA-4, Syntenin-1, and low levels of Cytochrome C, validating their exosomal identity. **Conclusions:** Overall, the successful isolation of hTDCs-derived exosomes establish a

critical step for Nano-Tenosome platform and their potential in future magnetically guided tendon inflammation.

Keywords: Exosomes, NF-kB pathway, Inflammation, Iron oxide nanoparticles, Magnetic fields

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24309 | From Bisphenol A to Its Alternatives: Hidden Risks for Human Neutrophils and Immunotoxicological Effects

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Background & Aim: Bisphenol A (BPA) is an industrial chemical frequently used in the production of polycarbonate polymers and epoxy resins in several applications such as baby bottles, water bottles, and thermal receipts. Given the well-established endocrine-disrupting properties and associated toxicity of BPA, structural analogues bisphenol F (BPF), bisphenol AF (BPAF), and bisphenol AP (BPAP), have been progressively employed in industrial applications [1]. However, their safety profile, particularly regarding their impact on the human immune system, remains unclear. Reactive species (RS) are pivotal mediators produced by neutrophils during the immune response. Nevertheless, excessive RS production can lead to oxidative damage and tissue injury. This study aims to evaluate the in vitro effects of BPA and its structural analogues (BPF, BPAF, and BPAP) on human neutrophils by assessing cellular viability and RS production. **Methods:** Human neutrophils were isolated from healthy donors by density gradient centrifugation. Cell viability was first assessed using the trypan blue exclusion assay. Subsequently, reactive species (RS) production during the oxidative burst was evaluated using the fluorescent probe dihydrorhodamine 123 (DHR 123). **Results:** BPA, BPF, and BPAF did not significantly affect neutrophil viability up to the highest tested concentration (100 µM). In contrast, BPAP exhibited pronounced cytotoxicity, inducing extensive cell death at higher concentrations (75 and 100 µM). Furthermore, exposure to BPAP and BPAF modulated RS production, indicating dysregulation of the oxidative burst and suggesting potential immunotoxicological effects. **Conclusions:** The findings of this work suggest that structural analogues of BPA may not be inherently safer than BPA itself, highlighting the need for further toxicological assessment and careful consideration before their widespread commercial use.

Keywords: Bisphenols; Human Neutrophils; Oxidative Burst; Cytotoxicity.

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24313 | Plant-Based Diets and Body Image: A Systematic Review

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Background & Aim: While Plant-based (PB) Diets are increasingly adopted for health and sustainability reasons, their association with Body Image (BI) remains unclear. We aimed to systematically review the evidence on differences in BI between individuals adhering to PB Diets and those following other dietary patterns. **Methods:** A systematic search was conducted in Medline, Web of Science, Scopus and EBSCO, yielding 18 eligible observational studies. **Results:** Findings were heterogeneous, with most studies reporting non-significant or mixed results. However, some trends emerged: vegans showed a higher drive for thinness, and semi-vegetarians appeared to be at higher risk of self-objectification. The motivation for dietary adherence - particularly weight control versus ethical reasons - emerged as a key moderating factor. **Conclusions:** Current evidence does not support a consistent association between PB diets and BI. Further research is needed, using standardised definitions and methodologies to clarify this relationship and its psychological implications.

Keywords: Plant-based Diets; Body Image; Systematic Review

24826 | Infrared Spectroscopy Analysis of Saliva from Patients with Prosthetic Stomatitis- a case-control study

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Background & Aim: Denture stomatitis occurs in patients wearing dental prosthesis. Ill-fitting dentures occlude the oral mucosa and reduce salivary flow, creating favorable conditions for *Candida* proliferation (1). Clinically, the mucosa appears erythematous and edematous and may be asymptomatic or cause pain and burning, especially in tissues in contact with dentures, such as the palate (1). Mid- infrared spectroscopy, such as Fourier-Transform Infrared Spectroscopy (FTIR), offers an innovative approach for disease identification by monitoring molecular vibrations in biological samples and identifying unique biochemical signatures. These site-specific vibrations can reveal pathological molecular alterations and potential biomarkers (2,3,4). This project aims to identify biochemical alterations in the saliva of patients with denture stomatitis and explore FTIR as a tool for pathology identification. **Methods:** A case-control design was used: the study group included patients with denture stomatitis associated with *Candida*, and the control group consisted of denture wearers without stomatitis. Saliva samples were collected following adapted published protocols (2,4). Spectral acquisition was performed using a PerkinElmer FTIR spectrometer with an attenuated total reflection (ATR) accessory, depositing a drop of saliva directly onto the ATR crystal. The spectra from both groups will be compared to identify peaks indicative of biochemical alterations (5,6). **Results:** This study is expected to reveal biochemical alterations in saliva, possibly involving changes in pro-inflammatory factors, as well as characteristic spectral patterns (7). Chemometric tools, including PCA and PLS-DA, will be used to analyse and identify relevant molecular changes. **Conclusions:** The anticipated findings may support the clinical application of FTIR as an auxiliary tool for monitoring denture stomatitis and contribute to the development of non-invasive salivary biomarkers.

Keywords: Prosthetic Stomatitis; Oral Candidiasis; Fourier Transform Infrared Spectroscopy; Saliva Analysis; Biochemical Markers

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24468 | LABORATORY APPROACHES IN THE OPTIMIZATION OF A VACCINE FOR TOXOPLASMOSIS PREVENTION

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Background & Aim: Toxoplasmosis is a highly prevalent worldwide infectious disease caused by *Toxoplasma gondii*, which can lead to miscarriage or severe, permanent fetal damage when primary infection occurs during pregnancy, and its reactivation in immunocompromised individuals may have life-threatening outcomes [1]. Although approximately one-third of the world's population is infected with *T. gondii,* there is currently no approved, safe, and effective vaccine for human use to prevent disease [2]. Previous work from our group showed that intranasal immunisation of BALB/c mice with a protein subunit vaccine composed of *T. gondii* membrane proteins (TGMP) plus CpG oligodeoxyribonucleotide (CpG ODN) adjuvant conferred protection against infection, which was associated with the induction of granzyme B (GrB)–producing cytotoxic spleen $CD8^+$ T cells, a key mediator of this protection. This study aimed to evaluate whether GrB expression in the lungs of immunised mice upon infection, one of the organs that showed reduced parasite burden, and to assess mouse lung histopathology in controls and immunised mice. It was also our goal to identify the TGMP immunogenic proteins. **Methods:** The histological analysis of the lungs was performed in hematoxylin-eosin-stained tissue sections, and GrB expression was evaluated by immunohistochemistry. The evaluation of TGMP immunogenic proteins was done by immunoblotting. **Results:** Preliminary GrB immunohistochemistry results revealed similar nuclear immunostaining in lung cells from animals of both immunised groups**.** Three immunogenic bands (22, 34, and 39 kDa) were identified in TGMP, confirming previous results and corresponding to dense granule proteins,

surface antigen-related sequences, and other proteins involved in host cell invasion and parasite persistence, reinforcing its potential as a vaccine candidate. A significant increase in lung cell aggregate number was observed in TGMP plus CpG ODN immunised mice compared to CpG ODN immunised animals, upon infection. **Conclusions:** Further optimisation of the methodologies will be necessary to better determine GrB expression in lung cells, to characterise the cells forming the aggregates found in the lungs, and to more clearly define which vaccine components are essential for inducing effective immunity against *T. gondii* infection.

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24368 | Bioprospection of Actinomycetota for the discovery of new antibiotics under One Health approach

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Background & Aim: Antimicrobial resistance (AMR) threatens human and animal health by compromising the efficacy of antibiotics [1]. Within the One Health framework, the development of new antibiotics is a crucial strategy to overcome this crisis [2]. Notably, Actinomycetota display significant potential to produce bioactive compounds with antibiotic properties [3]. This study aims to evaluate the antimicrobial activity of 10 actinobacterial isolates obtained from caves in Bosnia and Herzegovina, targeting future veterinary applications. **Methods:** Cultures were subjected to organic extraction twice with methanol/acetone and the resulting extracts were analysed for their antimicrobial activity. The extracts were screened using disk diffusion and broth microdilution assays against a panel of seven strains, comprising both reference and clinically resistant strains. Halos of inhibition, minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined. For resistant strains, potential synergistic effects with antibiotics, to which these isolates exhibited resistance, were first assessed using disk diffusion synergy assays and subsequently evaluated by determining antibiotic MICs in the presence of a fixed subinhibitory concentration of the compound. **Results:** The extracts exhibited no antimicrobial activity in disk diffusion and MIC/MBC values exceeded the maximum concentration tested. No potential synergy was observed with cefotaxime against ESBL (Extended Spectrum Beta-Lactamase) *Escherichia coli* (SA/2), nor with vancomycin against VRE (Vancomycin-Resistant Enterococci) *Enterococcus faecalis* (B3/101). **Conclusions:** Although antimicrobial and synergistic effects were not detected under the tested conditions, future studies will explore alternative biological activities, including biofilm inhibition, to further evaluate the biotechnological potential of cave-derived Actinomycetota as a resource for antimicrobial discovery addressing AMR.

Keywords: Antimicrobial resistance, secondary metabolites, Bosnia and Herzegovina caves, Actinomycetota

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24812 | Development and optimization of a LAMP-based test for early detection of sepsis

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Background & Aim: Sepsis is a global life-threatening condition caused by a dysregulated response to infection that can rapidly progress to septic shock and multi-organ dysfunction (1). Early diagnosis is critical but current diagnostic approaches rely on non-specific symptoms and laboratory tests, with time-consuming techniques like PCR, ELISA, and Western blotting, which also require specialized and costly equipment (2). Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification technique performed at a constant temperature (60-65ºC) using four to six primers, enabling amplification within 30-60 minutes. Thus, it is a rapid and sensitive method that can be performed using minimal equipment such as a heat block or incubator (3). To enable early sepsis detection, this project aims to develop and optimize a rapid, sensitive, and specific LAMP-based assay targeting the *CXCL8* gene, which encodes interleukin-8 (IL-8), a pro-inflammatory cytokine elevated in septic patients. **Methods:** The first phase of the workflow consisted of retrieving the *CXCL8* transcript from NCBI (NM_000584.4) and designing primers using the *NEB LAMP Primer Design Tool*. The primer regions were screened for the presence of genetic variants using *Ensembl* and evaluation for hairpin and primer-dimer formation was assessed through *OligoAnalyzer*. The second phase, currently underway, focuses on optimizing the LAMP reaction. **Results**: Of five candidates primer sets, one was selected for having the lowest values across dimerization energy and possibility for hairpin formation, with absence of sequence polymorphisms within primer binding region. The optimization of the LAMP assay is focusing on reaction conditions, including temperature, reaction time and primer concentrations. **Conclusions:** Once optimized, the LAMP assay could enable rapid, sensitive, and specific detection of *CXCL8* in resource-limited settings, supporting earlier sepsis diagnosis, faster clinical decision-making, and potentially reduce mortality.

Keywords: Sepsis; IL-8; isothermal reaction; LAMP method;

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24508 | Evaluation of Novel Organic Compounds and Synergistic combinations against *Candida albicans*

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Background & Aim: Invasive candidiasis is a major global health threat caused predominantly by *Candida albicans* [1]. The increasing incidence of antifungal resistance (e.g., azoles and polyenes) highlights the urgent need for new therapeutic strategies. This study aimed to evaluate the antifungal activity of novel organic derivatives synthesized from bioactive natural compounds, including Naphthalene, Betulinic Acid (BA) [2]; Protocatechuic Acid (PCA) and Gallic Acid (GA), alone, or in combination with conventional antifungals to investigate synergistic effects that enhance their efficacy. **Methods:** Antifungal susceptibility assays were performed in *C. albicans* ATCC 10231 using the broth microdilution method, according to CLSI M27 guidelines, to determine Minimum Inhibitory Concentrations (MICs) and Minimum Fungicidal Concentrations (MFC). The interaction between BA derivatives and amphotericin B was assessed by checkerboard assay and fractional inhibitory concentration index (FICI). **Results:** Naphthalene, PCA and GA derivatives showed low antifungal activity, falling within the moderate to weak bioactivity range (MIC ≥ 100 µg mL^{-1}; [3]) (data not shown). Among BA derivatives, BA-4 and BA-5 exhibited the best antifungal activity, with MIC values of 0,95 and 1,91 µg mL^{-1}, respectively, achieving 99% fungicidal activity at the same concentration (Fig. 1). Furthermore, in combination assays, both compounds enhanced Amphotericin B activity, showing synergistic effects (FICI = 0.56 and 0.62), resulting in a decrease in MIC (Fig. 1). **Conclusions:** Overall, while newly synthesized compounds exhibited limited antifungal potential, BA-4 and BA-5 demonstrated very strong bioactivity and fungicidal effects against *C. albicans*. Their synergistic interaction with Amphotericin B highlights their potential as

antifungal adjuvants and supports the further development of Betulinic acid derivatives as promising approaches to address antifungal resistance.

Keywords: *Candida albicans*; Antifungal therapy; Synergistic effect; Bioactive natural compounds

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Figure 1: MIC and MFC values of synthesized compounds against *C. albicans* (Robin) Berkhout ATCC 10231. Effect of BA and BA derivatives, alone or in combination with Amphotericin B (n=2).

Compound	MIC (μg mL^{-1}) Range	MIC (μg mL^{-1})	MFC (μg mL^{-1})	MFC/MIC ratio
Betulinic Acid	0,072-4,567	>4,567	-	-
BA-1 (RFAN1)	0,084-5,39	>5,39	-	-
BA-3 (RFAN3)	0,088-5,66	>5,66	-	-
BA-4 (RFAN4)	0,119-7,63	1,91	1,91	1
BA-5 (RFAN5)	0,12-7,60	0,95	0,95	1
Amphotericin B	0,0625-4	0,125	0,25	0,5

Drug/drug combination	MIC (μg mL^{-1})		FIC (μg mL^{-1})	ΣFIC	Remarks
	Alone	Combination			
Amphotericin B	0,125	0,0625	0,5	0,56	Synergism
BA-4	1,91	0,119	0,06		
Amphotericin B	0,125	0,0625	0,5	0,626	Synergism
BA-5	0,95	0,12	0,126		

24229 | Harnessing Microbial Power for Sustainable Soil Bioremediation

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Background & Aim: Hydrocarbon contamination in coastal ecosystems remains a significant environmental challenge. Microbial biosurfactants enhance hydrocarbon solubilization and degradation, making them promising tools for sustainable bioremediation. Marine microorganisms associated with macroalgae are naturally adapted to saline and polluted environments, representing a valuable source of robust producers. However, industrial application is still limited by low yields and high production costs.[1,2] The main goal of the present study is to identify novel biosurfactant-producing strains from coastal environments and optimize their growth and production yields using sustainable substrates. **Methods:** Microorganisms from four macroalgae samples collected in hydrocarbon-contaminated coastal areas were inoculated into Luria-Broth and onto Sabouraud Dextrose, Cetrimide, and Starch Casein Nitrate Agars. Isolates were taxonomically identified via *16S rRNA* gene sequencing.[3] Biosurfactant production was optimized through batch fermentation using industrial green liquid waste as substrate, with and without 2% (w/v) glycerol supplementation.[4] Biosurfactants were extracted via acid precipitation followed by ethanol purification.[5] **Results:** Preliminary characterization identified 11 isolates belonging to the genera *Bacillus*, *Shewanella*, *Lysinibacillus*, *Staphylococcus*, and *Cobetia*. Growth assays confirmed glycerol as an effective co-substrate, leading to a 10-fold increase in biosurfactant yield when combined with the liquid waste. These results highlight the efficacy of supplementing industrial wastes to overcome yield limitations. **Conclusions:** Indigenous coastal bacteria can effectively valorize industrial waste for biosurfactant production. Supplementation with glycerol significantly enhanced yields, supporting a cost-effective and scalable circular economy approach to bioremediation.

Keywords: Marine Microorganisms, Bioremediation, Biosurfactants, Agro-industrial Waste

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24650 | Microbiome Composition and Functioning of Environmental Cyanobacterial Biofilms

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Background & Aim: Cyanobacteria are photosynthetic microorganisms that are prolific producers of natural products. In nature, they are usually found as part of phototrophic biofilms, composed of multi-cyanobacterial species encapsulated in extracellular polymeric substances. This polymeric matrix also creates a favorable microenvironment for microbial communities to grow – the cyanosphere. In the laboratory, cyanobacteria are typically maintained as non-axenic unicyanobacterial cultures, due to their inability to grow axenically, highlighting the interactions between cyanobacteria and the cyanosphere microbiome. A lack of systematic analysis limits our understanding of how microbiome composition and microbial interactions influence cyanobacterial cultivability and chemical diversity. In this project we aim to unravel the composition and chemical interactions of cyanobacteria-dominated biofilm microbiomes. **Methods:** To achieve this, we integrated multidisciplinary approaches targeting the metagenome, metatranscriptome and metabolome of 32 cyanobacteria-dominated biofilms collected from distinct environments. This was complemented with cyanobacterial isolation and co-cultivation studies. **Results:** Preliminary metabolomic analyses revealed distinct metabolic profiles across the sampled biofilms, including features unique to some samples, without matches in public databases. Ongoing co-cultivation studies coupled with metatranscriptomic and metabolomic analysis will provide additional cues on how interactions affect the cyanobacterial biosynthetic gene clusters expression and metabolites production. **Conclusions:** Main findings are expected to contribute for advancing biotechnological prospection of cyanobacteria including the disclosure of key culturing interveners and bottlenecks that limit the growth of cyanobacteria in laboratory and Identifying triggers for cyanobacterial BGCs expression and metabolites production.

Keywords: Cyanobacteria; Microbiome; Natural Products; Multi-omics

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24695 | Monitoring Oral Staphylococcus Strains Over Time: Insights from IR Biotyper® Spectral Analysis

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Background & Aim: *Staphylococcus aureus* and *Staphylococcus warneri* are frequently detected in the oral cavity and may be associated with both colonization and infection (1, 2). Understanding the temporal stability of oral *Staphylococcus* strains is relevant for epidemiological surveillance and infection control. This study aimed to evaluate the applicability of the IR Biotyper® for differentiating Staphylococcus strains and assessing their persistence in the oral cavity over time (3, 4). **Methods:** A longitudinal study was conducted with 34 dental students from the Faculty of Dental Medicine, University of Porto. Oral samples were collected at four timepoints using a sterile swab applied in the oral cavity for one minute. Samples were cultured on mannitol salt agar and, after 24 hours, isolates were subcultured on brain heart infusion agar. Identified *S. aureus* and *S. warneri* strains were analyzed using the IR Biotyper®, based on Fourier-transform infrared spectroscopy, following standardized spectral acquisition and clustering protocols. **Results:** At this preliminary stage, 31 *S. aureus* and 13 *S. warneri* isolates were obtained from 28 participants. All isolates yielded high-quality, reproducible infrared spectra. Hierarchical cluster analysis revealed a very low spectral distance between 2 *S. warneri* isolates obtained from the same participant at the same timepoint, suggesting they represented the same strain. In addition, 2 *S. aureus* isolates collected from the same participant at different timepoints clustered closely, indicating a high degree of spectral similarity and suggesting strain persistence over time. **Conclusions:** Preliminary findings indicate that the IR Biotyper® is a promising tool for rapid differentiation and longitudinal monitoring of oral *Staphylococcus* strains. The observed clustering patterns suggest its potential to assess strain

stability and dynamics within individuals. Further analyses are ongoing to confirm these results and validate the method.

Keywords: *Staphylococcus aureus*; *Staphylococcus warneri*; IR Biotyper®; Fourier-transform infrared spectroscopy; Strain typing

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24412 | Mycobacterium tuberculosis isolates modulate levels of protective IL-1β in vivo impacting disease severity

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Background & Aim: Tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis* (Mtb), is responsible for approximately 10 million new cases and 1.4 million deaths annually[1]. Clinically, TB presents as a spectrum of severity ranging from mild to severe pulmonary manifestations, reflecting a high heterogeneity in outcomes[2]. This variability results from the diversity of the pathogen and the host's immune response. Our research group has shown that different clinical isolates modulate the immune response in distinct ways: Mtb recovered from patients diagnosed with mild TB induce robust cytokine responses in macrophages, for example, interleukin-1 (IL-1), while strains isolated from severe TB cases evade innate recognition, decreasing cytokine levels[3].**Methods:** Building upon this study, mice were infected with selected clinical isolates of Mtb ("4I2" from patients with mild TB; " 6C4" from patients with severe TB) to study the impact on disease outcome. At day 12, 30, and 75 days post-infection, infected lungs were collected for bacterial loads quantification and histopathology analysis. **Results:** Our results show that infection with Mtb 6C4 presents more bacterial loads and lower IL-1β compared to Mtb 4I2 infection across all timepoints. Our histology analysis shows that Mtb 6C4 produce higher number of lesions, yet the cellular composition of lesions is similar between the two infections. Through optical and fluorescence microscopy, we are characterizing the spatial distribution of the bacteria and their dynamics during different phases of infection. **Conclusions:** Our results showed the mouse model recapitulated the differential outcome seen in TB patients, whereas Mtb infection associated with lower IL-1 induced higher bacterial burden and more lesions compared to Mtb 4I2. These data reveal the importance of pathogen diversity in determining the severity of the disease.

Keywords: Tuberculosis; Pathogen Diversity; Infection; Histopathology

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24466 | Taxonomic Identification of Marine Actinomycetota by MALDI-TOF Mass Spectrometry

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Background & Aim: Marine Actinomycetota have high ecological and biotechnological relevance. Rapid and reliable identification at the genus and species levels is essential for prioritizing strains for further studies. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) is a fast and affordable tool for bacteria identification, however its use in environmental microbiology is limited, due to underrepresentation of environmental bacteria in commercial databases and the complex cell wall of Actinomycetota, which requires optimized protein extraction protocols (1). In Addition, culture conditions may influence protein expression and identification results. This study aims to standardize the MALDI-TOF MS identification of marine Actinomycetota and evaluate its robustness under various growth conditions, contributing to the development of a reference database. **Methods:** Ten marine Actinomycetota strains, belonging to the genera *Brevibacterium, Micromonospora, Streptomyces, Nocardiopsis, Arthrobacter, Rhodococcus, Cellulosimicrobium* and *Microbacterium*, were selected. Protein extraction using formic acid and acetonitrile protocol was compared between two different biomass sources: colonies from agar medium and cell pellet obtained from liquid culture (2). Strains were cultivated on TSA, MA, ISP2 and GYM to assess spectral variation from different culture media. **Results:** Preliminary results show that extraction from agar colonies generated spectra with more peaks and higher quality. Comparative analysis among culture media is ongoing to determine whether spectral variability affects consistent strain identification. The impact of salinity and temperature on identification robustness will be assessed subsequently. **Conclusions:** Optimizing growth conditions and sample preparation is crucial to improve MALDI-TOF MS reliability for marine Actinomycetota identification and to support its broader application in environmental microbiology.

Keywords: Marine Actinomycetota; MALDI-TOF; Taxonomic Identification

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24721 | The Role of DGAV in the Export Approval of Food of Animal Origin to Third Countries: A Comparative Analysis

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Background & Aim: International trade in food of animal origin relies on robust official control systems ensuring sanitary guarantees. Within the European Union, access to third-country markets requires export approval procedures that vary according to the importing country's regulatory model and arrangements. This study analyses the role of the National Competent Authority, Direção-Geral de Alimentação e Veterinária, in export approval through a comparative assessment of regimes applicable to Japan, Canada, Turkey and Cuba. **Methods:** An observational qualitative comparative study was conducted during a curricular internship at the Direção de Serviços de Alimentação e Veterinária da Região Norte. The methodology involved documentary analysis of EU and national provisions on official controls and export certification, examination of approval and pre-listing procedures, and comparison of four third-country regimes. Regional certification data (2025) were analyzed to contextualize activity. **Results:** The regimes exhibit distinct recognition models. Japan applies product-dependent models combining country-level recognition with mandatory listing for specific categories. Canada recognizes the equivalence of the EU framework while imposing additional product-specific requirements. Turkey introduced a mandatory pre-listing system for selected products alongside the EU-coordinated certification framework. Cuba maintains an establishment-based approval model through direct on-site evaluation missions. Certification data indicate that procedural complexity does not necessarily correlate with volume. **Conclusions:** Export approval regimes reflect heterogeneous institutional recognition and risk management models. DGAV plays a structuring role in adapting the Portuguese official control system to diverse international requirements. By ensuring compliance, DGAV strengthens trust in the Portuguese control system and facilitates access to third-country markets while safeguarding public health.

Keywords: Food of animal origin; Exports to third countries; Export approval; Official controls; National Competent Authority

24629 | Low-level laser therapy as a strategy for treating dentin hypersensitivity after tooth whitening: a systematic review

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Background & Aim: This systematic review aimed to evaluate, based on randomized clinical trials, the effectiveness of low-level laser therapy (LLLT) in reducing dentin hypersensitivity (DH) associated with dental bleaching procedures. **Methods:** The review was conducted according to PRISMA guidelines and registered in PROSPERO. Electronic searches were performed in PubMed, Scopus, and Web of Science using the descriptors “low-level light therapy” AND “dentin hypersensitivity,” adapted for each database. Only randomized clinical trials applying LLLT before, during, or after bleaching were included. Control groups consisted of placebo, no treatment, or alternative desensitizing therapies. The primary outcome was pain reduction assessed by Visual Analog Scale (VAS) or equivalent scales. Risk of bias was assessed using RoB 2, and certainty of evidence evaluated by GRADE. **Results:** A total of 555 records were identified, and after removal of duplicates, 340 studies were screened. Twenty-seven full-text articles were assessed for eligibility, and 14 randomized trials were included. Most studies reported reduction in intensity and/or duration of post-bleaching DH when LLLT was applied, especially after bleaching sessions. Some studies showed no significant differences compared to placebo, revealing heterogeneity in laser parameters (wavelengths from red to infrared), irradiation protocols, and bleaching techniques. Studies combining LLLT with desensitizing agents showed reduced sensitivity, although results were inconsistent. None of the studies affected bleaching efficacy; color change outcomes similar between groups. **Conclusion:** LLLT appears a promising strategy to reduce DH after bleaching without compromising whitening outcomes. Nevertheless, variability in irradiation parameters, treatment intervals, and standard protocols limits clinical recommendations. Standardized, well-designed randomized trials are needed to establish optimal treatment protocols worldwide.

Keywords: Low-level laser therapy; Dentin hypersensitivity; Tooth whitening

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24965 | A cadaveric study of human masseter muscle perimeter: assessment of possible associations with laterality and sex

António Pereira Rodrigues[1], João Cordeiro Mendes[1], Marta Tavares[1], Miguel Laires[1], Hélio Alves[1], André Rodrigues Pinho[1,2,3,4], Maria Dulce Madeira[1,2,3], Pedro Alberto Pereira[1,2,3]

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Background & Aim: The masseter muscle, a powerful quadrangular muscle of mastication, elevates the mandible to occlude the teeth in mastication and has a small effect in side-to-side movements, protraction and retraction [1]. Importantly, the muscles of mastication are commonly involved in temporomandibular disorders (TMD's) [2]. The present study was designed to investigate the perimeter of the masseter muscle in human donor bodies and assess potential associations with laterality and sex. **Methods:** Sixteen formalin-embalmed adult human Portuguese donor bodies were subjected to routine dissection of the head and neck. The masseter muscles were bilaterally dissected and cut along its superior fixation in order to measure the perimeter of the muscles 2 centimetres above and parallel to the lower border of the mandibular ramus. **Results:** We found no statistically significant differences regarding laterality. Regarding sex, although the median male values were greater than those found in the females, the difference was not statistically significant. **Conclusions:** This study strived to increase the knowledge of masseter anatomy. Our results are in line with those obtained by other authors which found, for instance, no significant differences in the masseter muscle thickness regarding laterality [3] and sex [4]. Our findings are especially important since there are studies reporting, for example, differences in laterality [5] and sex [6] in patients with TMD's regarding the masseter muscle thickness. It is important to highlight that these cited studies were done in live patients by using imagological methods. We acknowledge some limitations of the present study: the relatively small sample of donor bodies (limiting statistical power to detect subtle differences), the changes induced by death and embalming procedures in the

volume and general tissue morphology, and lack of clinical information related to the studied donor bodies that hinders correlations between anatomical and clinical data.

Keywords: Masseter, Perimeter, Human donor bodies, Dissection, Anatomy

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24983 | Characterization of epigenetically-regulated BEGAIN, a protein putatively involved in the pathophysiology of depression, in glial cells

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Background & Aim: Major depressive disorder (MDD), commonly referred to as depression, is a highly prevalent, heterogeneous psychiatric condition with a substantial health impact and economic burden. Converging evidence links glial dysfunction to the pathophysiology of depression, yet the role of specific candidate proteins within these glial-mediated pathological pathways remains poorly defined. Hypermethylation of Brain-Enriched Guanylate Kinase-Associated Protein (BEGAIN) gene at exon 3 has been identified in the human brain prefrontal cortex, and more specifically ascribed to astrocytes, of patients diagnosed with depression. This epigenetic modification inversely correlates with the expression levels of BEGAIN, which are significantly decreased. We aimed to characterize Begain expression patterns and subcellular localization in glial cells of the mouse cerebral cortex. **Methods:** To that effect, we quantified the two main *Begain* isoforms in cultured glial cells by RT-qPCR. In parallel, we used immunocytochemistry to study the expression and subcellular localization of the endogenous and transfected Begain protein in astrocytes and microglia in vitro. **Results:** Quantitative data from qPCR show robust expression of *Begain* in astrocytes and substantial differences in *Begain* isoform expression. Immunocytochemistry results confirmed the robust expression of Begain in astrocytes and provided relevant information about its subcellular location, with the protein appearing to concentrate almost exclusively in the nucleus. Microglial expression, on the other hand, was residual. **Conclusions:** Despite the small number of samples and the absence of formal quantification of the immunocytochemistry data, these results provide initial but critical insights into Begain biology in glial cells. Future work with a larger sample size and additional quantitative approaches will validate these results and provide further insight into the role of Begain in the context of depression.

Keywords: BEGAIN, Epigenetics; Gene expression; Depression; Glial cells.

24663 | Accounting for long-term culture effects reveals specific cisplatin resistance mechanisms in FaDu cells

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Background & Aim: Head and neck squamous cell carcinoma (HNSC) is commonly treated with cisplatin, however, therapeutic resistance remains a significant clinical challenge [1]. Although *in vitro* models of chronic cisplatin exposure are widely used to study resistance, prolonged culture itself may induce independent molecular changes [2]. The aim of this study was to generate and characterise a cisplatin-resistant HNSC cell line, and to distinguish cisplatin-specific effects from long-term culture adaptations. **Methods:** The FaDu parental cell line was exposed to increasing cisplatin concentrations up to 2 µM (FaDu Cis), while a passage-matched control was maintained without drug (FaDu control). Cisplatin sensitivity was assessed by resazurin-based viability assay with IC_{50} determination. Molecular characterisation was performed by RT-qPCR, and gene expression differences were analysed by one-way ANOVA with Tukey's multiple comparisons test. **Results:** Cisplatin caused a concentration-dependent decrease in resazurin reduction, with a rightward shift in FaDu Cis (IC_{50}=5.370×10^{-6} M) and control cells (IC_{50}=7.233×10^{-6} M), compared with the parental line (IC_{50}=2.885×10^{-6} M), indicating increased resistance. FaDu control cells exhibited increased CD44 expression (1.47±0.17, n=3, $p < 0.05$) and decreased E-cadherin expression (0.75±0.17, n=3, $p < 0.05$). In contrast, FaDu Cis cells showed markedly increased N-cadherin expression (13.89±3.18, n=3, $p < 0.05$) while maintaining E-cadherin levels (0.96±0.04, n=3). The expression of Nrf2, ABCB1, and ALDH3A1 were significantly reduced in both the FaDu control cells and FaDu Cis cells. **Conclusions:** These results highlight the importance of distinguishing culture-induced adaptations from drug-specific resistance by including passage-matched controls. Considering this control, it was visible that cisplatin resistance in this model is associated with a hybrid epithelial–mesenchymal phenotype, whereas other transcriptomic changes reflect long-term culture.

Keywords: Head and neck squamous cell carcinoma; cisplatin resistance; hybrid EMT phenotype; long-term culture adaptations.

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24268 | Acute Pancreatitis Associated with PD-1 Inhibitors: A Disproportionality Analysis of Global Pharmacovigilance Data

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Background & Aim: PD-1 inhibitors are immune checkpoint inhibitors (ICIs) used to treat various advanced cancers. Although effective, concerns have been raised about rare but serious immune-mediated adverse events, particularly pancreatitis. This study aims to assess the potential association between PD-1 inhibitors and acute pancreatitis using real-world post-marketing data from *VigiBase*. **Methods:** A disproportionality analysis was conducted using Individual Case Safety Reports (ICSRs) from VigiBase up to 16 June 2025, accessed via VigiLyze. ICSRs were included if a PD-1 inhibitor (nivolumab, pembrolizumab, cemiplimab, or dostarlimab) was listed as suspected or interacting. Reporting Odds Ratios (ROR) and Information Components (IC) were calculated with 95% confidence (CI) and credibility intervals (CrI), respectively. Additional stratified and sensitivity analyses were performed to assess signal robustness across drugs and Preferred Terms. **Results:** We retrieved 1,578 ICSRs involving PD-1 inhibitors and pancreatic adverse events, primarily associated with nivolumab (53.6%) and pembrolizumab (46.3%). Most cases involved male patients (55.2%) aged 45–74 years and were classified as serious (97.8%), including fatal (7.0%), life-threatening (6.1%) and hospitalisation-requiring events (54.8%). Pancreatitis (61.1%), immune-mediated pancreatitis (21.1%) and acute pancreatitis (17.0%) were the most frequently reported terms. Statistically significant disproportionality signals for acute pancreatitis were observed for all four PD-1 inhibitors, with stronger associations for nivolumab (ROR: 3.70; IC: 1.87) and pembrolizumab (ROR: 3.57; IC: 1.82), and emerging but significant signals for cemiplimab and dostarlimab. **Conclusions:** The study identified potential safety signals linking PD-1 inhibitors to pancreatitis, particularly nivolumab and pembrolizumab. Findings underscore the importance of continued pharmacovigilance and further studies to confirm and quantify these associations.

Keywords: Pharmacovigilance; Spontaneous Adverse Drug Reaction Reporting Systems; Immune Checkpoint Inhibitors; Drug-Related Side Effects and Adverse Reactions; Programmed Cell Death 1 Receptor (PD-1)

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24779 | Adiposity Indices as Discriminators of Metabolic Health Phenotypes Before and After Bariatric Surgery

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Background & Aim: Excess body fat and it's central distribution impair metabolic health (MetH). Bariatric surgery reduces adiposity and improves MetH. We assessed the distribution and evolution of 5 adiposity indices (exclusively anthropometric or combined anthropometric–metabolic) before and after surgery. We also evaluated their ability to discriminate MetH phenotypes and the impact of surgery type on discrimination. **Methods:** Metabolically healthy and unhealthy (MH/MUH) phenotypes were defined according to Karelis (2008) within 1,216 individuals (CRI-O, Portugal; 82.2% women; 42.6±5.5kg/m²; 43.8±10.5y) undergoing Roux-en-Y gastric bypass (RYGB) or sleeve gastrectomy (SG). Percentage of MH/MUH individuals above the established cut-off values were assessed, at baseline (B_0) and annually up to 4y post-surgery (F_{1-4}), for lipid accumulation product (LAP) and visceral adiposity index (VAI; mixed anthropometric–metabolic indices) as well as waist-to-hip ratio (WHr), abdominal volume index (AVI) and body adiposity index (BAI; purely anthropometric) (Li, 2022). **Results**: At B_0, >75% of individuals had values above cut-offs for all indices that were significantly different for MH *vs* MUH for VAI–RYGB and VAI–SG (81.6% *vs* 96.2%, $p<0.001$, and 80.0% *vs* 95.9%, $p<0.005$, respectively), LAP–

RYGB (84.2% *vs* 96.4%, $p<0.001$), WHr–RYGB (83.3% *vs* 93.1%, $p=0.033$), and WHr–SG (76.9% *vs* 95.0%, $p=0.007$). At follow-up, analyses were stratified by normal BMI and overweight/obese BMI groups. For the latter, a similar pattern was observed for LAP–RYGB and LAP–SG at F_1 (44.3% *vs* 84.8%, $p<0.001$, and 54.3% *vs* 83.8%, $p=0.007$, respectively), for LAP–RYGB at F_2 (42.2% *vs* 84.6%, $p=0.007$), for BAI–SG at F_1 (77.1% *vs* 94.3%, $p=0.040$), and for BAI–RYGB at F_2 (52.2% *vs* 84.6%, $p=0.036$). In the post-surgery normal BMI group, discrimination of MetH phenotypes by adiposity indices differed. **Conclusion:** Discrimination of MetH phenotypes by adiposity indices is feasible but depends on the index, BMI category, type of surgery, and time point.

Keywords: Metabolic phenotype, Obesity, Bariatric surgery, Adiposity indices

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24623 | An Automated Pipeline for 3D Structural Modelling of DNA Probes for Pathogen Detection

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Background & Aim: Pathogen detection in hospital and environmental settings is critical, particularly because respiratory diseases affect populations worldwide. Conventional diagnostics are slow and can promote the use of broad-spectrum antibiotics, contributing to antimicrobial resistance [1]. DNA probes offer a rapid, specific alternative for detection [2]. Their secondary and tertiary structures strongly influence hybridization efficiency and binding specificity [3]. This study aims to develop an automated pipeline for predicting the 3D structure of single-stranded DNA probes, facilitating studies of probe binding to target DNA sequences. **Methods:** An automated workflow was developed to predict 3D structures of DNA probes targeting bacterial and viral pathogens. Secondary structures were predicted using ***RNAfold*** with DNA-specific thermodynamic parameters and minimum free energy (MFE) calculations. Predicted secondary structures were converted into 3D models using the ***NSP toolkit*** through knowledge-based fragment assembly. Conformational optimization and clustering were performed to obtain representative structures, which were then reconstructed at the all-atom level and energy-minimized using the ***AMBER OL15*** force field. Five independent predictions were generated per probe to assess structural variability. **Results:** The workflow successfully

generated realistic 3D conformations of DNA probes. Stable secondary structures were predicted, followed by clustering, energy minimization, and ranking of probe stabilities using total potential energy values. The refined structures will be prepared for future docking studies against bacterial and viral genomic targets. **Conclusions:** The automated pipeline integrates thermodynamic prediction, 3D model generation, conformational refinement, and energy minimization for structural modeling of DNA probes. This approach enables improved probe design and supports the development of rapid, reliable pathogen detection strategies.

Keywords: DNA Probes; Bioinformatic Pipeline; Three-dimensional Structure Prediction; Pathogen Detection;

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24660 | Assessing Study Design of Probiotic Clinical Trials in Children with ADHD

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Background & Aim: Attention-Deficit/Hyperactivity Disorder (ADHD) is a neurodevelopmental condition affecting 5-7% of children globally. Limitations of pharmacological treatments have increased interest in non-pharmacological interventions. The gut–brain axis modulates cognition and behaviour, with gut dysbiosis being associated with ADHD. Probiotics have potential as adjunct therapies, with some studies testing their efficacy. This study compiles and critically analyses clinical studies on probiotics for young patients with ADHD, highlighting similarities and differences between them. **Methods:** A literature search was conducted using the PubMed and Google Scholar databases. Articles were evaluated following specific criteria, including study type, population and age range, inclusion/exclusion criteria, evaluation/intervention period and procedures, and biological samples collected. **Results:** Ten studies have been included. Nine were randomized controlled trials, with one single-arm study. Heterogeneity was observed across trials including age ranges, sex distribution (one study included only boys), and selection criteria (one study excluded any ADHD-medicated participants, and others required a washout period). Intervention/evaluation periods differed in duration, number of timepoints, and probiotic strains used. Study procedures also diverged in the cognitive/behaviour tests and questionnaires used, and only 2 studies considered dietary habits. In sample collection, only 4 studies collected blood, and 3 collected faecal samples. Mixed results were found with some showing improvement in ADHD symptoms (e.g. hyperactivity) and others opposite/neutral results. These differences highlight a lack of standardization, potentially limiting the robustness of the results when applied to the entire young ADHD population. **Conclusions:** There is an urgent need for more robust, standardized, and inclusive study protocols when testing probiotic treatments in ADHD

Keywords: ADHD; Gut-brain Axis; Clinical Trials; Probiotics.

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24986 | Assessment of vegetarian option availability in "Dish of the Day" menus at commercial establishments around the University of Porto

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Background & Aim: Vegetarian dietary patterns are associated with several health benefits and the availability of such options may influence food choices, particularly among university students. Higher education institutions are required to provide a vegetarian option in their food services. This study aimed to characterize the availability of vegetarian options in "Dish of the Day" menus offered by public catering establishments located near the University of Porto and to explore associations with other menu characteristics. **Methods:** Data were collected through a questionnaire assessing establishment type and menu characteristics, including soup availability, vegetarian option and average menu price**.** Statistical analysis were conducted using IBM SPSS 30.0. **Results:** The study included 82 establishments, of which 42 (51%) were restaurants, 36 (44%) were cafés/snack bars/bakeries/pastry shops, and 4 (5%) offered fast-food/takeaway services. Overall, 55 establishments (67%) offered soup and 23 (28%) offered a vegetarian option. The availability of vegetarian options differed significantly according to the type of establishment ($p = 0.002$), being higher in fast-food/takeaway establishments (75%) and restaurants (38%) than in cafés (11%). No significant association was observed between the availability of vegetarian options and soup ($p = 1.000$). The average menu price was €9.95 in establishments offering a vegetarian option and €8.50 in those without it, but the difference was not statistically significant ($p = 0.051$). **Conclusions:** The availability of vegetarian options in "Dish of the Day" menus varies according to the type of establishment surrounding the University of Porto. However, it was not associated with soup availability. There was a tendency for menus with a vegetarian option to be more expensive. Studies with larger and more representative samples should be carried out.

Keywords: Vegetarian option, catering establishments, university students.

24475 | Between Cilia and Genes: Structural and functional study of Primary Ciliary Dyskinesia in an affected family

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Background & Aim: Primary ciliary dyskinesia (PCD) is a rare genetic disease caused by structural and functional defects in motile cilia, leading to impaired mucociliary clearance and dysfunction of other cilia-dependent processes such as left-right patterning and fertility [1,2]. The aim of this study was to evaluate the ciliary structure and function in a patient clinically diagnosed with PCD. **Methods**: Using ciliated cells obtained by nasal brush [3], we evaluated ciliary function by Highspeed videomicroscopy (HSVM) of cultured cells [4,5], the ultrastructure of cilia by transmission electron microscopy (TEM) [5,6], the exome by next generation sequencing (WES) [7], protein expression by immunofluorescence (IF) [5,8], and protein structure by structural models obtained by X-ray crystallography and cryo-electron microscopy [9]. The family was screened by Sanger sequencing [10], HSVM and IF. **Results**: The patient exhibited total ciliary

immotility, a predominance of outer dynein arm absence, two heterozygous missense variants of unknown significance in genes *CCDC40* (c.1575T>G p.(His525Gln)) and *GAS2L2* (c.1780G>A p.(Gly594Arg)), and a significant decrease in CCDC40 expression but not of GAS2L2. Regarding protein CCDC40 (assembly and organization of the internal dynein arms and of the nexin-dynein complex), the substitution of His525 by Gln was predicted to affect the binding of the lateral chains, weakening the stabilization of the complex. The semen analysis revealed normal semen parameters. The family study displayed altered HSVM values (increased in the father, decreased in the mother), decreased CCDC40 expression in the father, with Sanger sequencing detecting the two variants in the father. **Conclusions:** Given these results, the expected situation of compound heterozygosity remains in question. To discover the cause of the disease, sequencing of the entire *CCDC40* gene and its mRNA is underway, with the aim of deciphering the mechanism underlying this unexpected genotype-phenotype association.

Keywords: Primary ciliary dyskinesia; CCDC40; GAS2L2; Cilia

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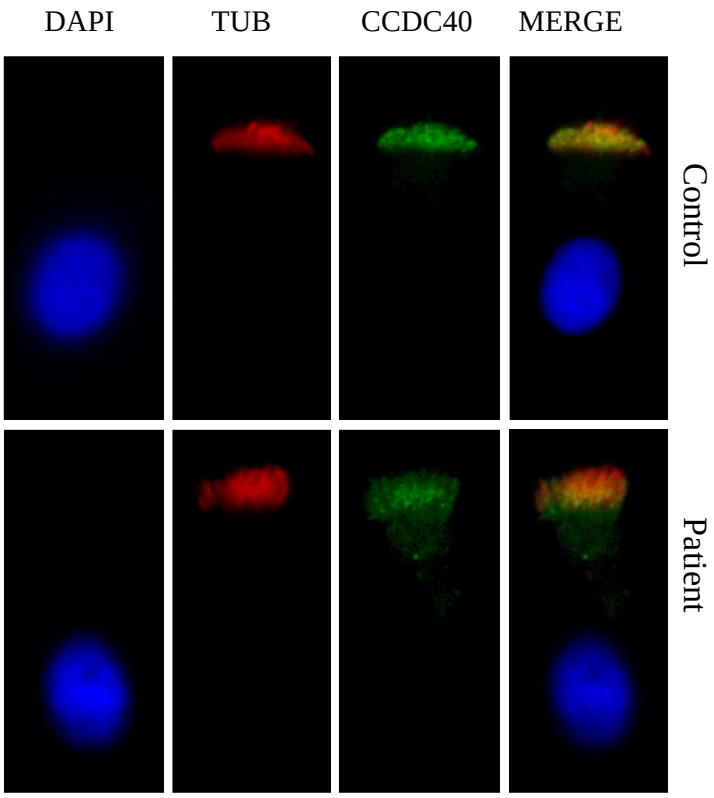

Figure 1. Immunofluorescence analysis of respiratory epithelial cells from the patient. Cells were stained with DAPI (blue, nuclei), anti-acetylated tubulin (TUB, red, ciliary axonemes), and antibodies against the corresponding axonemal protein (green). In all panels, the Merge images showed co-localization. In the patient, fluorescence localization of CCDC40 was preserved at the distal cilia region and at the basal body region, but additionally presented fluorescence signals in the cytoplasm; the fluorescence intensity of CCDC40 was significantly reduced.

24425 | Beyond therapeutic effects: An ex vivo approach to assess the impact of corticosteroids on bone tissue

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Background & Aim: Corticosteroids are widely used in clinical practice due to their anti-inflammatory, immunosuppressive, and antiproliferative effects [1]. However, their prolonged use, particularly via systemic administration, is associated with alterations in the bone tissue, including reduced bone mineral density [2]. Inhaled corticosteroids, such as fluticasone, exhibit high local anti-inflammatory potency and low systemic bioavailability and are frequently prescribed for asthma and chronic obstructive pulmonary disease [3]. Nevertheless, their impact on bone tissue remains controversial. This study aims to comparatively evaluate the effects of fluticasone and dexamethasone – both often prescribed for asthma - on bone tissue, hypothesizing that corticosteroids employed in distinct routes of administration may induce different effects. **Methods:** An ex vivo organotypic embryonic chicken femur model will be used. Femora will be cultured at the liquid–gas interface and exposed to logarithmic concentrations of fluticasone or dexamethasone. Histomorphometric analysis will be carried out using micro-computed tomography to quantify tissue volume, bone volume, trabecular thickness, and bone mineral density. Histological analysis will include observation of cartilaginous and collagenous tissues using Alcian blue and Sirius red staining, respectively. Quantitative data will be analyzed using one-way ANOVA ($p < 0.05$). **Results:** Alterations in bone mineral density and mineralized tissue volume are expected in corticosteroid-exposed groups, potentially in a concentration- and drug-dependent manner. **Conclusions:** This study may clarify the impact of corticosteroids administered via different routes on bone tissue, highlighting their potential relevance in Dentistry. Since alterations in maxillary bone mineral density can aggravate periodontal disease and compromise osseointegration, prolonged inhaled corticosteroid therapy may be an additional risk factor to consider in clinical practice.

Keywords: bone mineral density; asthma; fluticasone; dexamethasone; embryonic chicken femur

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24503 | BIOLOGICAL IMPACT OF LISDEXAMFETAMINE DIMESYLATE IN C. ELEGANS: A STUDY ON SURVIVAL, DEVELOPMENT, AND LONGEVITY

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Background & Aim: Lisdexamfetamine dimesylate (LDX) is a prodrug of *d*-amphetamine used to treat attention-deficit/hyperactivity disorder (ADHD) [1]. Despite its clinical use, some knowledge gaps remain regarding its toxicity. To address these gaps, we used *Caenorhabditis elegans*, a rapid, tractable model with a short lifespan, transparent body, and well-characterized development, which allows efficient preliminary toxicological assessment [2]. **Methods:** Synchronized L1-stage animals of the *C. elegans* DC19 [bus-5(br19)] strain (~200/condition) were exposed, in liquid medium (48-well plates), to increasing concentrations of LDX (from 10 µM to 10 mM in the survival assay and 0.5 and 1 mM in the development and lifespan assays). Following a 72-h incubation in M9 buffer containing OP50 bacteria as a food source, worms were analyzed using a stereomicroscope. Survival was assessed by counting the number of live and dead worms after the exposure period. Development was evaluated by measuring the body length using Fiji software by tracing a segmented line along the longitudinal axis of each animal. For lifespan analysis, animals were monitored every two days throughout their lifespan, and dead animals were counted over time. **Results:** Exposure to LDX did not significantly reduce animal survival at concentrations up to 2 mM. In contrast, exposure to 3, 4, 5, and 10 mM LDX significantly decreased animal survival in a concentration-dependent manner [percentage of surviving animals: 0 mM=96,98±4,1% (p>0,9999); 3 mM=47.71±26.2% (p=0.0157); 4 mM=42.87±29.1% (p=0.0138); 5 mM=40.37±27.2% (p=0.0003); and 10 mM=1.663±3.426% (p<0.0001)]. No significant alterations in development were observed in F0 animals exposed to

sublethal concentrations of 0.5 and 1.0 mM of LDX over time or in their F1 progeny, nor did such exposure result in a significant reduction in animal lifespan. **Conclusions:** Overall, the results across the different assays indicate that LDX is relatively well tolerated at low doses in this experimental model.

Keywords: Stimulant; Amphetamine; Attention-Deficit/Hyperactivity Disorder (ADHD); Toxicity; Prodrug

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24866 | Biomimetic Bacterial Nanocellulose Scaffold Designed for Osteochondral Applications Ensures Progenitors Cytocompatibility

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Background & Aim: Osteochondral defects pose a significant clinical challenge, commonly associated with age. Such conditions are related to the wearing down of articular cartilage and subchondral bone in joints, thus leading to pain, disability and socio-economic burdens. Current medical devices face structural and functional limitations in replicating the complex mechanical gradients and biological properties of osteochondral tissues [1]. Taking advantage of bacterial nanocellulose (BNC) as a biocompatible hydrogel option to mimic the extracellular environment, this study aims to replicate bioinspired microarchitectures to further enhance outcomes in bone and cartilage repair [2]. **Methods:** Laser-structured BNC scaffolds with controlled gradient porosity were used as 3D substrates for mesenchymal progenitor cell culture. After seeding, cell viability was assessed through Live/Dead assay (Cell Tracker Red CMTPX and Zombie Green™) under various differentiation conditions and timepoints. In order to explore scaffold's cytocompatibility during differentiation, Z-series images were acquired via confocal microscopy, and then analysed using Image J. **Results:** The results reflect high cell viability, homogeneous infiltration and distribution within the scaffold porous microarchitecture, as well as cell morphology and matrix characteristics consistent with osteochondral commitment. **Conclusions:** These findings demonstrate the aforementioned scaffold exhibits good cytocompatibility and provides a supportive microenvironment, proving its potential for osteochondral tissue engineering strategies.

Keywords: Scaffold, Bacterial nanocellulose, Osteochondral, Regeneration, Cell viability.

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24921 | Caregiver knowledge and oral hygiene practices in patients with cerebral palsy: an exploratory study

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Background & Aim: Cerebral palsy (CP) is the most common cause of motor disability in childhood. Neuromuscular limitations frequently compromise oral hygiene autonomy, contributing to a high prevalence of periodontal disease [1–4]. Due to the functional dependence of many individuals with CP, caregivers play a crucial role in maintaining oral health. However, their effectiveness may be limited by behavioral barriers and insufficient oral health literacy [2,5,6]. This exploratory study aimed to describe oral health knowledge and difficulties with hygiene practices among caregivers of individuals with CP at the Cerebral Palsy Association of Vila Real (APCVR). **Methods:** This study is part of an ongoing cross-sectional study conducted at APCVR. Considering the current sample size (n = 20 caregivers) and the preliminary nature of the data, an exploratory analysis was performed based on responses to a self-administered questionnaire. Variables included oral health knowledge, hygiene practices, and perceived barriers to oral hygiene. Descriptive statistical analysis was performed using IBM SPSS Statistics® version 29.0 (IBM Corp., Armonk, NY, USA). **Results:** The sample (n = 20) consisted predominantly of females (80.0%), most of whom were family members (75.0%). Most caregivers (85.0%) considered oral health extremely important. Regarding knowledge, almost all participants (94.4%) identified gingival bleeding as a sign of disease, although only 33.3% recognized bacterial plaque as the main cause of gingival inflammation. Regarding practices, 70.0% reported performing oral hygiene at least twice daily. Manual toothbrushes were the most commonly used tool (65.0%), while dental floss use was limited (10.0%). The most frequently reported physical barriers were patient head movement (50.0%) and resistance to mouth opening (25.0%). Despite these challenges, 70.0% of caregivers reported feeling adequately trained to perform oral hygiene care. **Conclusions:** This exploratory analysis highlights gaps in oral health knowledge and interdental hygiene practices despite the high perceived importance of oral health. These findings suggest the need for targeted, practical training programs to overcome physical barriers and improve caregivers' oral health literacy.

Keywords: Cerebral Palsy; Periodontal Health; Caregivers; Knowledge; Oral Health

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24557 | Characterization of human AC16 cardiomyocytes during differentiation with horse serum

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Background & Aim: *In vitro* models of cardiomyocytes are scarce. AC16 cardiomyocyte-like cells have human origin; however, under standard conditions, they maintain a proliferative phenotype that does not reflect the adult myocardium's quiescent status. Although differentiation strategies aim to promote a more mature phenotype, the molecular and metabolic adaptations underlying these protocols remain insufficiently characterized. Thus, this study aimed to characterize one of the AC16 differentiation protocols described by Davidson *et al.*, focusing on intracellular ATP content, cell number, total protein levels, and total glutathione (tGSH) over the time course of differentiation. **Methods:** AC16 cells were maintained in medium supplemented with 12.5% fetal bovine serum (proliferative state) and then differentiated by replacing it with 2% horse serum (HS). In both states, evaluations were carried out. Intracellular ATP levels were quantified using a bioluminescence assay, while tGSH levels were determined using a kinetic spectrophotometric assay at the latest point (72 h in proliferative cells and 48 h in differentiated cells). The Sulforhodamine B (SRB) spectrophotometric assay (absorbance at 510 nm) and nuclear morphology assessment by Hoechst 33258 fluorescence staining are being performed at extended time points. Data were analyzed using the Mann–Whitney test or two-way ANOVA. **Results:** Differentiated cells with HS maintained ATP levels despite reduced serum levels, suggesting maintenance of cellular energy homeostasis. Furthermore, differentiated cells displayed significantly higher tGSH levels, indicating enhanced antioxidant defense capacity. **Conclusions:** The adaptation of the differentiation protocol with 2% HS results in a significant increase in cellular antioxidant capacity while maintaining vital energy levels. Future analyses

will further elucidate the maturation state of these cells, providing a more accurate model to mimic the adult myocardium in future cardiotoxicity screenings.

Keywords: AC16 cells differentiation; proliferative state; differentiation state

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24269 | Characterization of N88S Seipin-containing IB formation and chaperone interaction network in the yeast model of human N88S seipinopathy

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Background & Aim: Seipinopathies are autosomal dominant motor neuron diseases caused by toxic gain-of-function mutations (N88S and S90L) in the endoplasmic reticulum (ER) protein Seipin (1). Misfolded Seipin accumulates in cytosolic inclusion bodies (IBs), disrupts ER proteostasis, and chronically activates the unfolded protein response (UPR), ER-associated degradation (ERAD), and molecular chaperone systems. In a humanized yeast model of N88S seipinopathy, Seipin IBs colocalize with Kar2p and the disaggregase Hsp104p, indicating coordinated protein quality control to manage misfolded Seipin. However, IB composition and associated chaperone interaction networks remain undefined. We aimed to characterize N88S Seipin IB formation, ultrastructure, and the corresponding proteostasis networks.

Methods: Stress signaling was quantified using stress responsive PACE- and CCAAT-driven mCherry gene reporters. Quantitative proteomics compared global protein abundance in WT and N88S strains, with or without proteasome impairment (*rpn4*Δ), to identify differentially enriched chaperones and stress-response factors. Hsp104-myc nanotrap pull-downs coupled with mass spectrometry identified IB-associated proteins. Transmission electron microscopy with Hsp104 immunogold labeling assessed ER and lipid droplet (LD) morphology and IB ultrastructure in WT and N88S mutant cells.

Results: N88S seipin expression significantly increased stress reporter activity, indicating elevated proteotoxic stress. N88S seipin-expressing cells exhibited enlarged LDs, increased ER stacking, and altered IB subcellular distribution. Proteomic and pull-down analyses identified key chaperones, co-chaperones, and proteostasis regulators associated with N88S IBs, supporting coordinated disaggregation, refolding, and degradation mechanisms.

Conclusions: This work defines conserved chaperone networks involved in managing the N88S-induced loss of proteostasis, paving the way for identifying human orthologs as potential therapeutic targets.

Keywords: Seipin, N88S seipinopathy, Endoplasmic Reticulum (ER) stress, Protein Quality Control (PQC), Chaperones.

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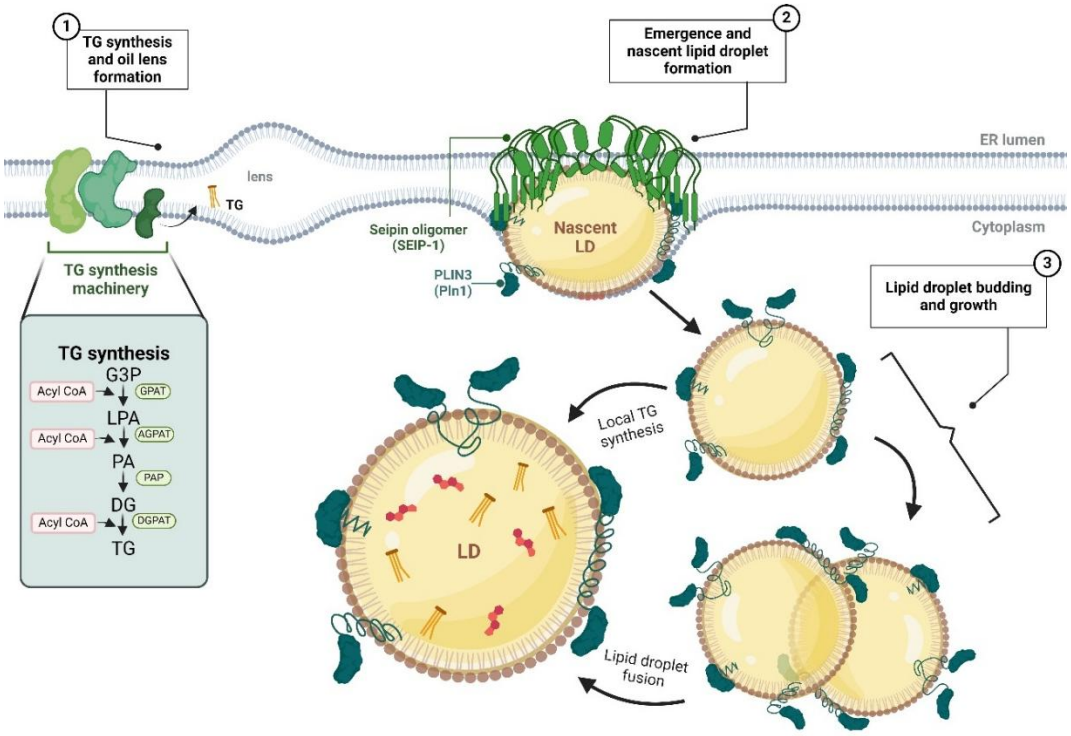

Figure 1. Schematic representation of major stages in lipid droplet (LD) biogenesis. Lipid droplets are derived from the ER through three key stages. The first stage involves TG synthesis (shown in the figure) and cholesterol esters by specific enzymes, which concentrate NL between the layers of the ER membrane. Once NL concentrations increase, they merge into a highly condensed lens structure. The second stage involves the recruitment of several proteins, including **seipin,** to the forming lens, thus promoting the emergence of the nascent LD. Finally, the third stage involves LD bud off from the ER by merging with other LDs/local TG synthesis. AGPAT, acylglycerolphosphate acyltransferase; DG, diacylglycerol; DGAT, acyl-CoA: diacylglycerol acyltransferase; G3P, glycerol-3-phosphate; GPAT, glycerol-3-phosphate acyltransferase; LPS, lysophosphatidic acid; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PLIN, perilipin.

24776 | Characterization of the extracellular matrix properties of spinal cord injury site in Acomys versus Mus

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Background & Aim: Spinal cord injuries remain a major unmet medical condition, affecting millions of people worldwide. The primary challenges in treating these injuries are the intrinsic inability of adult neurons to regenerate and the formation of a scar at the injury site, creating an inhibitory barrier. Even though this is the rule in mammals, the African spiny mouse (*Acomys dimidiatus)* is an extraordinary exception, being capable of regenerating tissues and recovering function after a complete spinal cord transection [1]. Evidence shows that in *Acomys,* a distinct ECM remodeling occurs after injury, creating a pro-regenerative environment. The aim of this study is to further characterize this remodeling process and improve understanding of spinal cord regeneration in *Acomys* in order to translate this knowledge to non-regenerative species. **Methods:** An *in silico* analysis of bulk and single-cell RNA sequencing data was performed to identify differentially regulated ECM-related transcripts and the corresponding activated cell types, after injury, in *Acomys* and the non-regenerative species *Mus musculus*. Given that differences in the ECM are likely to alter tissue stiffness, the mechanical properties of the spinal cord, before and after injury, were assessed in both species using atomic force microscopy. **Results:** *Acomys* shows a distinct ECM transcriptional response after injury, with increased expression of collagen-degrading metalloproteinases and anti-fibrotic proteins. In contrast, fibrosis-associated proteoglycans are expressed at lower levels compared to *Mus.* Microglia, oligodendrocytes, and endothelial cells are the main contributors to this ECM profile. Alongside this response, spinal cord stiffness in *Acomys* is reduced at 1 week post-injury. **Conclusions:** The ECM signature and the soft mechanical environment observed at the *Acomys* spinal cord injury site may contribute to a permissive environment for axonal regeneration.

Keywords: Spinal cord injury, Regeneration, *Acomys dimidiatus*, Extracellular matrix, Atomic force microscopy

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24295 | Cogni Party: Enhancing ABI Rehabilitation through Adaptive AI and Multiplayer Serious Games

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Background & Aim: Acquired Brain Injury (ABI) causes severe cognitive and psychosocial deficits, with spontaneous recovery and pharmacological treatments showing limitations [1]. Traditional rehabilitation struggles with adherence due to monotonous exercises lacking ecological validity [2]. While serious games (SGs) encourage the intensive practice required for neuroplasticity [3], current solutions often lack real-time difficulty adaptation and and frequently require patients to train alone. This study aims to develop a multiplayer SG with adaptive Artificial Intelligence (AI) to support ABI cognitive and psychosocial rehabilitation. **Methods:** *Cogni Party* will be a local multiplayer PC game emphasizing social interaction through party-style mini-games. It will be developed using Godot and Blender to ensure lightweight performance and accessibility across a wide range of hardware. The core innovation will integrate AI agents trained through Reinforcement Learning using the PPO algorithm and Godot RL Agents, enabling adaptive and intelligent in-game interactions. A co-design approach will be adopted, involving patients, families, and healthcare professionals through interviews, workshops, and usability testing. This process will ensure that the platform strictly meets clinical standards and accessibility requirements. **Results:** Expected outcomes include a platform that promotes social interaction through multiplayer games. In the absence of human peers, AI bots simulate opponents with similar cognitive skills. This dynamic adaptation prevents frustration, sustaining the high engagement needed to drive tangible clinical improvements. **Conclusions:** This project provides an innovative ABI rehabilitation tool combining evidence-based therapeutic principles with engaging social party game dynamics. By transitioning therapy into a shared, adaptive digital environment, it actively reduces social isolation, boosts motivation, and promotes accessible home-based training, serving as a robust complement to traditional therapies.

Keywords: Acquired Brain Injury, Cognitive Rehabilitation, Serious Games, Artificial Intelligence, Reinforcement Learning, Co-design.

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24558 | Combined Senolytic and Lipolytic Treatment Reverses Aging-Associated Changes in Human Subcutaneous Adipose Tissue

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Background & Aim: Adipose tissue (AT) is a dynamic endocrine organ essential for energy homeostasis [1,2], whose disruption marks a key step in the development of metabolic dysfunction. During aging, AT undergoes structural and functional changes, including extracellular matrix remodeling, immune cell infiltration, and oxidative stress, leading to chronic inflammation known as adipaging, which mirrors the premature dysfunction observed in obesity [1,3]. In both aging and obesity, this persistent low-grade inflammation promotes the accumulation of senescent cells that, through the release of pro-inflammatory factors, disrupt lipolysis, thermogenesis, and adipogenesis, further compromising tissue function. This study evaluates a novel pharmacological approach, combining two senolytic drugs, Desatinib and Quercetin (D+Q) and one lipolytic agent, the melanocortin α-MSH (M) or the β-adrenergic agonist Isoproterenol (I) to reverse age-related AT dysfunction. **Methods:** Human subcutaneous AT explants from individuals aged 50 or above with metabolic dysfunction were treated *ex vivo* with D+Q plus M or I for one and two weeks. Senescence, inflammation and lipid metabolism markers were evaluated by Western-blotting and PCR techniques. **Results:** Both D+Q+M and D+Q+I combinations modulated the expression of lipolytic enzymes after two weeks of treatment. D+Q+M significantly increased the total and phosphorylated forms of HSL compared to controls (~2-fold increase) whereas ATGL expression increases significantly with D+Q+I (~3-fold increase) combination. Additionally, the expression levels of senescence and inflammation-related proteins, such as LamB1, IL-6 and PAI-1, were significantly reduced at one and two weeks of treatments. Furthermore, D+Q+I treatment also reduced APOJ and TGF-β mRNA expression levels. **Conclusions:** These findings suggest that simultaneously targeting cellular senescence and lipid metabolism represents a promising strategy to reverse AT aging, restoring metabolic function.

Keywords: Adipose Tissue; Senescence; Browning; Senolytics; Aging

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24751 | Creation and validation of a questionnaire on the impact of dental aesthetics on the personal and professional lives of public-facing employees

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Background & Aim: The impact that dental aesthetics often has on social life is widely recognized. But does it also have an impact on professional life? The aim of this study was to create and validate a questionnaire that would enable us to ascertain whether dental aesthetics interferes with the personal and professional lives of public-facing employees in Portugal and Brazil, and whether there is a difference between the two countries. **Methods**: The questionnaire formulated for this research was based on two specific questionnaires published in the literature, the "PIDAQ-P" and the "OHEE-Q" [1, 2]. The questionnaire consisted of 21 anonymized questions that assessed aesthetic perception and its influence on interpersonal and professional relationships. For its validation, it was submitted to 5 dental professionals from both countries to determine its validity. After the clarity and objectivity were validated by the experts, the questionnaire was administered to at least 50 individuals in each country to assess its comprehensibility. The instrument was considered inadequate if 15% or more of answered "I did not understand the question" for any item. In such cases, the question would be reformulated and a new pre-test conducted. **Results:** In Portugal, the pre-test had a total of 53 responses, and only 50 were valid according to the inclusion and exclusion criteria. We had 6 questions that raised doubts among respondents, with a maximum rate of "I did not understand the question" for one question being 6%. In Brazil, 54 responses were obtained, all of which were valid and none of which were "did not understand the question," resulting in a comprehension rate of 100%.**Conclusions:** The development and validation of a single questionnaire on the impact of dental aesthetics on personal and professional life will enable further research on this topic, particularly a comparative study between Portugal and Brazil on how dental aesthetics affect the personal and professional lives of public-facing employees.

Keywords: Dental Aesthetics; Quality of Life; Psychosocial Impact; Public Service; Interpersonal Relationships.

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24341 | Deciphering Ubiquitin and Hippo Pathways in Familial Thyroid Cancer

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Background & Aim: Thyroid cancer (TC) is the most common endocrine malignancy [1]. Around 10% of all TC cases are hereditary, however their genetic basis and clinical behavior remain poorly characterized [2]. Among them, non-syndromic familial non-medullary thyroid carcinoma (NSFNMTC) represents a rare and heterogeneous entity [2]. In a NSFNMTC family, our team identified a germline *USP42* variant *(p.Gly486Arg)* associated with alterations in cell morphology, proliferation, apoptosis and cell cycle control, suggesting a relevant role for this deubiquitinase (DUB) in thyroid cell biology [3]. This study also revealed that USP42 overexpression increases p53 expression, supporting a potential link between USP42 and p53 regulation. The Hippo Signaling pathway, regulated by DUBs through the YAP/TAZ complex, has emerged as a key player in tumorigenesis but its contribution to TC and its clinical impact remain largely underexplored [3-5]. Therefore, this project aims to investigate how USP42 and other DUBs may modulate thyroid tumor biology through modulation of the Hippo Signaling pathway, as shown in other tumor models. **Methods:** Immunohistochemistry (IHC) was performed on FFPE thyroid tissues from familial and sporadic TC cases to access the expression of DUBs, p53 and key Hippo pathway components, YAP and p-YAP. In parralel, *USP42* silencing is being carried out in Nthy-ori 3-1 cells using siRNA, followed by mRNA and protein analysis of YAP, TAZ and LATS by RT-PCR and Western blot. **Results:** Preliminary IHC results using the YAP antibody showed the expected nuclear and cytoplasmic staining. The staining appeared to be stronger in tumor tissue than adjacent tissue, and more intense in familial cases than sporadic cases. In contrast, p-YAP, expected to stain only the cytoplasm, showed unexpected nuclear staining. **Conclusions:** With this work we expect to advance our understanding of TC biology and identify novel molecular targets for therapeutic strategies in NSFNMTC.

Keywords: Familial Thyroid Cancer, USP42, Hippo Signalling Pathway, p53 pathway

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24823 | Defining IL-17A-associated meningeal myeloid responses in neonatal Group B Streptococcus meningitis

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Background & Aim: Neonatal bacterial meningitis remains a major cause of mortality and long-term neurodevelopmental impairment. Group B *Streptococcus* (GBS) remains the leading bacterial cause worldwide. The meninges are enriched in immune cells critical for neuropathophysiology, yet the neonatal meningeal immunity is poorly defined. Using a clinically relevant mouse model of vertical GBS transmission, our group mapped the neonatal meningeal immune landscape and observed an increased IL-17A pathway during infection, associated with bacterial brain invasion. As border-associated macrophages (BAM) are the dominant meningeal immune population and central to antimicrobial defence, we hypothesized that IL-17A may reprogram their responses. **Methods:** We profiled neonatal meninges by single-cell RNA sequencing (scRNAseq), followed by downstream differentially expressed gene assessment in BAM and monocytes clusters to identify IL-17A-responsive populations. We then modelled IL-17A signalling *in vitro* using bone marrow-derived macrophages (BMDMs), stimulating with different IL-17A concentrations and quantifying phagocytic uptake of GBS. **Results:** scRNAseq revealed dynamic myeloid remodelling during meningitis, with BAM, neutrophils and classical monocytes showing an increase in the IL-17 signalling pathway. Interestingly, our preliminary *in vitro* data show that IL-17A stimulation leads to a significant increase in phagocytic uptake compared with unstimulated controls, although no clear dose-response correlation was established**.** No differences were further observed in the bacterial control over time. **Conclusions:** While still preliminary, our results suggest a potential role for IL-17A in regulating macrophage phagocytic responses, highlighting IL-17 as a possible contributor to the modulation of a proinflammatory meningeal environment. Further studies are required to confirm these observations and to better characterize the underlying mechanisms.

Keywords: Group B *Streptococcus*; neonatal meningitis; border-associated macrophages.

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24263 | Development and Characterisation of PLGA Nanoparticles Encapsulating Rivastigmine for Alzheimer's Disease Therapy

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Background & Aim: Alzheimer's disease (AD) is an irreversible and progressive neurological disorder characterised by neuronal degeneration, cognitive decline, amyloid plaques, and neurofibrillary tangles, accounting for 60–80% of dementia cases worldwide and projected to reach 131.5 million by 2050 (1). A major limitation in AD therapy is the blood–brain barrier (BBB), which prevents more than 98% of small-molecule and nearly all large-molecule drugs from entering the brain (2). Rivastigmine, a clinically approved acetylcholinesterase inhibitor, presents low bioavailability due to poor solubility and rapid metabolism (3). To overcome these limitations, this study aims to develop and optimise rivastigmine-loaded PLGA nanoparticles (NPs) as a non-invasive brain targeting strategy. **Methods:** PLGA NPs were prepared by solvent displacement and electrospraying. Solvent-displaced NPs were characterised by DLS (size, PDI, zeta potential), DSC, and TEM. Electrosprayed NPs were analysed by SEM and FTIR. Encapsulation efficiency (EE) was estimated by UV–Vis spectroscopy. **Results:** Solvent-displaced NPs exhibited mean diameters around 100 nm, low PDI (<0.25), and negative zeta potential (approximately –27 mV), confirmed by TEM. DSC showed a typical amorphous PLGA profile with a glass transition within the expected temperature range and no distinct melting peak. Electrosprayed blank NPs measured 198 nm, while rivastigmine-loaded NPs averaged 220 nm. FTIR of rivastigmine and PLGA was analysed. FTIR spectra of loaded NPs closely resembled PLGA, showing no drug-specific bands, indicating successful encapsulation. EE is expected to exceed 50%. **Conclusions:** These PLGA NPs exhibited physicochemical properties suitable for effective brain delivery and good physical stability, highlighting their potential as a brain-targeted drug delivery system.

Keywords: Alzheimer's disease, Nanoparticles, Blood-brain barrier, Treatment

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24296 | Development of a magnetically responsive Nano-Tenosome system for targeted tendon inflammation

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Background & Aim: Tendinopathy is a prevalent musculoskeletal disorder characterized by chronic inflammation and impaired tissue regeneration that lacks efficient therapies. The NF-kB pathway plays a central role in tendinopathy, making it a potential target in the development of innovative therapeutic strategies[1]. Exosomes are small vesicles secreted by cells, which are emerging as effective delivery systems for therapeutic molecules[2]. Recent evidence suggests that hypoxia enhances exosome release and alters their molecular cargo, which is involved in regulation of inflammation. Moreover, superparamagnetic iron oxide nanoparticles (SPIONs) have shown potential for remote control of inflammatory processes under external magnetic fields (MF), representing a promising strategy for tendon regeneration. Therefore, we have designed a magnetically responsive Nano-Tenosome system, integrating exosomes with SPIONs functionalized with an NF-kB inhibitor peptide to modulate inflammatory responses. **Methods**: Exosomes were isolated from human tendon-derived stem cells (hTDCs) cultured under hypoxic and normoxic conditions and analysed for exosomal markers (CD63, CD81, CD9) and associated molecules (VLA-4, Syntenin-1, Cytochrome C) by Western Blot and ELISA. SPIONs-peptide were incorporated into exosomes via magnetofection and characterized by SEM and zetasizer. Their therapeutic potential was evaluated in an inflammatory co-culture model of hTDCs and macrophages under MF, and cellular responses assessed by RT-qPCR, Western Blot, and cytokine profile for inflammatory and macrophage phenotype markers. **Results:** Preliminary results confirmed the successful isolation of exosomes, evidenced by the presence of CD63, CD81, CD9, VLA-4, Syntenin-1, and low levels of Cytochrome C, validating their exosomal identity. **Conclusions:** Overall, the successful isolation of hTDCs-derived exosomes establish a

critical step for Nano-Tenosome platform and their potential in future magnetically guided tendon inflammation.

Keywords: Exosomes, NF-kB pathway, Inflammation, Iron oxide nanoparticles, Magnetic fields

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24205 | Neurofilament light chain in plasma: diagnostic and prognostic value in schizophrenia - a systematic review

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Background & Aim: Schizophrenia is a chronic mental illness, with functional/structural brain repercussions and serious impact on those affected. The use of biologic markers in Neurology has been established, but its utility in the psychiatric field remains uncertain. This systematic review aimed to determine whether the measurement of neurofilament light chain (NfL), a marker of axonal damage, in the plasma of schizophrenic patients could have diagnostic and prognostic value in schizophrenia. **Methods:** Literature review was conducted using the electronic databases Pubmed, Scopus and Web Of Science (last searched on December 8th, 2025). The inclusion criteria were: cross-sectional studies, case-control studies, randomized controlled trials and case reports, which determined NfL plasma values in individuals with diagnosed schizophrenia, schizophrenia spectrum disorders or first psychotic episode and were *in vivo* human studies. Newcastle-Ottawa Quality Assessment Scale (adapted for cross-sectional studies) was used to assess the quality of the included studies. **Results:** A total of 11 articles were included in this review, with a total of 3026 participants. Marked heterogeneity between studies was noted. Significant positive correlations were found between higher NfL plasma values and treatment resistance/more severe schizophrenia cases. Higher serum NfL levels were shown to predict lower cortical thickness in the left and right insula, and left inferior temporal regions, in treatment resistant patients, translating more severe neurodegeneration. Significant differences were also found between NfL plasma levels in schizophrenic patients *versus* patients with neurodegenerative conditions, but not when compared to healthy individuals, denoting a potential role in differential diagnosis. **Conclusions:** Evidence found in this review solidifies NfL as a possible future marker in psychiatric clinical practice for the diagnosis, prognosis and therapeutic approach in schizophrenia.

Keywords: Schizophrenia, Neurofilament light chain (NfL), Diagnosis, Prognosis.

24221 | Impact of Non-Nutritive Sweeteners on Placental Function: An In Vitro Study Using HTR-8/SVneo Cells

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Background & Aim: Consumption of non-nutritive sweeteners (NNS) has been increasing over the years, yet clear safety guidelines for their use during pregnancy are lacking. [1,2] Proper placentation involves extravillous trophoblasts (EVTs) invasion of the maternal uterus, and any disfunction in this process can compromise fetal growth and pregnancy outcomes. [3] NNS are generally considered safe to consume during pregnancy, although recent studies suggest that these compounds can cross the placental barrier and may correlate with adverse pregnancy outcomes. [4] Therefore, this research aims to determine whether these compounds can effectively compromise the behavior of invasive EVTs. **Methods:** The effect of a 24h-exposure of the HTR-8/SVneo cell line (a model of **human first-trimester EVTs)** to three NNS (acesulfame-K, saccharin and sucralose) on cell viability (MTT and LDH assays), proliferation (^{3}H-thymidine incorporation), migration (wound healing), oxidative stress (TBARS), and glucose (^{3}H-deoxy-D-glucose) and methionine (^{14}C-methionine) uptake was evaluated. **Results:** While metabolic activity (MTT assay) was not affected by the NNS, all the NNS, and particularly sucralose, induced membrane damage, as shown by increased LDH release. Specific effects of the NNS on lipid peroxidation were observed (TBARS assay), with sucralose increasing and acesulfame K and saccharin reducing MDA levels. Cell migration was significantly reduced by saccharin and sucralose at the highest concentrations, whereas acesulfame K had no significant effect. Cell proliferation was differentially affected, with sucralose enhancing and saccharin reducing DNA synthesis at higher concentrations. Finally, these NNS slightly reduced ^{3}H-deoxy-D-glucose uptake, and increased ^{4}C-methionine uptake. **Conclusions**: Our findings suggest that NNS interfere with multiple aspects of extravillous trophoblast functions and homeostasis, which may be of relevance for placentation.

Keywords: Non-nutritive sweeteners; Extravillous trophoblasts; Placentation.

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24241 | Neuromodulation of orbitostriatal circuitry during contextual time-reward information processing in chronic pain

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Background & aim: Impaired delay gratification has been reported in chronic pain patients, leading to a preference for smaller immediate rewards over delayed ones. Alterations in the orbitofrontal cortex (OFC) and nucleus accumbens shell (NAcSh) have been described in chronic pain. However, the contribution of the OFC-NAcSh circuit to time-reward associations remains unclear. Here, we investigated the role of glutamatergic OFC-NAc projections in decision-making using optogenetics. **Methods:** To address this, male rats were trained in a two-lever operant task: one lever delivered a smaller reward (1 pellet) after a 3-second delay, while the other delivered a larger reward (3 pellets) after a 6-second delay. Rats underwent 5-day training period under immediate reinforcement contingencies. Rats with 80% or higher completed trials were selected for stereotaxic surgery for viral vector delivery and optic fiber implantation. Subsequently, neuropathic pain was inducted using the spared nerve injury (SNI) model. Control rats underwent identical procedures without nerve injury. Performance was evaluated during days 21 and 28 post-surgery, with optogenetic stimulation. Finally, brain tissue was later collected to verify viral transfection and optic fiber placement. **Results:** Results indicate that SNI rats showed a preference for the smaller immediate reward compared to control rats. Optogenetic stimulation of OFC-NAc projections did not significantly alter lever preference in SNI rats, but control rats increased preference for immediate rewards during stimulation. **Conclusion:** The findings indicate that optogenetic activation of OFC-NAcSh projections modulates reward-based decision-making, particularly in control rats. Furthermore, the differential effects observed in neuropathic pain conditions suggest that chronic pain alters the

functional contribution of this circuit to time-reward associations and highlight its potential as a target for therapeutic strategies aimed at maladaptive decision-making.

Keywords: Delayed gratification; Orbitofrontal cortex; Nucleus accumbens shell; Neuropathic pain

24243 | Hydrolysed vs. Non-Hydrolysed Rice Flours: Effects on Glycaemic Carbohydrate Release under Infant and Adult In Vitro Digestion

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Background & Aim: The nutritional quality and metabolic impact of cereal-based porridges depend on flour processing and digestive capacity. While hydrolysed flours are commonly used to enhance sweetness and digestibility, the combined effects of processing, product format, and digestive physiology on glycaemic response remain underexplored. This study quantitatively compared the digestion kinetics, carbohydrate bioaccessibility, and estimated glycaemic response of rice-based formulations to assess suitability for different target populations. **Methods:** Four industrial rice-based formulations were prepared with non-hydrolysed and hydrolysed flour (NHF, HF), and non-hydrolysed and hydrolysed instant meals (NHM, HM). Samples were digested under both INFOGEST 2.0 protocol and an adapted infant model. Soluble carbohydrates were analysed by HPLC-RI, and digestion kinetics used to calculate hydrolysis index (HI) and estimated glycaemic index (eGI). Water absorption and solubility indices (WAI, WSI) were also evaluated. **Results:** Digestive physiology was the main determinant of saccharides' bioaccessibility, followed by processing and preparation format. In the infant model, HM yielded a higher glucose release and eGI (53.4) than NHM (52.0), showing that enzymatic hydrolysis improves digestibility under immature digestive conditions. In contrast, in the adult model, NHF showed the highest eGI (104.1), while HM reached only 94.1, suggesting that pre-hydrolysis may not always enhance glycaemic response in mature systems. Differences in WAI and WSI among samples explained variations in hydration and enzymatic accessibility. **Conclusions:** Hydrolysed formulations may benefit infants, elderly individuals, patients with impaired digestion, and those requiring rapid energy availability, while non-hydrolysed options may be preferable for healthy adults or individuals managing postprandial glycaemia. This work supports the formulation of rice-based products adapted to specific physiological and nutritional needs.

Keywords: Rice, Hydrolysed Flour, *In Vitro* Digestion, Glycaemic Index, Carbohydrates

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24272 | Priming and metaplasticity in Transcranial Magnetic Stimulation across neuropsychiatric disorders – a review.

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Background & Aim: Transcranial Magnetic Stimulation (TMS) has been considered a promising non-invasive treatment for multiple neuropsychiatric disorders. Several techniques have been developed in recent years to improve its therapeutic efficacy. An example of this approach is priming, a procedure administered immediately before TMS to potentiate the neural response through neuromodulatory effects. Although very promising, priming is a paradigm that requires further investigation. **Methods:** A systematic review was performed using PubMed, Web of Science, Scopus, and LILACS, in accordance with PRISMA 2020 guidelines. Electronic databases were systematically searched through January 2026 to identify studies assessing the effects of priming and metaplasticity in transcranial magnetic stimulation across neuropsychiatric disorders. Inclusion and exclusion criteria were established a priori according to the PICOS framework. **Results:** The search found 132 records, and after eliminating duplicates, 69 articles remained, of which 21 reports were selected for full-text review. Considering the inclusion and exclusion criteria, 19 studies were included in this systematic review. Among these, 12 were randomized controlled trials, 2 were retrospective observational studies, 1 was an open-label pilot study, 1 was a pseudo-randomized trial, 1 was a preliminary report, and 1 was a case study. Overall, priming interventions across magnetic, electrical, and pharmacological strategies showed the capacity to modulate cortical excitability and enhance rTMS-induced neuroplasticity. **Conclusions:** Priming neuromodulation appears to be an encouraging strategy to enhance the neurophysiological and clinical effects of rTMS across neuropsychiatric disorders, supporting the idea of neuromodulation guided by metaplasticity. Although promising, differences in stimulation protocols and study designs require further investigation using standard protocols and larger controlled trials to identify the best priming methods and their specific therapeutic benefits for each disorder.

Keywords: Transcranial Magnetic Stimulation (TMS); priming; metaplasticity; neuropsychiatric disorders.

24309 | From Bisphenol A to Its Alternatives: Hidden Risks for Human Neutrophils and Immunotoxicological Effects

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Background & Aim: Bisphenol A (BPA) is an industrial chemical frequently used in the production of polycarbonate polymers and epoxy resins in several applications such as baby bottles, water bottles, and thermal receipts. Given the well-established endocrine-disrupting properties and associated toxicity of BPA, structural analogues bisphenol F (BPF), bisphenol AF (BPAF), and bisphenol AP (BPAP), have been progressively employed in industrial applications [1]. However, their safety profile, particularly regarding their impact on the human immune system, remains unclear. Reactive species (RS) are pivotal mediators produced by neutrophils during the immune response. Nevertheless, excessive RS production can lead to oxidative damage and tissue injury. This study aims to evaluate the *in vitro* effects of BPA and its structural analogues (BPF, BPAF, and BPAP) on human neutrophils by assessing cellular viability and RS production. **Methods:** Human neutrophils were isolated from healthy donors by density gradient centrifugation. Cell viability was first assessed using the trypan blue exclusion assay. Subsequently, reactive species (RS) production during the oxidative burst was evaluated using the fluorescent probe dihydrorhodamine 123 (DHR 123). **Results:** BPA, BPF, and BPAF did not significantly affect neutrophil viability up to the highest tested concentration (100 µM). In contrast, BPAP exhibited pronounced cytotoxicity, inducing extensive cell death at higher concentrations (75 and 100 µM). Furthermore, exposure to BPAP and BPAF modulated RS production, indicating dysregulation of the oxidative burst and suggesting potential immunotoxicological effects. **Conclusions:** The findings of this work suggest that structural analogues of BPA may not be inherently safer than BPA itself, highlighting the need for further toxicological assessment and careful consideration before their widespread commercial use.

Keywords: Bisphenols; Human Neutrophils; Oxidative Burst; Cytotoxicity.

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24343 | Gelled microemulsions containing *G. nigrolineata* bark extract for cosmetic applications

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Background & Aim: Plants of the genus Garcinia (Family Clusiaceae) have gained popularity in recent decades due to the chemical constituents of their extracts, such as xanthones, flavonoids, and benzophenones, which exhibit a variety of biological activities that are advantageous to the health of the skin. For example, the various sections of *G. nigrolineata*, including its bark, leaves and twigs, contain distinct compounds with antioxidant activity (1). However, to the best of our knowledge, there is no evidence of the use of *G. nigrolineata* in cosmetics. Thus, the main objective of this work was to develop and characterize gelled micromemulsions containing *G. nigrolineata* bark extract, intended for cosmetic application. **Methods:** Micromemulsions with and without the extract were prepared and the size of oil droplets were evaluated by laser diffractometry. Then, microemulsions were gelled using Carbopol®. The pH, color, viscosity and texture properties were evaluated to compare the differences between formulations with and without the extract. **Results:** The results of this study showed that microemulsions with and without extract presented oil droplets with sizes between 200 and 300 nm. Both gelled microemulsions presented pH values suitable for skin application and, as expected, the formulations containing the extract showed higher values of the chroma parameter. Regarding the texture parameters, the addition of the extract did not cause a significant change in firmness and adhesiveness. However, the addition of the extract caused a slight decrease in the viscosity of the gelled microemulsion. **Conclusions:** The developed gelled microemulsion incorporating the bark extract of *G. nigrolineata* showed good physicochemical and mechanical properties for cutaneous application, constituting a promising antiaging cosmetic formulation due to the antioxidant properties of the extract.

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24344 | The T Cell Glycocalyx in cancer immunotherapy: mechanisms and clinical applications

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Background & Aim: Immunotherapy has improved cancer treatment; however, its efficacy in colorectal cancer (CRC) remains limited, underscoring the need to uncover new mechanisms of immunoregulation in the patient's immune system and tumor microenvironment (TME)(1). Protein glycosylation is a post-translational modification, and its alteration is considered a hallmark of cancer(2-4). In CRC, intratumoral $CD8^+$ T cells showed altered glycosylation, particularly increased β1,6-GlcNAc *N*-glycans, associated with exhausted phenotypes and higher PD-1 and TIM-3 expression(3, 5, 6). The deletion of this suppressive branched *N*-glycosylation layer on therapeutic $CD8^+$ T cells by CRISPR/Cas9, mediated by *Mgat5* knockout ($^-/^-$) enhances T cell activation and antitumor responses, including in human CAR-T cells(5). However, the impact of *MGAT5* on T cell glycocalyx modulation remains unclear. Thus, this project aims to determine how differential glycogene expression reshapes the T cell glycocalyx in the CRC TME and evaluates glycan signatures as potential biomarkers of immunotherapy response. **Methods:** $CD8^+$ T cells from *Mgat5* $^-/^-$ and wild-type (WT) OT-I mice were isolated, activated, and co-cultured with MC38-OVA, followed by flow cytometry analysis for lectins. Further, MC38 tumors were established in *Mgat5* $^-/^-$ and WT mice, followed by tumor digestion and flow cytometric analysis of T cell glycocalyx. **Results:** Preliminary results from *in vitro* and *in vivo* experiments show alterations in overall *N*-glycosylation, mainly in PD-1^+CD8^+T cells. Strikingly, *in vivo*, these differences in T cell glycocalyx were observed only in the tumor microenvironment, consistent with decreased tumor growth but not in the draining lymph. **Conclusions:** Our results suggest a different glycan pattern/ arrangement on the surface of mouse $CD8^+$ T cells upon *Mgat5* $^-/^-$. indicating that *Mgat5* contributes to T cell glycocalyx remodeling. PD-1 expression and signals from the TME also play critical roles in shaping the T cell glycocalyx in CRC.

Keywords: colorectal cancer; *N*-glycosylation; T cell glycocalyx; T cell exhaustion; Immunotherapy

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24364 | Identification of pain-related markers of delayed gratification in rodents via ventral striatum characterization

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Background & Aim: Alterations in mesolimbic pathways have been reported in several neurological conditions, including chronic pain. Disruption of nucleus accumbens shell (NAcSh) during painful states is considered a potential source of decision-making deficits, particularly in time-reward association mechanisms. However, the impact of chronic pain on NAcSh function and delayed gratification remains poorly understood. This study aimed to explore the role of the NAcSh in delayed gratification and to characterize pain-related structural changes in NAcSh neurons. **Methods:** For this, male CD rats were evaluated on their behavioral performance on a two-lever task with distinct contingencies: an immediate reward (1 pellet) delivered when the rat pressed the lever 3 s after trial start, and a delayed reward (3 pellets) when the lever was pressed 6 s after start. After a 5-day learning curve with only immediate contingency, animals completing ≥ 80% of trials were selected for further evaluation on delayed gratification mechanisms and subjected to the spare nerve injury (SNI) model of neuropathic pain. Control rats underwent similar procedures without nerve injury. Performance and mechanical nociceptive thresholds were assessed on days 21 and 28 post-surgery. Finally, immunohistochemical analyses were performed to evaluate morphological alterations in the NAcSh of control and SNI animals. **Results:** Results showed that SNI rats developed mechanical allodynia, evidenced by reduced mechanical threshold. Moreover, SNI animals showed a strong preference for smaller immediate rewards, whereas control animals maintained a preference for delayed larger rewards. **Conclusions:** These findings indicate that neuropathic pain impairs

time-reward decision-making and support a critical role for the NAcSh in self-control mechanisms under chronic pain.

Keywords: Nucleus Accumbens Shell (NAcSh); Chronic pain; Spare Nerve Injury (SNI); Delay gratification

24365 | Oral health outcomes in paediatric patients with cystic fibrosis – a systematic review

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Background & Aim: Cystic fibrosis (CF) is an autosomal recessive hereditary disease caused by mutations in the CFTR gene, leading to impaired ion transport and thick secretions affecting multiple organs. In individuals with CF, salivary gland dysfunction, frequent antibiotic use and specific diet may alter salivary composition and the oral microbiota, potentially influencing caries, periodontal status and enamel development. However, evidence remains heterogeneous. This systematic review aims to critically analyze the available scientific evidence on oral health outcomes in children and adolescents with CF compared with healthy peers, focusing on dental caries prevalence, periodontal status, salivary alterations and enamel defects. **Methods:** This review was conducted in accordance with PRISMA guidelines and registered in PROSPERO (CRD420251166781). The research question was structured using a modified PICO strategy. Electronic searches were performed in PubMed, Scopus, Web of Science and LILACS without time restriction. Observational full-text studies assessing dental caries, periodontal parameters, salivary characteristics or developmental enamel defects in individuals ≤18 years with CF were included. Study selection was performed in three stages and risk of bias was assessed using the Newcastle–Ottawa Scale. **Results:** Of 126 studies identified, 12 studies met the eligibility criteria. It is expected to demonstrate that children and adolescents with cystic fibrosis may present a higher prevalence of dental caries and gingivitis, as well as alterations in salivary flow and composition and an increased occurrence of developmental enamel defects. **Conclusions:** Children with CF may present distinct oral health patterns, particularly regarding periodontal and salivary parameters. However, further well-designed studies are required to clarify disease-specific oral risks and to support preventive strategies.

Keywords: Cystic Fibrosis; Oral Health; Dental Caries; Periodontal Disease; Enamel Defects.

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24367 | Insulin Regulation of Fatty Acid Uptake and Lipid Droplet Dynamics in Sertoli Cells

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Background & Aim: Diabetes Mellitus (DM) is a metabolic disease with increasing global prevalence and is associated with complications that include male infertility. Sertoli cells (SCs) are the "nurse cells" of the testis, providing essential metabolic support for germ cell development. This study investigated the role of insulin in modulating fatty acid (FA) metabolism in SCs, a critical energy source for maintaining spermatogenesis. **Methods:** The immortalized TM4 Sertoli cell line was cultured with or without insulin (10 µg/mL) for 18 hours. To assess metabolic health and lipid dynamics, we measured the mitochondrial membrane potential (ΔΨm) using the JC-1 assay, and lipid droplet content through Oil Red O staining. Additionally, a functional fatty acid uptake assay was performed to determine the rate of lipid entry into the cells. **Results:** The results showed that while mitochondrial function remained preserved, lipid droplet content was significantly reduced in the absence of insulin. This alteration was associated with a decreased rate of fatty acid uptake. **Conclusions:** Insulin regulates the entry of fatty acids into Sertoli cells, but does not appear to directly modulate their mitochondrial oxidation under these conditions. These results suggest that insulin deprivation, characteristic of poorly managed Diabetes, disrupts lipid homeostasis in SCs, potentially starving developing germ cells and contributing to male infertility.

Keywords: Testicular metabolism, Sertoli cells, diabetes mellitus, insulin signaling, lipid metabolism.

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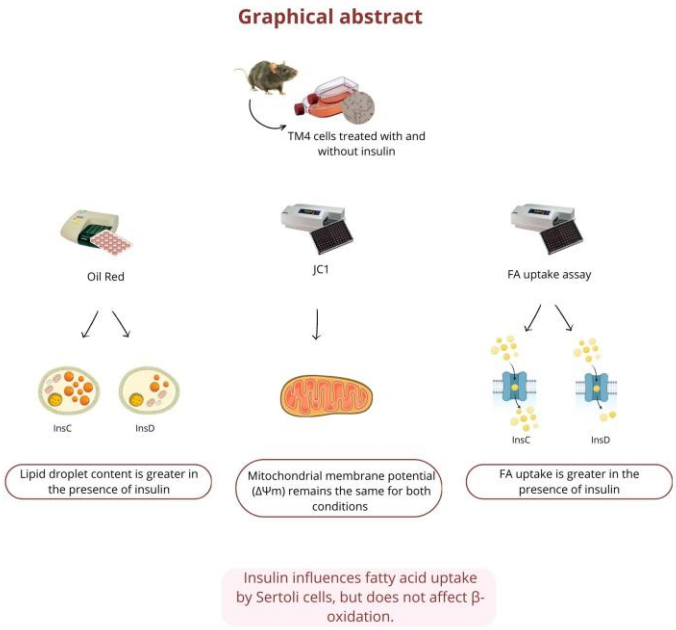

Figure 1. **Graphical Abstract: Insulin promotes fatty acid uptake and storage.** Experimental results show that while mitochondrial function (ΔΨm) is preserved, insulin is essential for the uptake of fatty acids and the formation of lipid droplets in Sertoli cells.

24386 | Impact of Extraction Methods on the Vitamin E Profile and Neuroprotective Potential of Grapeseed Oils

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Background & Aim: Grapeseed oil (GSO) is a relevant source of vitamin E, whose levels vary with grape material and extraction. Beyond nutritional value, vitamin E – particularly γ-tocotrienol – has been associated with neuroprotective effects. This preliminary study aimed to characterize the vitamin E content and profile of GSOs obtained from three grape seed lots using different extraction methods, and to explore their protective effect against oxidative stress in neuronal cells. **Methods:** Oils were obtained with hot hexane (Soxhlet), cold cyclohexane/isopropanol (Smedes), and by direct centrifugation (simulating physical pressing). Vitamin E (tocopherols and tocotrienols) was quantified by NP-HPLC-FLD. All analyses followed validated and routinely implemented laboratory protocols. SH-SY5Y cells were pre-treated with non-cytotoxic concentrations of GSO extracts (4 h) and challenged with TBHP (10–50 µM, 2 h). Intracellular ROS production was assessed using DCFH-DA fluorescence. **Results:** Extraction yields were 7.1-11.8% for Soxhlet, 7.7-9.2% for Smedes and 1.5-4.6% for solvent-free extraction. Total vitamin E ranged 517-1311 mg/kg oil, depending on grape lot and method. The vitamin E profile was dominated by γ-tocotrienol, reaching 716 mg/kg oil (75%), followed by α-tocopherol and α-tocotrienol, representing ~70-85% of total vitamin E. In SH-SY5Y cells, GSO extracts (<25 mg/L) were non-cytotoxic and reduced TBHP-induced ROS production in a concentration-dependent manner. **Conclusions:** Extraction method influenced oil yield and vitamin E recovery, with γ-tocotrienol predominating across samples and extraction methods. Also, preliminary data support antioxidant protection by tocotrienol-rich grapeseed oils in neuronal cells.

Keywords: Grapeseed oil; Vitamin E; Tocotrienols; Oxidative stress; SH-SY5Y cell model

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24388 | Digital violence and its impact on victims' mental health: development of a research protocol using the Experience Sampling Method

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Background & Aim: Digital violence (DV) has emerged as a significant social and public health concern due to the widespread use of digital technologies. It encompasses behaviors such as cyberbullying, cyberstalking, non-consensual sharing of intimate content, and technology-facilitated surveillance, which are often associated with interpersonal and domestic violence. Evidence indicates that digital victimization is associated with anxiety, depression, emotional distress, and social isolation. However, most studies rely on cross-sectional and retrospective designs, limiting understanding of temporal dynamics and immediate psychological effects due to recall bias and low ecological validity. The Experience Sampling Method (ESM) enables real-time assessments in natural contexts, offering a promising alternative. This study develops an ESM-based protocol to examine the association between DV episodes and momentary changes in victims' mental health. **Methods:** This ESM-based research protocol entails a pilot phase in which participants exposed to DV are asked to complete self-assessment questionnaires throughout the day using the *m-Path* application. Data will be collected during a predefined monitoring period through app prompts, enabling real-time recording of DV exposure, contextual variables, and emotional states. **Results:** This work represents the initial phase of protocol development, with data collection underway. Expected outcomes include identifying temporal patterns of digital victimization and associated emotional fluctuations that are difficult to capture using traditional methods. **Conclusions:** This protocol seeks to address limitations of traditional research by adopting an ecologically valid, real-time approach, enhancing understanding of the immediate and cumulative impact of DV on mental health and supporting more effective prevention and intervention strategies.

Keywords: Digital violence; Experience Sampling Method; Technology-facilitated abuse; Real-time approach; Mental health outcomes

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24415 | Setting the Threshold of mRNA Surveillance: The Role of Nonsense-Mediated Decay in CTNNA1-Associated Hereditary Diffuse Gastric Cancer

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Background & Aim: Hereditary diffuse gastric cancer (HDGC) is linked to *CDH1* and *CTNNA1* germline variants that create premature termination codons and trigger nonsense-mediated mRNA decay (NMD), causing complete E-cadherin and αE-catenin loss [1]. While the *CDH1* NMD activity threshold is defined at amino acid (aa) 836 [2], this remains unclear for *CTNNA1,* despite theoretical prediction near aa 795. We aim to define the *CTNNA1* NMD boundary to improve *CTNNA1* variants clinical classification in HDGC. **Methods:** We analyzed the frequency of 315 clinical phenotypes from *CTNNA1* truncating variant carriers in NMD-competent and incompetent regions. We then used CRISPR/Cas9 to generate isogenic gastric cancer (GC) cell lines with different homozygous truncating *CTNNA1* variants in these regions. We characterized and validated by sanger sequencing, reverse transcriptase qPCR, flow cytometry, and immunocytochemistry. *CTNNA1* mRNA and αE-catenin protein levels will be measured upon NMD inhibition with cycloheximide. **Results:** HDGC-associated phenotypes are 3-fold more frequent if variants occur before *aa* 795, in the NMD-competent (n=74; one variant per 10.7 aa) than in the NMD-incompetent (n=4; one variant per 28 aa) region. All other phenotypes show similar frequencies in both regions (one variant per 3.7 aa(n=217) vs one variant per 5.6 aa(n=20), respectively). We generated 31 genotyped CRISPR/Cas9 GC cell mutants, one confirmed to carry a homozygous variant in *CTNNA1* exon 15, resulting in a functional *CTNNA1* knockout in the NMD-competent region, currently under RNA and protein validation. Ongoing work aims to expand GC cell lines harboring *CTNNA1* truncating variants near the predicted NMD boundary. **Conclusions:** We demonstrate that HDGC-phenotypes are more common in carriers of *CTNNA1* truncating variants in the NMD-competent region. Further studies will extend the generation of mutant GC cells lines to unveil the true *CTNNA1* NMD boundary, ultimately improving clinical interpretation of *CTNNA1* variants in HDGC.

Keywords: *CTNNA1*; Hereditary Diffuse Gastric Cancer; Nonsense-mediated mRNA decay

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24429 | MRP8⁺ Myeloid Cells Orchestrate Tumor-Promoting Inflammation in Pancreatic Cancer

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Background & Aim: Pancreatic ductal adenocarcinoma (PDAC) remains largely refractory to immunotherapy, underscoring the need to identify tumor-promoting inflammatory circuits that can be therapeutically disrupted. While myeloid infiltration is a hallmark of PDAC, the specific subsets that actively drive disease progression remain poorly defined. Our preliminary data identify MRP8⁺ myeloid cells as a clinically significant and previously underappreciated inflammatory population. High intratumoral MRP8⁺ infiltration strongly correlates with reduced overall survival in PDAC patients. **Methods:** In genetically engineered mouse models, accumulation of MRP8⁺ cells trigger a pathogenic inflammatory cascade that reshapes the tumor microenvironment toward a pro-tumorigenic immune state and accelerates disease progression. **Results:** We hypothesize that MRP8⁺ cells function as central orchestrators of PDAC-promoting inflammation rather than passive bystanders. To establish causality, we will perform adoptive transfer of purified MRP8⁺ cells into PDAC models and longitudinally assess tumor growth and metastatic dissemination by MRI. High-dimensional immune profiling will define the downstream immune circuits rewired by MRP8⁺ cells. **Conclusions:** This project moves beyond descriptive immunophenotyping to directly test the functional and therapeutic relevance of a discrete inflammatory subset in PDAC. By establishing MRP8⁺ cells as drivers of tumor progression, we aim to uncover a tractable inflammatory vulnerability and provide a conceptual framework for precision immunomodulation in PDAC.

Keywords: Tumor Microenvironment; MPR8; Inflammation; PDAC

24437 | Differential Effects of Avasimibe-Mediated ACAT1/2 Inhibition on Cell Proliferation and Neutral Lipid Dynamics in MCF-7 and MDA-MB-231 cell lines

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Background & Aim: Altered lipid metabolism plays a key role in breast cancer progression. Cholesterol esterification by acyl-CoA:cholesterol acyltransferases 1 and 2 (ACAT1/2) into cholesteryl esters promotes lipid droplet (LD) formation and supports tumor cell survival. It has been shown that ACAT1 is overexpressed in several malignancies, including breast cancer[1]. Avasimibe, an inhibitor of ACAT1/2, disrupts this process and exhibits antitumor activity[2]. The aim of this work is to get insights on the differential effects of avasimibe, in two breast cancer cell lines of distinct intrinsic molecular subtypes: MDA-MB-231 (triple negative, metastatic) and MCF-7 (luminal A, non-metastatic). **Methods:** MCF-7 and MDA-MB-231 cells were seeded on 96-well plates at cellular densities of $2.5x10^4$ and $1.5x10^4$cells/mL, respectively. After cell adhesion, avasimibe (10 µM) was added and cells were incubated for 24h. Cell viability was determined using the Presto Blue™ reduction assay and proliferation by counting cells using High Contrast Brightfield (HCB). Cells were stained with the fluorescent dyes Nile Red and Hoechst 33342 to label LDs and nuclei, respectively. Images were acquired using a Lionheart FX microscope. CellProfiler™ software was used to quantify total neutral lipid content (LDs)/cell. Results are expressed as mean ± SD (n≥3). Statistical significance was assessed using Student´s t-test. **Results:** Preliminary results suggest that avasimibe reduces cell proliferation and viability, more pronounced in MCF-7 comparatively to those seen in MDA-MB-231 cells. Moreover, it was possible to observe an increase in neutral lipid content, in both cell lines. **Conclusions:** Our results are in line with the reported anti-tumor effect of avasimibe. However, the observed increase in neutral lipid content was not expected, as ACAT1/2 activity contributes to the total neutral lipid load. These findings may be explained by the activation of other mechanisms in response to stress caused by non-esterified cholesterol.

Keywords: Lipid Droplets; breast cancer; ACAT1/2; avasimibe; cholesterol

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24438 | in vitro Model to Study Tendon Calcifications

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Background & Aim: Tendon calcification is associated with aberrant osteogenic differentiation of tendon-derived cells (TDCs) and dysregulated crosstalk between BMP/TGF-β, Hedgehog and mechanosensitive pathways such as Piezo1, which are all implicated in pathological ossification and tendon homeostasis. This project aims to develop a 3D *in vitro* model of TDCs to dissect how biochemical and physical cues jointly drive osteogenic versus tendinous fates relevant to tendon calcification.[1] **Methods:** Human TDCs will be cultured in osteogenic medium with or without supplementation of calcium chloride to mimic a pro-calcifying environment in a 2D cell culture and, later, assembled as 3D spheroids, alone and combined with magnetic stimulation via a bioreactor (MICA biosystems) to simulate mechanical and pathological loading. Readouts will include mineralization assays (Alizarin Red S, von Kossa, ALP), ELISA for SMAD1 and immunocytochemistry for RUNX2, tendon markers (Scleraxis, Tenomodulin), Hedgehog transcriptional effectors (GLI1/2) and Piezo1. **Results:** MICA stimulation is anticipated to modulate Piezo1 activity and differentially tune BMP/TGF-β and Hedgehog outputs, revealing context-dependent effects of mechanical loading. Spatial organization/assembly, biochemical and mechanical cues may synergize or oppose each other in directing hTDCs fate. **Conclusions:** The proposed formulated model will serve to clarify how BMP/TGF-β, Hedgehog and mechanosensitive signalling intersect to control hTDCs osteogenic differentiation under pathological, calcifying conditions. Establishing this controllable *in vitro* platform will provide a mechanistic basis to study tendon calcification and to test future targeted interventions that favour tendon-like over osteogenic phenotypes.

Keywords: Tendon-derived cells; BMP/TGF-β; 3D *in vitro* model; magnetic stimuli; Calcific tendinopathy

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24442 | Enhancement of the Immune Response and Antitumor Effect of Immunotherapy by BBIT20 in Pancreatic Cancer

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Background & Aim: Pancreatic cancer (PC) is a highly aggressive neoplasia with limited therapeutic options [1]. Immune checkpoint inhibitors (e.g., anti-PD-1) show poor efficacy due to an immunosuppressive tumor microenvironment [2]. Targeting DNA damage response represents a promising strategy to enhance tumor responsiveness to immunotherapy [3]. BBIT20 is a monoterpene indole alkaloid previously identified as an inhibitor of homologous recombination DNA damage repair pathway [4,5]**.** This study aims to evaluate whether BBIT20 can inhibit tumor growth by activating immune-related features, alone or combined with anti-PD-1 immunotherapy, in a syngeneic PC model. **Methods:** KPC pancreatic cancer cells were subcutaneously implanted in C57BL/6J mice. Animals received vehicle, BBIT20 (2mg/kg), anti-PD-1 (100 µg/animal), or the combination. Tumor growth was monitored by caliper measurements. At endpoint, tumors were collected for tissue analysis. Immunohistochemistry has been used to evaluate PD-L1 expression and immune cell populations, including cytotoxic T lymphocytes and macrophage subsets, as well as inflammatory mediators. **Results:** BBIT20 reduced tumor progression compared with the vehicle, whereas anti-PD-1 showed only a modest inhibitory effect. Notably, in combination, BBIT20 significantly enhanced the antitumor effect of anti-PD-1, suggesting a synergistic therapeutic response compared with either treatment alone. Immunohistochemical analysis is underway to evaluate treatment-associated changes in immune cell activation and infiltration in PC tumors, including the quantification of PD-L1, CD8, and CD206, among others. Notably, a reduction in PD-L1 expression was already

observed in BBIT20-treated tumors. **Conclusions:** BBIT20 decreases tumor growth and PD-L1 expression and enhances the antitumor activity of anti-PD-1 in PC. Together, these results support the enhancement of immunotherapy efficacy by BBIT20 through an immunostimulatory mechanism.

Keywords: Pancreatic cancer; DNA damage repair; Anti-PD-1; Immune system

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24447 | Extracellular Vesicles in Gastric Cancer as Circulating Biomarkers of Disease Progression

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Background & Aim: Gastric cancer (GC) is a serious global health problem, with high incidence and mortality rates [1]. Late diagnosis and disease heterogeneity complicate treatment and patients' survival, highlighting the need for better screening methods and novel non-invasive biomarkers. Circulating extracellular vesicles (EVs) from cancer patients are known to carry biomolecules that reflect the biological state of tumour cells, making them promising candidates for liquid biopsy biomarkers [2]. The tumour antigen sialyl-Tn (STn) is an aberrant glycan present in several epithelial tumours, including GC [3], and has already been detected in GC EVs [4]. This study aims to isolate and characterise EVs from the plasma of GC patients and evaluate the association between their STn status and their proteomic content for biomarker discovery. **Methods:** EVs were isolated from the plasma of GC patients and healthy controls by size exclusion chromatography (SEC) and characterised by transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA). The presence of EV markers, plasma proteins, and the STn antigen was evaluated by western blotting. The proteomic analysis of these EVs by LC-MS/MS is planned. **Results:** Preliminary results confirm the isolation of particles with morphology and size distribution compatible with EVs, together with the presence of the classic EV marker syntenin-1, validating an enrichment of EVs in our samples. STn was detected in a subset of samples from GC patients, but not in controls, confirming the feasibility of detecting tumour-derived glycoproteins in circulating EVs from GC patients. **Conclusions:** EVs were successfully isolated from plasma samples, and the presence of glycoproteins displaying STn was detected only in the circulating EVs from GC patients. The STn status will be further combined with an EV proteomic signature and patients' clinical data to identify putative novel markers of tumour progression.

Keywords: extracellular vesicles; gastric cancer; biomarkers; sialyl-Tn; liquid biopsy

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24461 | IDH1 mutation as a mechanism of cellular competition in AML

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Background & Aim: Acute myeloid leukemia (AML) is a malignant and heterogeneous disease characterized by the abnormal expansion of immature myeloid progenitors in the bone marrow (BM) [1] , marked by frequent relapse. Recruitment and hijacking of hematopoietic stem cell (HSC) niches in the BM contribute to chemoresistance in AML [2]. There is an unmet need for novel therapies targeting relapses in AML. IDH1 mutations are common in AML and produce the oncometabolite 2-hydroxyglutarate (2-HG) that promotes leukemogenesis [3]. IDH1 mutations drive AML proliferation and survival, and seem to have a competitive advantage over non-mutated AML cells. We hypothesized that IDH1-mutated cells will have a competitive advantage over chemoresistant AML (AML-CR) cells. We focused on the competitive dynamics between $IDH1^{mut}$ AML and AML-CR cells. **Methods:** Murine MLL-AF9 $iDTR^{+}$ $IDH1^{wt}$ and $IDH1^{mut}$ AML cells were used. *In vitro* assays to assess the effect of extrinsic 2-HG on $IDH1^{wt}$ and $IDH1^{mut}$ cells' viability and survival were measured by CCK-8 and flow cytometry. *In vitro* co-culture models of $IDH1^{wt}$, $IDH1^{mut}$, and AML-CR cells were used to study clonal competition by establishing different co-culture ratios with MLL-AF9 $dsRed^{+}$ cells with results assessed by flow cytometry. **Results:** 2-HG negatively affected survival of both $IDH1^{wt}$ and $IDH1^{mut}$ cells after 48 hours of exposure, with no significant differences between groups. In the co-culture assay, $IDH1^{mut}$ cells had a lower proliferation rate compared to $IDH1^{wt}$ cells but showed higher efficiency in outcompeting and inhibiting MLL-AF9 $dsRed^{+}$ cell proliferation. **Conclusions:** $IDH1^{mut}$ cells show promising results in outcompeting MLL-AF9 AML cells *in vitro*. Further studies will include AML-CR cells, and AML mouse models will be used for *in vivo* spatial localization of competing clones by intravital microscopy. With this work we aim to provide novel insights into leveraging clonal dynamics as a therapeutic strategy to overcome chemoresistance and prevent AML relapse.

Keywords: Acute Myeloid Leukemia (AML); Chemoresistance; Cell Competition; Isocitrate dehydrogenase-1 (IDH1); 2-hydroxyglutarate (2-HG);

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24491 | Development of Sensory-Friendly Skincare Formulations

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Background & Aim: Sensory processing abnormalities, including skin hyperreactivity, can affect daily function and well-being (1). The emerging field of neurocosmetics explores how active ingredients can modulate neural pathways involved in skin sensation and perception (2). Despite its relevance, this neuro-sensory dimension remains largely overlooked in commercial skincare (3). To fill this gap, this study aims to develop sensory-friendly body moisturizers. **Methods:** Two O/W emulsions (CML14 and CML15) were prepared based on a Quality by Design approach, in which Cetaphil® Moisturising Lotion was benchmarked to guide the Target Product Profile (TPP) and the Quality TPP definition. Active ingredients with "Sensory-friendly", "Neurocosmetic" and "Sensitive skin" claims were selected using Coptis® database. The formulations were characterised for short-term physical stability and physicochemical, rheological, and textural properties. An affective sensory evaluation (n=36) was done following ethics committee approval (Ref.05-06-2025), to rate appearance, texture, skin feel, and moisturizing properties**.** **Results:** All formulations exhibited a homogeneous whitish appearance, pH in the range 5.5-7.0, with no phase separation after centrifugation. Rheological data confirmed their shear-thinning behaviour. CML15 exhibited the lowest consistency coefficient, the easiest spreadability, and the lowest adhesiveness, supporting its selection for sensory testing. CML15 achieved sensory ratings comparable to those of Cetaphil® across all attributes, demonstrating the successful development of a sensory-pleasing moisturiser. Further studies are warranted to confirm the effect on the skin neuropathways. **Conclusions:** This study underscores the relevance of developing skincare products that address both functional and affective dimensions of skincare.

Keywords: Sensory-friendly skincare; Sensitive skin; Neurocosmetics; Body moisturizer.

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24756 | Oral Cryotherapy for the Prevention of Mucositis in Adult Oncology Patients Undergoing Chemotherapy: Reducing the Gap Between Evidence and Clinical Practice – A Scoping Review

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Background & Aim: Oral mucositis is a frequent complication of antineoplastic treatments and is associated with pain, functional impairment and reduced tolerance to chemotherapy, making the implementation of effective preventive strategies essential. Oral cryotherapy has been described as a low-cost, simple and safe intervention and has become increasingly relevant in nursing practice. The aim of this review was to map and synthesize the available scientific evidence on the use of oral cryotherapy for the prevention of oral mucositis in adult oncology patients undergoing chemotherapy, considering application protocols, effectiveness, safety, and factors influencing its clinical implementation. **Methods:** A scoping review was conducted in accordance with the methodology proposed by the Joanna Briggs Institute (JBI). The search was carried out in the CINAHL Complete (via EBSCO) and MEDLINE (via PubMed) databases, including studies published in the last 10 years in Portuguese and English. Studies involving the use of oral cryotherapy in hospital and outpatient settings, in adult patients undergoing chemotherapy, were considered. After completion of the search, the Rayyan software platform was used to remove duplicate records and to independently screen the remaining articles using the blind-on feature. **Results:** The evidence suggests that oral cryotherapy should be initiated 5 to 10 minutes before chemotherapy and maintained for a minimum of 30 minutes during the infusion. It should be performed in all chemotherapy cycles, according to patient tolerability. In specific cases, such as longer chemotherapy regimens, oral cryotherapy after chemotherapy administration is suggested, with continuous monitoring using mucositis and pain assessment scales. A clear reduction in the incidence of oral mucositis was observed, particularly in patients with solid tumors treated with 5-fluorouracil (5-FU). Oral cryotherapy is a low-cost intervention with few reported adverse effects. **Conclusions:** The findings unequivocally support oral cryotherapy as a safe, low-cost, and highly effective non-pharmacological intervention for reducing the incidence and severity of oral mucositis. Its physiopathological mechanism is well established, involving local vasoconstriction induced by temperature reduction, which

decreases tissue exposure to cytotoxic chemotherapy agents. In addition to minimizing patient discomfort and pain, it enhances treatment adherence, supporting continuity of chemotherapy cycles and potentially improving prognosis and therapeutic outcomes. Regarding application protocols, considerable variability and heterogeneity were observed in terms of timing and delivery methods (from crushed ice to innovative devices). There is a need for standardized protocols that systematize the existing evidence**.** The translation of evidence into clinical practice requires a multifactorial approach and should be supported by targeted team training. The adoption of standardized protocols based on current scientific evidence is therefore essential. However, initiation prior to chemotherapy infusion appears to be a key determinant of preventive effectiveness.

Keywords: Oncology, Oral cryotherapy, Oral mucositis, Chemotherapy

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24516 | Digital Violence in Intimate Partner Relationships: Perspectives of Perpetrators

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Background & Aim: The ubiquity of digital technologies has expanded Intimate Partner Violence (IPV) into the digital realm, challenging legislation. The Directive (EU) 2024/1385 criminalizes cyberviolence and requires Member States to harmonize their laws by June 2027. Research highlights a "digital dualism" where online abuse is perceived as less serious or more acceptable than offline violence. This study examines how perpetrators perceive digital violence, focusing on legitimation and normalization. **Methods:** The study adopts a qualitative design to explore perpetrators' subjective experiences. A purposive sample is being recruited from the Portuguese prison system, in collaboration with prison services in the North of Portugal. Eligible participants are adults convicted for domestic violence. Individuals are identified through institutional records and invited to participate voluntarily. Data collection through semi-structured interviews is ongoing. The interview guide was designed to identify which online behaviors are recognized as abusive/violent and to compare them with those similarly identified in face-to-face contexts. Reflexive thematic analysis (Braun and Clarke, 2023) will be employed to analyse the data. **Expected Results:** Preliminary analysis suggests the prevalence of "digital dualism" in perpetrators' narratives, whereby online behaviors (e.g., cyberstalking) are distinguished from "real" (physical) violence. Participants are expected to minimize abuse, framing digital control as a normal relationship component or as a "proof of love." Furthermore, results are expected to reveal specific legitimation mechanisms where technology is framed as the trigger for conflict (e.g., "social media creates distrust"), shifting responsibility. **Conclusions:** The study is expected to demonstrate a gap between the legal severity introduced by Directive (EU) 2024/1385 and the subjective trivialization of these acts by perpetrators. Such findings highlight the need for prevention programs that deconstruct the "harmlessness" of digital violence.

Keywords: Digital Violence; Intimate Partner Violence; Legitimation Strategies; Normalization; Perpetrators.

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24753 | Dissecting the Clinical Implications of Platelet Genetic Variation in Cervical Cancer: Insights from a Portuguese Cohort

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Background & Aim: Cervical cancer (CC) is a major global health concern, particularly in regions with limited access to screening programmes and healthcare resources. It is currently the fourth leading cause of cancer-related death among women worldwide. Despite advances in prevention and treatment, morbidity and mortality rates remain high. Furthermore, CC patients are frequently diagnosed with cancer-associated thrombosis (CAT), which further deteriorates their prognosis, highlighting the urgent need for improved predictive, diagnostic, and prognostic biomarkers to improve cancer management. Hence, this study aims to identify genetic variants associated with CC prognosis and susceptibility to CAT, thereby contributing to improved patient outcomes. **Methods:** A retrospective study will be conducted involving patients with a confirmed CC diagnosis from the northern region of Portugal. Peripheral blood samples were collected for DNA extraction and subsequent analysis. Selected genetic polymorphisms known to influence platelet activity will be analysed to investigate their potential role in CAT development and cervical tumourigenesis. Polymorphism genotyping will be performed by real-time PCR, using a TaqMan allele discrimination methodology. **Results:** We anticipate identifying specific genetic variants linked to thrombotic risk and the prognosis of CC patients. **Conclusions:** This preliminary study may uncover clinically relevant genetic markers. The findings may represent an important step toward the development of personalised strategies for thrombotic risk monitoring and prognostic assessment in patients with cervical tumours.

Keywords: Cervical cancer; Venous Thrombosis; Polymorphism, Single Nucleotide.

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24827 | DO ALL IMMUNOGENIC COMPOUNDS PERFORM THE SAME IN BONE TISSUE? AN EX VIVO ORGANOTYPIC STUDY

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Background & Aim: Inflammation is an essential defense mechanism that, when not effectively resolved, may progress to chronic low-grade inflammation, contributing to the pathogenesis of conditions such as osteoarthritis, atherosclerosis, and autoimmune diseases [1]. Lipopolysaccharide (LPS) is a potent immunogenic stimulus that activates Toll-like receptors and the NF-κB signaling pathway, promoting the release of pro-inflammatory cytokines and disrupting bone metabolism by increasing osteoclastogenesis and inhibiting osteoblastic differentiation [2]. Similarly, monosodium urate (MSU) crystals induce acute and chronic inflammatory responses that compromise bone tissue integrity [3], while lysophosphatidic acid (LPA) regulates bone remodeling via its receptors, influencing osteoclast formation [4]. Iodoacetate (MIA) and sodium arsenite (AS) also represent relevant models of inflammation and bone degeneration, being associated with inflammasome activation and pro-inflammatory pathways such as ERK, negatively impacting bone homeostasis [5,6]. In the context of bone inflammation, the use of more complex models, such as organotypic ex vivo femur cultures, provides a controlled, biologically relevant platform for dissecting inflammation-driven molecular mechanisms [7]**.** Therefore, this study aims to evaluate the effects of different immunogenic compounds on bone tissue by characterizing gene expression and inflammatory markers using an ex vivo organotypic model. **Methods:** Embryonic chicken femora (day 11) will be cultured for 10 days at an air–liquid interface at 37 ºC and subsequently exposed for 24 h to LPS, MSU, LPA, MIA, or AS. Total RNA will be extracted, converted into cDNA, and inflammatory and bone-related gene expression will be quantified by RT-qPCR using the $2^{-\Delta\Delta Ct}$ method. Statistical analysis will include ANOVA or Kruskal–Wallis tests ($p < 0.05$). **Results:** Exposure to immunogenic compounds is expected to significantly upregulate pro-inflammatory gene expression compared to control cultures. Distinct molecular expression patterns are anticipated for each compound, reflecting differences in pathway activation, cytotoxicity, and modulation

of bone metabolism. **Conclusions:** This work will contribute to clarifying how distinct immunogenic stimuli modulate inflammatory signaling in bone tissue and interfere with bone metabolic regulation, thereby strengthening the experimental foundation for future studies addressing inflammatory bone disorders.

Keywords: Inflammation; Chicken femora model; Gene expression; Bone; Ex vivo organotypic culture.

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24833 | Early-Life Exposure to Stevia: Long-Term Impact on Skeletal Muscle Metabolism and Morphology

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Background & Aim: The consumption of non-sugar sweeteners (NSS) has increased in recent years, even among pregnant women (1, 2), due to their ability to provide sweetness without caloric contribution (3). Although approved as food additives (4), the WHO recently advised against the use of NSS for weight control due to potential adverse cardiometabolic effects, highlighting the need for studies on the consequences of exposure during pregnancy and childhood (5, 6). In the MHSWEET project, we have shown a clear fetal programming effect of female adult dysmetabolism induced by maternal consumption of Rebaudioside A (RebA) (7), the main NSS component of Stevia. This study aims to investigate the long-term effects of perigestational exposure to RebA, specifically on skeletal muscle (SKM) morphology, metabolism and function in the adult female offspring of the MHSWEET project. **Methods:** Female Sprague-Dawley rats (G0) were given RebA in drinking water (4 mg steviol equivalents/kg/day, n=8) or regular water (Control, C, n=8), during mating, pregnancy and lactation. Offspring (G1) were weaned onto a standard diet (STD) until 8 months of age, when they were switched to a high-fat diet (HFD) or continued on STD until 10 months of age, raising 4 experimental groups: C/STD, C/HFD, RebA/STD and RebA/HFD. In this work, SKM will be used for histological analysis (H&E, ATPase and SDH staining) and for gene expression (RT-PCR) of metabolic, mitochondrial, and oxidative stress markers. **Results:** From this experimental approach, we expect to determine whether perigestational RebA exposure induces, in the SKM of the offspring: a) changes in muscle fiber cross-sectional area and fiber type distribution, b) redox balance alterations and c) disturbance of key metabolic and mitochondrial pathways. **Conclusions:** This work will help to understand the mechanisms involved in the observed fetal programming of metabolic dysfunction of female offspring induced by maternal consumption of RebA during the perigestational period.

Keywords: Rebaudioside A; Fetal programming; Skeletal muscle; Oxidative stress; Metabolic dysfunction

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24773 | Epigenetic dysregulation and lifespan in the yeast model of Niemann-Pick type C disease

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Background & Aim: Niemann-Pick type C (NPC) is an autosomal recessive neurodegenerative disorder caused by loss-of-function mutations in lysosomal proteins NPC1 or NPC2, leading to defective intracellular cholesterol trafficking, lipid imbalance, and impaired mitochondrial function, autophagy and stress responses.[1,2] Altered histone acetylation or methylation are implicated in ageing and neurodegeneration, and inhibition of histone deacetylases has protective effects in NPC by reducing lipid accumulation and improving proteostasis.[3,4] Yeast lacking Ncr1p (NPC1 homolog) recapitulates NPC features, namely sphingolipid accumulation, iron overload and mitochondrial dysfunction.[2,5,6] This project aims to elucidate the mechanistic link between histone modifications and *ncr1*Δ aberrant phenotypes. **Methods:** *Saccharomyces cerevisiae* was used as experimental model. Gene deletions of selected histone-modifying enzymes, previously implicated in ageing, were generated in *ncr1*Δ cells. Chronological lifespan (CLS) was assessed by monitoring colony-forming units (CFUs) in stationary phase cultures. Histone H3 levels were analyzed by Western blotting. Spot assays were performed to evaluate respiratory competence, oxidative stress sensitivity, and stress resistance. **Results:** The deletion of the histone deacetylase *HDA1* partially rescued the shortened lifespan of the *ncr1*Δ mutant, suggesting a role for histone H3 acetylation in modulating cell survival. Total histone H3 levels were similar between wild-type and *ncr1*Δ strains, indicating that the observed effects are likely due to alterations in post-translational histone modifications. Evaluation of specific histone acetylation and methylation marks is currently underway**. Conclusions:** Our findings suggest that epigenetic modulation influences survival and cellular fitness in the yeast model of NPC1 and identify histone-modifying enzymes as key regulators and potential targets to ameliorate NPC cellular dysfunction.

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24862 | Establishing a *S. aureus* Infection Model in the Chorioallantoic Membrane (CAM)

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Background & Aim: The chorioallantoic membrane (CAM) is a highly vascularized extra-embryonic structure widely used to study angiogenesis, oncology, and tissue engineering (1).Due to its rapid vascular development and ethical advantages compared with traditional animal models, CAM represents a promising platform for infection research, as current in vitro setups do not replicate the dynamics of clinical infectious conditions (2). However, standardized and validated protocols for studying bacterial infections in this model remain limited. *Staphylococcus aureus*, a major human pathogen involved in several clinical conditions (3), is a suitable candidate for establishing a reproducible infection model. This study aims to characterize CAM alterations following *S. aureus* inoculation, evaluate bacterial behavior within this highly vascularized environment, and optimize the model as a validated preclinical infection platform. **Methods:** Fertilized chicken eggs are incubated under controlled conditions, and a window will be created at a defined embryonic day to access the CAM. A standardized inoculum of *S. aureus* will be applied to establish infection. Vascular and inflammatory changes will be assessed. Evaluation of the host response to bacterial infection is assessed through embryo survival and colony forming units (CFUs) present in CAM and the embryo. Appropriate controls and statistical analyses will be applied to ensure reproducibility and model validation. **Results:** Preliminary findings demonstrate that *S. aureus* inoculation induces measurable host response, decrease in embryo viability and CAM inoculation allows to fast bacterial growth within the embryonic and extra-embryonic compartments. The adjunct use of minocycline also demonstrates the sensitivity of the study platform to the environment. These results are expected to support the feasibility and reproducibility of the CAM infection model. **Conclusions:** The establishment of a standardized S. aureus infection protocol in the CAM model may validate this platform as a reliable and cost-effective preclinical tool for studying host–pathogen interactions in a highly vascularized and more complex. This work contributes to refining new

approach methodologies and expands the application of CAM in infectious disease research, potentially impacting the development and preclinical testing of antimicrobial and tissue-engineering therapies.

Keywords: Chorioallantoic membrane; angiogenesis; staphylococcus aureus; pre clinical model

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24633 | Establishing Ethical Foundations: A Code of Ethics for Students in Digital Health

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Background & Aim: Digital technologies in healthcare education are expanding faster than student-orientated ethical guidance for undergraduate Digital Health training. While codes exist in medicine, biomedical engineering, and data science and AI governance, there is no widely adopted framework designed to address the specific ethical challenges faced by Digital Health and Biomedical Innovation students. We aim to develop a Code of Ethics grounded in comparative normative analysis, tailored to Digital Health education. **Methods:** We conducted a comparative normative analysis of selected professional codes of ethics and international guidance documents relevant to medicine, biomedical engineering, data science, and AI governance, including the Portuguese National Code of Ethics for Medical Students, the Declaration of Helsinki, and the General Data Protection Regulation (GDPR). Core ethical principles, professional duties, and technology-related responsibilities were comparatively examined and mapped against key challenges in Digital Health education, including data governance, privacy and consent, algorithmic accountability, human oversight of AI-enabled systems, and early industry partnerships. Areas of convergence, divergence, and omission were then used to inform the development of a student-orientated Code of Ethics for undergraduate Digital Health training. **Results:** The analysis revealed three key gaps in current frameworks: limited attention to student-specific responsibilities when handling sensitive health data and developing prototypes; insufficient operational guidance on algorithmic accountability and responsible data stewardship; and weak safeguards for managing conflicts of interest arising from early industry engagement. Based on these findings, we propose a Code of Ethics built around five core principles: Beneficence, Non-maleficence, Autonomy, Responsibility, and

Justice, adapted to student-relevant duties in hybrid clinical-technical educational environments. **Conclusions:** This framework addresses a structural gap in Digital Health training and offers a transferable model for institutions engaged in Digital Health training. Embedding sound ethical principles and professionalism from the outset of training is essential to ensure that technological innovation remains aligned with patient rights, professional integrity, and public trust in healthcare systems.

Keywords: Digital Health; Bioethics; Students; Biomedical Innovation; Data Privacy

24892 | Evaluation of Pyrimidine-based Derivatives as Potent Antitumor Agents to Overcome Cancer Multidrug Resistance

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Background & Aim: Pyrimidine-based derivatives are well-established bioactive molecules, with many substitution patterns on the pyrimidine core, which are associated with several properties including antitumoral activity.[1,2] In particular, derivatives of 2-aminopyrimidine have been identified as promising tyrosine kinase inhibitors with potential to target ATP-dependent proteins.[1,3] One such proteins is P-glycoprotein (P-gp)[4], responsible for multidrug resistance (MDR) in cancers such as non-small cell lung cancer (NSCLC) and colorectal cancer (CRC).[4,5] The aim of this work was to study the antitumor and anti-P-gp activity of eight newly synthesized pyrimidine-based derivatives, in counterpart pairs of sensitive and MDR NSCLC and CRC cell lines. **Methods:** Two pairs of drug-sensitive and MDR counterpart NSCLC (A549 and A540-CDR2) and CRC (DLD1 and DLD1-TxR) cell lines were treated with different concentrations of the eight compounds for 48h. Concentration-response curves were obtained with the SRB viability assay and the GI_{50} concentrations (that inhibit the cell growth by 50%) were calculated. **Results:** Compounds **2**, **3**, **6**, and **8** showed low GI_{50} concentrations in the sensitive A549 (9.8 µM, 8.7 µM, 10.1 µM and 8.2 µM, respectively) and in the MDR A549-CDR2 (12.4 µM, 9.0 µM, 11.6 µM and 7.1 µM, respectively) cells. Similar GI_{50} concentrations were obtained in the sensitive DLD1 (10.8 µM, 13.7 µM, 11.5 µM and 8.2 µM, respectively), and in the MDR DLD1-TxR (10.6 µM, 14.4 µM,

10.1 µM and 10.2 µM, respectively) cells. In contrast, compounds **1**, **4**, **5**, and **7** demonstrated higher GI_{50} concentrations, ranging from 13 µM to 39 µM in both pairs of cell lines. Further studies on the effect on P-gp activity are being conducted with the most promising compounds. **Conclusions:** Our results suggest that all compounds have similar activity in sensitive and MDR counterpart cell lines. Notably, compounds **2**, **3**, **6**, and **8** emerge as promising "hit" candidates for further functional and mechanistic studies against MDR in cancer.

Keywords: Pyrimidine-based derivatives; Multidrug Resistance; P-glycoprotein; non-small cell lung cancer; colorectal cancer

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24875 | Evaluation of the Accuracy of the Woodpex V Apex Locator in the Presence of Different Irrigating Solutions – A Laboratory Study

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Background & Aim: Accurate working length (WL) determination is crucial for successful root canal treatment, allowing effective chemomechanical preparation while preventing over- or under-instrumentation. Although electronic apex locators (EALs) are highly reliable, their accuracy may be affected by the irrigant present in the canal [1,2]. Evidence regarding newer devices, such as the Woodpex V, under different irrigating conditions remains limited. This study aims to assess the accuracy of the Woodpex V in determining WL in the presence of five endodontic irrigants: sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), citric acid, Dual Rinse® HEDP and chlorhexidine. **Methods:** This laboratory study followed PRILE 2021 guidelines. Sixty extracted human single-rooted teeth (Vertucci type I; apical foramen ≤ #20 diameter) were randomly allocated to six groups (n=10): NaOCl 3%, EDTA 17%, citric acid 10%, Dual Rinse® HEDP, chlorhexidine, and dry canal. Actual length was established by advancing a #10 K-file until its tip became visible at the apical foramen. For electronic WL determination, teeth were embedded in fresh alginate, canals were filled with the assigned irrigant, and a #15 file was advanced until the Woodpex V displayed a stable "0.0/apex" reading for 5 s. Measurements were recorded using the digital caliper. **Results:** The accuracy of the Woodpex V is expected to vary according to the irrigant. Solutions with more stable electrochemical behavior, such as HEDP, may provide readings closer to actual length [1,2], whereas NaOCl may show a higher frequency of measurements outside the ±0.5 mm tolerance [1,2]. These expectations align with reports demonstrating increased out-of-range values with NaOCl and improved accuracy in the presence of certain chelating or mixed solutions. **Conclusions:** The findings are expected to clarify the influence of various irrigants on Woodpex V accuracy, helping optimize WL determination protocols and promote more consistent, evidence-based endodontic practice.

Keywords: Eletronic Apex Locator; irrigation solutions; root canal treatment; working length

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24564 | Evaluation of the antitumor potential of isoquinolinequinone N-oxides in pancreatic cancer cell lines

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Background & Aim: Pancreatic cancer is the 12th most diagnosed malignancy worldwide and the sixth leading cause of cancer-related death, accounting for nearly 470,000 deaths and over 500,000 new cases in 2022 (1). Its dismal prognosis is mainly driven by intrinsic and acquired drug resistance, resulting in a mortality rate exceeding 90%. Current treatments rely largely on gemcitabine, alone or combined with paclitaxel (2,3), highlighting the urgent need for new anticancer agents, particularly ones capable of overcoming chemoresistance. Recently, isoquinolinequinone (IQQ) *N*-oxides showed promising antitumoral activity in counterpart pairs of sensitive and multidrug-resistant (MDR) lung and colorectal cancer cell models (4,5). This study aims to evaluate the antitumor and chemosensitizing effects of seven IQQ *N*-oxides in (sensitive and MDR) pancreatic cancer (PDAC) cell lines and to characterize their biological impact. **Methods:** MiaPaCa-2, Capan-1, BxPC-3, Panc-1 and Panc-1-CDR (MDR) cells were treated with nine IQQ *N*-oxides (RK1 to RK9) or gemcitabine for 48 h. Cell viability was assessed using the SRB assay. Long-term clonogenic capacity was evaluated by colony formation assay (2 or 6 days of treatment). Anti-migratory effects were analyzed using wound healing assay for 48 h (Figure 1). **Results:** RK2 and RK3 emerged as the most potent derivatives, displaying GI_{50} values between 0.80 to 2.35 µM across multiple cell lines. Both compounds were also effective against a gemcitabine-resistant cell line (Panc-1-CDR) presenting GI_{50} concentrations of 1.41 and 1.14 µM, respectively. Clonogenic assays in Panc-1 cells with both compounds demonstrated a marked concentration and time dependent suppression of colony formation, with near complete inhibition by day 6. Moreover, 1.0 µM of RK2 and RK3 significantly impaired migratory

capacity, restricting wound closure to ~60% and ~51% at 48 h, respectively, compared to 80% wound closure in the control. **Conclusions:** IQQ *N*-oxides RK2 and RK3 effectively decreased PDAC cell growth, including in a gemcitabine-resistant (MDR) cell line, and suppressed key malignant traits, such as self-renewal and migration, supporting their further study as promising therapeutic leads for PDAC treatment.

Keywords: Chemoresistance; Isoquinolinequinone *N*-oxides; Pancreatic Cancer

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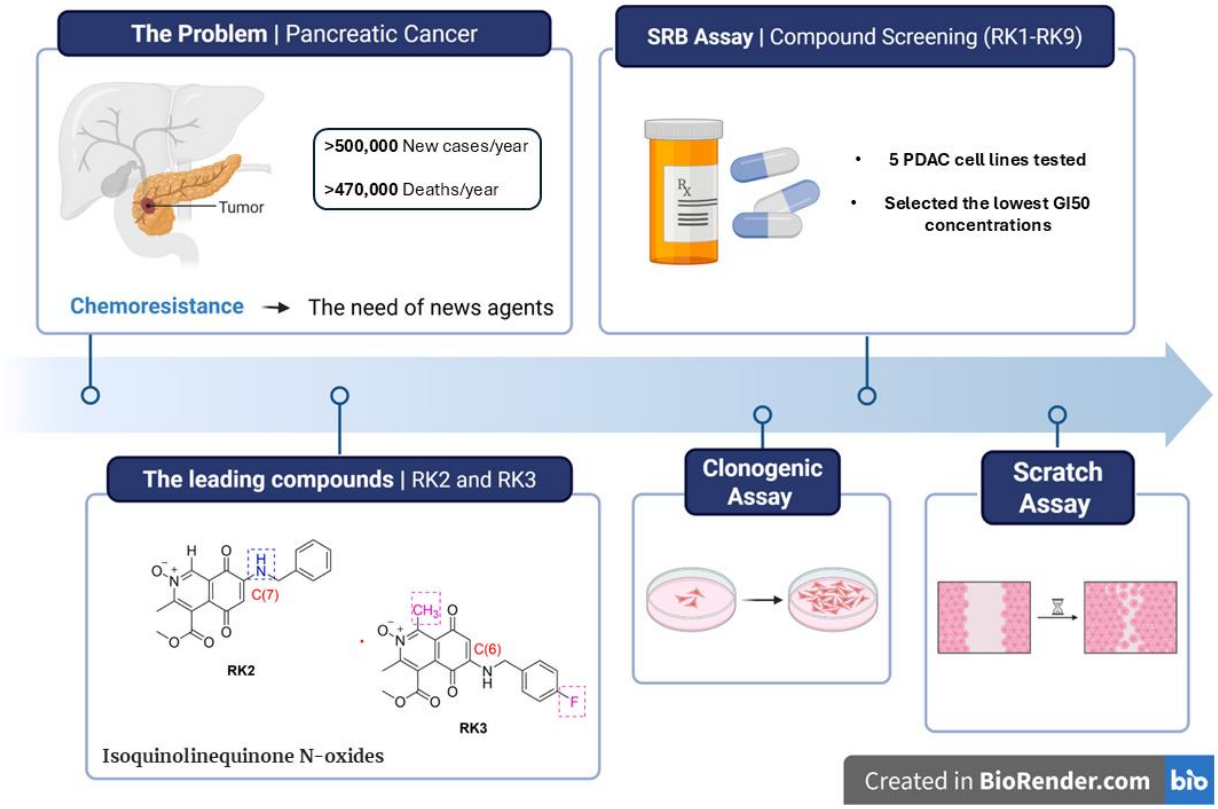
Figure 1: Novel Antitumor Agents RK2 and RK3: Inhibiting Cell Growth and Migration in Pancreatic Cancer cell lines.



24519 | Evolutionary and molecular studies of the obesity-associated gene DENND1B

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Background & Aim: Obesity is a chronic multifactorial disease characterized by excessive fat accumulation and influenced by both environmental and genetic factors [1-3]. Recent genome-wide association studies have identified DENND1B as a novel obesity-associated gene in both dogs and humans [4]. DENND1B encodes a guanine nucleotide exchange factor for the small GTPase RAB35, which is a key regulator of MC4R-mediated melanocortin signaling, contributing to the hypothalamic control of energy homeostasis [5]. This study aimed to investigate the molecular evolution of DENND1B and to optimize the molecular analyses of a DNA fragment containing the obesity-associated variant rs6702421. **Methods:** Protein sequences of DENND1B orthologues from various placentals mammals were retrieved from Ensembl [6] and subjected to selection and filtering criteria. Multiple sequence alignment (MSA) was performed on the selected few using Clustal Omega [7] and Jalview [8]. Variants were collected from ClinVar [9] and GnomAD [10]. For the experimental work, primers flanking the rs6702421 variant were designed for PCR amplification and subsequent Sanger sequencing analyses. **Results:** Preliminary comparative analyses of DENND1B orthologues identified both the highly conserved and non-conserved regions, with several missense variants reported in humans showing CADD scores>20, showing these positions to be functionally relevant to the gene. The genomic region containing the obesity-associated variant rs6702421 was successfully targeted allowing specific PCR amplification of the expected fragment. **Conclusions:** This work provides an evolutionary and molecular framework for the study of DENND1B, supporting its functional relevance in obesity-related pathways. The optimized molecular strategy establishes a basis for future genetic screening and functional characterization of obesity-associated variants in mammals.

Keywords: Obesity, rs6702421; comparative genomics, molecular analyses

Acknowledgments: This study was supported through national funding from the Foundation for Science and Technology – FCT (Portuguese Ministry of Education and Science) within the projects: UID/215/2025, UID/PRR/00215/2025, LA/P/0064/2020 and the PhD studentship 2024.05899.BD.

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24658 | Exploring the Olfactory Receptor OR51E2 in Triple-Negative Breast Cancer Hallmarks

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Background & Aim: Triple-negative breast cancer (TNBC) is an aggressive subtype lacking ER, PR and HER2 expression, limiting targeted therapies [1]. Olfactory receptors (ORs) are G protein-coupled receptors (GPCRs) and comprise ~400 members in the human genome [2, 3]. Beyond olfaction, ORs are often ectopically expressed in several cancers. OR51E2 specifically modulates prostate tumor aggressiveness [4, 5]. β-ionone, a plant-derived volatile isoprenoid found in violets, fruits and perfumes, is a natural ligand and activates OR51E2 [5]. Our group is interested in the overall ectopic function of sensory receptors/ORs [2]. We recently demonstrated that taste receptors participate in glioblastoma signaling [6, 7]. This study aims to explore how OR51E2 expression and stimulation influences key tumor hallmarks, namely proliferation, migration and apoptosis. **Methods:** Two TNBC cell lines were used: MDA-MB-157 and MDA-MB-231, with distinct OR51E2 expression profiles. OR51E2 expression was assessed by polymerase chain reaction (PCR) and reverse transcription-quantitative PCR (RT-qPCR). Baseline proliferation was assessed with MTT and crystal violet stain, migration by scratch assay and apoptosis by Annexin V/PI. Cells were then treated with β-ionone at various concentrations to evaluate dose-dependent effects. Subsequently, OR51E2 was downregulated with siRNA, and proliferation, migration and apoptosis were reassessed. Post-silencing, cells will also be treated with β-ionone to determine receptor-specific ligand responses. **Results:** Preliminary results confirmed OR51E2 expression in both TNBC cell lines, with higher expression in MDA-MB-157. Baseline functional characterization was established. β-ionone treatment induced a dose-dependent reduction in cell viability/growth. **Conclusions:** Ongoing studies combine differential expression analysis, dose-escalation and density-controlled β-ionone treatments with siRNA-mediated OR51E2 downregulation to dissect expression level-dependent and receptor-specific effects on proliferation, migration and apoptosis. These experiments aim to establish a

mechanistic framework for evaluating OR51E2 as a potential therapeutic target for triple-negative breast cancer.

Keywords: Triple-negative breast cancer; OR51E2; Olfactory receptors; β-ionone; Cancer hallmarks

Acknowledgments:

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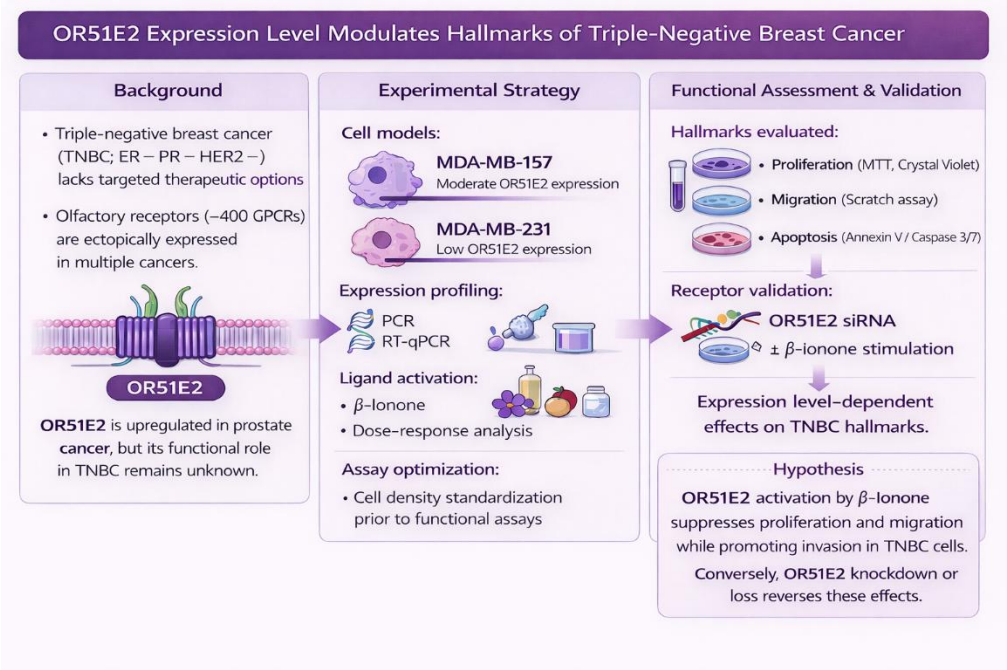

Figure 1: Schematic overview of the experimental design investigating OR51E2 in triple-negative breast cancer (TNBC).

24733 | Family Health Nursing in Families Facing Older Adult Dependence

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Background & Aim: Functional dependence in older adulthood frequently coexists with socioeconomic vulnerability, environmental risk and caregiver burden, challenging primary health care responses. Although systemic Family Nursing frameworks are associated with relational reorganization and measurable health gains, evidence in structurally vulnerable contexts remains limited. This study aimed to analyse health gains resulting from a specialized systemic family nursing intervention grounded in the Dynamic Model of Family Assessment and Intervention proposed by Figueiredo (2012), applied to a family experiencing functional dependence and cumulative vulnerability. **Methods:** A single-case study was conducted in a primary health care setting, including six home visits and three telephone contacts. Assessment and intervention were theoretically and operationally guided by the Dynamic Model of Family Assessment and Intervention[1], integrating transitional and resilience-oriented perspectives. Systemic family interview techniques and motivational interviewing strategies were used. Nursing diagnoses were monitored across structural, developmental and functional domains, enabling comparison between baseline and post-intervention indicators. **Results:** Initial assessment identified unsafe environmental conditions, residential neglect and dysfunctional family processes linked to caregiver burden. Post-intervention, environmental safety improved, household organization was partially restored and family roles were renegotiated. Behavioural changes included smoking reduction and renewed engagement with health monitoring. Following the death of the dependent member, adaptive grief integration was supported. **Conclusions:** Systemic family nursing, operationalized through the Dynamic Model of Family Assessment and Intervention [1], produced measurable health gains, particularly in environmental risk reduction and relational reorganization. Persistent socioeconomic constraints limited change, highlighting the interaction between clinical action and structural determinants. The findings support the model's relevance for outcome-oriented Family Nursing in vulnerable contexts.

Keywords:

Family Nursing; Dynamic Model of Family Assessment and Intervention; Functional Dependence; Primary Health Care; Vulnerability

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24563 | From Digital Transformation and Innovation to Universal Health Coverage: Ethical Guardrails for Equity and Accountability

Catarina Faria[1]; Maria Beatriz Pinto[1]; Margarida Dias[1]; Marcelo Brasiela[1]; Ivone Duarte[2,3,4]; Rui Nunes[2,3,4]; Rui Amaral Mendes[3,4,5]

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Background & Aim: Digital access is increasingly framed as a key enabler of fundamental rights and equitable healthcare. Social determinants such as income, education, geography, age, and housing shape who benefits from digital tools. This narrative review examines how Digital Health innovations may both mitigate and intensify inequities and identifies ethical and governance principles to steer a fair digital transformation. **Methods:** A narrative review was conducted drawing on recent scientific literature, policy documents, and institutional reports on digital health equity, AI, telemedicine, and digital literacy. Sources were analyzed thematically to map recurrent inequities, ethical risks, and proposed strategies. **Results:** Digital health, AI, and telemedicine can expand access to care; however, poorly designed or unevenly implemented technologies may reinforce disparities, deepening inequities, particularly for minorities and the social-economic fringes of society. Key challenges include non-representative datasets, algorithmic bias, limited digital literacy, inadequate infrastructure, and accessibility barriers affecting marginalised groups. Promising approaches include participatory design, proportionate regulation and ethical governance frameworks, improved connectivity, and targeted digital literacy initiatives. **Conclusions**: An ethically grounded digital transformation requires representative datasets, robust bias mitigation, inclusive design, and sustained attention to literacy and accessibility. Ensuring equitable access to digital health and inclusive models is an ethical imperative rooted in justice, equity, beneficence, and non-maleficence. Addressing digital inequities and steering accountability mechanisms is essential if digital health is to fulfil its potential to improve outcomes for all, thus securing de facto Universal Health Coverage in the age of digital transformation.

Keywords: Digital Health; Digital Transformation; Bioethics; Social Determinants; Universal Health Coverage

24861 | Gradient bacterial nanocellulose scaffolds support deposition of cartilage-specific matrix

Lígia Costa[1], Jorge Moura[2], Lara Santos[1], Pedro Almeida[1], Alexandre Carvalho[2], Pedro Granja[1], Fernando Dourado[3], António Fernandes[2], Paulo Antunes[2], José Eduardo Pereira[4], Florinda Costa[2#], Miguel Gama[3#], Sílvia A. Ferreira[1,5#]

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Background & Aim: Osteoarthritis is a degenerative joint disease characterized by the progressive breakdown of articular cartilage that affects millions of people worldwide [1]. This project aims to develop a bacterial nanocellulose (BNC) scaffold with a gradient porosity via laser technology to stimulate osteochondral repair. The scaffolds were engineered to mimic the extracellular matrix (ECM) and cellular organization of the native cartilage and bone. **Methods:** Scaffolds were seeded with human bone marrow mesenchymal stem/stromal cells (hBM-MSCs) and then cultured for 21 days. The chondrogenic differentiation potential was assessed by examining the production of cartilage-specific matrix. We evaluated the production of aggrecan - a proteoglycan present in the articular cartilage [2] - collagen type I, collagen type II and collagen type X - markers for fibrocartilage, articular cartilage and hypertrophic cartilage, respectively [3]. In order to visualize matrix distribution, the samples were subjected to immunofluorescence staining. Posteriorly, confocal laser scanning microscopy was used to acquire images of cell-laden scaffold that were then processed and analysed using ImageJ software. **Results:** Qualitative evaluation of the confocal images revealed expression of the desired ECM components, namely aggrecan and collagen type II. This distinct matrix profile suggests a successful hBM-MSC differentiation into a stable chondrogenic phenotype, encouraging the formation of hyaline-like cartilage instead of unwanted fibrocartilage or hypertrophic tissue. **Conclusions:** Altogether, these results reveal that BNC gradient scaffolds were able to provide a supportive microenvironment for the differentiation of hBM-MSCs into chondrocytes. By successfully leading to the production of specific cartilage ECM components,

this approach showcases the potential of the developed scaffolds as a tissue engineering strategy for osteochondral repair.

Keywords: bacterial nanocellulose; osteochondral; biomimetic; laser technology; gradient scaffolds

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24949 | How Does 5-Fluorouracil Affect non-tumoral Angiogenesis? Bolus vs Metronomic Regimens in the Chick Chorioallantoic Membrane (CAM) Model

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Background & Aim: 5-Fluorouracil (5-FU) is widely used in the treatment of several cancer types. Although its cytotoxic mechanisms are well known, increasing evidence suggests that its effects may go beyond direct tumor cell inhibition, potentially affecting endothelial function and angiogenesis in a dose-dependent manner (1). Nevertheless, most studies focus on the tumor microenvironment, and its impact on tumoral angiogenesis. In non-tumoral context it remains poorly explored. Considering the fundamental role of vascularization in tissue repair and regeneration, understanding how different 5-FU administration regimens affect non-neoplastic angiogenesis would improve the scope of is clinical application. **This study aims to compare the effects of bolus versus metronomic administration schemes of 5-FU on non-tumoral angiogenesis using the chick chorioallantoic membrane (CAM) model. Methods:** Fertilized chicken eggs (*Gallus domesticus*) were incubated under controlled conditions and prepared for CAM access at embryonic day 3 (2). Four experimental groups were established: a positive control, a negative control, a single-dose bolus 5-FU group, and a metronomic low-dose 5-FU group. The drug was topically applied using the CAM with predefined concentrations. Angiogenesis was assessed through quantitative and qualitative parameters. **Results:** Preliminary observations suggest that bolus administration of 5-FU alters the dynamics of vascular formation within the CAM, producing more irregular and transient angiogenic responses. In contrast, metronomic dosing appears to modulate vascular development in a more controlled manner, favoring the establishment of a more organized and stable vascular network. **Conclusions:** 5-FU may exert distinct effects on non-tumoral angiogenesis depending on the administration regimen. Metronomic dosing may modulate vascular organization rather than simply suppress vessel formation. These findings broaden the understanding of 5-FU biological effects and may translate into clinical applications for tissue repair and therapeutic optimization.

Keywords: 5-Fluorouracil; Angiogenesis; CAM model; Metronomic regimen; Vascular normalization

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24850 | Lighting up nature's defense: Can Brazilian green propolis activated by photodynamic therapy outsmart Enterococcus faecalis in endodontic biofilms?

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Background & Aim: Successful endodontic treatment depends on effective elimination of intracanal microorganisms.[1] Persistent infections, particularly those associated with *Enterococcus faecalis*, remain a major cause of treatment failure.[2] This Gram-positive bacterium survives adverse conditions, forms biofilms, and resists conventional irrigants, including NaOCl, creating a microbial profile that challenges standard irrigation solutions. Despite being the gold standard, NaOCl's efficacy is limited by cytotoxicity and poor dentinal tubule penetration to reach bacteria, making Photodynamic Therapy (PDT) a promising alternative.[4, 5, 6] In this context, Brazilian green propolis (BGP), activated at 400-450 nm, is a promising natural photosensitizer with strong antimicrobial properties, no reported microbial resistance, and enhanced performance as a PDT adjunct.[5, 6] However, its synergistic potential in endodontic irrigation is unexplored. This study assesses the antimicrobial efficacy of BGP-PDT against *E. faecalis* biofilm and its cytocompatibility. **Methods:** Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations of BGP against *E. faecalis* followed CLSI broth microdilution guidelines. BGP-PDT (150 s; 458 nm) synergy against 48-hour *E. faecalis* biofilms was assessed using adapted published protocols.[5, 6] A 5.25% NaOCl solution served as the positive control. Resazurin assays and colony-forming unit counts assessed antimicrobial activity. Cytocompatibility will be evaluated in human gingival fibroblasts per ISO 10993. Statistical analysis is performed using SPSS. **Results:** BGP MBC was 4.7 mg/mL, while the intrinsic turbidity of the BGP solution limited MIC determination. BGP demonstrated concentration-dependent antimicrobial effects in 48-hour biofilms, and BGP-PDT synergistically reduced viability. Cytocompatibility results are expected to confirm favorable cytocompatibility for both BGP and BGP/PDT groups. **Conclusions:** Photoactivated BGP is a promising biocompatible adjuvant for root canal disinfection, reducing reliance on cytotoxic irrigants and broadening endodontic strategies.

Keywords: Endodontic irrigation; Brazilian green propolis; Photodynamic therapy; Antimicrobial activity; Cytocompatibility.

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[6] DOI: 10.1016/j.sajb.2022.08.018

24353 | Defining and Enhancing NK Cell Control of Metastasis in PDAC

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Background & Aim: Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy with limited therapeutic options [1]. We previously demonstrated that Natural Killer (NK) cells are key regulators of PDAC progression (unpublished data), yet their therapeutic potential remains unexplored [2]. NK-92 cells infiltrate patient-derived organoids, induce caspase-3/7-dependent apoptosis, and suppress their growth. Proteomic analyses showed enhanced NK cytotoxic and metabolic programs upon tumor engagement, whereas PDAC cells activated stress-response and immune-evasion pathways (unpublished data). Based on these findings, we aim to establish the preliminary steps required to develop a PDAC-optimized CAR-NK cell. **Methods:** Candidate tumor-associated surface antigens were identified by mass spectrometry and validated by immunohistochemistry in patient-derived xenograft (PDX) tissues. Functional validation includes inhibition of tumor-associated pathways using neutralizing antibodies or siRNA, alongside engineering NK-92 cells to overexpress NK-enhancing factors to improve persistence, cytotoxicity, and metabolic fitness. Top-performing engineered NK-92 cells will be co-cultured with PDX organoids, and cytotoxicity will be assessed by granzyme B and perforin expression, caspase-3 activation, multiparametric flow cytometry, and cytokine/interleukin secretion (IFN-γ, TNF-α, IL-12, IL-15, IL-18). **Results** PDAC-associated targets and candidate NK-enhancing factors have been identified and are undergoing PDX validation. Target inhibition is expected to reduce tumor cell viability, while NK-enhancer overexpression should increase NK persistence and cytotoxicity, maintaining functional activity during organoid co-culture. **Conclusions:** This work provides a translational framework for the rational development of optimized CAR-NK cells with enhanced cytotoxicity, persistence, and metabolic adaptability, supporting the advancement of next-generation NK cell-based therapies for PDAC.

Keywords: Pancreatic ductal adenocarcinoma; Natural Killer cells; NK-92 cells; CAR-NK therapy; Patient-derived organoids

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24842 | Improving hair graft viability and trichogenic capacity for hair transplant

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Background & Aim: In hair restoration surgery, prolonged ex vivo storage of hair follicle (HF) grafts during megasessions (up to 10h) increases the risk of ischemic injury and oxidative stress, potentially compromising graft survival and trichogenicity. Here we evaluate whether Solution X®, used for organ preservation, improves HF viability and maintains the hair-inductive transcriptome compared to saline, the standard holding solution. Additionally, the study compares post-implant HF viability using: the conventional Choi vs the semi-automated HairBOT implanter developed by Insparya to optimize hair microtransplant (FUE). **Methods:** Anagenic HFs from the occipital region of n=6 patients undergoing FUE were stored in Saline or Solution X® for 0h, 4.5h, and 9h, and used for: i) morphological analysis (stereomicroscopy) and viability analysis (immunohistochemistry with proliferation/apoptosis markers); ii) ex vivo culturing to measure HF growth and time to catagen onset; iii) dermal papilla (DP) microdissection and analysis of trichogenic gene expression using RNA-Seq and bioinformatic analysis. For device comparison, HFs from n=8 patients were extracted and checked for structural integrity; then implanted using either Choi or HairBOT. HFs were then re-extracted for morphological and histological analysis, as previously described. **Results:** Solution X® is expected to mitigate ischemic injury, preserve anagen and maintain the DP transcriptomic signature more effectively than saline. Preliminary RNA processing confirmed successful DP microdissection and RNA extraction. Concerning the implanter devices, morphological analysis showed no significant differences in the percentage of viable HF between Choi (94.1%) and HairBot (95.2%). Histological staining revealed similar proliferation patterns. **Conclusions:** Solution X® shows potential as a superior holding solution to minimize HF deterioration and improve clinical outcomes. Additionally, HF quality is preserved when using the HairBOT implanter.

Keywords: Hair transplant; Hair follicle; Holding solution; Trichogenic capacity; Semi-automated implanter

24517 | Integrative transcriptomic analysis of smoking-related cancers for the identification of common mRNA signatures and regulators

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Background & Aim: The tobacco epidemic is identified as a premier global health crisis, claiming over 7 million lives annually [1]. The co-transcriptional processes, 3' untranslated region alternative polyadenylation (3'UTR-APA) and intronic polyadenylation (IPA) produce mRNA isoforms of varying lengths due to the use of multiple polyadenylation sites throughout a gene [2]. In this work, we aim to use bioinformatics tools to obtain smoking-specific mRNAs produced by 3'UTR-APA and IPA in lung, bladder and head and neck cancers that may serve as potential risk biomarkers and therapeutic targets, and to identify common regulators of these signatures. **Methods:** We previously identified 460 smoking-associated mRNA signatures exclusive to smokers with lung, bladder and head and neck cancers. Subsequently, a bioinformatic pipeline was developed to remove possible false positives emerging from overlapping genomic regions with neighboring genes. The corrected dataset will then be used for motif discovery with Homer and MEME Suite. **Results:** Of the 460 initial smoking-associated signatures, 384 were retained as high confidence genes with the pipeline built. From these, 366 were filtered for 3'UTR-APA (84.7%) and 18 for IPA (69,2%). Then we will analyse the 3'UTR of these signatures using Homer and MEME Suite and from the analysis of both tools, we expect to determine whether common *cis*-regulatory motifs are shared across most of these mRNAs or whether they can be classified into distinct co-regulated groups. It will also be possible to identify RNA-binding proteins that regulate the expression of these signatures. **Conclusions:** Overall, this now filtered dataset of 384 smoking-specific mRNA signatures represents a promising resource for the identification of future risk biomarkers and therapeutic targets in smoking-related cancers. Moreover, this work will identify common *cis*-regulatory sequences and regulatory networks that can be used to modulate the mRNA signatures expression in these cancers.

Keywords: 3'-UTR alternative polyadenylation, intronic polyadenylation, mRNA signatures, transcriptomic analysis

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24821 | Iodine Status in Pregnancy and Offspring Anthropometric and Adaptive Outcomes: Evidence from the IoMum Cohort

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Background & Aim: In Portugal, low compliance to iodine supplementation (IS) has been associated with mild-to-moderate iodine deficiency (ID) in pregnancy (1). Importantly, benefits of this measure in populations with mild-to-moderate ID are currently a matter of debate (2). This study explored associations between 1st-trimester maternal iodine status or IS and child physical growth or adaptive behavior (AB) up to 36 months. **Methods:** This prospective study included 279 mother-child pairs from the IoMum cohort (3). Maternal urinary iodine concentration (UIC) was measured in the 1st trimester by ICP-MS and IS recorded. Anthropometry was assessed from birth until 36 months and AB at 36 months. Multivariable logistic and linear regression models were used. **Results:** Maternal iodine status and IS were not associated with pregnancy complications or delivery mode. In girls, severe maternal ID (UIC <50 µg/L) was associated with lower weight and BMI z-scores at birth (p=0.009; p=0.018) and lower height z-scores (p=0.021) at 12 months. Significant interactions between iodine status and growth trajectories were observed for height z-scores in boys (p=0.030) and BMI z-scores in girls (p=0.035). No significant associations were found between maternal IS or iodine status categories and AB domains in boys or girls. **Conclusions:** In this cohort, although 1st-trimester maternal iodine status was not associated with pregnancy complications, delivery mode, or AB at 36 months, severe ID was associated with poorer early anthropometric indicators, particularly in girls, and sex-specific interactions with growth trajectories were observed up to 36 months. These findings suggest that severe ID early in pregnancy may influence early child growth. Conversely, IS in a setting of mild-to-moderate iodine deficiency did not associate with clear benefits for offspring growth or AB, underscoring the importance of continuous monitoring and strategies to optimize iodine status during pregnancy.

Keywords: Iodine; Pregnancy; Child growth; Adaptive behavior; Iodine supplementation

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24494 | Is von Willebrand factor a Biomarker of Paravalvular Leak After Transcathether Aortic Valve Implantation (TAVI)?

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Background & Aim: The von Willebrand factor (vWF) plays a crucial role in hemostasis and undergoes structural changes due to aortic stenosis (AS). Transcatheter aortic valve implantation (TAVI) has been associated with modifications in the multimetric profile of vWF, which may reflect the severity of stenosis and the occurrence of possible post-procedural complications, such as periprosthetic leak (PPL). The aim of the study was to determine whether changes in vWF multimers are associated with PPL and the severity of AS. **Methods:** Platelet-poor plasma samples were analyzed from a longitudinal prospective cohort of 48 patients, before and after TAVI. The study was a multicenter prospective observational investigation. Total vWF levels were determined by ELISA. The multimeric profile was characterized by immunoelectrophoresis on agarose gels. vWF and high-molecular weight multimer (HMWM) percentages were compared between groups with and without PPL, using appropriate statistical tests. Correlations with clinical variables were assessed using Pearson or Spearman tests. Analyses were performed using R (version 4.5.1), and a $p<0.05$ was considered significant. **Results:** vWF immunoelectrophoresis showed well-defined multimeric profiles, with no band overlap that could compromise the analysis. No significant differences were observed in the distribution or intensity of the bands between groups with and without periprosthetic leak. vWF was not correlated with disease severity. HMWM (%) was inversely correlated with intervention

duration (r=-0.35, *p*=0.0171). **Conclusions:** This study allowed for the clinical and laboratory characterization of a longitudinal cohort of patients with AS undergoing TAVI, focusing on the occurrence of PPL and the vWF profile. Despite the lack of significant differences in the evaluated parameters, the results highlight the complexity of the relationship between the clinical, anatomical, and biochemical factors involved in the pathophysiology of PPL. The analysis of the vWF multimeric profile proved feasible and may represent a useful tool for future studies with larger and better stratified populations.

Keywords: Aortic valve stenosis; Transcatheter aortic valve implantation; Hemostasis; von Willebrand factor multimers; Paravalvular leak

24836 | Knowledge and Attitudes of Dental Students Regarding the Prevention and Detection of Domestic Violence - a systematic

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Background & Aim: Domestic violence is a concept commonly used to refer to a broad range of abusive behaviors physical, sexual, emotional, and financial directed at individuals who are or have been in an intimate relationship[(1)]. It is associated with multiple adverse health outcomes, highlighting the multidimensional impact of domestic violence on public health[(2)]. Since the face is often targeted in assaults, dental professionals can play an important role in identifying domestic violence[(3)]. Therefore, the primary aim of this systematic review is to assess the knowledge and attitudes of Dental Medicine students regarding the identification and management of domestic violence cases. **Methods:** This systematic review was conducted in accordance with *PRISMA* guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and registered on the PROSPERO plataform[(4)]. A comprehensive literature search was performed in PubMed, Scopus, LILACS, and Web of Science. The articles will be imported into EndNote® 21 software and duplicate entries will be removed. The remaining records will be screened according to the predefined inclusion and exclusion criteria. The quality assessment of the included observational studies will be conducted through risk-of-bias analysis, using the Newcastle–Ottawa Scale. **Expected Results:** The extracted data will contribute to analyze the level of preparedness of dental students to recognize, manage, and report cases indicative of violence in the clinical setting, as well as to identify potential gaps in their ability to detect signs suggestive of domestic violence in patients. **Conclusions:** Through this systematic review is expected to highlight the need to more consistently integrate domestic violence related content into the curricular programs of Dental Medicine faculties, promoting the development of competencies for professional practice and the protection of victims within the context of oral healthcare.

Keywords: Dental students, domestic violence, forensic dentistry, interpersonal violence.

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24524 | Knowledge and Attitudes of Orofacial Injuries in Roller Hockey Athletes: A Pilot Study

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Background & Aim: Roller hockey is a high-contact sport widely practiced in Portugal, involving high-speed play, physical contact, and the use of sticks and balls, which increases the risk of orofacial injuries. Despite recommendations for preventive measures such as custom-made mouthguards, their use remains inconsistent and frequently undervalued. This pilot study aims to characterize the knowledge and attitudes of athlete's orofacial injuries. Additionally, it seeks to assess perception of risk and management strategies following trauma. **Methods:** A cross-sectional pilot study was conducted through an online self-administered questionnaire on knowledge about orofacial trauma in roller hockey, distributed between December 2025 and February 2026 via LimeSurvey (University of Porto). The instrument was developed by the research team based on a review of the literature and validated questionnaires previously applied in roller hockey and analogous contact sports. Following institutional ethical approval, dissemination was carried out through official Portuguese roller hockey clubs and social media platforms. The target population comprised current and former federated athletes aged ≥16 years. Participation was voluntary and anonymous, with informed consent obtained prior to data collection. Descriptive statistical analysis is currently underway to address the study's aims. **Results:** In this study the identification of knowledge regarding prevention and management of orofacial injuries will be expected, besides a heterogeneous access of information are anticipated and a low prevalence of mouthguard use. Findings will support the need for structured educational and preventive strategies. **Conclusions:** New insights regarding orofacial trauma in roller hockey may be particularly relevant by the identification of gaps and preventive shortcomings that can improve educational programs and policy recommendations, ultimately optimizing preventive approaches in this high-risk sport.

Keywords: Rink Hockey; Orofacial Injuries; Dental Trauma; Mouthguard; Prevention

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24583 | Knowledge, attitudes, and challenges of parents/caregivers in promoting oral health in children and adolescents with autism spectrum disorders - A systematic review

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Background & Aim: Children with Autism Spectrum Disorder (ASD) often experience behavioral, sensory, and communication difficulties that negatively affect daily oral hygiene and access to dental care. Parents and caregivers play a crucial role in managing these challenges, and their knowledge and perceptions strongly influence preventive behaviors and health outcomes. Although research on oral health in children with ASD has increased, evidence regarding caregivers' attitudes, practices, and the main barriers to dental care remains fragmented. Therefore, this study aims to systematically review the literature on parents' and caregivers' knowledge, attitudes, and practices regarding oral health in children with ASD, as well as the barriers to dental care. **Methods:** This systematic review follows PRISMA guidelines. Searches will be conducted in PubMed, Scopus, LILACS, and Web of Science. Studies published in English, Portuguese, or Spanish will be considered for inclusion, regardless of study design. Studies addressing parents' and caregivers' knowledge, attitudes, and practices regarding oral health in children with ASD will be included. Risk of bias will be assessed using appropriate validated tools. **Expected results:** The analysis is expected to identify gaps in caregivers' knowledge and inconsistencies in oral health practices. Barriers are anticipated to include behavioral management difficulties, sensory sensitivities, limited professional training, and reduced access to specialized dental services. These findings are expected to highlight priority areas for intervention and education. **Conclusions:** This review is expected to identify evidence-based priorities for intervention and highlight the need for targeted educational programmes for caregivers and dental professionals, ultimately contributing to improved preventive strategies and better oral health outcomes for this vulnerable population.

Keywords: Oral health; Autism Spectrum Disorder; Parents; Caregivers; Knowledge

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24696 | Lemon verbena health products: Between authenticity and bioactivity

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Background & Aim: Metabolic disorders, particularly type 2 diabetes, are associated with oxidative stress and impaired postprandial glucose regulation. Polyphenol-rich natural products have gained attention as complementary strategies to modulate oxidative imbalance and carbohydrate digestion[1]. *Lippia citrodora* (Paláu) Kunth (lemon verbena) is traditionally used in Europe for mild stress and gastrointestinal complaints and has reported antioxidant and anti-inflammatory properties, mainly attributed to verbascoside[2; 3]. However, increasing commercialization of herbal products raises concerns regarding authenticity and phytochemical consistency. This study aimed to: (i) characterize the phenolic profile of *authenticated L. citrodora* samples (A–C) and commercial products (D–H); (ii) evaluate their antioxidant and α-glucosidase inhibitory activities; and (iii) compare their phytochemical composition and bioactivity. **Methods**: Ethanolic extracts (A–H) were analyzed by HPLC-DAD. Antioxidant activity was assessed by DPPH assay, and antidiabetic potential by in vitro α-glucosidase inhibition. **Results**: Verbascoside was the major compound in most samples, together with seven flavonoids. In samples F and G (blends of different plants with lemon verbena), only verbascoside was detected. Samples C (IC_{50} = 0.17 ± 0.03 mg/mL) and E (IC_{50} = 0.20 ± 0.04 mg/mL) showed the strongest antioxidant activity. Samples A, F, and G inhibited α-glucosidase in a concentration-dependent manner, with F being the most potent (IC_{50} = 6.17 ± 0.47 mg/mL). **Conclusions**: *L. citrodora* extracts exhibited relevant antioxidant and moderate α-glucosidase inhibitory activities. Variability between authenticated and commercial samples highlights the need for phytochemical standardization to ensure quality and efficacy.

Keywords: *Lippia citrodora*; phenolic compounds; antioxidant; α-glucosidase

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24828 | Leveraging LLMs for Health Information Governance: Structuring Dispersed Knowledge in Complex Institutional Environments

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Background & Aim: The European Health Data Space (EHDS) makes secondary use of health data dependent on cross-border governance.[1] While FAIR principles and catalogue metadata improve dataset discovery, institutional governance artefacts remain fragmented and hard to query, reuse and audit.[2] This work asks how Large Language Models (LLMs) can structure and interlink such sources into semantically coherent, provenance-aware knowledge.[3,4] The aim is to identify gaps in current metadata models for representing organizational context and derive requirements for LLM-supported governance knowledge integration aligned with EHDS principles.[5] **Methods:** The project follows a mixed-methods approach combining conceptual/documentary analysis of scientific literature, institutional reports and EU regulatory sources (EHDS, GDPR, NIS2, AI Act) to identify gaps in metadata representation and organizational context, and a structured survey of health data professionals (researchers and institutional representatives) analyzed with descriptive statistics and qualitative content analysis. **Results:** Preliminary findings from the literature-based analysis indicate that EHDS readiness for secondary use of data is constrained by institutional fragmentation and heterogeneous governance practices across countries and organizations. A structured survey targeting researchers and institutional representatives has been designed to capture perceptions on health data use across professional profiles and organisational contexts. Its results will identify priority contextual elements and governance information to inform requirements for knowledge structuring and decision support under EHDS secondary use. **Conclusions:** Governance knowledge structuring is a missing layer between EHDS interoperability standards and real-world secondary-use decisions. This work frames

governance knowledge as an operational interoperability layer for EHDS secondary use and specifies requirements for structuring dispersed artefacts in a traceable way.

Keywords: Large Language Models (LLMs); European Health Data Space (EHDS); Secondary Use of Health Data; Interoperability; Metadata.

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24754 | Mapping the Glycan-Mediated Interactions Between Cancer and T-cells using GlycoAvatar Models

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Background & Aim: Gastric cancer remains one of the leading causes of cancer-related mortality worldwide and is characterised by multiple genetic, epigenetic, and post-translational alterations (1). Among these, aberrant glycans, particularly truncated O-glycans, such as Tn and sialyl-Tn (STn), are recognised glycosignatures associated with tumour progression and immune evasion (2-5). This immunomodulation is largely mediated through interactions between tumour-associated glycoproteins and glycan-binding receptors on immune cells, including T cells (6-9). However, many of the receptors involved in these glycan-mediated interactions remain unidentified. This project aims to understand how the abnormal expression of short-chain *O*-glycans directly interacts with and modulates T cells. **Methods:** To achieve this, we will employ the GlycoAvatars method receptor-identification protocol previously developed by the group for receptor identification on myeloid cells (6). This approach uses magnetic beads coated with plasma membrane extracts from glycoengineered cancer cell lines to capture T-cell surface receptors that bind tumour-associated glycans. Pulled-down complexes will be analysed by mass spectrometry, and candidate receptors linked to immunosuppressive T-cell phenotypes will undergo orthogonal validation through binding specificity and receptor-blocking functional assays. **Results:** Co-culture of pre-activated T cells with glycoengineered gastric cancer cell lines (AGS and MKN-45) showed that C1GALT1-knockout tumour cells reduced expression of CD25 and CD279 (activation-induced

markers) and the proliferation marker CD71 in both $CD4^+$ and $CD8^+$ T cells compared with wild-type controls. Plasma membrane extracts from the cancer cell models were used to construct GlycoAvatars for subsequent receptor identification. This study will aid in mapping cancer glycoproteins-T cell interactome, ultimately identifying novel glycan-binding receptors as potential immunotherapeutic targets.

Keywords: Gastric cancer, Tn antigen, STn antigen, T cell, GlycoAvatars

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24610 | Modulating immune transendothelial migration to enhance immunotherapy in B-cell lymphomas

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Background & Aim: Relapsed/Refractory diffuse large B-cell lymphoma (DLBCL) remains difficult to treat despite major advances with immunotherapies such as CAR-T cells and immune checkpoint inhibitors. Although these strategies are increasingly recognized, many patients fail to respond due to insufficient immune cell activation and tumor homing (1). High endothelial venules (HEVs) regulate lymphocyte trafficking within lymph nodes but remain largely unexplored in DLBCL. There are reports of HEV regression in DLBCL patients' lymph nodes (2). We hypothesize that modulation of HEV-mediated transendothelial migration can enhance T-cell trafficking and improve immunotherapy efficacy in DLBCL. **Methods:** We used the syngeneic Eμ-Myc mouse model of aggressive B-cell lymphoma. Lymphoma cells were injected intravenously, leading to systemic disease within 15 days. Two-photon intravital microscopy was performed in popliteal lymph nodes to analyze T- and B-cell trafficking through HEVs. HEVs will be blocked using MECA-79 antibody or stimulated using LIGHT fusion proteins/LTβR agonists. Combination therapy with PD-L1 antibody will assess synergy with immunotherapy. Disease progression and survival will be evaluated. Human DLBCL samples will be analyzed for HEV density, immune infiltrate, and PD-1/PD-L1 expression, correlating with clinical outcomes. **Results:** Intravital imaging of lymph nodes demonstrates progressive HEV regression as disease advances. Ongoing analyses aim to delineate alterations in HEV structure and lymphocyte trafficking dynamics throughout disease progression. We anticipate that HEV stimulation will increase T-cell transmigration and improve therapeutic response. **Conclusions:** This study investigates HEV modulation as a novel adjuvant strategy to enhance immunotherapy in DLBCL. By restoring or amplifying lymphocyte trafficking within lymphoma-bearing lymph nodes, HEV targeting may overcome resistance and provide a translational strategy to improve outcomes in R/R DLBCL patients.

Keywords: B-cell lymphoma; high endothelial venules; immunotherapy.

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24951 | Nanoparticle-Mediated Delivery of Temozolomide and O6-BG: towards improving Glioblastoma Treatment

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Background & Aim: Classified as a Grade IV central nervous system tumor by the World Health Organization, glioblastoma (GB) is the most common and most aggressive primary brain malignancy in adults, with a two-year survival rate <30%[(1)].

Current standard treatment encompasses surgical resection, radiotherapy and temozolomide (TMZ) chemotherapy. A key determinant of GB therapy response is the methylation status of the MGMT promoter, as active MGMT mediates resistance by repairing TMZ-induced DNA damage. To overcome this, O^6-benzylguanine (O^6-BG) has been proposed as a chemosensitizing agent that mimics TMZ-induced DNA lesions and irreversibly binds MGMT, depleting its active form and thereby enhancing TMZ cytotoxicity. Thus, the combined use of TMZ and O^6-BG[(2)] represents a promising strategy to counteract MGMT-driven chemoresistance and improve therapeutic efficacy. In this context, nanoparticle (NP)-based drug delivery systems offer advantages for GB treatment by enhancing drug stability and bioavailability, promoting targeted tumor accumulation, facilitating blood–brain barrier penetration, and reducing systemic toxicity. By evaluating the cytotoxicity, permeation and bioavailability of drug-loaded NPs *in vitro* and *in vivo*, we aim to define an optimized therapeutic strategy that enhances TMZ efficacy while minimizing off-target toxicity to healthy tissues. **Methods:** GB patient derived cells will be used to evaluate *in vitro* cytotoxicity and permeability of TMZ and O^6-BG-loaded NPs. They

will also be used to establish an orthotopic model in NSG or Nude CBA mice for *in vivo* biodistribution studies of the drug loaded-NPs. **Results:** Preliminary data from orthotopic GB inoculation in Nude CBA mice show tumor growth for ongoing study of NPs biodistribution. **Conclusions:** This work supports the development of NP mediated TMZ and O6-BG co-delivery to overcome chemotherapy resistance and improve outcomes in GB patients with low MGMT promoter methylation.

Keywords: Glioblastoma; MGMT; O6-BG; Nanoparticle; PDX.

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24487 | Obesity-associated Breast Cancer Secretome Modulates Endothelial Cell Activity in vitro

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Background & Aim: Obesity is a key modulator of angiogenesis and breast cancer (BC) development, contributing to tumor etiopathogenesis and alterations to the angiogenic process. Although anti-angiogenic therapies are used in BC treatment, their clinical benefit is limited by resistance and eventual therapeutic failure. However, the potential role of obesity in angiogenic therapy resistance remains unclear. This study investigates whether BC cell lines exposed to obese (Ob) or healthy (H) human serum differentially affect endothelial cell (HMVEC) responses. **Methods:** Secretome from BC cell lines of distinct molecular subtypes (MCF-7, MDA-MB-231, Sk-Br-3) was obtained by incubating cells with a 20% concentration of culture medium containing H or Ob human serum for 24 hours. These conditioned media were then used for treatment of HMVEC cells to evaluate cell migration, with an injury assay, cell proliferation with a BrdU assay and cell viability by MTT assay. Treatments were performed with a 20% concentration of BC cell lines secretome in HMVEC growth medium, for 24 hours. **Results:** Preliminary results show that HMVEC cell migration is diminished after 17 and 24 hours, when treated with either H or Ob-treated MCF-7 cells secretome, with a higher reduction effect observed in cells treated with Ob secretome compared to cells treated with H secretome. Results also point to a reduction in HMVEC cell proliferation when incubated with the 3 BC cell line secretome, except for MDA cells exposed to H serum. Furthermore, for each cell line, the secretome of cells exposed to Ob serum induces an even greater inhibitory effect on cell proliferation than their H counterpart, except for the Sk-Br-3 cell line. Findings also suggest that Ob secretome from MCF7 reduce HMVEC cell viability compared to their H counterpart. **Conclusions:** These preliminary findings suggest that, in general, Ob and H BC secretome alter HMVEC behavior, with a higher effect observed in Ob secretome treatment.

Keywords: Breast cancer; Angiogenesis; Obesity; *in vitro*

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24588 | Optimizing Melanoma Screening: AI-Based Risk Stratification for Efficient Patient Prioritization

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Background & Aim: Melanoma is a malignant neoplasm derived from melanocytes that is highly curable if detected early, but 5-year survival drops from 99% to 35% when it is diagnosed in late stages [1,2]. It is also established that despite legal time limits, Portugal's public health care system (SNS) waiting lists face constrictions that compromise the critical treatment window. [3] Herein, the development of an intelligent Clinical Decision Support System for dermatologists, using a dual-stage deep-learning-based pipeline [4] to pre-classify melanoma risk in dermoscopic images from primary health care, while optimizing patient prioritization. **Methods:** The image classification methodology employs a two-layered Python TensorFlow machine learning computer vision approach, trained on the open-sourced ISIC 2020 and HAM10000 skin-lesion datasets. Firstly, skin lesions are identified, isolated and segmented by a U-Net deep learning algorithm. Afterwards, segmented masks are classified by an EfficientNet algorithm to generate a malignancy probability. These models were later embedded into a real-time web application based on Python's Reflex framework. **Results:** Preliminary results show that the U-Net architecture effectively segments the lesion, preventing EfficientNet from analyzing irrelevant artifacts. After U-Net segmentation, the EfficientNet classifier achieved an accuracy of 98.67% and an area under the curve of 99.8% on a test set of 4500 dermoscopic images. Crucially for a clinical screening tool, the model demonstrated a 0.99 recall (sensitivity) for malignant lesions, yielding only 17 false negatives out of 2250 malignant cases. **Conclusions:** The proposed two-step deep-learning pipeline functions as a support for dermatologists. By delivering automated and interpretable risk assessments, it helps physicians feel confident prioritizing urgent cases. This tool augments clinical workflows, offering a solution to reduce diagnostic delays in the public healthcare system.

Keywords: Melanoma, Deep Learning, Dermatology, Triage

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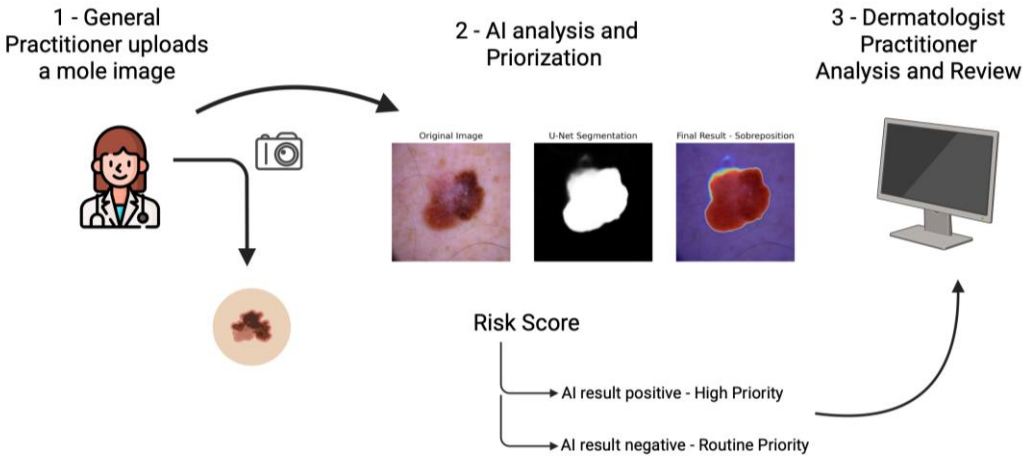

Figure 1: Proposed workflow of the AI-powered Clinical Decision Support System. General practitioners upload dermoscopic images (1), which are automatically processed by the two-step deep-learning pipeline for segmentation and risk stratification (2). Patients are then prioritized based on the calculated risk score, optimizing the final review by specialized dermatologists (3).

24835 | Oral Health and Associated Behaviours in Individuals from Roma Communities: a Cross-Sectional Study

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Background & Aim: Despite global oral health progress, marked inequalities persist among socially marginalized groups, particularly Roma communities, who face structural barriers including poverty, discrimination, and limited access to healthcare [1, 2]. These determinants contribute to poorer health indicators than the general population [2]. The aim of this study is to characterize the oral health status and related behaviors of Roma communities residing in the municipality of Maia, Portugal. **Methods:** A cross-sectional study was performed in collaboration with the "Projeto Maia Melhor" and the "Grupo de Atividade Comunitária-Investigação e Promoção da Saúde Oral da Faculdade de Medicina Dentária da Universidade do Porto". A structured questionnaire collected data on sociodemographic characteristics, oral hygiene behaviours, dental care access, and oral health perceptions. Clinical variables were assessed through oral examinations. Data collection procedures were conducted anonymously. This Study was conducted and reported in accordance with the STROBE guidelines for cross-sectional studies [3]. Descriptive statistical analysis was performed using SPSS. **Results:** Twenty-four questionnaires and twenty-six clinical examinations were obtained. Most participants were unemployed (95.8%), and 41.7% had no formal education. One-third (33.3%) had never brushed their teeth. Dental visits were irregular: 45.8% had last visited a dentist more than 2 years ago, 8.3% had never visited, and high cost was reported as the main reason (50.0%). 87.5% had missing teeth, and self-rated oral health was fair or poor (75.0%). Gingival inflammation in 81.5% and gingival recession 59.3% were the most frequent findings. **Conclusions:** Significant oral health problems and insufficient preventive behaviours were identified among Roma communities. Systematic implementation of preventive examinations and hygiene programmes, combined with equitable access to oral care is needed to promote oral health and reduce inequalities.

Keywords: Oral health, Roma communities, Ethnic minorities, Health inequalities, Social disadvantages.

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24526 | Oral health in figure skating athletes: Impact on sports and preventive measures. A cross-sectional study

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Background & Aim: High-performance athletes are a vulnerable population for oral health problems due to risk factors associated with sport practice, such as frequent intake of carbohydrate-rich foods and acidic sports drinks, mouth breathing, and physical and psychological stress. These factors may increase the risk of dental caries, erosion, periodontal disease, and bruxism, potentially affecting sports performance. Although oral health has been studied across several sports disciplines, artistic roller skating remains underrepresented. This study aimed to characterise the self-reported oral health status of competitive artistic roller-skating athletes, identify associated risk factors, and propose measures to promote better oral health and, consequently, optimise sports performance. **Methods:** A descriptive cross-sectional study is being conducted among federated artistic roller-skating athletes registered in clubs affiliated with the Portuguese Skating Federation. Data are being collected via an anonymous, semi-structured online questionnaire covering sociodemographic data, oral health history, hygiene habits, dietary and hydration practices, and knowledge of dental trauma and mouthguard use. Data will be analysed using IBM SPSS Statistics 31, with descriptive statistics calculated, associations assessed using the chi-square test, and Bonferroni correction applied for multiple comparisons. This study was approved by the Ethics Committee of the Faculty of Dental Medicine and by the Porto University Data Protection Commission. **Results:** Data collection is currently ongoing. Results are expected to characterise self-reported oral health status among competitive artistic roller-skating athletes and to identify links between oral health and sports performance. **Conclusions:** This study may raise awareness among athletes, coaches, and technical staff about the importance of oral health in sports, supporting preventive actions and guiding future research and oral health policies.

Keywords: Athletes; oral health; sports performance; sports dentistry

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24573 | Polysaccharide Hydrogels for Vaccine Delivery in Cancer Immunotherapy

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Background & Aim: Cancer remains a major health burden, with current therapies struggling to prevent cancer recurrence(1, 2). Protein glycosylation plays a central role in tumour progression, making it an attractive target for immunotherapy(3, 4). Glycovaccines based on tumour-associated carbohydrate antigens offer a promising approach to induce durable anti-tumour immunity, yet there is an opportunity to enhance their immunogenicity(5, 6). Injectable hydrogels based on polysaccharides and thermoresponsive polymers provide a suitable depot to control delivery and potentiate the immune response to glycovaccines(7, 8). This project aims to develop a delivery platform capable of enhancing immune responses. **Methods:** The hydrogel will be synthetised using the "cold method" and polysaccharides will be properly characterised. Physicochemical characteristics, including gelation time, degradation rate and rheological behaviour will be evaluated. *In vitro* release kinetic, using model proteins and glycoantigens, will be assessed by MicroBCA assay. Furthermore, studies with dendritic cells and both cancer and healthy cells will be conducted to determine cytocompatibility (DAPI staining) and activation ($CD86^+$, $CD11c^+$, $HLA\text{-}DR^+$) of antigen-presenting cells (APCs) resorting to flow cytometry. Simultaneously, cytokines secretion will be analysed. **Results:** The hydrogel's gelation temperature is expected to be near the physiological temperature, showcasing a rapid gel formation. The platform is anticipated to protect the antigen and control its release over several

hours, preventing the transient antigen expression. Finally, the hydrogel is expected to be non-cytotoxic while capable of activating APCs. **Conclusions:** The hydrogel-based platform emerges as a powerful tool for glycovaccine delivery, combining biocompatibility, controlled antigen release, and immune activation. This integrated system holds strong potential to boost anti-tumour immunity and pave the way towards next-generation cancer immunotherapies.

Keywords: Cancer; glycovaccines; thermoresponsive hydrogels; polysaccharides

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24958 | Postprandial Glycemic Response Following Banana and Bread Co-Ingestion

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Background & Aim: Postprandial glycemic control is essential for metabolic health(1), yet the impact of fruit co-ingestion on carbohydrate-induced glycemic responses remains insufficiently characterized. Given the potential antidiabetic properties of banana (*Musa* spp.)(2), this study aimed to determine whether banana co-ingestion modulates the postprandial glycemic response to white bread. **Methods:** 51 healthy adults (66.7% female; median age 22.8 years; BMI 21.67 ± 1.76 kg/m²) completed three controlled feeding sessions. Standardized portions of white bread (WB, 45 g; control) and banana (BAN, 115 g), each providing 25 g of digestible carbohydrates, were used. Four conditions were evaluated: WB alone, BAN alone, half-combined portions (HCP; ½WB + ½BAN providing 25 g digestible carbohydrates), and full-combined portions (FCP; WB + BAN providing 50 g digestible carbohydrates). Participants fasted ≥ 6 h or had a standardized meal 4 h before assessments. Capillary glucose was measured using glucometers from 0–120 min. iAUC was calculated using the trapezoidal method(3). Values were analyzed with SPSS® v30 ($p < 0.05$). **Results:** BAN induced an earlier glycemic peak than WB (38 [30, 45] vs 60 [53, 82] min; $p < 0.001$) and a lower iAUC (1901 ± 946 vs 3104 ± 1144 mg.dL^{-1}.min; $p < 0.001$). HCP produced a glycemic curve similar to BAN, with an earlier peak (45 [30, 45] min; $p < 0.001$) and lower peak glucose than WB (120 ± 16 vs 128 ± 16 mg.dL^{-1}; $p = 0.037$). HCP also resulted in a lower iAUC than WB (2442 ± 1260 mg.dL^{-1}.min; $p = 0.007$). FCP resulted in a higher iAUC (4080 ± 1987 mg.dL^{-1}.min) than WB ($p = 0.013$) but a lower iAUC than the summed WB and BAN responses ($p = 0.022$). **Conclusions:** Banana ingestion induces a lower glycemic response than a carbohydrate-matched bread portion. Additionally, a combination of banana with bread does not seem to promote a better glycemic response when compared to bread alone and results in a non-additive glycemic response. The earlier glycemic peak when banana is co-ingested with bread may result from bread starch digestion by banana amylase. Further studies

incorporating insulin profiling and assessment of amylolytic and glucosidase-modulating activity in banana are needed to clarify the underlying mechanisms.

Keywords: Postprandial glycemic response; Banana (Musa spp.); Starch digestion; iAUC

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24867 | Psychosocial impact of major lower limb amputation: depression, suicide risk, body image, and caregiver burden

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Background & Aim: Major limb amputation has a significant physical, functional, and psychosocial impact, requiring individual and family adaptation. Evidence shows a high prevalence of depressive symptoms and body image disturbances, associated with increased psychological vulnerability and suicide risk. This study aims to evaluate psychological adjustment following amputation, analysing depressive symptoms, suicide risk, and body image in amputees, as well as emotional distress and caregiver burden. **Methods:** This is a quantitative, descriptive, and correlational cross-sectional study with two sub-studies: amputees and family caregivers. The sample includes adults with major lower limb amputation of vascular etiology and their caregivers. Data will be collected through interviews using a sociodemographic questionnaire and validated instruments (PHQ-9, C-SSRS, ABIS-20, Zarit). Data will be analyzed using descriptive and inferential statistics to explore associations between variables. **Results:** Preliminary results suggest the presence of clinically relevant depressive symptoms among amputees, accompanied by negative body image perception and indicators of psychological vulnerability. Furthermore, evidence indicates that up to 30-50% of amputees present with depressive symptoms, with an increased risk of suicide reported in this population. Trends suggest potential associations between depressive symptoms, body image disturbances, and suicide risk in amputees, as well as between caregiver burden and patient clinical characteristics. Final results are expected to confirm these associations and further clarify the interaction between psychological and clinical variables in both groups. **Conclusions:** These findings may enhance understanding of the psychosocial impact of major lower limb amputation, identifying vulnerability factors in amputees and caregivers, informing targeted clinical and nursing interventions, and promoting greater visibility of care for this population.

Keywords: Amputees; Mental Health; Suicide; Health Vulnerability

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24872 | Psychostimulants and neuroinflammation: Finding key players in the crosstalk between glial cells and neurons

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Background & Aim: Exposure to methamphetamine (Meth), a highly addictive widely used psychostimulant, is classically associated with damage to neuronal terminals, but its neurotoxicity can also be mediated via activation of the neuroinflammatory response. We have shown that acute methamphetamine (Meth) causes microgliosis and increases microglia activation through astrocytic-TNF release [1]. We are now interested in clarifying the progression of neuroinflammation under chronic drug exposure and how different brain and immune cells contribute to this inflammatory process. Our preliminary results showed that CD47, a "don´t eat me signal", was decreased after chronic Meth administration and that this was concomitant with an increase in microglia numbers and homeostasis dysregulation. In this regard, the main objective of this work is to evaluate the synaptic pruning after chronic Meth exposure and during an early and a late withdrawal period. **Methods:** C57BL/6 male mice were administered with escalating doses of Meth (1-6 mg/kg/day, IP, 10 days). The animals were evaluated at P60 (control), P70 (end of exposure), P72 (2 days withdrawal), and P80 (10 days withdrawal). Brains isolated from perfused mice were sliced in the cryostat and for immunostaining, sections at the level of dorsal hippocampus were processed in free floating, to evaluate microglial phagocytosis by confocal microscopy. To do so, a colocalization of synaptic markers with CD68 in Iba+ cells was performed using IMARIS 3 D reconstruction. **Results:** The preliminary results seem to indicate an increase in the microglia phagocytic activity after 2 days of withdrawal. Current analyses are set to determine whether the pruning of excitatory synapses is occurring, causing a dysregulation of synaptic transmission. **Conclusions**: This work will elucidate how chronic Meth exposure **impacts the synaptic remodeling, which may change the way that this process has been perceived under psychoactive drugs this far.**

Keywords: Addiction; Methamphetamine; Microglia; Synaptic pruning.

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24596 | Role of adenosine in cisplatin resistance in breast cancer

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Background & Aim: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype associated with poor prognosis [1,2]. Although platinum-based agents, such as cisplatin are used in TNBC treatment, their efficacy is often compromised by the development of resistance [3,4], highlighting the need for improved therapeutic strategies. Adenosine is abundantly present in the tumor microenvironment and activates A_1, A_{2A}, A_{2B} and A_3 receptors, promoting proliferation, angiogenesis, immunosuppression and overall tumor cell survival [5,6]. However, the role of adenosine signaling in cisplatin resistance in TNBC remains unclear. This study aimed to investigate the impact of adenosine-mediated signaling on proliferation of cisplatin-sensitive and -resistant TNBC cells. **Methods:** Cisplatin-sensitive and -resistant MDA-MB-231 cells were treated with Suramin (CD39 inhibitor and non-selective P2 antagonist), α,β-methylene-ADP (CD73 inhibitor) and BAY 60-6583 (selective A_{2B} agonist). Additionally, immunocytochemistry was performed to evaluate CD39, CD73 and ENT1 expression**. Results:** Suramin reduced proliferation in both cell lines, with a stronger effect in sensitive cells (decreasing to 50%, versus 78% proliferation in resistant cells at 72h, 200 µM). CD73 inhibition decreased proliferation in sensitive cells, ranging from 67 to 78% proliferation, while resistant cells exhibited 115-126% across the 72h period, at 10 µM. The A_{2B} activation promoted proliferation in resistant cells (117% at 48h, 1 µM), but reduced growth in sensitive cells (75% at 48h, 1 µM). Immunostaining assays, cisplatin-resistant cells showed reduced CD39 expression, no changes in CD73 levels and increased ENT1 staining when compared to cisplatin-sensitive cells. **Conclusions:** Cisplatin resistance in TNBC may involve purinergic rewiring, sustained A_{2B} signaling, reduced reliance on extracellular ATP hydrolysis and enhanced adenosine transport. Targeting A_{2B} and adenosine-regulating pathways may help overcome cisplatin resistance.

Keywords: Breast Cancer; TNBC; Adenosine; Cisplatin; Resistance

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24946 | ROS-Responsive Nanocarriers for Enhanced Therapy in ALS

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Background & Aim: Neurodegenerative disorders are progressively affecting a larger proportion of the population worldwide., with Amyotrophic Lateral Sclerosis (ALS) representing a rare, progressive, and fatal condition characterized by selective motor neuron degeneration. Mitochondrial dysfunction leads to downstream Amyotrophic Lateral Sclerosis (ALS) pathological features, namely oxidative stress, causing high levels of reactive oxygen species (ROS) in central nervous system, which ultimately contributes to neuronal dysfunction and cell death.[1] Despite that FDA approved drugs for ALS only act in the symptomatic relief, their inability to cross the blood-brain barrier (BBB) and short half-circulation time cause a lack of efficacy, promoting a short extension of patient's life.[2] These challenges highlight the need for advanced, targeted delivery systems to enhance the stability and brain bioavailability of these drugs, improving the overall therapeutic outcome. In this study, we propose the development of ROS-responsive polymeric nanoparticles as nanocarriers for the delivery of different ALS drugs or drug candidates. **Methods:** Nanocarriers based on thioether were prepares via Polymerization-Induced Self-Assembly (PISA),[3] and characterized in terms of drug encapsulation efficiency using an Ultra-High Performance Liquid Chromatography system, as well as regarding their particle size and polydispersity assessed by Dynamic Light Scattering. Surface charge was also measured by Electrophoretic Light Scattering. A preliminary evaluation of cytotoxicity and permeability was performed using neuronal and nasal epithelial cells, respectively. **Results:** The resulting nanocarriers were successfully prepared by using the PISA method with sizes lower than 150 nm and low polydispersity (PDI values < 0.2). Also, the ROS-responsive nanocarriers did not show cytotoxicity and were able to surpass the intranasal barrier. **Conclusions:** Overall, this study introduces a multifunctional, ROS-responsive and intranasal permeable nanoplatform designed to enhance brain delivery of ALS drugs or drug candidates.

Keywords: Amyotrophic Lateral Sclerosis; Reactive Oxygen Species; Nanotechnology; Drug Delivery

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24728 | School-Based Oral Health Promotion Programs in Children Aged 6-12 Years for Dental Caries Prevention

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Background & Aim: Dental caries remains a major public health concern, supporting preventive programs in school and community settings. Early intervention fosters oral health behaviours that may persist across the lifespan. This scoping review aimed to map interventions targeting dental caries prevention and reduction in children aged 6–12 years and to synthesise reported clinical outcomes. **Methods:** The review followed the Joanna Briggs Institute methodological guidance for scoping reviews and applied the PCC framework: Population — children aged 6–12 years; Concept — oral health promotion programs focused on dental caries prevention or reduction; Context — school-based or community interventions linked to the school environment. Searches were conducted in PubMed, Scopus and Web of Science. Six sources were included: three systematic reviews, one cluster randomised controlled trial, and two prospective non-randomised interventional studies reporting clinical caries outcomes (e.g., DMFT/dmft). **Results:** Eligible evidence, in accordance with the predefined inclusion criteria, identified two predominant types of intervention: education only approaches and structured multicomponent programs integrating educational and preventive elements. Educational interventions consistently improved oral health knowledge and hygiene behaviours, although clinical effects on caries indicators varied. Programs incorporating preventive components more frequently reported favourable trends in caries prevalence or incidence during follow-up. Heterogeneity in study design, follow-up duration and outcome definitions limited direct comparability. **Conclusions:** In children aged 6–12 years, structured multicomponent programs appear more consistently associated with favourable clinical outcomes. However, methodological heterogeneity and limited follow-up restrict interpretation. Further well-designed studies with harmonised clinical indicators and longer follow-up are needed to clarify effect magnitude and sustainability.

Keywords: oral health promotion; school-based intervention; dental caries; children; prevention.

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24960 | Sex-related differences in neurodevelopment outcome after neonatal Group B Streptococcal infection

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Background & Aim: Neonatal infection is a major risk factor for neurodevelopmental impairment. Group B *Streptococcus* (GBS) remains a leading cause of neonatal invasive disease, associated with neuroinflammation and altered brain development. Increasing evidence indicates that neurodevelopmental outcomes may differ between sexes, however these differences remain uncharacterized under neonatal GBS infection. Here, we investigated whether neonatal GBS infection induces sex-specific differences in early neurodevelopmental outcomes. **Methods:** BALB/c dams were randomly divided into non-colonized and colonized groups, and females in the colonized group were intravaginally inoculated with GBS. Neurodevelopmental milestones were assessed in pups from postnatal day (PND) 2 to PND21 using a battery of behavioral tests, including surface righting, negative geotaxis, cliff aversion, grasping, rooting reflex, auditory startle, and open field performance. **Results:** Infected pups exhibited significant delays in several developmental milestones compared with healthy controls within both sexes ($p<0.01$), confirming the detrimental impact of neonatal GBS infection on early neurodevelopment. Direct comparisons between males and females revealed only limited differences across behavioral tests, suggesting that the infection-induced delays occur largely independently of sex in this model. **Conclusions:** Together, these findings indicate that neonatal GBS infection disrupts early neurodevelopmental progression in both male and female pups. Although minor behavioral differences between sexes were observed in specific tests, the overall neurodevelopmental impairment associated with neonatal GBS infection appears to affect both sexes in a broadly similar manner.

Keywords: Group B Streptococcus; Neonatal sepsis; Sex differences; Neurodevelopmental milestones

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24767 | Strychnos Madagascariensis fruit components as skin care products

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Background & Aim: Cutaneous xerosis is a dry skin condition characterized by itchy, flaky, and rough skin. Atopic dermatitis, diabetes, and hypothyroidism are diseases that could exacerbate the condition. Therapy includes hydration and, in worst-case scenarios, the use of corticosteroids. The current formulations continue to use ingredients, such as *petrolatum,* a non-renewable fuel. Our aim is to develop a sustainable and eco-friendly water-based toner from *Strychnos madagascariensis*, an African fruit, to treat cutaneous xerosis. (1) **Methods:** Extraction of the different parts of the fruit at room temperature and storage at -20ºC. Contents were chemically characterized regarding their protein content, lipids, minerals and humidity; and a primary formulation will be developed. Water-based toner formulation stability and biocompatibility will be evaluated using cell cultures *in vitro* assays, namely human dermal fibroblasts (HDFs) and spheroids, through viability assays. (2) Statistical analysis will be performed using Graph Pad Prisma 10® and Image J, considering a p-value<0.5 for statistical significance. **Results:** Pre-formulation of water-based toner from *Strychnos madagascariensis* demonstrates biocompatibility and a richness in lipids that could show future efficacy in preventing cutaneous xerosis, by promoting skin tissue repair. **Conclusions:** The developed formulation reveals a potential in treating/preventing cutaneous xerosis, while being a more sustainable approach and thus less harmful to the environment.

Keywords: Cutaneous xerosis; Sustainability; Tissue regeneration; Water-based toner.

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24885 | Technical Quality of Root Canal Fillings Performed by Undergraduate Students

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Background & Aim: Radiographic evaluation of technical quality is essential for predicting the long-term success of root canal treatments. The quality of undergraduate students' root canal fillings often varies due to anatomical complexity, difficulties in determining working length, and differences in endodontic skills. This study aimed to assess radiographically the technical quality of root canal fillings performed by undergraduate students at the Faculty of Dental Medicine of the University of Porto. **Methods:** Ninety-four teeth treated endodontically by undergraduate students were evaluated using periapical radiographs. Quality was assessed based on length (adequate, short, overfilling) and density (adequate, inadequate). A filling was considered acceptable only if both criteria were adequate. Differences were analyzed using chi-square tests (α=0.05). **Results:** Overall, 36.2% of fillings were acceptable. Adequate density was observed in 68.1% of teeth, and adequate length in 54.3% of teeth. Anterior teeth showed a higher proportion of acceptable fillings (45.0%) than posterior teeth (29.6%), though not statistically significant ($p = 0.125$). Length adequacy was significantly better in anterior teeth ($p=0.031$), while density did not differ ($p=0.917$). Acceptable fillings were more frequent in the maxilla (44.7%) than the mandible (27.7%) ($p = 0.086$). **Conclusions:** The technical quality of root canal fillings performed by undergraduate students was moderate, mainly limited by inadequate length. Anterior and maxillary teeth tended to show better results.

Keywords: Endodontic treatment; Undergraduate students; Root canal fillings; Technical quality.

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24698 | The Impact of Child Behavior on Pediatric Oral Health Practices

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Background & Aim: Oral and general health are linked, with early childhood caries (ECC) affecting 37.2% of children under five in the EU (1). Beyond pain, ECC can impair growth and sleep (2). While twice-daily brushing is recommended, child behavior is critical for routine adherence. Hyperactivity and conduct problems are linked to inferior hygiene practices, while prosocial behavior is associated with better oral health (3). This study evaluates how children's behavioral dimensions contribute to their oral health habits. **Methods:** 70 mother-child dyads from the OralBioBorn cohort (24-36 months old) were evaluated. Child behavior was assessed using the Strengths and Difficulties Questionnaire, focusing on hyperactivity, conduct and peer relationship problems, emotional symptoms and prosocial behavior. Oral health was evaluated via maternal questionnaires regarding brushing frequency and habits. **Results:** Statistical analysis revealed that behavioral dimensions can affect oral hygiene consistency. A positive correlation was found between prosocial behavior and brushing frequency (r_s=0.271; p=0.028), specifically in the afternoon (r_s=0.333; p=0.021), suggesting higher social competence increases cooperation. Hyperactivity was correlated with nighttime eating habits (r_s=0.269; p=0.030), indicating impulsivity may increase dietary intake following oral hygiene. Furthermore, fewer conduct difficulties were associated with increased frequency of evening brushing (r_s=-0.280; p=0.023). **Conclusions:** This study identified modest but significant associations between child behavior and oral hygiene habits. Prosocial traits facilitate consistent brushing, while hyperactivity and conduct challenges hinder plaque control. Although causal relationships cannot be inferred, these findings support the relevance of considering behavioral profiles when

assessing risk in pediatric oral health. Further longitudinal studies are needed to clarify the directionality of these associations and clinical implications.

Keywords: Oral health; Child behavior; Tooth brushing; Health behavior.

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24575 | The importance of NRS-2002 and Handgrip Strength to assess Nutritional Risk in patients of a Central Hospital

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Background & Aim: Early nutritional assessment is essential to identify undernourished or at-risk inpatients and improve clinical outcomes. This study aimed to evaluate the nutritional risk of hospitalized adult patients in the Men's Medicine Department at Hospital São João using Handgrip Strength (HGS)[1] and the NRS-2002[2], and to explore their association. **Methods:** This cross-sectional observational study included 99 male inpatients aged ≥50 years, assessed within the first 72 hours after admission. Nutritional risk was evaluated using the NRS-2002 tool, and HGS was measured with a calibrated Gripwise® dynamometer. Statistical analysis was performed using SPSS software with a 95% confidence level. **Results:** According to NRS-2002, 91.9% of patients were at nutritional risk, and all patients aged ≥70 were classified as at risk. HGS was assessed in 78 patients, of whom 89.7% had values below 27 kgf, indicating a high prevalence of possible malnutrition. A weak but statistically significant negative correlation was found between HGS and NRS-2002 scores ($\rho = -0.259$, $p = 0.010$), and between HGS and nutritional deficit ($\rho = -0.216$, $p = 0.032$). No significant correlation was observed between HGS and age ($p = 0.396$) or disease severity ($p = 0.739$), nor between nutritional deficit and disease severity ($p = 0.750$). Most patients (95.2%) without HGS measurement were also at nutritional risk. **Conclusions:** A high proportion of hospitalized patients, particularly those aged ≥70, were at nutritional risk. HGS showed a significant inverse association with NRS-2002, suggesting that reduced muscle strength may reflect nutritional impairment. HGS appears to be a practical and useful tool for identifying patients at nutritional risk in hospital settings[3,4]. Early detection and nutritional intervention are crucial to improve outcomes and prevent malnutrition-related complications.

Keywords: malnutrition; nutrition-assessment; nutrition-risk; handgrip strength.

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24515 | The Influence of Maternal Mental Health on Children's Oral Health - A Systematic Review

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Background & Aim: The perinatal period is associated with an increased susceptibility to mental health disturbances.[1, 2] Approximately one in five mothers experiences a mental health disorder during this time[3], which may compromise maternal responsiveness and the promotion of healthy habits[4, 5], potentially impacting children's oral health.[5-7] The aim of this systematic review is to systematize how maternal mental health conditions may contribute to adverse oral health outcomes in children and to identify opportunities for early preventive intervention. **Methods:** This systematic review follows the PRISMA criteria and was registered in PROSPERO (CRD420251166596). The search question - "Can mothers' mental health influence their children's oral health?", was structured using a PCO strategy (Population: mothers; Context: maternal mental health; Outcome: children's oral health). The search was conducted in the databases PubMed, Web of Science, Scopus and Lilacs. Cross-sectional, case control and cohort studies published in the last seven years were included. Maternal mental disorders had to be diagnosed through medical records or validated self-report instruments, according to ICD-11 criteria. Outcomes included dental caries, dental trauma, bruxism, biofilm accumulation, gingivitis and periodontal disease. Qualitative studies, without full-text access, literature reviews and those involving children with medical conditions affecting their oral health were excluded. The quality of the studies will be assessed using the Newcastle-Ottawa scale. **Results:** Of 2884 studies, 17 met the inclusion and exclusion criteria. Children whose mothers have mental health problems are expected to present poorer oral health, with dental caries being the most prevalent condition. **Conclusions:** Synthesizing recent evidence on this association may support a multidisciplinary approach, integrating maternal mental health assessment into preventive strategies aimed at improving children's oral health outcomes.

Keywords: Mother; Mental disorders; Child; Oral health

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24898 | The role of iron in TET2 clonal hematopoiesis

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Background & Aim: Clonal hematopoiesis (CH) is the expansion of clones deriving from hematopoietic stem and progenitor cells (HSPC) carrying variants in genes such as the epigenetic regulator TET2. TET2 is an iron- and α-KG-dependent enzyme that catalyzes the oxidation of 5-methylcytosine, leading to DNA demethylation. Loss-of-function mutations of TET2 are related to myeloid malignancies and increased cardiovascular risk.[1] Despite being iron-dependent, the effects of iron modulation and its relationship to TET2 function in the context of hematopoiesis is not fully understood. There is also evidence that iron overload leads to a myeloid bias.[2-4] Mitochondrial dysfunction is also described to contribute to this myeloid skewing.[5] We hypothesize that iron excess and TET2 deficiency have cumulative effects on myeloid differentiation through mitochondrial impairment. **Methods:** HSC-*SCL*-Cre-ER^{T};Ai6$^{+/mut}$;*Tet2*$^{+/flox}$ or HSC-*SCL*-Cre-ER^{T};Ai6$^{+/mut}$;*Tet2*$^{+/+}$ bone marrow (BM) cells were transplanted into adult C57BL/6 (normal iron levels) and *Hamp1*-knockout (iron overload) mice (n=11-12 each group). After engraftment, *Tet2* deletion was induced specifically in HSCs by tamoxifen injection[6], and mice were monitored for 26 weeks. Serial blood collections were made for flow cytometry analysis and blood cell counts. At endpoint, BM analysis was performed by flow cytometry. **Results:** Our preliminary results show that HSPCs in the *Tet2*$^{+/flox}$ and *Hamp1*-KO mice exhibited an increased myeloid bias, demonstrated by a larger population of granulocyte-monocyte progenitors and multipotent progenitors biased towards myeloid. **Conclusions:** These findings suggest that iron excess amplifies the myeloid bias driven by TET2 deficiency, potentially through a shared pathway. Further research will focus on unraveling the mechanistic relation between iron and TET2. This investigation may provide novel insights on hematopoiesis, which

is expected to have clinical implications, such as the possibility of iron modulation in TET2-CH patients.

Keywords: iron, TET2, hematopoiesis, myeloid bias

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24647 | The Role of Rehabilitation Nursing in Promoting Sexuality After Spinal Cord Injury: A Scoping Review

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Background & Aim: Sexuality is considered a central dimension of identity, functionality, and quality of life. In people with spinal cord injury, it is often compromised by neurological, psychological, and relational changes. Despite the high prevalence of sexual dysfunction in this population, its management remains insufficiently integrated into rehabilitation programs. The Rehabilitation Nurse Specialist, through specific competencies, plays a key role in the structured, person-centered promotion of sexuality. This review's objective is to map the available scientific evidence on the contribution of the Rehabilitation Nurse Specialist to the promotion of sexuality in adults with spinal cord injury, in hospital and community contexts. **Methods:** A *scoping review* was conducted in November 2025, following the Joanna Briggs Institute methodology and PRISMA-ScR guidelines. Eligibility criteria followed the PCC framework (Population: adults with spinal cord injury; Concept: contribution of the Rehabilitation Nurse Specialist to the promotion of sexuality; Context: hospital and community). The search was carried out in the MEDLINE Complete®, CINAHL Complete®, Scopus®, and Psychology and Behaviour Sciences Collection® databases, without time limits applied, including full-text studies. Grey literature was also included. **Results:** Of the 916 studies identified, only 14 met the eligibility criteria. Findings indicate that educational, psychoeducational, digital, and rehabilitation-integrated interventions improved sexual function and satisfaction and reduced anxiety related to sexuality. Structured, person-centered programs showed greater impact than isolated interventions. **Conclusions:** Promoting sexuality in this population requires systematized, multimodal, and person-centered interventions within the relational context. The Rehabilitation

Nurse Specialist plays a central role in integrating sexual health into rehabilitation programs, improving quality of life and psychosocial well-being.

Keywords: Spinal cord injuries; Sexuality; Rehabilitation Nursing.

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24664 | TOXICOLOGICAL INTERACTION BETWEEN PARAQUAT AND 2,4-DICHLOROPHENOXYACETIC ACID IN CAENORHABDITIS ELEGANS

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Background & Aim: The intensive agricultural use of herbicides such as paraquat (PQ) and 2,4-dichlorophenoxyacetic acid (2,4-D) has been linked to an increased disease burden [1]. While their toxicity is often studied individually, real-world exposures involve mixtures, suggesting that isolated studies may mask interactive effects that amplify toxicity. This study investigated the toxicological interactions between PQ and 2,4-D using the *Caenorhabditis elegans* animal model [1]. **Methods:** Age-synchronized L1 animals of the DC19 strain [*bus-5(br19)*] were exposed in liquid medium to increasing concentrations of PQ (up to 10 mM) and 2,4-D (up to 40 mM), either individually or in combination (MIX), for 72h, and the survival rate was further assessed. Selected sublethal concentrations (0.5 mM) were then used to evaluate their impact on animal development and longevity. **Results:** Individual exposure to both herbicides caused a concentration-dependent decrease in animal survival, which became significant at concentrations above 1 mM for PQ and 10 mM for 2,4-D (p<0.001). Further experiments revealed that co-exposure to PQ (5 mM) and 2,4-D (0.5 mM) resulted in a higher survival rate compared to PQ alone (5 mM). A similar protective effect was observed when 2,4-D (25 mM) was combined with PQ (0.5 mM). In development assays, PQ (0.5 mM) exposed animals showed a slight increase in body length compared to controls, while 2,4-D (0.5 mM) and MIX (0.5 mM for each) groups showed values similar to controls after 72h of incubation. Longevity analysis revealed that exposure to PQ, 2,4-D, or the MIX significantly reduced the animal's lifespan. Ongoing studies are assessing locomotor and reproductive behaviors to further characterize potential neurotoxic effects. **Conclusions:** These findings indicate that the co-exposure to PQ

and 2,4-D elicits interactive effects beyond those observed with individual compounds, highlighting the importance of assessing the toxicology of pesticide mixtures under realistic exposure conditions.

Keywords: Paraquat; 2,4-Dichlorophenoxyacetic acid; Toxicological Interaction; Real Exposure Scenarios; *C. elegans*;

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24534 | Unsupervised Clustering of Maternal and Fetal Data to Identify Perinatal Profiles

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Background & Aim: Cardiotocography (CTG) is central to fetal surveillance, yet visual interpretation is subjective and may miss complex risk patterns. We will use unsupervised clustering of integrated maternal, pregnancy and CTG features to identify latent, explainable fetal profiles and explore associations with adverse perinatal outcomes such as prematurity, small-for-gestational-age birth, hypoxia surrogate markers and mode of delivery. **Methods:** Data come from a previously collected retrospective cohort of ~200 singleton pregnancies with longitudinal CTG recordings between 24 and 40 gestational weeks. For clustering, one CTG trace per pregnancy will be used, building two models: (i) 24-26 weeks and (ii) late pregnancy. The database has been cleaned (special codes removed; CTG event counts normalised by trace duration; features summarised) and includes maternal characteristics, pregnancy complications, neonatal outcomes and CTG-derived linear indices automatically computed using Omniview-SisPorto [1, 2]. Variables comprise mixed types (quantitative and binary), quantitative variables will be standardized (z-scores) and mixed-type dissimilarities will be computed using a suitable metric for mixed data types, such as Gower's distance. Clustering methods compatible with non-Euclidean dissimilarities (k-medoids/PAM and hierarchical agglomerative clustering) will be applied. Solutions will be evaluated using average silhouette width and within-cluster dissimilarity, and clusters will be compared on maternal factors and perinatal outcomes. **Results:** Preliminary descriptive analyses indicate a heterogeneous obstetric population with relevant burdens of gestational diabetes, hypertensive disorders and small-for-gestational-age fetuses, supporting feasibility and the potential for identifying distinct maternal–fetal patterns.

Conclusions: The study is expected to yield data-driven perinatal phenotypes beyond single CTG parameters informing personalised monitoring and decision support in obstetric care.

Keywords: Unsupervised clustering; Fetal heart rate; Cardiotocography; Maternal–fetal health; Perinatal outcomes.

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24743 | Unveiling Mechanisms of Intercellular Communication Mediating Drug Resistance in Non-Small Cell Lung Cancer

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Background & Aim: Lung cancer remains a major global health concern and the leading cause of cancer-related deaths worldwide. EGFR mutations are the most commonly occurring oncogenic alterations found in Non-small cell lung cancer (NSCLC). While these mutations are targetable by therapy, the development of resistance inevitably occurs. While the development of new mutations is the most common resistance mechanism, previous results from our group hint at the potential role of cellular communication in the establishment of resistance. This project aims to characterize the nature of communication established between therapy-resistant and therapy-sensitive cells that lead to the acquisition of mutation-independent resistance to EGFR TKIs. **Methods:** Extracellular vesicles (EVs) secreted by the human H1975 Erlotinib-resistant cells will be collected. EV isolation will be performed through ultracentrifugation and Size Exclusion Chromatography. Western Blot, LC-MS and Electron Microscopy techniques will be used to characterize EV content and morphology. Resistance to EGFR-TKI therapy will be induced by supplementing Erlotinib-sensitive lung cancer cells with different conditioned media fractions recovered from therapy-resistant cells to validate the role of EV communication in reprogramming recipient cells. **Results:** Preliminary data from our group showed that therapy-resistant mutations are only present in a fraction of relapsing tumor cells, and that EGFR-TKI resistance can be transferred from resistant to sensitive cells. This observation challenges the prevailing view of resistance development based on cell division and selection of mutated cells favoring the hypothesis that the transmission of resistance from mutant to non-mutant cells may occur through EV mediated intercellular communication.

Conclusion: This work aims to unveil the role of EV mediated communication in therapy failure, highlighting the importance of targeting intercellular communication to overcome EGFR-TKI resistance in NSCLC.

Keywords: Non-small cell lung cancer; EGFR; Extracellular vesicles; Therapy resistance.

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HUMANITIES AND SOCIAL SCIENCES

24591 | Is *CASA COMUM* our home? A Study on Cultural Mediation and Student Empowerment at the University of Porto

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Background & Aim: The contemporary university must transcend teaching and research to embrace a 'third mission' (Soeiro, 2011) of social and cultural outreach. This study focuses on Casa Comum, the University of Porto's cultural unit, analysing it as a third place for community building (Oldenburg, 1999). The study integrates Bourdieu's (1984) theories of distinction and Wacquant's (2023) perspective on how inequalities take physical form in urban morphology. The study aims to evaluate how student participation is fostered and implemented in order to transform students from passive observers into active 'prosumers' (Jenkins, 2006) and co-creators. **Methods:** A qualitative and exploratory approach (Creswell, 2014) was adopted. Data collection included a documentary analysis of strategic plans and activity reports (2018–2025), participant observation during institutional planning meetings and artistic workshops, focus groups with students, and semi-structured interviews with technical staff. Data analysis follows the content analysis procedures described by Carvalho Guerra (2006). **Results:** Preliminary findings from the analysis of documents (2018–2025) suggest that, although Casa Comum has a well-established programme, direct student-led initiatives are rare. Observations suggest that the institutional space still operates as a territory where symbolic social distance for students persists. However, initial monitoring of artistic workshops and planning meetings reveals significant latent interest in active student participation within the unit. **Conclusions:** The study concludes that active participation depends on overcoming structural barriers through effective cultural mediation. Transforming Casa Comum into a community-managed resource would reinforce the university's role as an 'Ecological University' (Barnett, 2017). This work contributes to the field by providing a model for democratic cultural governance and integral formation through student involvement.

Keywords: Student Engagement; Cultural Mediation; Sociology of Art; Third Place; Higher Education

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24694 | Traditional Portuguese Music and the Reconstruction of Social Bonds in Post-Modernity

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Background & Aim: This research examines Traditional Portuguese music as a contemporary form of community reconstruction. It explores how these practices foster social bonds, shared values, and collective memory (Branco & Pestana, 2022; Castelo-Branco & Toscano, 1988; Porto, 2014; Vasconcelos, 2001). In a postmodern context characterized by identity fragmentation, social isolation and unstable social ties (Bauman, 2001; Giddens, 2000; Harvey, 2000; Lipovetsky, 2010; Lyotard, 1986), the study argues that participation in these practices contributes to the re-composition of social values, functioning as spaces for encounter and mutual recognition. **Methods:** A qualitative approach (Creswell, 2014) was adopted, based on three case studies of Traditional Portuguese music groups from Porto. Data collection includes participant observation during rehearsals and performances, focus groups with group members, and documentary analysis. These methods allow an in-depth understanding of how macro-structural transformations are experienced at the micro-social level. **Results**: Preliminary findings indicate that participation in these groups serves as a meaningful space of social re-composition. Collective musical practice fosters dense interpersonal bonds that contrast with the fluid relationships typical of late-modern societies. Shared performance and repertoire transmission reinforce trust, reciprocity, and collective memory, thereby strengthening durable forms of belonging. **Conclusions:** The study suggests that these groups may function as spaces of symbolic resistance to the performative logic of late capitalism. Although not entirely detached from commodification, participants emphasize authenticity, collective effort, and non-market values. Traditional music emerges not merely as nostalgic heritage, but as an active site of social re-composition. This research contributes to debates on community reconstruction by highlighting the role of collective musical practices and social cohesion in contemporary societies.

Keywords: Traditional Portuguese Music; Identity; Belonging; Postmodernity; Community Reconstruction

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24802 | The relevance of the concept of risk in sexual health intervention: design of a sex education project in a public health third sector organization

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Background & Aim: Sexually Transmitted Infections (STIs) have been consistently rising in the European Union and Portugal(1). Although a comprehensive approach has been established as the standard, current sex education (SE) projects are overwhelmingly focused on the prevention of STIs and unplanned pregnancies(2,3), mostly ignoring other relevant aspects of sexuality. The rise in STIs suggests that, despite being risk-centered, the dominant approach to sexual and reproductive health (SRH) intervention hasn't been effective in raising awareness of the actual epidemiological risk, or fostering consistent condom use(4). We aim to explore the impact that different approaches to risk have in the effectiveness of sexual health intervention, through the design of a SE project in a public health third sector organization. **Methods:** Design of an action research SE project with teenage volunteers, to be carried out in the Portuguese League for Social Prophylaxis. Grounded in a peer education and leadership fostering approach, this project aims to produce a podcast, on which the teen participants interview SRH experts. The effectiveness of the project will be assessed through SRH knowledge surveys and a focus group. **Results:** Having identified the concept of risk as relevant to the analysis of SE projects, and considering the previous scientific validation of teen-led podcasts as effective in the promotion of SRH knowledge(5), we aim to test the potential of an approach that problematizes the notion of risk, through an educational method that, although focused on STI prevention, is anchored on a holistic view of SE. **Conclusions:** Although SRH intervention has been risk-focused, the prevalence of STIs suggests that this approach hasn't been effective in providing young people the tools to mitigate actual STI risk. Designed with this paradox in mind, this project aims to test the effectiveness of a SE approach that is focused on STI prevention without essentializing risk as the defining property of sexuality.

Keywords: risk; sexual and reproductive health; sex education; adolescent health

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24907 | Commuter students in the city of Porto: Impacts of spatial mobility on university students' academic, cultural and social practices

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Background & Aim: This research project aims to analyze the impact of daily commuting in the university experience and everyday life of higher education students in the city of Porto. Specifically, it's showcased how spatial and temporal dimensions of mobility can shape the commuter's student attitudes, engagement and practices towards the university context, operating as a decisive and structuring force on these formative years. On this topic, multiple analytical dimensions have been consistently brought up in scientific literature: integration and participation in the academic community [1][2]; academic success [3][4]; social inequality and exclusion [5][6]; physiological and psychological impact of daily commuting [7][8], *et cetera*. **Methods:** The research will conduct a hybrid methodology, gathering quantitative and qualitative data, proposing a complementary and confrontational dialogue between them. To this end, one survey and several semi structured interviews will be applied to the target population. **Results:** The need to commute and the forms of mobility practiced are expected to play a structural role in these students' academic and extracurricular lives. On the other hand, the study foresees these students developing personalized dispositions and strategies towards their mobility profile, that allow them to navigate their desires and expectations, revealing a more complex picture of the phenomenon. **Conclusions:** By highlighting daily commuting as an important component in university students' academic, cultural and social lives, this research hopes to bridge mobilities and higher education study fields, informing key findings and policy in the regional and national context.

Keywords: mobilities; commuter students; higher education; academic practices; student engagement

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24954 | Fashion and Youth Identity in School Contexts: A Sociological Perspective

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Background & Aim: In contemporary societies, fashion plays a significant role in the construction of youth identity. Within school environments, clothing and appearance function as symbolic resources through which young people negotiate belonging, distinction and social recognition. Schools are not only spaces of formal learning but also social settings where norms, hierarchies and identity processes emerge through everyday interactions. This research examines how fashion contributes to the construction of youth identities in school contexts and how aesthetic practices interact with institutional expectations and peer relations. **Methods:** The study adopts a qualitative ethnographic approach. Fieldwork will be conducted in a secondary school in Northern Portugal. Data collection combines participant observation of everyday school interactions, semi-structured interviews with students, and visual analysis of appearance-related practices, enabling the study to capture the meanings young people attribute to clothing and presentation in their daily experiences. **Results:** Preliminary observations suggest that fashion functions as a communicative and relational resource through which students express individuality while simultaneously negotiating conformity with peer group expectations and school norms. Clothing choices appear to reflect broader social dynamics, including processes of belonging, symbolic differentiation and recognition within youth groups. **Conclusions:** This research highlights the sociological relevance of fashion as a symbolic and relational practice within educational contexts. By integrating perspectives from the sociology of education and youth with fashion studies, the study contributes to a deeper understanding of how aesthetic practices shape identity formation and social interaction in schools.

Keywords: Sociology; Youth Identity; Fashion; School; Social Interaction

24971 | The New Face of Patriarchy? Maintaining Hegemony Trough Online Hate Speech and Violence Against Women in Portuguese

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Background & Aim: Antifeminism, and the inherent misogyny it displays, prevails in the digital formats within the manosphere- a space where diverse forms of violence are committed against women (Moloney and Love, 2018). Scientific knowledge of this phenomenon is mostly regarding the Anglo-Saxonist context. Therefore, this investigation seeks to address the gap in the Portuguese context. The main goal of this research is to explore the link between the Portuguese-language manosphere, the entrenchment of the patriarchal system and the maintenance of hegemonic white male identity, as conceived by Connell (1987). This study aims to demonstrate how the manosphere actively disguises its true intent- maintaining the patriarchal order in westernized societies by stripping women away from their voices and power in the public sphere (Barker and Jurasz, 2019). **Methods:** This research employs a mixed-methods approach. The qualitative component comprises three branches: semi-structured interviews with three Portuguese organizations possessing empirical knowledge of the manosphere; non participant observation of comment's section of five Portuguese journals on Instagram, as well as misogynistic posts on TikTok, Instagram and X (formerly Twitter) using relevant hashtags; and content analysis of the retrieved data. The quantitative approach aims to compare, via negative binomial regression in JAMOVI, the engagement metrics of 700 posts (350 feminist and 350 misogynistic) across TikTok, Instagram and X. **Results:** Preliminary results indicate that the Portuguese-language manosphere is similar to its transnational counterpart, being heterogeneous and composed of diverse sub-communities. Its members appear to belong to the anti-feminist counter-movement and frequently employ emotional appeals in their discourse. **Conclusions:** This research suggests that the manosphere represents a tangible danger, despite often being perceived as a niche or emerging issue.

Keywords: antifeminism; manosphere; misogyny; non participant observation; social media.

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24135 | Female Labour in Prehistory: The Matter on 7th Year Textbooks

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Background & Aim: This investigation examines the representation of prehistoric women within the Portuguese educational system, focusing on 7th-year textbooks - a subject that remains under-researched in Portugal. Given the central role that textbooks play in shaping students' worldviews, these themes must be addressed with scientific rigor and minimal bias. **Methods:** This research is based on visual and textual analysis from different history textbooks edited between the 1990s and the 2020s in Portugal. The analytical framework focuses on identifying gender-based patterns in iconography and narrative structures about Paleolithic and Neolithic periods. **Results:** A critical analysis reveals the systematic persistence of traditional binary gender roles; therefore, women are predominantly represented within the "domestic" or "maintenance" roles – associated with gathering, preparing food or caring for children – while men are represented hunting and associated with technological innovation and artistic expression. **Conclusions:** This paper argues that such representations diverge significantly from contemporary archaeological evidence and perpetuate pedagogical biases that distort children's understanding ofd these "gender roles". Therefore, the study emphasizes the need for a curricular update that incorporates the diverse contributions of women in early human societies.

Keywords: Prehistory; Economy; Gender Stereotypes; Textbooks; Female Represenation; 7th Grade.

24661 | A Critical Review of the Demographic Research and Funerary Inscriptions in the Roman Empire

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Background & Aim: This study chronologically presents the bibliography on demography in the Roman Imperial period using funerary inscriptions as a source. These monuments provide age at death and the sex of the deceased (Figure 1), serving as the basis for obtaining demographic indices. Previous studies have identified key limitations, such as group under-representation and asymmetries in the geographical distribution of the tombstones. It showed an evolution in the population sampling's methodologies as well as on the calculation of demographic indicators. This process established the state of the art and will constitute a fundamental framework for a future dissertation within a yet-to-be-studied geographical and population context. **Methods:** Since the 19th century, demographic studies have been conducted using diverse methodologies and purposes across the Empire's regions. The research targeted data regarding mortality rates, life expectancy, and sex ratio. The keywords used were demography, life expectancy, mortality, epigraphy, inscription, and tombstone; the scope included specialized literature, scientific articles, conference proceedings, theses, and congress reports. **Results:** A total of 25 works were analysed, including Karl Beloch's [1] pioneering 1886 study, which used scientific calculations to estimate the life expectancy of a Roman inhabitant. Information gaps were identified, along with their respective data mitigation methods. Applied methodologies presented concrete results within the Roman Empire for global life expectancy, age group, and sex. **Conclusions:** This study allows for the recognition of limitations for future investigations. Distinct approaches proved to be complementary and should therefore be used together. It has been demonstrated that demographic frameworks can be characterized within Roman populations. The analysed studies form a broad base of knowledge, particularly regarding the methodologies applicable during an investigation when facing new contexts.

Keywords: Roman Empire, Demography, Funerary Inscriptions

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Figure 1: Mérida 100-150 CE, Sacred to the Divine Spirits. To Lucius Julius Amoenus, 24 years old. Here he lies. May the earth rest lightly upon you. Cassia Amoena made [this monument] for her most devoted son.

24707 | Women on Trial: Crimes and Justice in 15th Century Portugal

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Background & Aim: In medieval Portugal, women faced profound legal and social subordination due to influences by Christian morality. Female criminality was described mostly of nature sexual and moral transgressions like adultery, concubinage, among others. This study intends to analyze the legal and normative framework of royal power, the restricted and definition of sexual crimes and their respective penalties, providing a detailed description. **Methods:** The methodology included bibliographic research and documentary analyses. Initially, a literature review was conducted using academic databases like UP and UNL and specialized scientific journals, focusing on keywords related to female criminality and transgression. Subsequently, an analytical grid was developed and applied to the *Ordenações Afonsinas* and the Chancelleries of Afonso V and João II. **Results:** The study combined bibliographic review with primary documentation analysis. This illustrates how the medieval society was articulated by the church doctrine with its own political, social and jurisdiction. The royal pardon letter by chancelleries of King Afonso V and King João II, integrated between theorical legal norms and actual judicial practice, to avoid physical punishment and exile. **Conclusions:** The justice under King João II and King Afonso V operated with a profound duality. While the law enforced strict moral codes, royal pardons functioned as a mechanism for social control and economic gain. The "Arca da Piedade" effectively transformed female "sins" into state revenue, revealing that the royal pardons were strategic tools for political centralization and fiscal policy rather than acts of mercy[1].

Keywords: Female Criminality; Royal Pardons; Medieval Justice; Concubinage.

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[1] DUARTE, Luís Miguel – "A boca do diabo": a blasfémia e o direito penal português da Baixa Idade Média. Lusitânia Sacra. 2.ª série. 4 (1992), p. 82

24771 | The «*Camisola Poveira*»: handcrafted clothing and tradition between the mountains, the folk ranch and the sea

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Background & Aim: This study aims to highlight the crucial importance of the «*Camisola Poveira*» (Figure 1) as a traditional handcrafted product, unequivocally associated with the cultural identity and heritage of the city of Póvoa de Varzim in Northern Portugal. Therefore, the author discusses its diachronic evolution in a period crystallised between the mid-19th century and the end of the 20th century, as well as the construction and transmission of vernacular know-how related to the various specificities of manufacture that elevate it as an icon of local culture. Ultimately, it seeks to clarify its cultural significance and its integration within and outside the community. **Methods:** This work is the result of an exhaustive documentation of the morphological and technical aspects of this structural piece of the traditional festive and pilgrimage costume worn by fishermen from Póvoa de Varzim [4]. This investigation is based on a careful interpretation of bibliographic information, from the diverse research contributions on the subject [1], [2], [3], [5], and complemented by fieldwork carried out through the gathering of photographic records from musealized pieces and oral memories. **Results:** It was possible to acquire a more solid comprehension of its technical transformations along the decades, its iconographic diversity, its process of cultural establishment in the territory and its definitive affirmation as cultural heritage. **Conclusions:** Overall, it is worth emphasizing the «*Camisola Poveira*» as a material testimony inseparable from the occupation of the Póvoa de Varzim coastline and its people. Widely associated with the technical knowledge built up and passed down through generations, this textile piece constitutes a rich cultural heritage that must continue to be valued and preserved.

Keywords: Contemporary Archaeology; Ethnography; Handicraft; Póvoa de Varzim; «Camisola Poveira»

Acknowledgments: I would like to express my deepest and most sincere gratitude to Dr. Deolinda Carneiro and Dr. Maria Cármen Flores Gomes Lopes of the Municipal Museum of Ethnography and History of Póvoa de Varzim (MMEHPV), whose guidance and support were absolutely essential to the completion of this study. Their generous sharing of experiences and materials was an invaluable contribution, greatly enriching the scientific and technical accuracy of this work. I would also like to thank the craftswomen I contacted for all their availability and motivation given in working on such a unique and captivating theme, which represents a significant contribution to the study of the cultural heritage of Póvoa de Varzim.

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Figure 1: The «*Camisola Poveira*». 20th century (2nd half). Donated by «*Rancho Poveiro*» to the Municipal Museum of Ethnography and History of Póvoa de Varzim (MMEHPV) © Dinis Duarte, captured on 16/01/2026.

24807 | Roman legal integration through the private sphere: An epigraphic analysis of individual agency in northwest Iberia

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Background & Aim: The ethical-legal concept of fides is fundamental to Roman law, as an invisible foundation of the bonds within these people[7]. In the northwest Iberia, Roman presence has been observed since the 2nd century BC; however, while *hospitium* and *clientela* existed among indigenous communities earlier, under Roman rule, evidence of individual agency becomes crucial for understanding how Roman law infiltrated the private sphere as an integration process[1,5,6]. This study explores how recording social bonds in tabulae and tesserae reflects a legal articulation independent of public administration, reinforcing individual autonomy[5,8]. **Methods:** The investigation analyzed three epigraphs "*Tabula* Clunia[2]", "*tessera hospitalis* Herrera de Pisuerga[3]", and "*Tabula* El Caurel[4]". It focused on an epigraphic study to understand communities' customs, along with an onomastic study to trace Roman integration among indigenous populations through the lens of Romanization and some extensive bibliographic work. **Results:** Results demonstrate that the appropriation of Roman law by indigenous communities occurred strategically and gradually, revealing a coexistence through the observation of indigenous anthroponyms (*Tillegus Ambati*) alongside Latin script and complex legal formulas. These elements place the indigenous subject as a protagonist in the use of language and law, linking indigenous lineage to Roman structures of representation[5,6.] **Conclusions:** Roman presence in the Northwest Peninsula also functioned in an interpersonal manner, as Latin transcends linguistic translation to become an agent of integration. By understanding that individuals, within their private sphere, resorted to Roman structures to validate pre-existing bonds, now granting them Roman legal protection, it becomes evident that integration did not imply the demolition of lineage, but rather a functional adaptation to a new system, observed even within the realm of private autonomy.

Keywords: Epigraphy; Northwest Iberia; *Hospitium;* Romanization; Private autonomy

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24982 | The influence of Brazilian gold on the minting series at the turn of the (17th) century.

An analysis of the monetary system of D. Pedro II of Portugal.

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Background & Aim: This article aims to present some research results and conclusions to be drawn from the analysis of the monetary system of the reign of D. Pedro II of Portugal (1683-1706), focusing on the impact that the gold from the former Brazilian colony had in this regard. To this end, different forms of expression will be addressed, always backgrounding its potential and limitations. Therefore, some of the main issues will be highlighted, which are visible in a set of complementary elements connected between them. **Methods:** This study results mainly from the reading and consultation of various studies. Thus, studying some bibliographically published coins, their main characteristics (e.g. obverse and reverse legend) associated with their historical and economic context, allowed a broader interpretative scope. **Results:** It became clear to check a diversity of consequences linked to the arrival of huge amounts of Brazilian gold in Portugal, so closely related to the coin minting. In addition to a monetary reorganization, an economic-financial upgrade came to light on the scale of the Portuguese overseas territories, showing not only the power but also the prestige of this former global Empire. At the same time, the economic connection and integration observed between the metropolis and Brazil was reinforced through the enormous flow of gold, which served as a source of profit used for the expenses and investments required by the nature and complexity of Portugal´s needs at the time. **Conclusions:** Finally, note the importance and dimension of Brazilian gold in relation to the minting processes that took place at the turn of the 17th to the 18th century, during the rule of D. Pedro II of Portugal, especially when it comes to the Numismatics field of study.

Keywords: Brazilian gold; D. Pedro II of Portugal; Monetary system; Coins; Numismatics.

Figure 1: **Obverses of gold coins from the regency of D. Pedro II of Portugal (1668-1683).** [1] Considering this image as a prelude of the presented theme, the coins are, in order: a *Moeda* (4.400 réis, 1668, hammered coin); a half *Moeda* (2.200 réis, 1668, hammered coin); a quarter of *Moeda* (1.100 réis, 1668, hammered coin); a *Moeda* (4.000 réis, c. 1678-1680, milled coin); and a half of *Moeda* (2.000 réis, c. 1678-1682, milled coin).

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24727 | Kant's Cosmopolitanism and its Classical precedents

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Background & Aim: Since Antiquity, the relationship with the foreigner has been problematized, with the idea of the "human as a citizen of the world" remaining highly relevant. The aim of this work is to explore Kant's cosmopolitan principles, primarily in *Toward Perpetual Peace* and *Idea for a Universal History* [2] in direct dialogue with classical antiquity thinkers, namely Cynics, Epicureans, Stoics, and the eclectic Cicero [1][3]. **Methods:** The methodology relies on comparative analysis, confronting Kant's thought with the perspectives of classical philosophers like Diogenes, Epicurus, Zeno, Chrysippus, Marcus Aurelius, and Cicero. The cosmopolitanism thought of each Hellenistic school, as well as the Kantian one, are first approached through a philosophical exegesis, and then compared. **Results:** The analysis resulted in four theoretical axes: pacifism, natural teleology, cosmopolitan right, and the idea of a political organization like a league of nations. Preliminary results show that universal law and harmony are transversal in Ancient Philosophy, such as Epicurean friendship and Stoic peace. However, Kant departs from classical resignation, adopting an optimistic challenge. He proposes a natural teleology where "unsocial sociability" forces humanity to seek peace and a just civil constitution. While universal law was explicit in Cicero, Kant innovates by elevating cosmopolitanism from a philanthropic ideal to a strict juridical principle. This is translated politically into a federation of free states and universal hospitality. **Conclusions:** Kantian cosmopolitanism transcends its classical antecedents by moving beyond mere ethical frameworks. While the ancients laid moral foundations, Kant institutionalized these notions into a structured system to guarantee Perpetual Peace.

Keywords: Immanuel Kant; Cosmopolitanism; Hellenistic philosophies; Stoicism.

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PSYCHOLOGY AND EDUCATION SCIENCES

24147 | EMOTIONS: DO THEY AFFECT MEMORY?

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Background & Aim: Emotions can interfere with memory in different ways (e.g., serving as contextual cues for encoding and retrieving information). One hypothesis is that memories are more retrievable if the emotional state is congruent between encoding and retrieval. However, it remains unclear under what conditions this effect occurs and which parameters (probability of memory retrieval and/or its accuracy) are affected. **Methods:** This study tested long-term visual memory for colored items (e.g., blue shoe, yellow cup) using a recall task where colors had to be marked on a hue ring. During encoding, negative or neutral emotional states (Study 1) and positive or neutral emotional states (Study 2) were induced by presenting emotional images before each colored item. In the retrieval phase of Study 1, half of the items were displayed after negative images and the other half after neutral images, creating congruent (encoding and retrieval in a negative state or encoding and retrieval in a neutral state) or incongruent (encoding in a negative state and retrieval in a neutral state or vice versa) contexts. In Study 2, the same strategy was applied to vary between positive and neutral states. The expected sample size per study is N = 60, currently N < 50. **Results:** Preliminary results indicate that the images elicited emotions consistent with their respective valences (negative, neutral, and positive). However, color recall did not vary with emotional state or with the congruence between encoding and memory retrieval. **Conclusions:** Images were able to evoke respective emotions in our sample (at least, based on self-report). Yet, retrieval did not vary with emotional state – showing no effects of congruency. Emotion effects are difficult to replicate?

Keywords: emotional context congruence; long-term visual memory; accuracy; probability of retrieval.

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Figure 1: *Illustration of the Flow of Events in the Encoding and Retrieval Phases for the Negative Group in Experiment 1*

24257 | Food Noise: Study Protocol of an Adaptation and Validation of Scale

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Background & Aim: Food noise is a recently introduced construct describing persistent food-related thoughts. Two instruments assess this concept: the Food Noise Questionnaire (FNQ) and the Ro Allison Indiana Dhurandhar Food Noise Inventory (RAID-FN). Based on distinct definitions, the FNQ targets intrusive thoughts interfering with daily functioning, whereas the RAID-FN captures unwanted and dysphoric food-related cognitions. This study aims to culturally adapt and validate the FNQ and RAID-FN for Brazilian and European Portuguese, as no validated Portuguese versions exist. Food noise has been considered an indicator of patient-reported outcomes in obesity treatment, and studying food noise may improve understanding of obesity management and treatment. **Methods:** This methodological study follows COSMIN (COnsensus-based Standards for the selection of health Measurement INstruments) guidelines for cross-cultural adaptation, including forward translation, synthesis, back-translation, expert review, and focus groups conducted in Brazil and Portugal. Participants (n=500), recruited from university and community samples, will respond online (LimeSurvey) to the Repetitive Eating Questionnaire, Three-Factor Eating Questionnaire, Clinical Impairment Assessment, and Depression Anxiety Stress Scales. Psychometric analyses will examine internal consistency, test–retest reliability, construct validity, convergent/discriminant validity, and factorial structure using exploratory and confirmatory factor analyses. Multi-group confirmatory factor analysis across countries, sexes, and clinical versus non-clinical groups will test measurement invariance. **Expected results:** Differential correlation patterns supporting convergent and discriminant validity, comparable reliability, and adequate fit across groups, with possible differences in factorial structure between instruments. **Conclusions:** This study will provide the first validated Portuguese measures of food noise, supporting cross-cultural and clinical research.

Keywords: Food noise; Psychometric validation; Cross-cultural adaptation; Portuguese language.

24393 | Promoting Written Mathematical Communication through Self-Assessment in Problem Solving: A Study in 11th-Grade Mathematics A

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Background & Aim: In problem-solving contexts, many students experience difficulties in clearly presenting and justifying their reasoning. It is within this framework that the present study, currently in progress, is situated. The research is conducted within the scope of the Supervised Teaching Practice and is integrated in the Master's Degree in Teaching Mathematics in Lower and Upper Secondary Education (FCUP). The main aim of this study is to examine how self-assessment can contribute to the development of written mathematical communication. **Methods:** This study adopts a qualitative research approach grounded in a pedagogical intervention focused on the topic "Sequences: Arithmetic and Geometric Progressions". The intervention involves 41 students from two 11th-grade classes enrolled in the Mathematics A and is structured into three phases:(i) independent problem-solving and the completion of a reflective questionnaire aimed at identifying perceived difficulties; (ii) whole-class discussion; (iii) self-correction of students' own work through the completion of a self-assessment rubric, with space for the identification of improvement actions. In this contest, four problems are implemented. Notably, the whole-class discussion of the first problem is based on anonymized student-written solutions, with the objective of identifying the characteristics of a complete, clear, and well-structured solution. Students' written productions, together with their questionnaire responses, are subjected to content analysis. Additionally, students' performance on a problem in a formal assessment context is examined to assess the extent to which the strategies developed throughout the intervention are mobilized and applied. **Expected Results:** It is anticipated that the pedagogical intervention will enhance students' ability to identify areas for improvement in their own written work. Consequently, improvements are expected in written mathematical communication, self-regulated learning and problem-solving skills.

Keywords: Written Mathematical Communication; Problems; Self-regulation

24419 | Artificial Intelligence and Audiobooks in online Book Clubs: The *Kitsch* in the Age of Social Media

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Background & Aim: *Kitsch*, according to the interpretation of Milan Kundera and Herman Broch, is something futile, ephemeral and incapable of reproducing the human spirit (Gonçalves, 2019). In times of Surveillance Capitalism (Zuboff, 2019), where workforce has ceased to be the main source of profits, the human being gradually becomes a product, whose surplus of energy is sold to companies (Coldry & Mejias, 2019). As a product that must be consumed quickly, *just in time* (Harvey, 2005), we don´t have time for leisure activities, such as reading. One of the ironies of the present day is that it is possible to verify an increase in book clubs, especially of the online groups, and a decrease in literacy levels (OECD, 2025). The use of artificial intelligence and audiobooks allow us to shorten reading time through book reading, and enables us to perform other activities while listening to the book's plot. These tools are becoming a common place in online book clubs. Therefore, the objective of our research is to understand the personal, economic, and social reasons for the use of artificial intelligence and audiobooks in online book clubs. **Methods:** For this purpose, a qualitative methodology research will be carried out using online ethnography to observed two online book clubs. Data collection will come from the observations of the discussions and interactions between the club´s members, as well as semi-structured interviews with some members**. Results:** The expected results are to identify and understand an increasingly "facilitation" in the practice of reading by the members, and how it affects the Adult literacy. **Conclusions:** Through a critical comparative analysis of the collected data and a review of the available bibliography, we imagine that we will deliver a brief philosophical, sociological, and educational reflection on the current state of the use of these instruments outside of formal educational institutions.

Keywords: Artificial Intelligence, Audiobooks, Literacy, Online Book Clubs, Surveillance Capitalism.

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24433 | The Mobilization of Strategies in Learning of Counting: A Study in the 11th Grade

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Background & Aim: This research, still in progress, is part of the Supervised Teaching Practice within the Master's Degree in Mathematics Teaching for Lower and Upper Secondary Education at FCUP. The study aims to identify and analyze the strategies used by students during and after the teaching and learning process of the topic *Counting*. **Methods:** This investigation is based on a qualitative approach of an interpretative nature. Data collection focused on students' written work and took place at five different moments during the 2025/2026 school year, involving 41 students from two classes at a school in the Porto Metropolitan Area: three moments throughout the unit and two after its completion. **Results:** This study showed that initially, students mainly used visual and schematic representations, which facilitated the understanding of concepts and contributed to distinguishing between the principles of addition and multiplication. The interactions established among students in group work encouraged discussion of ideas, generally enabling them to reach correct solutions. Subsequently, a transition to more algebraic representations was observed, which was associated with increased difficulties. After the introduction of the concept of combinations, some confusion between the fundamental counting principles emerged. The completion of individual tasks contributed to an increase in difficulties, as students no longer had access to the discussion space provided by group work. **Conclusions:** It was possible to verify that the different strategies used by students have a significant impact on their performance. When students work in groups and use more visual representations, they tend to better understand the problem statements and the procedures required to achieve the intended result. When they mainly rely on algebraic representations, they show a greater tendency toward misconceptions and difficulties in interpreting and structuring their reasoning.

Keywords: counting, mathematic representations, group work

24448 | How do adolescents think about ethical dilemmas? Correlations between adolescents' perspectives and self-reported ethical decision-making

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Background & Aim: In a world of increasing complexity and rapid change, teaching adolescents to make ethical and responsible decisions is a central educational goal[1;2]. Although research and educational frameworks position ethical decision making as a key process in development[3;4], little is known about how adolescents interpret and reason about ethical dilemmas. Within the scope of the project Schools2030, the present study addresses this gap by examining adolescents' perspectives on ethical dilemmas and their associations with their own perceived competence. **Methods:** Participants were 284 9th grade students (50% boys; M_{age} = 14.67; *SD* = 0.80; 84.2% born in Portugal) attending public schools in Portugal. Students answered to open-ended questions on a illustrated ethical dilemma depicting a real-life situation at school, being asked to describe what happened, how the protagonists were feeling, and possible solutions. Adolescents' responses were coded according to categories derived from the literature[3;4] capturing varied degrees of complexity in cognitive interpretations, emotional understanding, and resolution strategies. Participants also completed a self-report questionnaire on ethical decision-making skills. Correlations between the categories and adolescents' self-reported skills were computed. **Results:** We will share results based on the analyses on how adolescents interpret an ethical decision-making situation, identify emotions, and generate solutions (e.g., imposing decisions – decides according to what considers morally appropriate; ethical sensitivity – awareness of the consequences of the actions and balance between everyone's interests). We will explore correlations between perspectives and self-rated skills. **Conclusions:** Results will inform on the assessment and promotion of ethical decision-making during adolescence. Findings carry implications for the design of developmentally attuned interventions that target adolescents' interpretation and position on ethical dilemmas.

Keywords: Adolescence; Ethical decision-making; Ethical dilemmas; Socio-emotional competences.

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24463 | ChatGPT as a Mathematics Learning Tool: A Case Study on Challenges and Opportunities in the 9th Grade

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Background & Aim: This ongoing research is conducted within the framework of the supervised teaching practice for the Master's Degree in Mathematics Teacher Education for Middle and Secondary Schools (FCUP) during the 2025/2026 academic year. The study analyzes the pedagogical potential of ChatGPT as a supportive tool to enhance the mathematics teaching and learning, with the main aims: examine students' use of ChatGPT as a learning support tool in mathematics; analyze their perceptions of ChatGPT's mathematical errors; explore the pedagogical use of these errors as learning opportunities. **Methods:** This study adopts a qualitative approach within an interpretative paradigm, seeking to understand how familiar students are with ChatGPT. The study took place over four lessons focusing on the topic "Functions", within the context of a class of 19 students, in the 9th grade, at a school in the Porto region. Data collection relies on multiple sources, such as: diagnostic and final questionnaires, aimed at analyzing students' familiarity with AI tools and their perceptions regarding the use of ChatGPT; participant observation during lessons focusing on student-ChatGPT interactions, strategies adopted in task solving and difficulties encountered; and analysis of students' written productions, namely solutions to tasks and justifications and critical comments concerning the responses provided by ChatGPT. **Expected Results:** The expected results of this study include an increased student awareness regarding the errors and limitations inherent in ChatGPT. Furthermore, it is expected that students will develop the ability to critically analyze AI-generated responses, recognizing that the strategic use of these tools can significantly enhance their mathematical proficiencies.

Keywords: Artificial Intelligence, ChatGPT, Mathematics Education, Education Technology

24470 | Contributions to the understanding of the linguistic behavior of queer speakers of European Portuguese – an exploratory study

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Background & Aim: This paper aims at describing the linguistic behavior of queer European Portuguese (EP) speakers, particularly in the context of social media. For this purpose, we drew upon Queer Theory as proposed by Motschenbacher [1] and Motschenbacher & Stegu [2], in order to propose that the features often associated to the use of language by queer individuals are of a performative nature. Research suggests that this behavior has a parodic nature, aiming to satirize the very stereotypes this community is targeted by [3]. Besides, it highly depends on the social and discursive context, defined in terms of Communities of Practice (CoP) [4, 5]. **Methods:** Based on this framework, we analyzed two *corpora* composed of EP social media posts retrieved from two different social media platforms, to better understand the main features and the variability which emerge through their use. We thus have built a Reddit *corpus* consisting of 40 posts retrieved from the subreddit *r/lgbtportugal* within the last year and an X (Twitter) one consisting of 30 posts and *retweets* by social media personality Kiko is Hot from the year 2024. **Results:** The analysis of the two *corpora* provided some evidence both for the concept of CoP and for the concept of performative queer linguistic behavior: whereas the posts from Reddit, which deal with more serious subjects, showed little to none of the features outlined in the literature, the X (Twitter) posts, addressing more trivial subjects, showed a quite high occurrence. **Conclusions:** This data seems to corroborate the hypothesis that linguistic behavior stereotypically attributed to queer individuals emerges only in specific social and discursive contexts – namely, in CoPs that are typically informal and low-stakes – where such parodic behavior is encouraged. Furthermore, the study provides empirical data on queer linguistic behavior in European Portuguese, an area which remains significantly under-researched.

Keywords: Queer Linguistics, Queer Theory, Community of Practice, Socia Media, Sociolinguistics.

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24496 | From the field to life: injury as an existential threshold. A qualitative study on meaning-making and post-traumatic growth in an elite handball athlete.

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Background & Aim: Sport injury in elite athletes is a catalyst for physical and psychosocial consequences. Exclusive athletic identity [1] renders athletes particularly vulnerable to identity and psychological crises following severe injury, a process explained by the integrated model of response to sport injury [2]. Integrating a post-traumatic growth perspective [3] and Frankl's logotherapy [4], adversity may serve as a starting point for the discovery of new sources of meaning. This case study analysed the repercussions of a severe shoulder injury in an elite handball athlete. **Methods:** A qualitative case study design was adopted. Data were collected through semi-structured interviews and analysed using thematic analysis, focusing on cognitive appraisal, emotional response, and existential meaning-making throughout the injury and recovery process. **Results:** The injury acted as a catalyst for ethical transformation, enabling the athlete to detach from an instrumental identity and find in self-care a new biographical narrative. Failures in organisational support systems often neglected the injured athlete [5], highlighting the need for humanised leadership that recognises the athlete's full humanity beyond competitive utility. **Conclusions:** Severe injury can function as an existential threshold, promoting post-traumatic growth when supported by meaningful psychological intervention. Humanised organisational practices and logotherapy-informed approaches represent promising tools for supporting elite athletes through injury-related identity crises.

Keywords: athletic identity; handball; case study; post-traumatic growth; sport injury

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24528 | Students' difficulties in interpreting mathematical problems: an intervention with 9th grade students

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Background & Aim: Problem solving in mathematics is a fundamental cross-curricular skill, valued in essential learning in middle and secondary education. However, many students have difficulties understanding and interpreting statements, often associated with the language used, the representations involved, and the context of the problems. This poster presents preliminary research developed as part of the Supervised Teaching Practice of the Master's Degree in Mathematics Teaching (FCUP), which seeks to understand these difficulties by analyzing the influence of vocabulary, representations and context on the interpretation of statements. **Methods:** The study follows a qualitative approach and seeks to analyze in detail the difficulties that students encounter in interpreting mathematical problem statements. The work was developed with a group of six 9th grade students from a school in the Porto Metropolitan Area, through the analysis of task solutions with different representations and contexts and individual interviews focused on the strategies and difficulties experienced during problem solving. **Expected Results:** The analysis of written work and interviews will allow the identification of common difficulties in students' interpretation of mathematical vocabulary and representations. Differences in interpretation related to problem context and recurring errors associated with specific types of representation are expected. The interviews will also provide insight into the strategies students use when reading problem statements and the obstacles they perceive in this process.

Keywords: Interpretation of statements, Language, Representations, Context of the problem

24541 | Perspectives of Brazilian Higher Education Students and Support Structures on visual anti-immigration narratives on social media

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Background & Aim: The present research aims to understand how brazilian international students in Portugal construct meaning on their migration experience in light of visual anti-immigration narratives circulating in the public sphere, particularly on social media, as well as getting to know the perspectives of supporting structures of Higher Education Institutions. This research emerges from ongoing investigation on the relevance of the visual narrative[1] on the creation of identity[2];[3] and dessensitization to hate speech[4;[5], as well as a prior understanding of immigrant students´ vulnerabilities within their host institutions[6] and countries[7]. **Methods:** In order to accomplish the aforementioned goals, we contemplate two focal groups with higher education students, one with students from Arts and Humanities fields and another with students from STEM fields, as well as a total of five interviews with responsible members of supporting structures. These structures contemplate a central support service for international students, a '*Centro Local de Apoio à Integração Migrante*' integrated in a HEI and a student-lead group. The focus groups and interviews will contemplate a set of pre-selected and categorized images displaying three types of populist narratives: anti-elitist, nationalist and exclusionist[8]. The main goals of the focus groups and interviews will differ according to the role of the participants, while maintaining a common ground to allow cross referencing the collected data and compare perspectives from students and HEI institutional discourse. **Results:** Based on current readings, we expect to identify a multitude of meanings attributed to these narratives, anticipating a range from discomfort to desensitisation. **Conclusions:** The present work is trusted to allow comprehension of a less explored topic in regards to international students, which is the impact of hate speech in their academic and personal lives, and allow reflection on the potential protective role of HEI.

Keywords: Hate Speech; Social Media; Visual Narrative; Higher Education; International Students;

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24561 | Learning from mistakes: How does error analysis contributes to Problem Solving?

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Background & Aim: Problem solving is central in the mathematics curriculum, from primary to secondary schooling. Challenging problems enables students to mobilise their knowledge and, when they reach a correct solution, the resulting sense of achievement contributes to the development of a positive disposition towards the subject [1]. Error is an inherent element of the learning process and rather than being viewed as a failure, may constitute a valuable learning opportunity. Therefore, teachers should integrate them into the teaching and learning process [2]. When explored in the classroom, errors can function as a catalyst for learning by encouraging students to question their reasoning and reconstruct knowledge in a more meaningful way [3]. This study aims to explore how engaging students in error analysis can enhance their problem-solving skills. **Methods:** This study involves the implementation of a pedagogical intervention in a 9th-grade class. The intervention is structured in three phases. First, students solve three mathematical problems. Then, they analyse a worksheet containing the most common errors, working in groups and discussing their findings with the class. Last phase mirrors the first one but includes different problems. Data will be collected through students' written productions, audio recordings and direct observation. **Results:** Initial analysis shows that errors occur predominantly at the level of the problem-solving process, particulary in performing algebraic manipulations. Ongoing comparative analysis between the 1st and 3rd phases seeks to identify possible improvements in students' problem-solving strategies. **Conclusions:** Making mistakes is an inevitable part of the learning process, and involving students in the analysis of their own errors, instead of having the teacher simply point them out, promotes deeper reflection and metacognition. This approach helps students understand the nature of their mistakes and potentially improve future performance.

Keywords: error analysis; problem solving; formative assessment.

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24585 | Using assessment criteria and teacher feedback to promote students' written mathematical communication in problem solving

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Background & Aim: Written mathematical communication plays a central role in students' learning, particularly in problem solving [1, 2]. Formative assessment practices, including explicit assessment criteria and feedback, can support students in improving how they express mathematical ideas. However, students often experience difficulties in clearly explaining their solutions and using appropriate representations [3, 4]. Therefore, this study aims to understand how 11th-grade students appropriate and use assessment criteria to improve their written mathematical communication in problem solving, and to explore the role of pair work and teacher written feedback in that process. **Methods:** This qualitative study is based on a teaching intervention in which students work in pairs to solve problems in two phases. Firstly, they solve a problem, write an explanation of the solution process, and engage in self-assessment using assessment criteria. Then, the teacher provides written feedback, and students can later revise their productions. Data collection will include students' written productions, self-assessments, teacher feedback, audio recordings of pair interactions, and semi-structured interviews. Data analysis will focus on students' use of assessment criteria and the development of their written communication. **Results:** This study is ongoing. The data is expected to allow an analysis of how students engage with assessment criteria and feedback. The findings are expected to contribute to the knowledge of how the explicit use of assessment criteria, combined with pair work and teacher feedback, may support students in improving the clarity, organization, and adequacy of their written explanations and representations. **Conclusions:** This study is expected to contribute to a better understanding of how formative assessment practices support students' written mathematical communication, as well as to better inform teaching practices.

Keywords: Formative assessment; Written mathematical communication; Problem solving; Assessment criteria; Feedback; Pair work

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24587 | Exploring Mathematics Learning Through a Mathematical Trail in Grade 8

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Background & Aim: Research in mathematics education highlights the importance of connecting formal classroom learning with non-formal learning environments to promote students' engagement and meaningful understanding [1,2]. Mathematical trails have been identified as exploratory learning experiences that foster problem solving, collaboration, and the application of mathematical concepts in real-world contexts [3,4]. This study aims to analyse how students engage with mathematical tasks in a mathematical trail and how they reflect on those tasks afterwards, in a classroom discussion setting. It also aims to explore students' perspectives on this learning experience. **Methods:** The study follows a qualitative exploratory design. Participants are Grade 8 students involved in a mathematical trail implemented outside the school building, followed by a class discussion on the tasks completed along the trail. Data collection includes students' written productions developed during the trail, audio recordings of a post-trail classroom discussion, students' answers to a short post-trail questionnaire, semi-structured interviews to a small group of students, and field notes from observations conducted during both the trail and the discussion lesson. **Results:** As data collection is ongoing, preliminary results are expected to shed light on how students engage with mathematical ideas in outdoor contexts, and how they later connect those experiences in a classroom setting through the discussion of different approaches to the various tasks of the trail. **Conclusions:** The study is expected to contribute to a better understanding of the learning processes during a mathematics trail and of the educational potential of mathematical trails as complementary learning environments to formal settings. It is also expected to inform exploratory teaching practices and support the integration of non-formal learning experiences into mathematics education.

Keywords: Mathematical trails; Mathematics education; Non-formal learning; Exploratory learning

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24604 | How can mistakes be used as a teaching resource in mathematics education?

A study with 9th grade students in basic education

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Background & Aim: This research, still under development, within the scope of the Supervised Teaching Practice of the Master's Degree in Mathematics Teaching in the 3rd cycle of Basic Education and Secondary Education at FCUP, in the academic year 2025/2026, focuses on valuing error as an essential element of mathematical learning. Traditionally associated with failure or shortcomings, error is, however, recognized in Mathematics Didactics as an opportunity for knowledge construction. In this sense, this study aims to understand the errors made by students and analyze how teachers can use them as a learning resource. Specifically, it aims to identify the most frequent errors in mathematical tasks and their possible causes, understand the impact of reflection on error in the development of mathematical reasoning and self-regulation, and analyze the contribution of strategies such as oral feedback and collective discussion. **Methods:** The research will follow a qualitative and interpretative methodology, developed in a 9th grade class. Data collection will focus on students' written work, audio recordings, and direct observation in the classroom, including analysis of interactions during task completion, with a special focus on moments of collective discussion. The types of errors identified, student reactions, and teacher intervention strategies will be analyzed, with the aim of promoting reflection and overcoming difficulties. **Results:** The aim is to identify patterns of error and understand their causes, namely conceptual difficulties or weaknesses in the mobilization of prior knowledge. It is anticipated that guided reflection on errors will promote awareness of thought processes, encouraging self-regulation and the development of mathematical reasoning. It is also anticipated that the intentional use of oral feedback and group discussion will encourage the transformation of errors into learning opportunities, promoting a more constructive perception of them in class.

Keywords: Error, Mathematical Learning, Collective Discussion, Self-regulation

27362 | Students' difficulties in tasks involving mathematical reasoning

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Background & Aim: Mathematical reasoning is one of the transversal skills emphasized in the secondary mathematics curriculum, highlighting the importance of work that encourages the exploration of problematic situations, the use of heuristic approaches, the formulation and validation of conjectures, as well as the justification of the resolution processes used [1]. To address difficulties in mathematical reasoning and foster its development, carefully designed tasks play a central role in student success [2]. The types of tasks students engage in, the ways they engage with them, and the interactions that arise around these tasks are particularly relevant [3], especially when such tasks promote problem-solving, mathematical connections, communication, and argumentation [4]. This study aims to identify difficulties students express when performing tasks involving mathematical reasoning and to understand the underlying causes of these difficulties. **Methods:** The study, still in development, follows a qualitative and interpretative approach [5] and has as participants is being carried out with six 10th graders enrolled in Mathematics A. The data includes audio recordings of three individual task-based interviews, a final group interview and the collection of written productions. The selected students have different levels of academic performance and are communicative and able to express their thoughts throughout the task-solving process. **Results:** At the end of the first interview, it was possible to identify some significant difficulties. The students revealed, essentially, limitations in the generalization process, particularly in the transition from specific cases, involving numerical values, to the formulation of the general case, using algebraic language. **Conclusions:** Students do not yet have sufficient abstract thinking skills to understand complex concepts without concrete support, needing practical examples and guidance to build gradually their knowledge.

Keywords: mathematical reasoning; generalization; justification; difficulties; secondary school

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24697 | Problem Solving in Real-World Contexts: Contributions to Meaningful Mathematical Learning and to Students' Civic Education

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Background & Aim: This study, in its initial development phase, within the scope of the Master's Degree in Mathematics Teaching in the 3rd Cycle and Secondary Education (FCUP), aims to understand how problem solving in real-world contexts contributes to mathematics learning and to students' civic education. **Methods**: The research is qualitative in nature and is based on a pedagogical intervention carried out in the 2025/2026 school year with a 9th-grade class from a public school located in the Porto region, which includes two phases. Initially, there was an in-person discussion with a quadriplegic person to help students understand the difficulties of wheelchair mobility in cities that are not adapted to the needs of people with reduced mobility. The objective of this interview was to help students perceive the importance of building ramps that comply with regulations and to raise awareness among students. This interview served as the starting point for the presentation of the first mathematical problem, in which students had to calculate the orientation of some ramps at the school and identify whether they comply with current Portuguese legislation, using trigonometry concepts. In the second phase, continuing the problem initiated, another mathematical problem was applied in a real-world context, which is still under analysis. Thus, data collection included student work, audio recordings, videos, and an interview. **Expected Results:** With this study, it is expected that students will understand how mathematics is present in everyday life, its application, and its relationship to real life. Furthermore, students are expected to understand how problem-solving in real-world contexts helps them develop attitudes as more conscious and reflective citizens, while developing meaningful learning and mathematical skills.

Keywords: Problem-solving, real-world context, mathematics learning, citizenship education.

24189 | Quality of parent-child interaction: A piece of the puzzle or the key to positive parenting?

Parenting programs are known to bring parents and children multiple benefits, with one of the most important being improved quality of care for children. Most parenting programs are underpinned by a cognitive-behavioral framework that addresses parents' cognitions, perceptions, and behaviors, leading to improvements in parent-child interactions and, in turn, positively influencing children's development. Although parent-child interactions are widely recognized as key factors in children's development, they are rarely considered in the evaluation of the effects of parenting programs. We addressed this research gap by answering two research questions: 1) What are the most and least frequent characteristics in parent-child interactions within vulnerable families identified by Child Protective Services?; 2) What are the effects of the Standard Triple P (STP) parenting program on these interactions? Twenty-five parent-child dyads engaged with the CPS received either the STP or care as usual. All these dyads were videotaped twice while assembling a jigsaw, before and after the intervention. These interactions were observed and coded using micro- and macro-coding systems to quantify behaviors and educational dimensions. The results showed that parents were mainly focused on their own performance during jigsaw assembly, often working side by side with their child, although some parents observed their child's performance. Children were almost always engaged in the activity and interacted adequately with the parents. Although the STP was not found to be effective in improving the quality of parent-child interactions during jigsaw assembly, this study contributes to a better understanding of the dynamics and quality of parent-child interactions within vulnerable families. However, the assembly of a jigsaw may not have been representative of the families' daily interactions, and future research should include a broader range of tasks, especially more challenging ones.

27362 | Students' difficulties in tasks involving mathematical reasoning

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Background & Aim: Mathematical reasoning is one of the transversal skills emphasized in the secondary mathematics curriculum, highlighting the importance of work that encourages the exploration of problematic situations, the use of heuristic approaches, the formulation and validation of conjectures, as well as the justification of the resolution processes used [1]. To address difficulties in mathematical reasoning and foster its development, carefully designed tasks play a central role in student success [2]. The types of tasks students engage in, the ways they engage with them, and the interactions that arise around these tasks are particularly relevant [3], especially when such tasks promote problem-solving, mathematical connections, communication, and argumentation [4]. This study aims to identify difficulties students express when performing tasks involving mathematical reasoning and to understand the underlying causes of these difficulties. **Methods:** The study, still in development, follows a qualitative and interpretative approach [5] and has as participants is being carried out with six 10th graders enrolled in Mathematics A. The data includes audio recordings of three individual task-based interviews, a final group interview and the collection of written productions. The selected students have different levels of academic performance and are communicative and able to express their thoughts throughout the task-solving process. **Results:** At the end of the first interview, it was possible to identify some significant difficulties. The students revealed, essentially, limitations in the generalization process, particularly in the transition from specific cases, involving numerical values, to the formulation of the general case, using algebraic language. **Conclusions:** Students do not yet have sufficient abstract thinking skills to understand complex concepts without concrete support, needing practical examples and guidance to build gradually their knowledge.

Keywords: mathematical reasoning; generalization; justification; difficulties; secondary school

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24725 | Developing Computational Thinking in Mathematics through Scratch

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Background & Aim: Computational Thinking (CT) is defined as a transversal mathematical competence in the Portuguese Mathematics curriculum for Basic Education, structured around five practices: abstraction, decomposition, pattern recognition, algorithmic thinking, and debugging [1, 2, 4]. Despite its curricular recognition, CT is often addressed superficially in mathematics classrooms, frequently reduced to the instrumental use of digital tools without explicit attention to the cognitive practices involved [5]. Visual programming environments such as Scratch offer relevant didactic potential, as they require students to construct explicit algorithms, test and refine solutions, and make their reasoning visible through debugging [3]. This study aims to explore how the intentional integration of Scratch in 9th-grade mathematics lessons can promote the development of the five CT practices and support a deeper understanding of mathematical concepts. **Methods:** This qualitative study is based on a teaching intervention in a 9th-grade mathematics class. Mathematical tasks will be designed to explicitly foster the five CT practices using Scratch, articulating problem solving and programming. Data collection will include students' digital productions, written work, classroom observations, and teacher-researcher reflective notes. Data analysis will focus on identifying evidence of CT practices in students' mathematical activity and examining how programming mediates conceptual understanding. **Results:** The study is about to be implemented. Results are expected to allow a greater understanding of how students engage with each of the five CT practices during mathematical tasks and how Scratch supports those processes. **Conclusions:** This study is expected to contribute to a better understanding of how CT, supported by Scratch, can be intentionally integrated into mathematics teaching and developed as a mathematics transversal skill.

Keywords: Computational Thinking; Mathematics education; Scratch; Mathematics transversal skills; Problem solving

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24731 | The police and the radical right: A correlational study

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Background & Aim: In a time when democracies are being threatened by the rise of the radical right, national institutions may be influenced by this ideology and the populist beliefs associated with it. The police are closely linked to the political regime, since policing practices reflect broader forms of political authority (Bayley, 1985). The potential radicalization within law enforcement is particularly concerning as it may threaten public trust in the institution's impartiality and credibility, contributing to the weakening of democratic rule of law principles (Koehler & Filipps, 2024). In Portugal, the increase in parliamentary representation of a populist radical right party and its growing among the population normalizes their anti-democratic views. This party's leader shows strong public support for improvements in police working conditions, mental health support and public recognition. As a result, some police officers may feel more politically acknowledged and become more receptive to populist or authoritarian beliefs. Thus, the main objective of this study is to examine the influence the radical right can have within a security force and to explore the factors that may be associated with greater adherence to these ideologies. **Methods:** This study will use a quantitative research method based on an online survey administered to members of the *Polícia de Segurança Pública.* The survey will assess officers' perceptions of their professional conditions (e.g., job satisfaction) and ideological attitudes. The data will be analyzed by correlations. **Results:** It's expected that higher levels of perceived insecurity during police service as well as lower levels of job satisfaction and perceived job recognition will be associated with higher levels of populist beliefs and right-wing authoritarianism. **Conclusions:** This study examines how police officers' professional perceptions relate to their political attitudes and their potential implications for democratic institutions and public trust.

Keywords: Police officers; Radical right; Populism; Right-wing authoritarianism

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24732 | A Multidimensional Approach to Leader Prototypicality: Legitimacy and Affective Polarization in Political Contexts

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Background & Aim: In polarized political contexts, leader legitimacy is shaped by identity processes. Drawing on Social Identity Theory (Tajfel & Turner, 1979), leadership legitimacy emerges from perceived prototypicality - the extent to which a leader embodies ingroup attributes (Hogg, 2001). Because prototypes are flexible clusters of attributes, this work conceptualizes prototypicality as multidimensional, encompassing demographic (e.g., gender, ethnicity) and ideological-behavioral attributes (e.g., values, policy focus). Two models are proposed: (1) a cognitive-evaluative model in which ingroup leader prototypicality predicts legitimacy through perceived representativeness; and (2) an emotional model in which weak ingroup leader prototypicality elicits aversive reactions towards them that intensify affective polarization. Both models examine moderation by ingroup identification and outgroup leader prototypicality. **Methods:** A between-subjects experimental design will be employed. Model 1 uses a 2 (demographic prototypicality: low vs high) x 2 (ideological-behavioral prototypicality: low vs high) design. Model 2 uses a 2 (ingroup leader prototypicality: low vs high) x 2 (outgroup leader prototypicality: low vs high) design. Measures assess perceived representativeness, leader legitimacy, aversive emotions, affective polarization, and ingroup identification. **Results:** In model 1, ingroup leader prototypicality is expected to predict legitimacy through representativeness. In model 2, low ingroup prototypicality is expected to elicit aversive reactions that mediate increases in affective polarization. These effects should be stronger among high identifiers and amplified when the outgroup leader is highly prototypical. **Conclusions:** By distinguishing dimensions of prototypicality and integrating cognitive and emotional pathways, this research clarifies how identity-based leadership processes simultaneously structure legitimacy judgments and fuel political polarization in democratic societies.

Keywords: Leader prototypicality; Social Identity Theory; Leader legitimacy; Affective polarization

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24735 | “Primeiro estão os homens, depois logo se vê”: first-person experiences of female footballers

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Background & Aim: Integrated in the Sports, Education and Communities curricular unit (1st cycle, Educational Sciences), this qualitative research analyses the socio-educational trajectories of female athletes as a way of understanding the phenomenon of gender inequality in the world of football, in the various dimensions of human life. Historically, women's sport has been characterised by structural invisibility and devaluation, with football being socially understood as a male domain (Marivoet, 2002; Morgado, 2019; Morgado, 2021). **Methods:** Five semi-structured interviews were conducted with female football players to learn about their socio-educational and sporting backgrounds. **Results:** Their testimonies revealed the persistence of gender inequalities, both in sporting structures and in social discourse, evident in the discrepancy in resources, salaries and the experience of parenthood itself. However, they also highlight paths of resistance and social change, such as the increase in the number of female football players registered with the federation and the creation of female support networks that show that motherhood and a football career can coexist, with the media and federation support playing an extremely important role in promoting women's sport and resource equity, respectively. **Conclusions:** The full inclusion of women in football and sport in general requires more than their mere presence: it requires social and institutional changes, especially in terms of effective equality policies. The social and academic relevance of this phenomenon justifies further research in this area, within the scope of the Observatory of Sport, Education and Communities and the subsequent publication of an article specifically addressing issues of motherhood in football.

Keywords: inequality, gender, football, inclusion, sports

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24745 | Approximate Resolution of Equations in the 12th Grade: A Didactic Approach to Newton's Method and the Fixed Point Method

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Background & Aim: The approximate resolution of equations is part of the new Essential Learnings (Aprendizagens Essenciais) for Mathematics A in the 12th grade, introducing iterative methods, namely the Newton–Raphson method. As this is a topic that has recently gained prominence in the curriculum, questions arise regarding its conceptual, historical, and didactic framing, as well as the need to reflect on how it should be implemented and explored in the classroom. This research aims to analyse the historical and curricular framing of the topic, discuss the need for approximate methods in solving equations, and examine the potential of a didactic approach that introduces the Newton–Raphson method. The study seeks to understand how this approach contributes to students' comprehension, what difficulties emerge during the process, and what impact it has on the development of various skills. It also aims to assess whether identifying the limitations of Newton's method may justify, from a didactic perspective, the introduction of the fixed-point method as a more general framework for iterative methods. **Methods:** The study, still in progress, will follow a qualitative methodology, combining documentary analysis of the Essential Learnings and the literature characterising students' and teachers' perspectives, with the design, implementation, and analysis of a sequence of didactic tasks in a pilot 12th-grade class, where the topic will be taught for the first time. Data collection will be based on students' written work, classroom observation records, photographic records, audio recordings, interviews, and reflections on the learning achieved and difficulties experienced. **Results:** As this is still an ongoing study, it is expected to identify how students understand Newton's method as an iterative process, what difficulties arise in its application, and to what extent analysing these limitations contributes to understanding the fixed point method as a more general framework. **Conclusions:** It is anticipated that the results will make it possible to assess whether the proposed approach represents an added value in implementing

the new Essential Learnings and whether introducing the fixed point method is didactically relevant in the 12th grade.

Keywords: approximate resolution of equations; Newton–Raphson method; fixed point method.

24762 | Happy Birthday? Normative Scripts of a Social Ritual in Hollywood's Living Digital Archive

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Background & Aim: Birthdays are among the most universally practiced and varied social rituals, yet their normative representation in media, particularly in our digital age, remains understudied. Grounded in Social Representations Theory (SRT), this research embraces the theoretical appeal that media functions as a valuable medium for understanding the construction of social realities. Alongside Manovich's digital media studies, we examine predominant birthday scripts in Hollywood cinema to understand how contemporary media constructs and potentially disrupts the normative social meaning of this ritual. **Methods:** A corpus of 47 films was constructed from the top 50 IMDb titles tagged "birthday" by vote count — capturing relevant narratives persistently active in contemporary audiences. A codebook spanning 13 dimensions was developed through literature review, thematic analysis and human-AI collaboration (Claude and Gemini). Scene coding was conducted by Gemini and validated by human analysis in a human-in-the-loop protocol. **Results:** While films often depict a “generic” social staging of birthdays — featuring ample guests, generic décor, and material gift receiving — they systematically strip away core celebratory rituals like cake consumption, singing, and candle blowing. Within this hollowed-out setting, protagonists (often younger than old) face thwarted expectations and interpersonal conflict rather than meaningful self-reflection, transforming the ritual into a turbulent narrative catalyst. **Conclusions:** Rather than reinforcing cohesive rituals, Hollywood systematically encodes birthdays as crisis catalysts, constructing a turbulent normative script with potential consequences for how audiences experience real-life ritual expectations across different life stages. These findings illustrate the methodological opportunities of culturally-salient digital archives and the broader relevance of examining how mainstream media shapes social realities in the digital age.

Keywords: Birthdays; Social Rituals; Social Representations; Digital Media; Media Studies;

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24804 | Motivational regulation and psychological risk in a female football athlete during a triple career transition

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Background & Aim: The transition from Portugal to the United States of America poses a complex challenge for athletes managing multiple demands, particularly within a triple-career framework encompassing elite sport participation, academic responsibilities, and work commitments. This study aimed to examine the motivational regulation of a Portuguese female football athlete competing in NCAA Division I, while simultaneously balancing her roles as a student and worker. Additionally, the study sought to develop a theory-based psychological intervention to promote sustainable motivation and overall well-being. **Methods:** A mixed case study design was adopted. Quantitative data were collected using the Portuguese version of the Sport Motivation Scale-II, assessing motivational regulation along the self-determination continuum. Qualitative data were obtained through a semi-structured interview exploring perceived competence, motivational climate, and attributional patterns. Data integration enabled the creation of a comprehensive motivational profile. **Results:** The athlete demonstrated a predominantly self-determined profile, with high integrated regulation (M=7.00), identified regulation (M=6.67), and intrinsic motivation (M=6.33). Elevated introjected regulation (M=6.00) indicated significant internal pressure and self-imposed demands. External regulation (M=1.67) and amotivation (M=2.00) were low. Qualitative findings confirmed strong athletic identity and adaptive attributional tendencies alongside perceived performance pressure, suggesting a complex motivational profile combining protective and risk-related elements. **Conclusions:** Although autonomous motivation

was robust, elevated introjected regulation may constitute a long-term psychological vulnerability within the triple career context. A preventive intervention focused on autonomy support, cognitive restructuring, and adaptive attribution is proposed to preserve motivational quality and promote sustainable adjustment.

Keywords: self-determination theory; triple career; sport motivation; female football; NCAA

24843 | The role of rumination in the relationship between body dissatisfaction and Anorexia Nervosa symptomatology in adolescents

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Background & Aim: Anorexia Nervosa (AN) is a severe eating disorder that often emerges during adolescence and is characterized by persistent dietary restriction, intense fear of gaining weight, and body image disturbance. Body dissatisfaction has been consistently identified as a key risk and maintenance factor of AN symptomatology. Among the cognitive processes involved, rumination — a repetitive focus on negative thoughts — has been associated with increased negative affect and greater eating disorder symptom severity. This study aims to examine the mediating role of rumination in the relationship between body dissatisfaction and AN symptomatology in female adolescents. Specifically, the study aims to: (1) characterize levels of body dissatisfaction, rumination, and AN symptomatology; (2) analyse associations between these variables; (3) compare levels of body dissatisfaction and rumination between clinical and control groups; and (4) examine the mediating role of rumination in the relationship between body dissatisfaction and AN symptomatology. **Methods:** This cross-sectional study is part of the international I-CAN project and involves data collection in clinical settings (outpatient clinic, day hospital, and inpatient units) at Magalhães Lemos Hospital, ULS Santo António, Portugal. Main variables will be assessed using validated self-report instruments adapted for the Portuguese population: the Eating Disorder Examination Questionnaire (EDE-Q), the Perseverative Thinking Questionnaire – 15 (PTQ-15), and the Body Appreciation Scale-2 (BAS-2). Participants are female adolescents diagnosed with AN. Data analysis will include descriptive and correlational statistics and mediation models using IBM SPSS Statistics. **Results:** Data collection is currently ongoing, and no preliminary results are available. **Conclusions:** This study is expected to contribute to a better understanding of cognitive mechanisms underlying AN in adolescence, highlighting rumination as a potential target for psychological intervention.

Keywords: anorexia nervosa; body dissatisfaction; rumination; adolescence

24899 | Living with Early-Onset Parkinson's Disease: Experiences, Meanings, and Adaptation Strategies

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Background & Aim: Parkinson's disease is a neurodegenerative condition traditionally associated with older age; however, a subset of individuals develops symptoms earlier in life, a condition known as Early-Onset Parkinson's Disease. When diagnosed during active stages of adulthood, the disease may have a significant psychosocial impact, affecting occupational, family, and social roles. Despite these challenges, research on how individuals with early-onset Parkinson's disease perceive and experience their condition remains limited. Similarly, there is limited research on psychological interventions in this population, although such interventions are believed to play a central role in supporting emotional adjustment, the reconstruction of life plans, and the development of effective coping strategies in response to the demands imposed by Parkinson's disease. In this regard, the present study aims to understand the subjective experience of living with early-onset Parkinson's disease and to explore how participation in a therapeutic group may influence individuals' psychological adjustment to the disease. **Methods:** This study adopts a qualitative design. Data are collected through semi-structured interviews with approximately 10–15 individuals diagnosed with early-onset Parkinson's disease who participated in a therapeutic group programme. Participants are recruited with the support of Young Parkies Portugal, the organization responsible for disseminating the study. **Results:** Data collection is ongoing; therefore, preliminary results are not yet available. **Conclusions:** This study is expected to contribute to a better understanding of the psychosocial impact of early-onset Parkinson's disease, the specific needs of individuals diagnosed with it, and the role of participation in therapeutic groups in the process of adjustment to the disease.

Keywords: Early-onset Parkinson's disease; Process of adjustment; Therapeutic group

SPORT SCIENCES

24931 | A Double-Periodisation Macrocycle for Master Swimmers Based on Physiological Monitoring

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Background & Aim: Training periodisation should be structured with scientific grounding to maximise adaptation and competitive performance. We conducted a macrocycle for master swimmers based on training-related changes through aerobic capacity and power tests. **Methods:** 44 master swimmers (17 women and 27 men of 44.6±11.9y.o., 68.8±10.8kg and 171.0±9.9cm) were monitored using critical velocity (200 and 800m bouts) and T400 front crawl tests in each mesocycle for training control and load adjustment. **Results:** a double-periodisation was adopted to obtain the best possible results at the winter and summer national championships in january and July'26 (respectively). The first macrocycle (Figure 1) comprised six mesocycles of three-six microcycles (each one including four training sessions). Every session had dry-land and in-water exercises, differing their focus accordingly to the training phase of the macrocycle: (i) preparatory period, divided in general preparation (aiming to improve the muscular aerobic resistance through low-moderate intensity workouts) and specific preparation (resembling the most common competitive events of each swimmer and focusing on heavy-severe intensity exercises); (ii) competitive period (with extreme intensity bouts to mimic the main competitive efforts and with active rest sets just before the target competition) and (iii) transition period (with exercises of low load and different from swimming aiming to facilitate physical and mental recovery). Across each mesocycle, the velocities corresponding to anaerobic threshold and maximal oxygen uptake were determined to track swimmers' evolution and the training process. With that information, the coach adapted the planification according to the team's needs. **Conclusions:** systematic swimmers' evaluation supports the training load adaption across training phases and makes the performance improvement of master swimmers more efficient.

Keywords: periodization, macrocycle, swimmers, master

Figure 1: The winter macrocycle of a double-periodisation season of a master swimming team.

Planification 1st macrocycle 2025/2026

Dates		8-14 sep	15-21 sep	22-28 sep	29 sep - 5 oct	6-12 oct	13-19 oct	20-26 oct	27 oct - 2 nov	3-9 nov	10-16 nov	17-23 nov	24-30 nov	1-7 dec	8-14 dec	15-21 dec	22-28 dec	29 dec - 4 jan	5-11 jan	12-18 jan	19-25 jan	26 jan - 1 feb	2-8 feb	9-15 feb	16-22 feb
Period		General preparation									Specific preparation								Competitive period 1			Competitive period 2			Transition
Mesocycle		1				2					3				4				5			6			7
Microcycle		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Competitions									Trophy CFP					Winter territorial	European championship				CN Maia tournment		National winter championship			National long-distance championship	
Evaluations				T400	Critical velocity				T400	Critical velocity			T400	Critical velocity			T400	Critical velocity							
Training	Water Total	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4	3	4	3	4		3	4	4	4
	Dry Total	4	4	4	4	4	4	4	3	4	4	4	4	4	3	4	0	0	3	4	3	3	4	4	4
Training	Dry	General Strength/Mobility				Resistance Strength					Rapid, Elastic and Explosive Strength				Rapid, Elastic and Explosive Strength				Tapering			Tapering			Ballet
	Water	A1	A1/A2	A1/A2	A1 (A3/AP/LT)	A2	A2	A2/A3	A2/A3	A1 (A3/AP/LT)	A2/A3	A3/AP	LT/LP	A1 (A3/AP/LT)	AP + LT + LP	AP + LT + LP	LT + LP	A1+LP+V	LT + LP	LT + LP	LP + V + A1	A2	A2	A2	A1

Training aereas: A1 - Aerobic capacity 1; A2 - Aerobic capacity 2; A3 - Aerobic capacity 3; AP - Aerobic power; LT - Lactate tolerance; LP - Lactate power; V - Velocity

24858 | Adapted Physical Activity in a School Sport Context: Effects on Socio-Emotional Competencies in Students with Autism Spectrum Disorder

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Background & Aim: Autism Spectrum Disorder (ASD) involves difficulties in social communication and interaction, often with reduced self-control. In school, these challenges may limit participation and peer contact when teaching is not adapted. Physical Education and School Sport (SS) promote participation through structured, cooperative and rule-based tasks. Adapted physical activities (APA) may help students practice interaction and emotion control in a safe setting. This study analysed the contribution of PE-based APA to socio-emotional skills in students with ASD. **Methods:** Participants were two 17-year-old males with ASD. Adapted physical activities were delivered weekly over two months within a SS team (Boccia) by a preservice PE teacher during regular team training time. Sessions followed a structured format (warm-up, adapted tasks, formal game, reflection). Quantitative data were collected through systematic behavioural observation (voluntary and involuntary interactions; emotional reactions) validated by video recordings. Qualitative data were collected through observation notes and brief end-of-session questions, analysed using descriptive content analysis. **Results:** Both students showed patterns suggestive of improvement in social engagement and behavioural adjustment during APA, with increases in voluntary interactions and decreases in involuntary interactions, suggesting potential progress in social initiative, self-regulation and task engagement. Student A appeared to demonstrate greater social initiative and persistence, although turn-taking difficulties remained. Student B showed more consistent pattern of progress, with indication of sustained attention, higher motivation and improved rule-following. Both students reported generally positive perceptions of the sessions. **Conclusions:** Adapted physical activities implemented in a SS setting appeared to support intentional social interaction and self-regulation in students with ASD, although findings should be interpreted considering the study's scope and sample size.

Keywords: Autism; Socio-emotional skills; Adapted physical activities; Interactions; self-regulation.

24355 | Attentional Performance Following GAP and Pilates Group Exercise in Adult Women

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Background & Aim: Acute physical exercise may differentially affect attentional performance according to exercise intensity and cognitive demands. High-intensity activities may induce transient fatigue-related reductions, whereas modalities involving sustained postural control and attentional focus may modulate executive processes differently [1,2]. This study aimed to compare post-exercise attentional capacity in adult women participating in two distinct group exercise modalities (GAP and Pilates). **Methods:** Twenty-four adult women (Pilates n = 11; GAP n = 13; 42.71 ± 11.55 years) were evaluated in a cross-sectional design. Attentional performance was assessed immediately post-exercise using the Trail Making Test (TMT-A and TMT-B), which measures visual attention, processing speed, and cognitive flexibility. Performance was quantified by completion time (seconds). Between-group differences were analyzed using the Mann–Whitney U test ($p \leq 0.05$). Statistical analyses were performed using IBM SPSS Statistics (version 29.0). **Results:** Mean TMT-A completion times were 34.67 ± 6.84 s (GAP) and 32.88 ± 5.99 s (Pilates). For TMT-B, values were 74.85 ± 20.67 s (GAP) and 64.12 ± 18.58 s (Pilates). No statistically significant differences were observed between modalities in TMT-A ($p = 0.664$) or TMT-B ($p = 0.248$). **Conclusions:** Despite differences in exercise characteristics, both modalities elicited comparable acute attentional responses. These findings suggest that differences in physiological and motor demands do not necessarily translate into distinct immediate executive outcomes following recreational group exercise.

Keywords: Attentional capacity; Executive function; Group exercise; Adult women

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24261 | Beyond the Game: Dual Career Experiences of Portuguese Women's Youth National Team Football Players

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Background & Aim: The dual career framework, promoted across European sport policy, advocates the integration of elite sport and education. In football, however, structural demands frequently hinder this balance. This study aimed to examine Portuguese women's youth national team football players' perceptions of the conditions necessary to successfully reconcile elite sport participation and academic progression. **Methods:** A descriptive-exploratory design was adopted. A questionnaire was administered to 60 players (aged 15–21 years) from the Portuguese women's youth national football teams (under-17, under-19 and under-21). Quantitative data were analysed using descriptive statistics to identify the parameters perceived as most relevant for reconciling sport and education. **Results:** Approximately 80% of participants reported aspirations to complete a bachelor's or master's degree, underscoring the importance of ensuring viable dual career pathways. A substantial proportion identified greater timetable flexibility, increased academic tutoring, and access to online learning platforms as essential support mechanisms. Notably, nearly 80% indicated that no school mentor was available. Furthermore, around half of the respondents reported receiving no academic resources when absent due to sporting commitments. Across most of the proposed support measures, participants expressed a perceived need for institutional improvement. **Conclusions:** The findings highlight persistent gaps in institutional and pedagogical support for women's youth national team football players pursuing dual careers. Despite strong academic aspirations, participants reported insufficient structural adjustments to accommodate sporting demands. These findings emphasise the need to implement flexible and coordinated support mechanisms capable of promoting sustainable educational engagement and mitigating the risk of academic dropout.

Keywords: Dual Career; Student-Athletes; Women's Football; Youth Elite Sport; Educational Support

24602 | Characterization of the biomechanical profile of U16 and U18 competitive surfers: an exploratory study

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Background & Aim: Surfing has undergone significant professionalization in recent decades and is now recognized as an Olympic sport, receiving great media attention [1]. Although there are great studies on the surfing physiological characteristics [2], little is known about the biomechanical factors that characterize maneuvers and performance. Therefore, this exploratory study aimed to characterize the biomechanical profile of competitive surfers in the U16 and U18 categories. **Methods:** Eight male surfers competing in the regional surf circuit (2024-2025 season) were allocated to the U16 (n=3; 16.00±0.00 years; BM=69.70±10.54 kg) and U18 (n=4; 17.75±0.50 years; BM=74.38±10.79 kg) age groups. They attended two pool sessions (one week apart) to accomplish dryland and in-water protocols. Dryland testing included handgrip strength (HG), knee-to-wall test (KTW), core endurance (CORE), countermovement jump (CMJ) and Functional Movement Screen battery (FMS). The in-water assessments were performed on their regular surfboards and comprised sprint paddling to collect velocity (v) and stroke rate (SR), as well as tethered paddling to determine peak (Fpeak) and mean (Fmean) paddling force. **Results:** There was a slight trend for the U18 group exhibit better scores in the majority of the testing variables: HG_{U16}=40.11±6.30 vs HG_{U18}=42.63±5.23 kgf; KTW_{U16}=13.57±1.13 cm vs KTW_{U18}= 16.61±0.66 cm; $CORE_{U16}$=215.33±21.57 vs $CORE_{U18}$=230.75±58.85 s; FMS_{U16}=14.00±2.00 vs FMS_{U18}=17.50±1.91; CMJ_{U16}=35.15±5.15 vs CMJ_{U18}=39.52±3.04 cm; $Fpeak_{U16}$=257.94±56.18 vs $Fpeak_{U18}$=259.61±33.72 N; $Fmean_{U16}$=143.10±21.45 vs $Fmean_{U18}$=146.81±17.18 N; v_{U16}=1.62±0.09 vs v_{U18}=1.65±0.09 $m \cdot s^{-1}$; SR_{U16}=72.17±4.34 vs SR_{U18}=76.30±9.13 $cycle.min^{-1}$. **Conclusions:** Overall, these values cannot be categorized due to the absence of reference values. However, gathering descriptive data and grouping surfers by age can be a starting point for defining normative values to better develop the biomechanical profile of surfers through their youth.

Keywords: Surf; youth; training; in-water; dryland

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24376 | Differences in Simple Reaction Time and Static Balance Between Artistic Gymnastics and Competitive Swimming Athletes

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Background & Aim: Sport-specific training promotes distinct neuromuscular and sensorimotor adaptations that may influence fundamental motor capacities. Static balance is particularly relevant in gymnastics due to its technical demands [1], whereas reaction time plays a critical role in swimming performance, especially during start phases [2]. Despite these modality-specific motor requirements, comparative evidence between artistic gymnasts and competitive swimmers during adolescence remains limited. Hence, this study aimed to compare static balance and simple reaction time between athletes practicing artistic gymnastics and competitive swimming. **Methods:** Thirty-two athletes aged 12–16 years (14.38 ± 0.98 years) participated in this study, including 16 artistic gymnasts and 16 competitive swimmers. Static balance was assessed using the Flamingo Test from the EUROFIT battery [3], recording the number of falls from a 4-cm beam over one minute. Simple reaction time was evaluated using the Reflex Test (Thinkabout®), measuring response latency to visual stimuli. Statistical analyses were performed using the Mann–Whitney test in IBM SPSS Statistics, adopting a significance level of $p \leq 0.05$. **Results:** No statistically significant between-group differences were observed in static balance ($p = 0.622$). Swimmers recorded 6.50 ± 5.70 falls, whereas gymnasts presented 5.13 ± 4.18 falls. However, swimmers demonstrated significantly shorter simple reaction times compared to gymnasts (0.31 ± 0.25 s vs. 0.36 ± 0.06 s; $p = 0.007$). **Conclusions:** These findings suggested that sport-specific training may differentially influence selected motor capacities during adolescence. While static balance was comparable between modalities, competitive swimmers exhibited shorter simple reaction times. The results reinforce the role of training's specificity in shaping neuromuscular adaptations in young athletes.

Keywords: Artistic gymnastics; Competitive swimming; Simple reaction time; Static balance; Motor performance

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24315 | Do Long-Haul Travels and Jet Lag Affect Athletes' Physiological, Humoral and Performance Outcomes? A Systematic Narrative Review

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Background & Aim: Long-haul travel and jet lag can disrupt athletes' circadian, physiological, and performance systems, potentially impairing competitive outcomes. This review aimed to examine the effects of long-haul travel on athletes' health and performance, distinguish between travel fatigue and jet lag, and summarize mitigation strategies. **Methods:** A systematic narrative review was conducted following PRISMA 2020 guidelines. PubMed, Scopus, and Web of Science were searched for studies addressing jet lag, travel fatigue, and long-haul travel in athletes. Eligible studies reported physiological, hemodynamic, or performance outcomes in athletes of any level and sex. Data were extracted on travel characteristics, interventions, physiological and performance markers, and risk of bias. **Results:** A total of 284 records were identified, of which 89 studies were included. Travel directions were equally distributed between eastward and westward journeys (n=23), crossing 1–12 time zones. Interventions to mitigate travel effects were reported in 17 studies, mainly involving melatonin (n=16), caffeine (n=6), and light exposure (n=3). Common physiological changes included sleep disturbances (n=36), body temperature alterations (n=18), blood pressure changes, hormonal shifts (n=9), HRV (n=4), and immune alterations (n=4). Reported travel-related effects included fatigue (n=25), sleep disturbances (n=21), decreased physical performance (n=18), mood changes (n=15), and cognitive impairments (n=9). Physical performance outcomes frequently assessed were anaerobic power (n=18), strength (n=14), velocity (n=12), aerobic capacity (n=10), coordination (n=8), and reaction time (n=7). The risk of bias was low in 49% of studies, moderate in 17%, and high in 34%. **Conclusions:** Long-haul travel is associated with impairments across

multiple physiological and performance domains in athletes, particularly affecting sleep, hormonal balance, autonomic function, and physical performance. However, the magnitude of these effects seems to be influenced by travel direction, number of time zones crossed, and individual susceptibility. Eastward travel may be linked to greater circadian disruption and reductions in aerobic capacity and coordination, whereas westward travel often induces greater fatigue and adversely affects team-sport outcomes. Monitoring key markers such as HRV, sleep, and cortisol, combined with personalized strategies including circadian management, sleep hygiene, nutrition, recovery interventions, and training load adjustments may help mitigate travel-related effects.

Keywords: Long-Haul Travel; Travel Fatigue; Sports Performance; Circadian Rhythms; Hemodynamic Response

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24230 | Effects of Competitive Distance and Acute Fatigue on Start Reaction Time in Masters Swimmers

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Background & Aim: Start reaction time is a key determinant of swimming performance, acting as a critical performance indicator in events where the start represents a significant percentage of the total race time. Consequently, marginal gains in the start phase can significantly affect competitive outcomes. This study examined whether acute fatigue induced by simulated 200 and 800 **m** efforts alters start reaction time in master swimmers and whether baseline reaction time differs between distances. Specifically, we aimed to (i) compare reaction time before and after exertion within each distance and (ii) test for between-distance differences at baseline and post-exertion**. Methods:** A total of 27 competitive master swimmers participated in a pre-post experimental design. Reaction time was assessed immediately before and after a simulated 200 and 800 m front crawl trials. Start reaction time was obtained from high-speed video recordings and processed using Blender, with statistical analyses performed in Jamovi. Data normality was verified using the Shapiro–Wilk test and paired-samples Student's *t*-tests were applied ($p<0.05$). **Results:** No significant differences were observed after exertion in either the 200 and the 800 m conditions ($p=0.158$ and 0.335, respectively). However, differences were found between distances in both the pre- and post-tests ($p=0.011$ and 0.014, respectively), with faster reaction times consistently recorded in the 200 m condition. **Conclusions:** Competitive distance **influences** start neuromotor readiness **in** master swimmers. Shorter-distance conditions are associated with faster reaction times, possibly reflecting higher activation levels and event-specific energy management strategies. Acute fatigue did not significantly impair start reaction time, suggesting preserved neuromotor responsiveness.

Keywords: Swimming; Start reaction time; Neuromotor performance; Acute fatigue; Master swimmers.

24363 | Effects of Feedback Frequency on Non-Dominant Basketball Shooting Performance

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Background & Aim: The frequency of feedback is considered a critical variable in motor learning. Although higher feedback frequencies may enhance immediate performance, excessive feedback can promote dependency and limit retention, as proposed by the guidance hypothesis [1,2]. Evidence also indicated that regulating feedback provision can facilitate longer-term skill acquisition by promoting learner autonomy [3]. This study aimed to examine the influence of different feedback frequencies (25%, 50%, and 100%) on non-dominant hand shooting performance in young basketball players. **Methods:** Twelve male basketball athletes (15–18 years) were randomly assigned to three groups differing in feedback frequency (25%, 50%, or 100%). The protocol included three phases conducted in each group: pre-test (3 shots without feedback), acquisition (20 shots with eyes closed and frequency-specific descriptive feedback), and post-test (3 shots without feedback). Performance was assessed using a 1–3 ordinal scoring scale. Data were analyzed using non-parametric tests (Kruskal–Wallis and Mann–Whitney; $p \leq 0.05$). **Results:** No significant differences were observed between groups in the pre-test ($p = 0.470$). Significant differences emerged during acquisition ($H = 7.376$; $p = 0.025$), with the 50% and 100% feedback groups outperforming the 25% group. Pairwise comparisons revealed significant differences between 25% vs 50% ($p = 0.029$) and 25% vs 100% ($p = 0.018$), but not between 50% and 100% ($p = 0.457$). No significant differences were found in the post-test ($p = 0.199$) or in percentage improvement ($p = 0.699$). **Conclusions:** Higher feedback frequencies improved performance during practice but did not result in superior post-test performance, suggesting a transient facilitative effect rather than robust learning. These findings provide partial support for the guidance hypothesis and highlight the importance of strategically regulating feedback frequency in youth basketball training.

Keywords: Feedback frequency; Basketball; Motor performance; Youth athletes

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24869 | Enhancing Student Engagement in Physical Education Through Cooperative Learning: Insights from an Action-Research Intervention

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Background & Aim: Physical Education (PE) plays an important role in the development of social skills. However, low commitment, limited proactivity, and cooperation difficulties constrained the learning environment, motivating this study. The aim of this study was to analyse how Cooperative Learning strategies influence students' commitment, involvement, and cooperation in PE classes. Cooperative Learning is a student-centred pedagogical model in which students work in structured, heterogeneous groups based on five key principles: positive interdependence, social interaction, individual accountability, social skills development, and group processing [1]. **Methods:** The study was conducted in a Portuguese public school following an action-research design [2]. Twenty-five 12th-grade students (11 males, 14 females), aged 16-19, participated. Initial class characterisation used the *Meaningful Experiences in Physical Education Survey* (MEPES) [3]. Diagnostic results informed planning, and the intervention was structured using Cooperative Learning strategies during Volleyball and Badminton units (18 lessons). Data collection included students' self-assessments, teacher notes, a questionnaire and a final focus group. The questionnaire was specifically developed for this study and validated by the supervising teacher. It was based on the *Cuestionario de Aprendizaje Cooperativo* [4] and on the five key elements of Cooperative Learning [5], using a five-point Likert scale. **Results:** Initial observations showed difficulties forming heterogeneous groups, preference for affinity peers and gender resistance. MEPES results revealed a positive perception of PE, with social interaction as the most valued dimension. Weaknesses emerged in perceptions of challenge, along with feelings of exposure and comparison that could hinder engagement. Issues of motor competence and perceived task relevance were also noted. After three weeks, improvements were observed in heterogeneous grouping, participation, and enhanced cooperation quality. **Conclusions:** Early findings suggest improvement in commitment, involvement, and cooperation, reinforcing Cooperative

Learning as an effective pedagogical model in PE. Further analysis will clarify its impact on students' meaningful experiences.

Keywords: Cooperation; Student-centered teaching; Meaningful Experiences.

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24847 | From Direct Instruction to Peer Teaching: The role of student involvement in learning badminton

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Background & Aim: Different teaching models in Physical Education (PE) can influence student engagement in learning. Student-centered approaches, such as peer teaching (PT), are associated with greater participation, responsibility and quality of interactions, promoting more active and meaningful learning [1,2]. In the context of PT, this model is based on collaboration between students and the exchange of feedback, supporting the development of motor and social skills [3]. In contrast, the Direct Instruction (DI) model involves structured intervention by the teacher, focusing on demonstration and technical correction. This study analyses and compares students' perceptions of learning and engagement in badminton in these two teaching models. **Methods:** Sixteen secondary school students (aged 16 to 18) participated in a two-phase badminton unit: DI (six lessons) and PT (six lessons). During DI, the teacher prescribed tasks and provided all feedback. In PT, students worked in small groups, alternating between the roles of performer and observer and taking greater responsibility for task execution and feedback. Data were collected through a structured questionnaire (5-point Likert scale), developed based on key dimensions of learning, motivation, and student engagement, as proposed in the literature (Reeve & Tseng, 2011; Casey & Goodyear, 2015). The instrument's content was validated by two specialists in sport pedagogy. Quantitative data were analysed using descriptive statistics (means and dispersion). Additionally, qualitative data were collected through the teacher's reflective diary, lesson reflections, and semi-structured interviews, and will be subject to content analysis. **Results:** Both pedagogical models revealed high levels of perceived learning and engagement, with differentiated patterns. In the DI phase, students reported high scores in active participation (M = 4.94), clarity of teacher feedback (M = 4.94), and perception of a positive learning environment (M = 4.81), highlighting the role of structured guidance and explicit instruction.

In the PT phase, high levels of engagement were also observed, particularly in concentration (M = 5.00), comfort in receiving feedback (M = 5.00), and cooperation (M = 4.94). Higher values were also found in reflection (M = 4.88) and peer communication (M = 4.88), suggesting a shift towards more interactive and student-centered learning processes.

Overall, DI appears to be associated with clarity and teacher support, whereas PT shows stronger indicators related to collaboration, reflection, and peer feedback. **Conclusions:** Both pedagogical models support positive perceptions of learning and engagement, through distinct but complementary mechanisms. Direct Instruction enhances structure, clarity, and teacher support, whereas Peer Teaching promotes collaboration, autonomy, and active involvement. It is expected that the integration of quantitative and qualitative data will clarify how these approaches differently influence students' technical learning, engagement, and interaction, contributing to a more grounded understanding of pedagogical choices in PE.

Keywords: Student-centered Teaching; Student Engagement; Cooperation; Dialogic Feedback.

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24912 | Game design as a pedagogical tool for improving game understanding and performance levels in football

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Background & Aim: In Portugal, the Physical Education (PE) curriculum is strongly oriented towards team sports, used to promote inclusion and students' holistic development. Within this framework, engaging students in game design fosters active involvement, critical thinking and problem-solving (Butler, 2016; Hastie, 2010). This study analyses the impact of game design on students' game understanding and performance in football. **Methods:** An action research study was conducted during a school placement with 21 pupils across a 12-lesson unit. Diagnostic and final assessments employed the 4v3+GK and GK+4v4+GK formats, using Monteiro's (in press) observational instrument with six offensive and defensive parameters rated on a five-point scale (1 – no competence; 5 – excellent): possession, progression, creation of scoring opportunities, preventing progression, ball recovery and preventing scoring. A multiple-choice questionnaire assessed initial knowledge and informed content selection, followed by interviews exploring students' reasoning. Lessons were video-recorded. Post-unit interviews addressed three themes: Game Creation (GC), its relationship with football, and game modification. Motor performance was assessed through the Game Performance Assessment Instrument. A field diary complemented the data, analysed deductively and inductively. **Results:** Initially, students struggled to position themselves to create passing lines and retain possession, despite articulating appropriate tactical intentions. Preliminary findings indicate improvements in participation, game understanding, decision-making and motor performance.

Conclusions: Findings support GC as an effective pedagogical strategy for teaching football within student-centred PE approaches.

Keywords: Physical Education; Creating Games; Game Modification; Invasion Games; Problem-solving.

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24281 | Impact of Artificial Intelligence use in the theoretical assignments in physical education

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Background and aim: The increasing availability of Artificial Intelligence (AI) in education has intensified debate about its impact on student learning. Although AI may improve organisation and written output, concerns remain about whether it fosters deeper understanding, particularly in assessment contexts where higher performance may not reflect genuine learning. This study examined whether access to AI during theoretical assessment tasks influences students' assignment performance and content comprehension. **Methods:** Participants were 15 eleventh-grade vocational students enrolled in a swimming theoretical module, divided into four balanced groups based on academic performance. Two groups completed assignments with AI access, while other two groups worked without AI under identical conditions. Tasks were developed over five hours across three lessons. In the AI condition, students followed guidelines prohibiting copying generated content or requesting fully completed work. After each assignment, a mini-test assessed content understanding. Evaluation combined assignment quality and mini-test performance, with descriptive comparisons between conditions. Qualitative data from student reflections and teacher records were analysed thematically. **Results:** Students who completed the assignments without AI achieved higher average scores than those who used AI support, suggesting that working without AI may have promoted deeper content comprehension and learning. Qualitative data showed that AI helped clarify concepts and organise work, but sometimes led to superficial engagement when students relied on generated suggestions. Teacher observations also indicated stronger discussion and sustained reasoning in the non-AI groups. **Conclusions:** The findings suggest that AI's educational value depends more on pedagogical framing than on its access alone. Effective integration requires structured guidance and assessment focused on reasoning and conceptual understanding rather than output quality.

Keywords: Artificial Intelligence; Learning; Physical Education; Assignment.

24359 | Learning to Give Feedback in Physical Education: A Preservice Teacher in Soccer and Badminton Lessons

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Background & Aim: Teaching quality in Physical Education (PE) depends on clear instruction, class management, and a positive learning climate. Teacher feedback is a key tool to guide motor learning, support engagement, and shape task focus. Yet, preservice teachers often struggle to vary feedback type, timing, and target, especially across sports with different task demands. Understanding how feedback is used in real PE lessons may help improve teacher education and lesson design. The present study aimed to describe and analyze feedback provided by a preservice PE teacher during Soccer (team sport) and Badminton (individual sport) lessons. **Methods:** One 24-year-old preservice PE teacher was observed teaching an 11th-grade class (n = 23). Four 90-minute lessons were analysed (two Football, two Badminton). In each lesson, three 10-minute periods were coded: general warm-up, early main part, and late main part. Video and audio recordings were transcribed and coded using established feedback categories: instruction, prescriptive, interrogative, descriptive, evaluative, positive, negative, feedback cycles, and direction (individual vs group). Data were summarised using absolute and relative frequencies. **Results:** A total of 579 teaching events were coded, with prescriptive feedback and direct instruction as the dominant categories. More feedback was recorded in Badminton than in Football, particularly during the early main part of the lesson. Feedback direction differed by sport: Badminton was characterised by predominantly individual feedback, whereas Football showed a more balanced use of individual and group feedback. Across both sports, feedback was limited during warm-up, and positive feedback and feedback cycles were rarely observed. **Conclusions:** Feedback varied by sport and lesson phase. Initial teacher education should therefore support preservice PE teachers in moving beyond instruction and prescriptive feedback by using more positive, questioning, and interactive feedback strategies.

Keywords: Feedback; Physical Education; Initial Teacher Education; Systematic Observation; Team vs Individual Sports.

24599 | Motivational processes and intervention effects on exercise adherence: a self-determination theory case study

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Background & Aim: Exercise adherence is influenced not only by the level but also by the quality of motivation. Grounded in Self-Determination Theory (SDT), this study aimed to analyse motivational processes underlying exercise participation and to evaluate the effects of a theoretically informed intervention in a case study of a 41-year-old female exerciser undergoing a life transition due to immigration. **Methods:** A mixed-method case study design was adopted. The Behavioral Regulation in Exercise Questionnaire (BREQ-4) and the Basic Psychological Need Satisfaction and Frustration Scale in Exercise (BPNSFS-E) were administered pre- and post-intervention. A semi-structured interview was conducted to explore subjective motivational experiences. The intervention focused on autonomy support, competence enhancement, task-oriented goal setting, and relational support. **Results:** Post-intervention results showed an increase in identified regulation (3.50 → 4.00) and in introjected regulation (avoidance: 2.25 → 3.25; approach: 2.75 → 3.50), indicating greater commitment, although partially driven by internal pressure. Integrated regulation decreased (3.00 → 2.50), suggesting that full internalisation into identity was not yet achieved. Intrinsic motivation remained stable at moderate levels (1.75), and amotivation remained absent (0.00). Regarding psychological needs, competence satisfaction increased (3.25 → 4.25) and competence frustration decreased (2.50 → 1.50), representing the most significant improvement. Autonomy showed an ambivalent pattern, with decreased satisfaction (4.00 → 3.50) and increased frustration (4.00 → 4.25). Relatedness satisfaction decreased slightly (4.00 → 3.00), while frustration remained low (1.00). Qualitative findings supported these results, revealing improved exercise consistency, enhanced perceived competence, and increased valuation of exercise, although still experienced partly as a structured obligation. **Conclusions:** The intervention improved exercise adherence and strengthened motivational quality, particularly through enhanced perceived competence and

identified regulation. However, motivational internalisation remains ongoing, with persistent controlled forms of regulation and autonomy ambivalence. These findings highlight that structured, SDT-informed interventions can promote adherence even before full internalisation occurs, especially in contexts of life transition.

Keywords: self-determination theory; exercise adherence; motivation; basic psychological needs; case study.

24957 | Practice Effects on Bilateral Transfer Pass in Youth Football Players

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Background & Aim: In youth football, motor laterality often results in a predominant use of the preferred foot, potentially limiting the balanced technical development of young players. Although previous research has demonstrated interlimb transfer in various motor tasks, evidence regarding its manifestation in precision-based skills such as passing in early developmental stages remains limited. This study aimed to analyze whether unilateral practice of passing skill with the preferred or non-preferred foot promotes contralateral performance. **Methods:** Twenty male football players (7–9 years old) from Rio Ave FC and Gil Vicente FC participated in the study, divided into two transfer conditions: Group 1 (preferred–non-preferred foot) and Group 2 (non-preferred–preferred foot). A standardized passing accuracy protocol was implemented, consisting of: pre-test (5 trials), acquisition (25 trials with the contralateral foot), retention (5 trials with the acquisition foot) and transfer (5 trials returning to the initial foot). Performance was scored using a distance-based target system (0–5 points). Shapiro–Wilk test assessed data normality and the Mann–Whitney U test compared the groups ($p \leq 0.05$). **Results:** No significant differences were observed in the pre-test phase ($Z=-1.598$; $p=0.110$), confirming equivalent baseline performance. Significant differences emerged during acquisition ($Z=-2.163$; $p=0.031$) and retention ($Z=-2.069$; $p=0.039$), with Group 1 presenting superior mean scores in both phases. In the transfer phase, no statistically significant differences were found ($Z=-1.714$; $p=0.086$), although a tendency toward greater transfer effects was observed in Group 2. **Conclusions:** a tendency to bilateral transfer from the non-preferred to the preferred foot was identified, although not statistically significant. These findings suggest that unilateral training involving the non-preferred foot may contribute to balanced technical development in early stages of football practice. The limited sample size may have constrained the magnitude of detectable effects.

Keywords: Youth football; Bilateral transfer; Motor laterality; Motor learning; Passing accuracy

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24798 | Practice Order Effects on Bilateral Transfer in Youth Football Players

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Background & Aim: Bilateral transfer is defined as the improvement in performance of one limb following practice with the contralateral limb. In football, proficiency with both lower limbs is critical for technical efficiency and tactical adaptability [1,2]. Despite extensive research in motor learning, evidence regarding the directionality and phase-specific manifestation of bilateral transfer in youth football players remains limited. Therefore, this study aimed to examine the existence and direction of bilateral transfer by comparing performance between preferred and non-preferred limbs across distinct phases of motor learning. **Methods:** Thirty-two male youth football players (10.25 ± 1.11 years) with at least one year of federated training participated. Players were allocated into two groups according to practice order: G1 (non-preferred–preferred limb) and G2 (preferred–non-preferred limb). A standardized passing protocol was applied, including pre-test (5 trials), acquisition (20 trials), retention (5 trials after a 5-minute interval), and transfer (5 trials with the opposite limb). Accuracy was assessed using a target-based scoring system. Between-group differences were analyzed using the Mann–Whitney U test ($p < 0.05$). **Results:** Significant differences were observed in the pre-test ($p=0.013$), retention ($p = 0.002$), and transfer phases ($p = 0.020$), but not during acquisition ($p = 0.557$). G1 showed superior initial performance and greater transfer effects, whereas G2 demonstrated better retention. Practice order therefore influenced specific stages of learning. **Conclusions:** Bilateral transfer was confirmed, although its expression was asymmetric and phase-dependent. Initiating practice with the non-preferred limb enhanced transfer, whereas starting with the preferred limb favored retention. These findings highlight the relevance of limb dominance and practice sequencing in youth football training design.

Keywords: Bilateral transfer; Limb dominance; Motor learning; Youth football players

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24816 | Recovering from cancer: a mixed-study focused on the psychological processes of participants in a physical exercise program

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Background & Aim: Cancer remains one of the most significant public health challenges, with an increasing incidence exceeding 20 million new cases annually worldwide, and more than 60,000 per year in Portugal [1, 2]. Current scientific evidence demonstrates that physical exercise is a safe and effective adjuvant strategy [3, 4]. This study aimed to: (1) assess participants' mental health and subjective well-being; and (2) qualitatively explore perceived mechanisms of recovery and program adherence. **Methods:** A mixed-methods approach was employed with 12 participants, ranging from 33 to 74 years old (M_{age}= 63.3, SD=12.2) (92% female). Quantitatively, four self-report measures (i.e., Mental Health Inventory–5; Satisfaction with Life Scale; Positive and Negative Affect Schedule and Subjective Happiness Scale) were used to assess mental health and subjective well-being, while the qualitative approach applied an interview guide with four sections: (1) treatment side effects; (2) individual exercise adaptations; (3) structure and support; and (4) behavioral changes and maintenance of exercise behavior. In terms of the quantitative data, the statistical analysis included normality tests, descriptive statistics, and correlation tests using IBM SPSS Statistics. Regarding the qualitative component, semi-structured interviews were audio-recorded and transcribed verbatim to ensure integrity of the data. Data analysis incorporated deductive and inductive methods. **Results:** The participants evidenced high levels of psychological well-being, characterised by moderate to high levels of positive affect (3.77, SD=.48), satisfaction with life (3.35, SD=.92) and happiness (3.77, SD=.77) and reduced levels of negative affect (2.43, SD=.74) and distress (2.33, SD=.99). Interviews revealed that the participation in the program enhanced participants' self-esteem, confidence, motivation, social relatedness and reduced stress and feelings of isolation. **Conclusions:** Exercise programs may promote mental health, well-being, self-esteem, confidence and social relatedness in cancer survivors. Social support and adaptations to participants' conditions and abilities seems to be key factors of efficacy.

Keywords: Cancer; Exercise; Program; Psychological processes; Mixed-study.

Acknowledgments: The authors would like to thank all subjects who voluntarily participated in this study for their collaboration and availability.

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24348 | Technical Directors' Contradictory Perspectives on Early Diversification and Early Specialization in Portuguese Elite Youth Football

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Background & Aim: This study explored the perceptions of Technical Directors (TD) within elite Portuguese youth football academies regarding player developmental pathways, namely the Early Specialization (ES) and Early Diversification (ED) routes to talent development. **Methods:** A qualitative, interpretive approach was adopted, grounded in a constructivist-interpretivist paradigm. Participants included eight TD from academies awarded the "five-star" certification by the Portuguese Football Federation, with professional experience ranging from 6 to over 30 years. Data were collected through semi-structured interviews and subjected to thematic analysis, following Braun and Clarke's [1] six-phase framework. **Results:** The findings revealed a unanimous endorsement of ED as a cornerstone for holistic development. TDs emphasized that engagement in multiple "*donor sports*" enhances motor literacy, psychological resilience, and the transfer of tactical and cognitive competencies to football. Conversely, ES emerged as the prevailing and pragmatically accepted trajectory. While TD acknowledged risks such as burnout, overuse injuries, and dropout, ES was perceived as advantageous for the premature acquisition of complex, football-specific technical-coordinative skills. This duality of perspectives is further reinforced by external pressures from parents, coaches, and agents who prioritize immediate competitive results over long-term developmental sustainability. **Conclusions:** The study underscores a significant disconnect between theoretical developmental assumptions and the practical realities of elite youth football academies. Despite the recognized value of ED, the contemporary football ecosystem institutionalizes ES as a competitive requirement. These findings suggest a critical need to reformulate coach education and talent development models to prioritize holistic, sustainable, and context-sensitive approaches.

Keywords: Early specialization, Early diversification, Youth football, Developmental pathways, Talent development.

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24818 | The impact of emotions on competitive performance in karate athletes

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Background & Aim: Emotions are key psychological determinants of sport performance, particularly in combat sports such as karate, which involve high pressure and quick decisions. While research has mainly focused on negative emotions, recent evidence highlights the functional role of positive emotions and optimal arousal. However, studies examining multiple emotions simultaneously in karate athletes remain scarce. This study examined the relationship between pre-competitive emotions and competitive performance in karate athletes and compared emotional profiles between medal-winning and non-medal-winning competitors. **Methods:** A cross-sectional study was conducted with 16 karate athletes aged 12 to 65 years (31.25% female) across different competitive levels following a national competition. Pre-competitive emotions were assessed using the Sport Emotion Questionnaire (SEQ) [1], evaluating anxiety, sadness, anger, excitement, and joy. Competitive performance was measured using a weighted coding system based on medals obtained in regional, national, and international competitions, rated on a 1–10 Likert scale and combined into a single performance index. Participants were classified as medaled (n = 8) or non-medaled (n = 8). Statistical analyses included descriptive statistics, Pearson correlations, and Mann–Whitney tests. **Results:** Athletes showed a predominantly positive emotional profile, with higher levels of joy and excitement and low levels of negative emotions. Significant correlations were observed among negative emotions, indicating co-occurrence. Excitement was the only emotion significantly associated with performance (r_p = .55, p < .05) and the only variable differentiating medal-winning from non-medal-winning athletes (Z = -2.73, p = .006). No significant associations were found for anxiety, sadness, anger, or joy. **Conclusions:** Findings indicate that excitement plays a key role in facilitating competitive readiness and performance among karate athletes.

Keywords: excitement; anxiety; sports performance; karate; emotions.

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24961 | The Relationship Between Personal and Social Responsibility in Basketball and Swimming by Gender

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Background & Aim: In the sports context, personal and social responsibility are central to youth development. Personal responsibility relates to autonomy, adherence to rules, and individual commitment, whereas social responsibility involves respect, cooperation, and empathy in interpersonal relationships [1]. As such, sport provides a privileged educational setting for the simultaneous development of both dimensions. This study aimed to examine and compare levels of personal and social responsibility among athletes from team and individual sports, as well as between genders. **Methods:** The sample comprised 51 athletes (25 females) aged 15.08 ± 1.16 years, including 27 swimmers and 24 basketball players. Participants completed a demographic and sport self-report questionnaire, as well as the Personal and Social Responsibility Questionnaire among Athletes, adapted for athletic populations by Li et al. (2008) [2] and translated into Portuguese by Martins et al. (2015) [3]. Pearson correlations were computed separately by sex (male/female) and by sport (basketball/swimming) to examine the associations between personal and social responsibility within each subgroup. Group differences were then tested between sexes and between sport groups. **Results:** The results indicated different correlation patterns for boys and girls. In boys, a significant association was found between personal and social responsibility. Across sports, personal and social responsibility were moderately and positively correlated in both basketball and swimming. Regarding sex differences, a significant difference was observed in social responsibility, with girls reporting higher social responsibility. **Conclusions:** The results suggest a consistent positive link between personal and social responsibility across team and individual sports, with girls showing higher social responsibility. These findings highlight the relevance of considering gender when examining responsibility-related outcomes in youth sport.

Keywords: Personal Responsibility; Social Responsibility; Basketball; Swimming; Gender differences

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24282 | Traditional Teaching vs. Flipped Classroom: Effects on Student Motivation and Engagement in Theoretical Physical Education Classes

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Background & Aim: Low academic motivation is a challenge in professional secondary education, particularly in theoretical Physical Education (PE), requiring strategies to increase student's engagement. Student-centered approaches, like flipped classroom may promote motivation by fostering autonomy and active involvement. This study compared traditional teaching and a flipped classroom approach in a theoretical module of a professional secondary course, examining their effects on students' motivation and learning perceptions. **Methods:** Twelve male 12th-grade students from a Sports Management program participated in the study. A theoretical handball module was taught using a traditional teacher-centred approach supported by instructional resources, after which students completed a translated and adapted version of the Academic Motivation Scale (AMS) (Rufini, et al., 2011). A theoretical swimming module was then delivered using a flipped classroom approach, followed by re-administration of the AMS. Qualitative data were collected through semi-structured interviews and a reflective journal to gain deeper insight into students' perceptions and to explain the patterns observed in the quantitative findings. Quantitative data were analysed descriptively, and qualitative findings were examined thematically. **Results:** Motivation scores increased after the flipped classroom module, but amotivation also increased, indicating a mixed motivational response. Interviews showed that most students preferred traditional instruction due to unfamiliarity with the flipped model, reluctance to invest effort, and difficult adaptation to greater responsibility. Nevertheless, students recognised the potential value of flipped learning when expectations and guidance were clearly defined. **Conclusions:** Flipped learning may support motivation in theoretical PE contexts but can also generate resistance in students with limited experience of autonomous learning. Flipped approaches should be gradual and supported with clear structure, explicit expectations, and accountability.

Keywords: Traditional Classes; Flipped Classroom; Motivation; Professional Education; Physical Education.

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24553 | When losing also teaches: defeat and motivation in child athletes

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Background & Aims: Defeat is inevitable in youth sport and may shape how young athletes feel about and engage with their sport. Grounded in self-determination theory and achievement goal theory, this study examined how experiencing defeat relates to motivation in youth futsal. **Methods**: Eight futsal players (10-12 years) and two coaches from district competition participated in a mixed-method design. Data included one focus group with athletes, questionnaires (Sport Motivation Scale-II [2]; Youth Sport Environment Questionnaire [1]), and semi-structured coach interviews. Quantitative data were described with descriptive statistics and qualitative data were analysed through thematic analysis. **Results**: Athletes described sadness and frustration immediately after losses, but these emotions were often regulated through peer support and coach feedback. Motivation profiles showed higher intrinsic motivation (M=5.38) and integrated regulation (M=5.04), with lower amotivation (M=2.92). Although task cohesion scores were modest, qualitative accounts highlighted strong bonds and a sense of belonging that helped sustain engagement during a season marked by repeated defeats. **Conclusions**: Defeat did not necessarily undermine motivation when the environment emphasised learning, effort and personal growth. An applied team-based intervention with three activities was proposed to strengthen collaboration, emotional regulation and collective commitment.

Keywords: youth sport; defeat; motivation; self-determination; futsal

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