ARTIGO DE REVISÃO BIBLIOGRÁFICA

MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

EFEITO ANTIMICROBIANO DO GRAFENO NOS MATERIAIS POLIMÉRICOS EM MEDICINA DENTÁRIA - UMA REVISÃO / ANTIMICROBIAL EFFECT OF GRAPHEN ON POLIMERIC MATERIAL IN DENTISTRY - A REVIEW

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EFEITO ANTIMICROBIANO DO GRAFENO NOS MATERIAIS POLIMÉRICOS EM MEDICINA DENTÁRIA - UMA REVISÃO / ANTIMICROBIAL EFFECT OF GRAPHEN ON POLIMERIC MATERIAL IN DENTISTRY - A REVIEW

Artigo de Revisão Bibliográfica

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Abstract

Research on functionalized polymeric biomaterials with different types of graphene is advancing into promising scaffolds for use in clinical procedures in dental medicine. This hybrid material embodies physical, chemical, mechanical, and biological properties. The resistance, hardness, and surface roughness give the graphene polymer a greater clinical longevity. This material also exhibits adequate biocompatibility, low cytotoxicity to living tissues, and high antimicrobial potential against all types of microorganisms that comprise the oral ecosystem.

Therefore, the objective of this review is to explore, in the mechanical and biological context, the advances of graphene-functionalized polymers aiming to combine antimicrobial efficacy with the functional properties of this biomaterial.

For this review, three databases, Clarivate, PubMed and Scopus, were searched using specific keywords and inclusion and exclusion criteria.

In vitro studies confirm the enhanced antimicrobial activity of graphene when coated on the surface of the polymer in suspension form. The gap in current studies highlights the need for more targeted investigation into the integration of graphene materials in polymer-based dental formulations for broader antimicrobial protection.

Keywords

Antimicrobial effect, Graphene, Polymeric materials, dental medicine, nanomaterials.

Resumo

Os estudos sobre biomateriais poliméricos funcionalizados com diferentes tipos de grafeno estão a contribuir para a criação de dispositivos promissores para utilização em procedimentos médico dentários. Este material híbrido possui distintas propriedades físicas, químicas, mecânicas e biológicas. Particularmente, a resistência, a dureza e a rugosidade da superfície conferem ao polímero de grafeno uma maior longevidade clínica. Este material apresenta ainda uma adequada biocompatibilidade, baixa citotoxicidade para os tecidos vivos e elevado potencial antimicrobiano contra todo o tipo de microrganismos que compõem o microbioma oral.

Neste sentido, o objetivo desta revisão é explorar, no contexto mecânico e biológico, os avanços dos polímeros funcionalizados com grafeno com o intuito de aliar a eficácia antimicrobiana às propriedades funcionais deste biomaterial.

Para esta revisão, foram pesquisadas três bases de dados, Clarivate, PubMed e Scopus, utilizando palavras-chave específicas e critérios de inclusão e exclusão.

Estudos in vitro confirmam o aumento da atividade antimicrobiana do grafeno quando revestido na superfície do polímero em forma de suspensão. Atualmente, a evidência científica evidencia a necessidade de desenvolvimento de estudos mais específicos sobre a integração de materiais de grafeno em formulações dentárias à base de polímeros para uma proteção antimicrobiana mais ampla.

Palavras-chave

Efeito antimicrobiano, grafeno, materiais poliméricos, medicina dentária, nanomateriais.

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List of Abbreviations

ATCC 10231	American Type Culture Collection, a specific strain of Candida albicans.
CFU	Colony-Forming unit.
Ct	Control test.
EDTA	Ethylenediaminetetraacetic Acid.
ERG11	Ergosterol biosynthesis gene 11.
EtBr	Ethidium Bromide.
f-GO	functionalized Graphene Oxide.
Flu	Fluconazole.
Gc	Group control.
GO	Graphene Oxide.
GNPs	Graphene Nanoplatelets.
GNRs	Graphene Nanoribbons.
GOns	Graphene Oxide nanosheets.
GQDs	Graphene Quantum Dots.
Gram-	Gram-negative bacterium.
iRBC	infected Red Blood Cells.
MCM	Malaria Culture Medium.
MNTC	Maximum Non-Toxic Concentration.
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
PBS	Phosphate-Buffered Saline.
PEG	Polyethylene Glycol.
PLGA	Poly(Lactic-co-Glycolic Acid).
PMMA	Polymethyl Methacrylate.
PVA	Polyvinyl Alcohol.
Ra	Average roughness.
RBC	Red Blood Cells.
rGO	reduced Graphene Oxide.
ROS	Reactive Oxygen Species.
RPMI	Roswell Park Memorial Institute medium.
SEM	Scanning Electron Microscopy.
SMRGO	Sulfonated Magnetic nanoparticles functionalized with rGO.
SW480	Human-derived colorectal cell line.
w/v	Weight/volume.
XTT	Tetrazolium salt.

1. Introduction

Carbon is a special element that can form various structures due to its ability to create strong covalent bonds with itself. Graphene is one of the many allotropes (different nanostructural forms) of carbon. It consists of a nanosheet of a single layer of carbon atoms arranged two-dimensionally in a hexagonal honey-cube structure [1] (Figure 1).

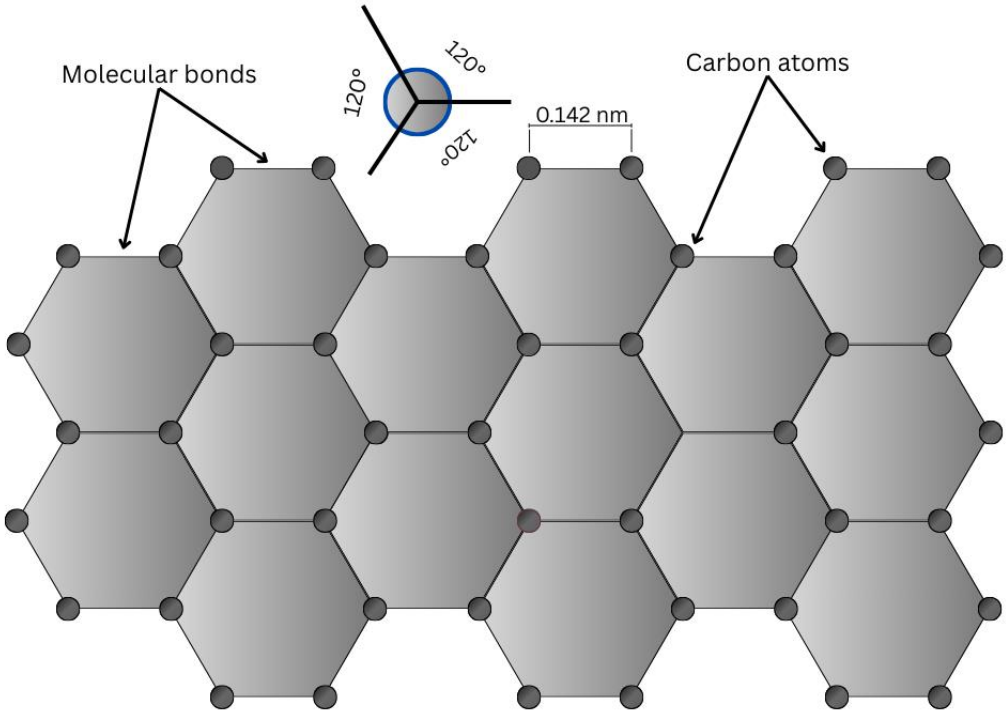

Figure 1: Depiction of a typical sheet of graphene. Adapted from Roberts *et al*. (2010). No authorized.

Graphene is an exceptionally strong material, while remaining lightweight, making it ideal for applications in electronics and nanotechnology. It also possesses outstanding conductivity and exhibits excellent biocompatibility and intrinsic antimicrobial properties. [2 - 6]. This exceptional material was first isolated in 2004 by Novoselov *et al*. [7], and it includes various derivative forms. Among the most prominent for material functionalization are graphene oxide (GO), reduced graphene oxide (rGO), and graphene nanoplatelets (GNPs).

GO and rGO are particularly valued due to their higher hydrophilicity and stability in biological environments compared to pure graphene [8 - 14], and have been explored for various biomedical applications, including drug delivery, tissue engineering, and biosensors [12, 13].

GNPs, on the other hand, represent a structurally distinct form of graphene-based materials. They are composed of stacks of few-layer graphene sheets with relatively large lateral dimensions and a thickness ranging from a few layers (~0.34 nm) to several tens of nanometres [15]. While GNPs have a lower content of oxygen-containing groups compared to GO or rGO, which limits their ability to generate reactive oxygen species (ROS), they retain excellent mechanical strength, thermal stability, and electrical conductivity. These properties make them attractive for applications in composite materials, electronic devices, and structural reinforcement. Their large surface area and layered morphology also contribute to their moderate antimicrobial activity, mainly through physical disruption of microbial membranes. However, because of their larger size and relatively lower hydrophilicity, direct microbial contact can be reduced. To enhance their antimicrobial effects, GNPs are often functionalized or combined in hybrid nanocomposites [16, 17].

Building upon these properties, GO and rGO stand out for their functional versatility and distinct antimicrobial behaviours. Both forms exhibit antimicrobial activity, yet the mechanisms and effectiveness differ due to variations in surface chemistry, oxidation level, and interaction with microbial membranes.

Notably, GO demonstrates intrinsic antimicrobial activity through multiple mechanisms. It can exert antimicrobial effects including physical disruption of microbial membranes (e.g., piercing or deformation by nanostructures), chemical damage via the generation of ROS, physical isolation of pathogens through nanowrapping, interference with genetic or metabolic processes, and modulation of biological pathways such as gene expression, enzyme activity, or quorum sensing. These properties also enable GO to synergize with conventional antimicrobial agents by loading or conjugating drugs, enabling targeted and controlled release. This makes GO effective against bacteria and fungi [18, 19], parasites [20 - 27] and viruses [28 - 30] when appropriately functionalized.

In contrast, rGO, having fewer oxygen groups and a more restored graphene lattice, tends to exhibit higher conductivity but lower hydrophilicity, which may reduce its

antimicrobial action in some settings. However, rGO still demonstrates significant bactericidal effects, especially through physical disruption of microbial membranes [31].

These distinctions make the choice between GO, rGO, and GNPs critical depending on the intended application in dental materials (Figure 2, Figure 3), where antimicrobial performance, cytotoxicity, and compatibility with the material matrix must all be considered. GO, due to its abundance of oxygen-containing functional groups, is more easily functionalized and can be used in lower concentrations enhancing biocompatibility while still delivering strong antimicrobial effects when incorporated into polymeric systems. In contrast, rGO and GNPs may require higher concentrations to achieve similar functional results, which can increase the risk of cytotoxicity and limit their biomedical applicability unless functionalized or incorporated into hybrids.

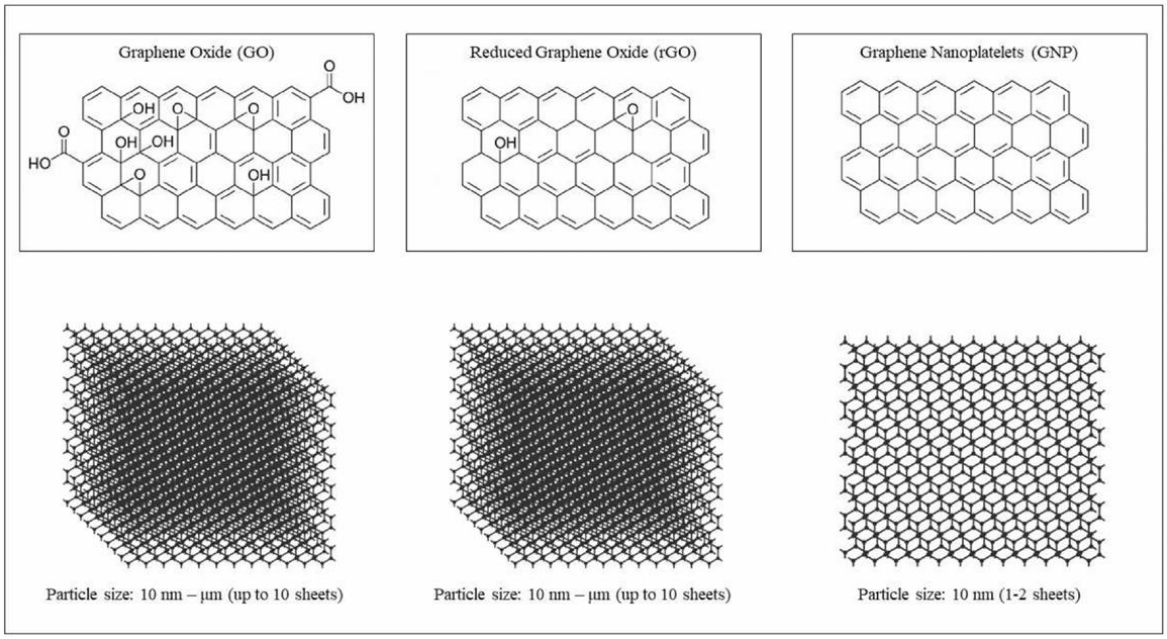

Figure 2 - Differences between graphene compounds. Adapted from Sahm BD *et al.* (2023). No authorized.

Graphene composites	Presence of oxygen(O)	Solubility	Surface area	Electrical conductivity
GO	●●●●●	●●●●●	●●●●●	●●●●●
rGO	●●●○○	●●●○○	●●●○○	●●●●●
GNP	○○○○○	○○○○○	●●●●●	●●●●●

Figure 3 - Inherent characteristics of graphene oxide (GO), reduced graphene oxide (rGO) and graphene nanoplatelets (GNP). Adapted from Sahm BD *et al.* (2023). No authorized.

Moreover, the selection of the graphene type often depends on the nature of the polymer used. Hydrophilic polymers such as polyvinyl alcohol (PVA), chitosan, or polyethylene glycol (PEG) tend to favour interactions with GO due to its surface polarity, while more hydrophobic polymers may be better suited for interaction with rGO or GNPs. Depending on whether surface coating or bulk incorporation is intended, the ease of functionalization and dispersion within the matrix will vary. Thus, the physicochemical compatibility between graphene type and polymer plays a key role in optimizing both the functional properties and the safety profile of the final material.

Graphene derivatives encompass a diverse family of materials, including GO, rGO, GNPs, graphene quantum dots (GQDs), graphene nanoribbons (GNRs), and graphene hybrids or nanocomposites. Among these, graphene hybrids incorporated into polymer matrices tend to show the highest antimicrobial efficacy, primarily due to their increased specific surface area and improved biointerface interactions that facilitate stronger contact with microbial cells, enhancing bactericidal effects. Other forms like GQDs [32, 33] and GNRs [34, 35] may also possess antimicrobial potential, but they typically require functionalization or hybridization to be effective within polymer systems.

In summary, the antimicrobial effectiveness of graphene-based materials in polymers strongly depends on their surface chemistry, morphology, and ability to form efficient biointerfaces, with hybrid graphene materials generally outperforming individual derivatives.

Although graphene and its nanoderivatives demonstrate promising antimicrobial potential in dental polymeric biomaterials, their biological interactions during clinical procedures are critical because, according to the results evaluated in studies, they may pose occupational, and handling risks due to their cytotoxicity. Factors such as graphene nanoflakes size and morphology, concentration, exposure time, control of oxidative stress, electrical conductivity, thermo-photo irradiation, exposure area size, types of graphene and types of functionalization, as well as biocompatibility with living tissues, significantly influence the cytotoxicity of the polymeric biomaterial [36 - 38]. These concerns require careful evaluation of the ideal dose relationship for long-term biocompatibility, cytotoxicity, and the potential for tissue regeneration.

Thus, understanding the unique antimicrobial behaviours of types of graphene and its derivatives lays the foundation for evaluating their potential roles in advancing dental materials and improving clinical outcomes.

While this monograph primarily focuses on the antimicrobial effects of GO, rGO, and GNPs when incorporated into polymeric materials in dentistry, it is important to note that many existing studies on their activity against fungi, parasites, and viruses have been conducted in non-polymeric environments, such as aqueous dispersions or drug-loaded delivery systems. These findings are included not to deviate from the central theme, but to underscore the broad-spectrum antimicrobial potential and versatility of these graphene derivatives, which may support their future integration into dental polymer systems. Understanding their mechanisms across different biological contexts provides a valuable foundation for tailoring their properties to dental applications, where research is still in early stages.

1.1. Objectives

The main objective of this study is to identify and evaluate the antimicrobial properties of different forms of graphene when functionalized into polymeric materials commonly used in dentistry, with particular emphasis on GO, which has currently shown the most consistent efficiency and efficacy. This includes its potential to partially or completely eliminate bacteria, fungi, viruses, and parasites.

2. Methods and Materials

For the development of this review, the bibliographic research will be based on a research question formulated according to the PICO acronym (Table 1).

Table 1: Description of the research question according to the PICO acronym.

Population	*Polymeric Nanomaterials*
Intervention	*Incorporation of Graphene*
Comparison	*Polymeric nanomaterials without graphene or with other incorporated materials*
Outcome	*Antimicrobial effect of graphene in polymeric nanomaterials.*

The bibliographic research will be conducted in the Clarivate, PubMed, and Scopus databases.

The inclusion of studies will be based on the following criteria: year of publication (1990–2025), reviews, publications in English only, and publications with open access only.

The exclusion criteria will include systematic reviews, meta-analyses, books and documents, randomized controlled trials, publications with restricted access, and those that are not related to the established objectives of this literature review.

2.1. Search method:

Boolean operators used is “AND”.

2.2. Inclusion and Exclusion Criteria

Query chosen ‘’graphene and antimicrobial and effect’’: 2533 articles.

- Range year filter (1990 – 2025) → articles remain: 2523
- Free full text filter → articles remain 561
- English language filter → articles remain 554
- Review filter→ articles remain 69
- Articles selected for study: 20

3. Development

Graphene derivatives have demonstrated antimicrobial activity against a wide range of pathogens, including bacteria, fungi, viruses, and even parasites. These effects are influenced by the material's physicochemical properties such as surface chemistry, size, and oxidation state. In this section, selected experimental studies are presented to illustrate the antimicrobial potential of some of the most widely studied graphene derivatives. The focus on GO, rGO and GNP reflects their prevalence in the literature and practical relevance, while the decision to highlight only representative experiments ensures clarity and avoids unnecessary repetition.

3.1. Graphene-Based Materials as Antibacterial Agents

Dental caries is a complex, multifactorial disease largely driven by acid-producing bacteria within oral biofilms, which lower the local pH and lead to the demineralization of dental hard tissues such as enamel and dentin. This acidic environment gradually weakens the tooth structure and can result in decay. The potential of GO and rGO against bacteria which are the primary contributors to oral diseases, with *Streptococcus mutans* being one of the most prevalent species, is demonstrated through different experiments. Some of these are described below according to their effect.

3.1.1. Remineralization and Protective Coatings

Recent research in-vitro has highlighted the potential of functionalized graphene oxide (F-GO) as a novel protective agent that goes beyond antimicrobial effects. In the described experiment [39], F-GO acts as a mineral shield by forming a stable, polymer-functionalized coating on dentin surfaces. This coating serves as a physical barrier that continuously interacts with minerals in the oral environment, effectively substituting lost mineral content caused by acid attacks and promoting ongoing remineralization. By wrapping the dentin and limiting acid penetration, f-GO helps maintain mineral homeostasis, thus reducing demineralization and preserving tooth integrity. This approach offers a promising strategy for preventing caries progression through continuous dentin remineralization and acid protection. In this experiment [39], five f-GO nanocomposites were synthesized: GO–silver (Ag), GO–Ag–calcium fluoride (CaF_2), GO–CaF_2, GO–zinc (Zn), and GO–tricalcium phosphate ($Ca_3(PO_4)_2$). These nanocomposites were applied directly onto hydroxyapatite plates and human dentin

slices as surface treatments. The study evaluated their antibacterial activity, particularly against *S. mutans*, their ability to form a physical barrier by sealing dentin tubule orifices, their impact on tooth colour, and their cytotoxicity in cell culture media. The antibacterial efficacy of each nanocomposite was tested against *Streptococcus mutans*, a key pathogen implicated in dental caries. Among the tested materials, GO–Ag and GO–Ag–CaF_2 demonstrated high antibacterial activity, particularly the GO-Ag-CaF_2, where the colony-forming unit (CFU) was reduced by 98%, highlighting their potent antimicrobial properties even superior than Saforide (silver diamine fluoride) [40], however, they provided a slight yellowish coloration. Other composites such as GO–CaF_2, GO–Zn, and GO–$Ca_3(PO_4)_2$ exhibited moderate antibacterial activity, lesser than the GO alone but no visible colour change. The inhibitory activity of f-GO at different concentrations is illustrated in Figure 4.

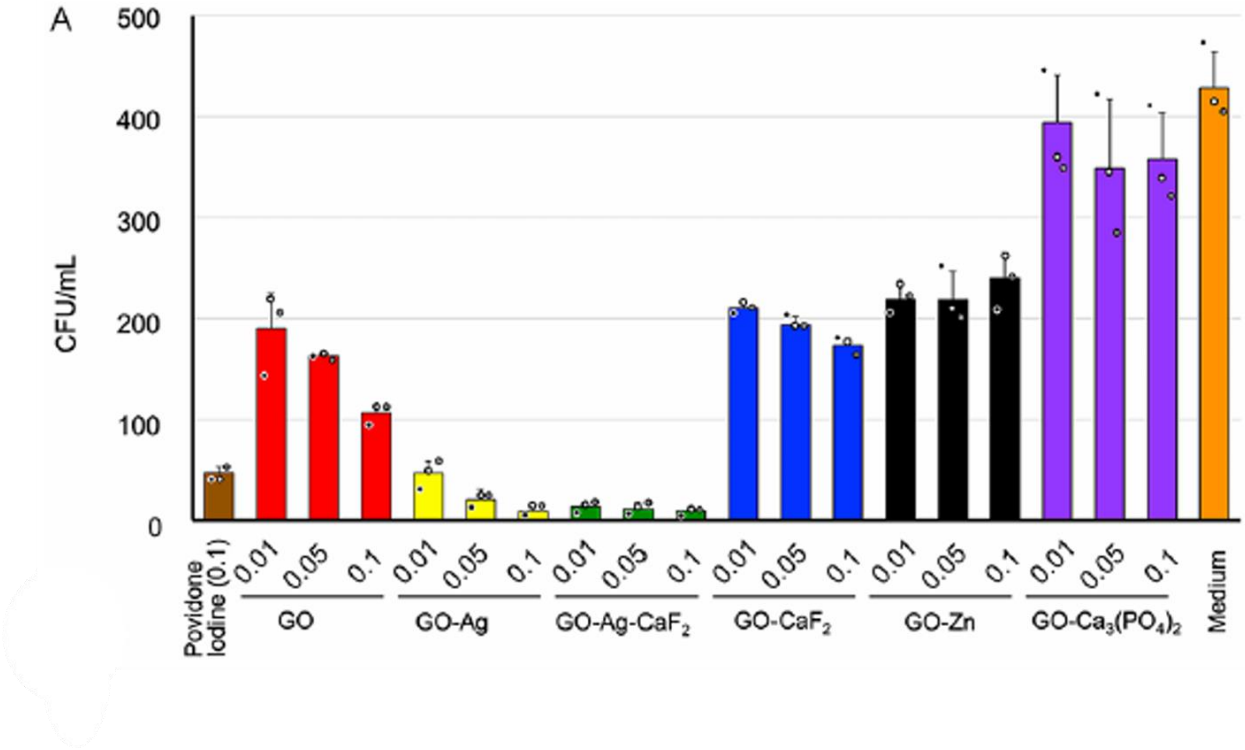

Figure 4: Concentration-dependent inhibition of *Streptococcus mutans* growth by f-GO. Adapted from Nizami *et al.* (2020), no authorized.

To evaluate the protective role of functionalized graphene oxide (f-GO) composites against dentin demineralization, dentin slices were coated with various f-GO formulations and subjected to acid exposure. Initially, the samples were immersed in

Ethylenediaminetetraacetic acid buffer (EDTA) to simulate chelating and erosive effects similar to those occurring during clinical dental treatments. Scanning electron microscopy (SEM) revealed that the f-GO coatings formed a thin protective layer over the dentin surface, with partial to complete sealing of the dentinal tubules. Notably, dentin coated with GO–Ag, GO–Ag–CaF_2, GO–CaF_2, GO–$Ca_3(PO_4)_2$, and GO–Zn showed tubule occlusion after EDTA exposure, whereas dentin treated with Saforide, despite its clinical use, still presented many open dentinal tubules. To further assess acid resistance, the same specimens were immersed in citrate buffer (pH 6.0), mimicking the mildly acidic conditions found in early caries development. SEM analysis showed that GO–Ag, GO–Ag–CaF_2, GO–CaF_2, and GO–$Ca_3(PO_4)_2$ maintained a stable, continuous coating that effectively occluded dentinal tubules, demonstrating strong resistance to acid-induced erosion. In contrast, dentin coated with GO–Zn displayed numerous reopened tubules and degraded intertubular areas, indicating inferior stability under acidic conditions. Additionally, the Saforide-treated dentin showed a rough, irregular surface, likely due to aggregation of silver diamine fluoride particles upon acid exposure. These findings suggest that while Saforide provides some protective effect, f-GO composites — especially those functionalized with silver, calcium fluoride, or phosphate — form more uniform and acid-resistant barriers. Their ability to seal dentinal tubules effectively supports their potential for reducing hypersensitivity and preventing demineralization in cariogenic conditions.

Cytotoxicity was evaluated on human epithelial cells across varying concentrations. At low concentrations, all functionalized GO (f-GO) composites exhibited minimal cytotoxic effects, remaining within acceptable biocompatibility thresholds. However, at higher concentrations (notably 0.1% w/v), slight cytotoxicity was observed in GO–Ag and GO–Ag–CaF_2 formulations. These findings suggest that while f-GO materials are generally biocompatible, especially at clinically relevant concentrations, concentration-dependent effects must be considered to ensure epithelial tissue safety (Figure 5).

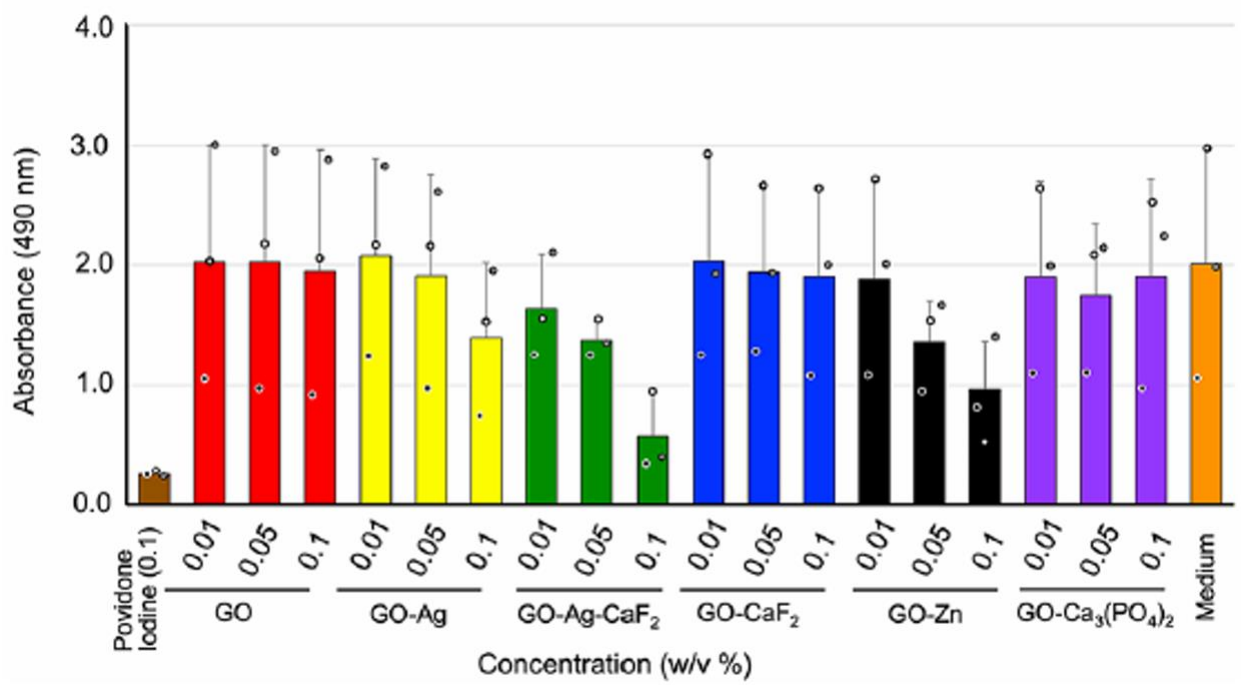

Figure 5: Concentration-Dependent Cytotoxicity of Various f-GO Composites on Human Epithelial Cells. Adapted from Nizami *et al.* (2020). No authorized.

In summary, specific functionalized graphene oxide (f-GO) composites—particularly GO–Ag, GO–Ag–CaF_2, and GO–CaF_2—offer promising antibacterial action, acid protection, and dentin tubule sealing. While GO–CaF_2 maintains good aesthetic compatibility, GO–Ag and GO–Ag–CaF_2 may cause slight yellowish discoloration, which could limit use in visible restorations. These composites also show low cytotoxicity except at high concentrations (0.1 w/v%). Further in vivo studies are needed to confirm their safety and effectiveness for preventive and restorative dentistry.

3.1.2. Improvement of the antibacterial performance of polymethyl methacrylate (PMMA) resin against *Streptococcus mutans (S. mutans)*

The application of composite materials with other components are necessary to meet the specific requirements of dental applications. Therefore, researchers have exploited the antimicrobial properties, ultra-high surface area, the presence of functional groups, and the ease of modification of graphene in combination with other biomaterials and biomolecules to assist the development of new dental applications.

Microbial biofilm formation on the surface of dental prostheses can lead to irritation of the underlying oral tissues, resulting in erythema, discomfort, and inflammation, a condition clinically recognized as prosthetic stomatitis. Salgado *et al.* (2022) explored the potential of enhancing the antibacterial performance of PMMA, a material widely used in denture fabrication, by incorporating graphene-based additives, specifically graphene nanoplatelets (GNPs), while aiming to preserve the material's structural and functional properties. GNPs were incorporated into PMMA resin at varying concentrations (0.01%, 0.1%, 0.25%, and 0.5% wt). The resulting 3D-printed composite samples were incubated with *S. mutans* cultures to evaluate antibacterial activity after 24 and 48 hours. A significant reduction in *S. mutans* colony formation was observed after 48 hours for samples containing 0.01%, 0.1% and 0.25% GNPs. Notably, a significant decrease was detected at 24 hours only in the specimens with the highest concentration (0.5% wt) (Figure 6). These findings suggest that lower GNP concentrations exert a bactericidal effect over extended exposure, while higher concentrations act more rapidly. Although further cytotoxicological studies will be required to fully evaluate the biocompatibility of these graphene-enhanced materials, particularly at higher concentrations.

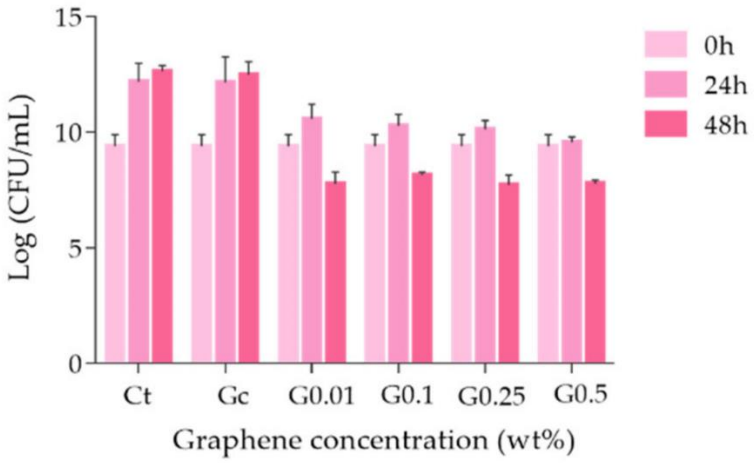

Figure 6: Growth inhibition of *S. mutans* DSM20523 in the presence of the graphene–PMMA resin specimens after 24 and 48 h of incubation. The values are expressed as the means of three independent experiments; error bars indicate the standard deviation. Adapted from Salgado *et al*. (2022). No authorized.

The other experiment done by Salgado et al. involved 3D printing PMMA resins incorporated with different concentrations of GNPs (0.01%, 0.1%, 0.25% and 0.5% wt) to evaluate their surface characteristics and how this influence microbial adhesion. The incorporation of GNPs increased the surface roughness of the printed material, and this roughness increases with the increase in the graphene concentration as observed through SEM (Figure 7). Eventually, it is well established that rough surfaces exhibit increased retention due to their physical properties. This phenomenon also facilitates improved adhesion of microorganisms such as bacteria [42, 43].

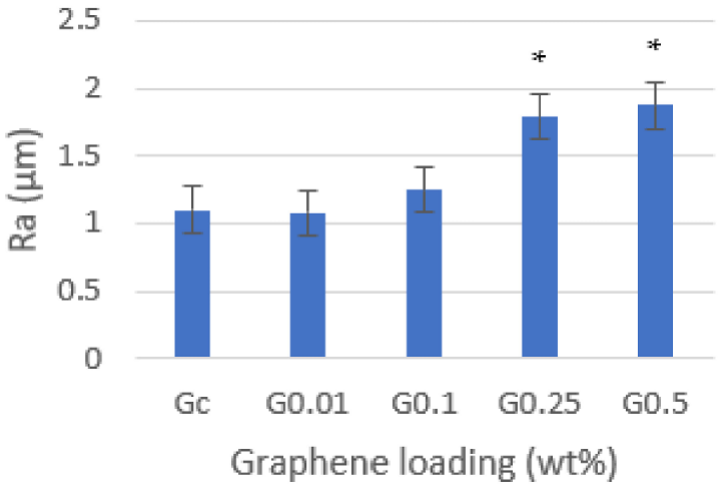

Figure 7: Effect of graphene on PMMA resin's surface roughness. Mean results (± standard deviation) are presented as Ra (µm) measured using contact profilometer. Asterisks (*) indicate statistical significance compared to Gc ($p < 0.05$). (Adapted from Salgado *et al*. (2022). No authorized.

However, despite the rougher surface, the formation of microbial biofilm, particularly by *S. mutans*, was significantly reduced. This suggests that the antimicrobial properties conferred by GNPs outweighed the increased surface roughness, effectively inhibiting bacterial adhesion and biofilm development. This review and experimental study support the viability of graphene-enhanced PMMA as a next-generation dental material, combining antimicrobial activity, and 3D-printing adaptability. The material shows promise in preventing oral infections associated with dental prostheses, representing a step forward in the development of advanced functional biomaterials in dentistry.

3.1.3. Improvement of the antimicrobial performance of acrylic resins against oral pathogens:

To evaluate the antimicrobial efficacy of rGO as a nanomaterial additive in dental materials, Sahm *et al.* (2024) conducted a detailed study incorporating rGO into self-cured and heat-cured acrylic resins. The aim was to determine whether rGO could reduce microbial activity and biofilm formation on the surface of the polymer, particularly targeting common oral pathogens.

Acrylic resin specimens were prepared with four different rGO concentrations: 0% (control), 0.5%, 1%, and 3%. These were tested against four microorganisms of clinical relevance in dentistry: *Streptococcus mutans (S. mutans)*, *Staphylococcus aureus (St. aureus)*, *Candida albicans (C. albicans)*, and *Pseudomonas aeruginosa (P. aeruginosa)*. The study employed three complementary methods to assess antimicrobial activity: CFU counting to determine viable microorganisms, tetrazolium salt (XTT) assays to measure metabolic activity, and SEM to visualize surface colonization.

The most notable finding was the strong antimicrobial effect of rGO, especially at 0.5% concentration, against *S. mutans*, a primary contributor to dental caries. Both CFU counts and metabolic activity for *S. mutans* showed significant reduction, particularly in self-cured resins. This indicates that rGO disrupts bacterial viability and energy metabolism at relatively low concentrations.

St. aureus and *C. albicans* also showed some reduction in metabolic activity and growth, although results were less consistent. Conversely, *P. aeruginosa* showed no significant decrease in either metabolic activity or CFU counts (Table 2). In fact, CFU values slightly increased at higher rGO concentrations, suggesting that rGO's mechanism—believed to involve oxidative stress and membrane disruption—was ineffective against this highly resistant Gram-negative bacterium (Gram -) [44].

Table 2 - Summary of Antimicrobial Effects of rGO-Incorporated Acrylic Resins Against Oral Pathogens Based on CFU and Metabolic Activity (XTT) Assays.

Microbe	CFU Reduction with rGO	Metabolic Activity	Clinical Relevance
Streptococcus mutans	☑ Significant	☑ Reduced	rGO is effective in preventing caries.
Staphylococcus aureus	⊗ No reduction	◉ Slightly reduced	Possible partial inhibition.
Candida albicans	⊗ No reduction	◉ Slightly reduced	Uncertain; may vary with resin type.
Pseudomonas aeruginosa	⊗ No effect or worse	⊗ No reduction	rGO not suitable against this strain.

These findings suggest that rGO has a selective antimicrobial effect, most effective against *S. mutans*, which supports its potential application in the development of caries-preventive dental materials. However, its limited efficacy against *P. aeruginosa* underscores the need for further optimization or combination with other agents to expand its antimicrobial spectrum.

3.2. Graphene-Based Materials as Antifungal Agents

3.2.1. Improvement of the antifungal performance of polymethyl methacrylate (PMMA) resin against *C. albicans*

Salgado *et al*. (2022) also extended their investigation to evaluate the antifungal performance of GNP-doped PMMA against *C. albicans*, a key fungal species involved in denture-associated infections such as prosthetic stomatitis. The 3D-printed PMMA specimens incorporating graphene nanoplatelets, at concentrations of 0.01%, 0.1%, 0.25% and 0.5% by weight, were incubated with *C. albicans* for 24 and 48 hours. Across all tested concentrations and both time points, a significant reduction in fungal biofilm formation was observed when compared to the control group (Figure 8).

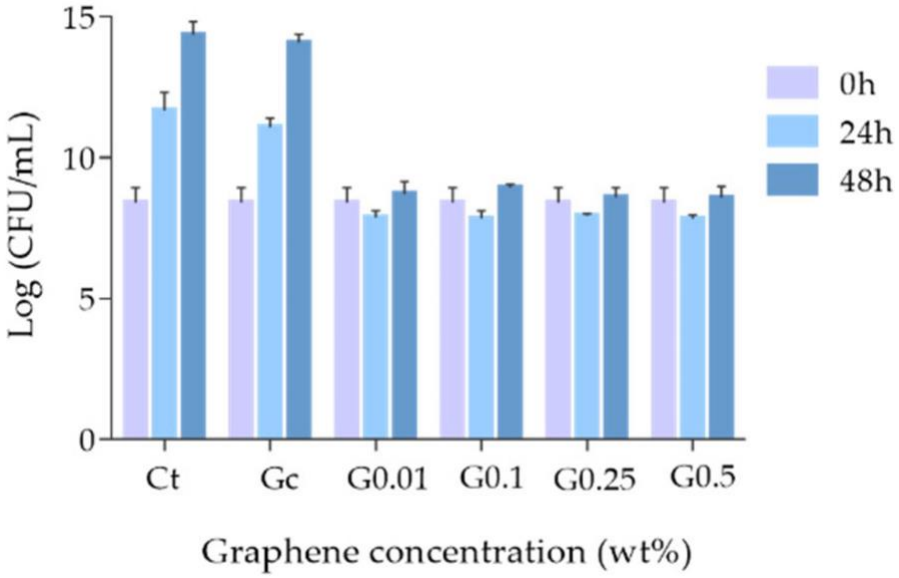

Figure 8: Growth inhibition of *C. albicans* ATCC 11225 in the presence of the graphene–PMMA resin specimens after 24 and 48 h of incubation. The values are expressed as the means of three independent experiments; error bars indicate the standard deviation. Adapted from Salgado *et al.,* (2022). No authorized.

Interestingly, all tested concentrations of graphene nanoplatelets demonstrated comparable antifungal efficacy against *C. albicans*, with no statistically significant differences observed between them. This indicates that increasing the graphene content did not enhance the inhibitory effect. Therefore, even low concentrations of graphene appear to be sufficient for effective antifungal activity, which means the amount of graphene can be minimized potentially, reducing cytotoxic effects while maintaining antimicrobial performance [41]. These findings demonstrate that the antifungal effect of GNPs is consistent and effective, regardless of the concentration used, highlighting their potential for reducing fungal colonization on prosthetic surfaces.

3.2.2. Improvement of the antifungal role of GO in combination with Fluconazole (Flu) against *C. albicans*

Azole antifungals, particularly Flu, are widely used to treat *Candida* infections. However, the rising incidence of resistance, especially in *C. albicans*, presents a significant therapeutic challenge. Resistance mechanisms commonly involve either overexpression or point mutations in the *ERG11* gene, which encodes lanosterol 14α-demethylase, the target enzyme of azoles. These changes lead to reduced Flu efficacy by either increasing

the amount of the enzyme (overexpression) or altering its structure (mutation), thereby decreasing drug binding.

To overcome this resistance, recent studies have investigated the synergistic use of GO with fluconazole [45, 46]. GO exhibits intrinsic antifungal properties, such as membrane disruption, oxidative stress induction and biofilm inhibition. When combined with Flu, GO enhances drug uptake and restores susceptibility in fluconazole-resistant strains. One of those studies [47] demonstrated that the *GO/Flu* nanocomposite effectively neutralized fluconazole-resistant *C. albicans* (ATCC 10231), showing a significant reduction in fungal viability compared to fluconazole alone. This confirms GO's potential to act as a sensitizing agent, offering a promising strategy to combat azole-resistant fungal infections.

In the same study, the *in vitro* release study of Flu , showed that the amount of Flu released from the GO/Flu nanocomposite over time was lower than that released from Flu alone in phosphate-buffered saline (PBS) at pH 7.4, a solution commonly used to simulate physiological conditions. This slower and more controlled release is beneficial, as it sustains the minimum effective concentration required for synergistic antifungal activity against *C. albicans* while minimizing potential toxicity. Moreover, MTT-based cytotoxicity assays, a standard method for assessing cell metabolic activity and viability, revealed that GO/Flu exhibited a dose-dependent manner on the viability of human-derived SW480 colorectal cell line, with significantly lower cytotoxicity compared to either GO or Flu alone. This finding is particularly relevant given that *GO* alone exhibited greater cytotoxicity than Flu. In adhesion assays, the *GO/Flu* compound significantly reduced the ability of *C. albicans* to adhere to SW480 cells, compared to treatment with either Flu or GO alone. This suggests that the nanocomposite effectively inhibits fungal attachment to host cells, potentially improving antifungal efficacy. Lastly, the DNA fragmentation assay revealed that *GO/Flu* significantly enhanced DNA degradation in *C. albicans* relative to *GO*, Flu, and untreated controls. This indicates a superior antifungal effect via induction of DNA damage, confirming the potent anti-*Candida* activity of the GO/Flu nanocomposite [47]. In conclusion, the findings of this study demonstrate that the GO/Flu nanocomposite exhibits effective antifungal activity against *Candida albicans* with minimal cytotoxicity toward the SW480 cells. These promising results support its potential as a therapeutic agent and warrant further *in vivo* studies to confirm its safety and efficacy.

While this study focused on the nanocomposite in its pure form, without embedding it into a polymeric matrix, the findings are highly relevant to dental materials research. GO-based nanocomposites like GO/Flu can potentially be incorporated into commonly used dental polymers, such as hydrogels, acrylic resins, or soft liners, to provide targeted antifungal properties in clinical settings. This approach could be particularly beneficial in preventing denture stomatitis or managing patients with recurrent oral candidiasis, offering a smart integration of nanotechnology into polymer-based dental applications.

3.3. Graphene-Based Materials as Antiviral Agents

3.3.1. Against *herpes simplex virus Type-1(HSV-1)*

Different antiviral mechanisms are described against herpes simplex virus Type-1(HSV-1) namely by inhibition of viral attachment, photothermal antiviral activity and through nanocomposite hydrogels.

3.3.1.1. Inhibition of Viral Attachment

particularly the GO, exhibits antiviral potential against HSV-1 by competitively binding to viral attachment sites. HSV-1 typically attaches to host cells via heparan sulphate receptors. GO mimics these receptors, thereby inhibiting viral entry without affecting cell-to-cell spread. Importantly the GO demonstrated high biocompatibility and showed no cytotoxic effects on host cells, positioning it as a promising candidate for therapeutic applications, such as antiviral coatings or topical treatments [28].

3.3.1.2. Photothermal Antiviral Activity

GO exhibits antiviral potential due to its surface functional groups, including hydroxyl (-OH), epoxy (-C–O–C), carboxyl (-COOH), and carbonyl (-C=O) groups. These groups interact with viral surface proteins through electrostatic interactions and hydrogen bonding, disrupting viral attachment and entry into host cells. While hydroxyl, epoxy, and carboxyl groups directly participate in binding, the carbonyl group enhances these interactions by increasing surface polarity and supporting hydrogen bond formation [48, 49]. Deokar et al. (2021) developed sulfonated magnetic nanoparticles functionalized with reduced graphene oxide (SMRGO) to capture and photothermally destroy HSV-1. Upon near-infrared irradiation, the SMRGO demonstrated significant antiviral activity, achieving

approximately 99.99% inhibition of HSV-1. This effect is attributed to the composite's ability to capture the virus and convert light into heat, effectively inactivating the virus [50].

GO itself can still achieve photothermal effects on its own. The role of nanoparticles, like sulfonated magnetic ones, is usually to improve light absorption efficiency, facilitate targeting, or aid in the heating process.

3.3.1.3. Nanocomposite Hydrogels

A recent study [51] developed a nanocomposite hydrogel with antiviral potential against *Herpes Simplex Virus type 1 (HSV-1)* with 86 % of effectiveness. The hydrogel - referred to as TA@ZnO/GO/CS nanocomposite hydrogel - was formulated using a combination of GO, chitosan nanoparticles, zinc oxide (ZnO) nanoparticles (NPs), and tannic acid (TA), each chosen for its specific contribution (Figure 9).

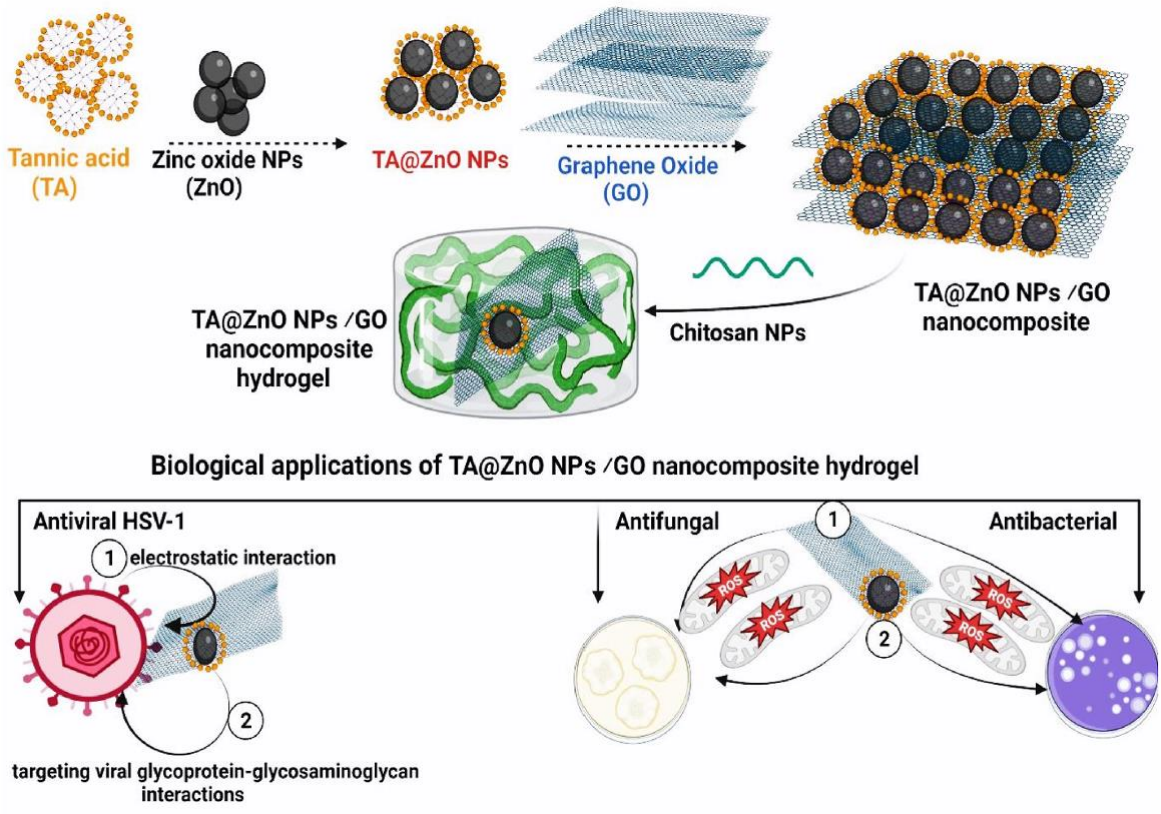

Figure 9: Graphical illustration of the formation of TA@ZnO/GO/CS nanocomposite hydrogel. Adapted from Salama *et al*. (2023). No authorized.

The antiviral activity of the hydrogel is attributed to the synergistic effect of its components. GO disrupts viral surface proteins through electrostatic and hydrogen bonding interactions, while ZnO nanoparticles generate ROS that damage viral

membranes and genetic material. TA enhances dispersion and contributes to antimicrobial effects, and chitosan provides a positively charged matrix that interacts with negatively charged viral envelopes. Together, these actions result in enhanced inhibition of viral entry and replication (Table 3).

Table 3: Functional Roles of Components in the Nanocomposite Hydrogel. Salama *et al*. (2023).

Abbreviation	Full Name	Function
GO	Graphene Oxide	Viral protein interaction, physical disruption.
ZnO NPs	Zinc Oxide Nanoparticles	ROS generation, membrane damage.
TA	Tannic Acid	Green crosslinker, antimicrobial, nanoparticle binder.
CS	Chitosan	Biocompatible hydrogel matrix, mild antimicrobial effect.

TA was used as a green crosslinker for the hydrogel structure, a binder for ZnO NPs to enhance their dispersion and functionality, and as an antimicrobial agent. GO sheets acted as stabilizers for ZnO NPs by preventing their agglomeration and served as a physical scaffold (supporting matrix) for the hydrogel. GO also engaged in non-covalent interactions (hydrogen bonding and π–π stacking) with TA@ZnO, contributing to the formation of a stable 3D structure, while providing its own intrinsic antimicrobial activity. ZnO NPs were incorporated primarily for their known antimicrobial properties. CS functioned as the main hydrogel matrix, offering biocompatibility, 3D structure, additional antimicrobial effects, and improved mechanical strength (Figure 9).

The resulting hydrogel was tested for cytotoxicity and antiviral activity using Vero cells. A series of dilutions were applied to the cells, and cell viability was evaluated using the MTT assay to determine the maximum non-toxic concentration (MNTC). The hydrogel showed pH-responsive swelling behaviour, expanding more in acidic environments, which could aid in targeted antiviral delivery. Results indicated that the formulation had significant antiviral activity by inhibiting viral entry and replication without harming host cells [51].

3.3.2. Against *coronavirus* disease 2019 (*COVID-19*)

Activity against *coronavirus disease 2019* was evaluated through molecular docking analyses that were conducted to investigate how GO sheets interact with three key biological structures: the SARS-CoV-2 spike protein (in both its open [6VYB] and closed [6VXX] conformations), the human ACE2 receptor (1R42), and the ACE2-spike protein complex (6M0J). GO demonstrated a strong affinity for the surface of all three structures. However, comparative analysis revealed that GO binds more strongly to the spike protein or ACE2 individually than to the ACE2-spike complex. The interaction prevents the virus from attaching to host cells, thus reducing the viral entry and replication. GO's surface properties, including its abundant oxygen-containing functional groups and nanoscale dimensions, allow it to interact strongly with viral particles, especially by blocking viral attachment sites and thus preventing entry into host cells. When GO is incorporated into polymeric matrices, these interactions can be maintained or even enhanced, depending on the polymer–GO interface [52]. This antiviral mechanism contributes significantly to the material's protective function.

Although the reviewed studies were not conducted directly on polymeric substrates or within a dental context, they provide valuable insight into the antiviral mechanisms of graphene-based materials. Mechanisms such as viral attachment inhibition, surface protein disruption, and photothermal inactivation reveal how graphene can be strategically used to combat viral infections. These principles are relevant for the future development of graphene-functionalized polymeric materials in dentistry, especially for antiviral coatings on dentures, orthodontic appliances, or liners, where preventing viral adhesion and survival is critical for oral health and infection control.

3.4. Graphene-Based Materials as Antiparasitic Agents

3.4.1. Against Malaria parasite *Plasmodium falciparum* (*P. falciparum)*

Plasmodium falciparum is the main parasite responsible for severe malaria and remains a global health challenge [53, 54]. While GO has demonstrated antimicrobial properties against various pathogens, including parasites, research specifically investigating GO's role against parasites within polymeric materials, especially in dental applications, is still limited.

The study by Kenry *et al.* (2017) suggests that GO nanosheets can interact with malaria parasites by adsorbing essential nutrients and physically trapping merozoites, thereby inhibiting parasite growth. These findings highlight GO's potential as an antiparasitic agent through mechanisms such as nutrient depletion and physical immobilization of the parasite.

In the context of dentistry, understanding these mechanisms is crucial for exploring how GO can be functionalized or incorporated into dental polymers to extend antimicrobial protection beyond bacteria and fungi to include parasitic organisms. Although the current evidence is mostly theoretical, this line of investigation offers promising perspectives for future polymeric dental materials with broad-spectrum antimicrobial effects.

Therefore, this section aims to explore the potential pathways through which GO could combat parasites when integrated into polymeric systems, laying the groundwork for future experimental studies focused on dental materials.

Given the established reactivity and biological interactions of GO and considering its minimal reported toxicity [55 - 58], a theoretical framework is proposed wherein GO nanosheets (GOns) could be introduced into the bloodstream to selectively interact with malaria-causing agents. This concept leverages GO's high surface area and its proven ability to bind biological molecules, which may allow it to capture or disrupt *Plasmodium* parasites during critical stages of their lifecycle. In addition to acting as a passive yet selective trap for merozoites, GO may contribute to nutrient depletion in the local microenvironment by adsorbing proteins and their derivatives, such as amino acids or peptides. Since *P. falciparum* parasites are highly dependent on exogenous protein sources, this localized deprivation could impair their growth and replication, thereby reducing parasitic load and inhibiting host cell invasion. Together, these mechanisms suggest the potential of GO-based materials in future malaria control strategies [Figure 10].

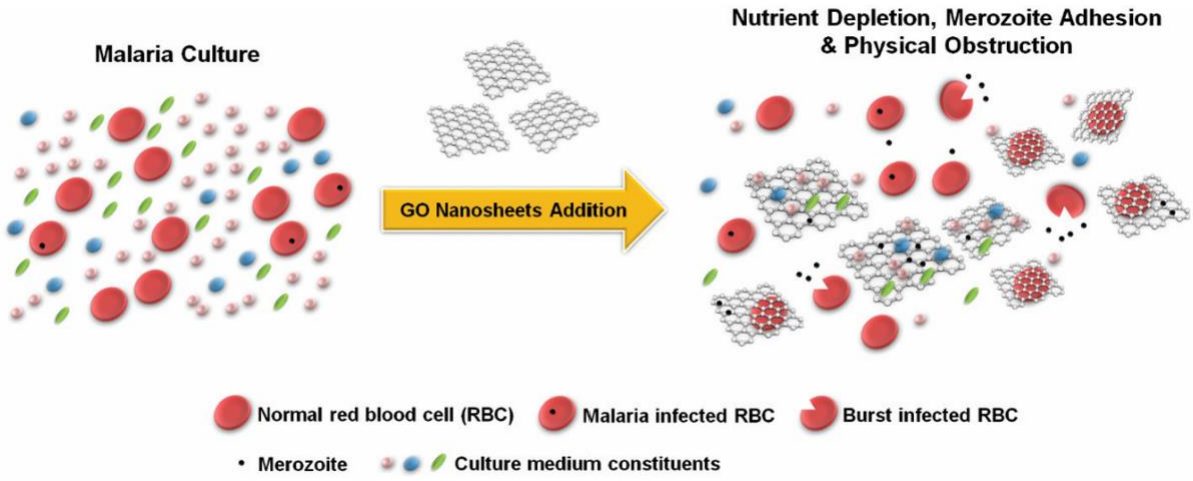

Figure 10: A graphical illustration depicting how GOns act as a physical barrier by binding to merozoites, leading to nutrient depletion through adsorption, thereby preventing their growth and multiplication. Adapted from Kenry *et al.* (2017), no authorized.

Before proceeding with any other experiments, it is crucial to assess the hemocompatibility of GOns. In the experiment, healthy red blood cells (RBCs) were exposed to GOns at a concentration of 6.25 µg/mL and the structural integrity of the RBCs was carefully monitored. The results demonstrated that GOns exhibited a high degree of hemocompatibility, as the RBCs maintained their structural integrity and did not undergo haemolysis. Notably, the sharp edges of the GOns did not cause any damage to the RBC membrane nor did they penetrate the cell, allowing GOns to smoothly conform to the shape of the RBCs. These observations highlighted the excellent hemocompatibility of GOns, suggesting that they can interact with blood cells without causing adverse effects, such as cell lysis or membrane disruption [27] [Figure 11].

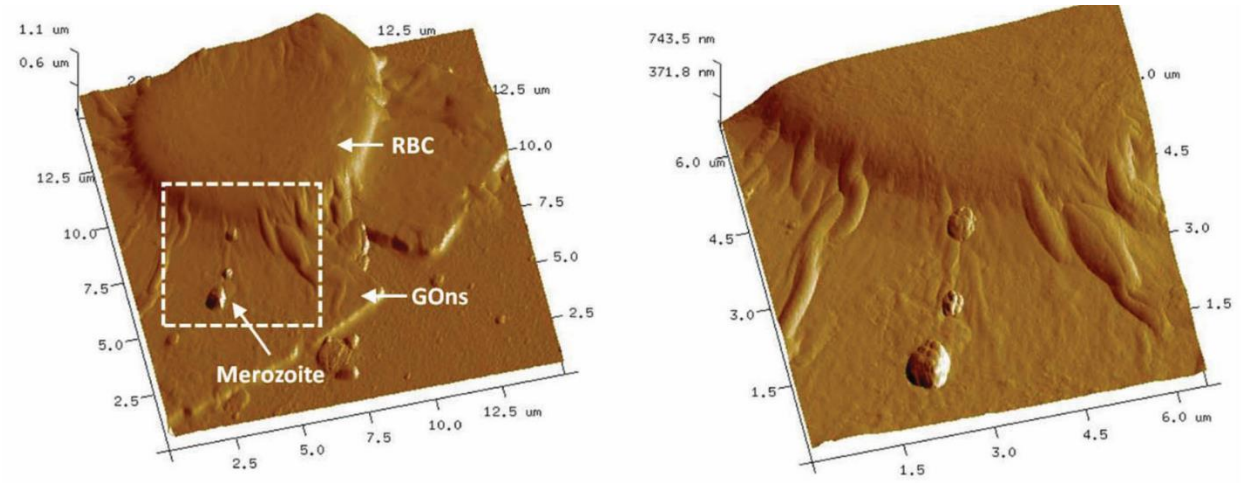

Figure 11: A graphical illustration depicting physical interactions between GOns, RBCs, and malaria parasites. Adapted from Kenry *et al*. (2017). No authorized.

Building on this, the next phase of the experiment aimed to evaluate the effects of GOns on the growth and behavior of Plasmodium parasites under in-vitro conditions. The Malaria Culture Medium (MCM) used for the experiment consisted of RPMI (Roswell Park Memorial Institute medium), a tissue culture medium providing essential nutrients necessary for Plasmodium growth. To support optimal parasite development, MCM was supplemented with several key components. Albumax II, a serum albumin substitute, was used to carry lipids in the blood and provide proteins and lipids essential for the parasite's metabolism. Hypoxanthine, a purine derivative, was critical for nucleic acid synthesis and energy metabolism in the parasites, enhancing their propagation by supplying building blocks for DNA and RNA synthesis. Gentamicin, an aminoglycoside antibiotic, was added to prevent bacterial contamination by inhibiting bacterial protein synthesis, ensuring a sterile environment for Plasmodium culture [27]. To further investigate the interaction between GOns and the malaria parasites, researchers incubated infected red blood cells (iRBCs) with GOns deposited on glass coverslips. Upon rupture of the iRBCs, the released parasite contents, particularly the merozoites, were stained with ethidium bromide (EtBr), a fluorescent dye that binds to DNA. Confocal microscopy revealed strong EtBr fluorescence on the surface of all GOns, indicating that the released merozoites were physically attracted to or retained by the GOns. This finding supports the hypothesis that GOns can effectively capture and immobilize the infectious agents following the lysis

of host cells, potentially interfering with the parasitic life cycle and providing a basis for further therapeutic exploration [27].

To assess the impact of GOns on parasite growth, the size of the graphene oxide nanosheets was varied using ultrasonication for different durations (0, 10, 60 and 120 minutes). This created GOns of varying lateral sizes, which allowed researchers to test whether size influences the ability of GOns to inhibit Plasmodium growth. The results demonstrated that larger GOns were more effective at trapping the malaria parasite, absorbing nutrients and inhibiting its growth. This highlights the importance of nanosheet size in the potential effectiveness of GOns as an antimalarial agent [27]. Additionally, experimental results showed that GOns effectively depleted key components in MCM, particularly proteins and their derivatives, starving the parasite and ultimately inhibiting its growth. By reducing the availability of essential nutrients, GOns played a significant role in limiting parasite propagation. This, combined with the ability to immobilize merozoites, positions GOns as a promising candidate for further therapeutic investigation in malaria treatment [27]. The presence of GOns has been shown to attract merozoites following the rupture of iRBCs. Future research aimed at exploring whether GOns can interact with or attract merozoites prior to iRBC rupture could represent a significant advancement, potentially allowing for intervention before host cell damage occurs. Taking into consideration the GO sheets sizes, it is important to note that the cytotoxicity of GO increases with higher concentrations. Therefore, further studies are necessary to determine the optimal GO dosage that achieves effective antimalarial activity while maintaining minimal cytotoxic effects on host cells.

3.4.2. Against protozoan parasite (*Acanthamoeba*)

A recent study by Jabri *et al.* (2024) developed a novel graphene oxide–chitosan (GO–CS) nanocomposite to enhance the anti-amoebic effects of doxycycline (doxy) and pentamidine (pent) against *Acanthamoeba* species [Figure 12].

Chitosan is a natural polymer widely studied in biomedical fields, including dentistry, for its biocompatibility, biodegradability, and antimicrobial properties [Rinaudo, 2006]. In this composite, graphene oxide (GO) sheets were incorporated into the chitosan polymer matrix, forming a stable three-dimensional nanostructure [59]. GO served as a high-capacity drug carrier due to its large surface area and layered structure, enabling the adsorption of drug molecules primarily through π–π stacking, hydrogen bonding and

electrostatic interactions. These interactions are reversible, allowing for controlled and sustained release of the drugs under physiological conditions, such as acidic pH or varying ionic strength, which can modulate drug delivery in the oral environment [59].

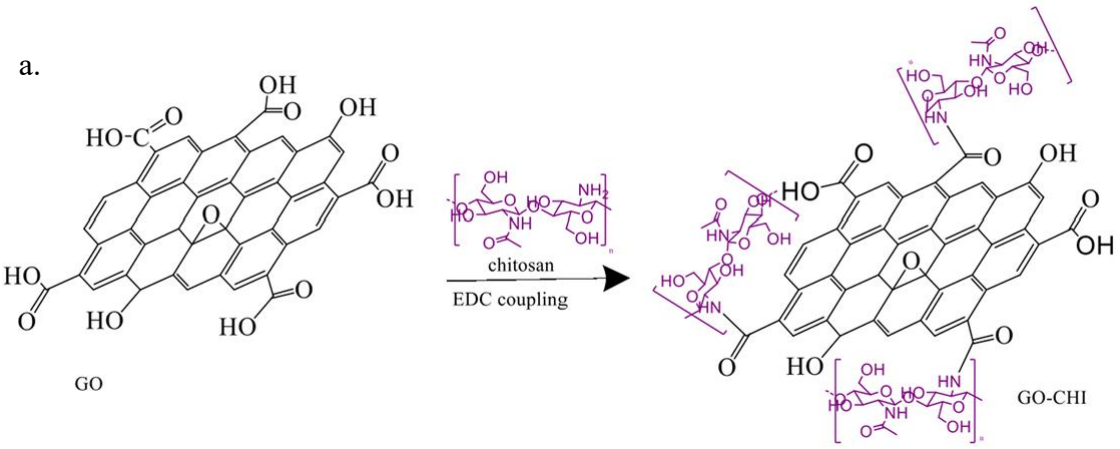

Figure 12: a- Synthesis of GO-CHI. Adapted from Jabri *et al*., (2024). No authorized.

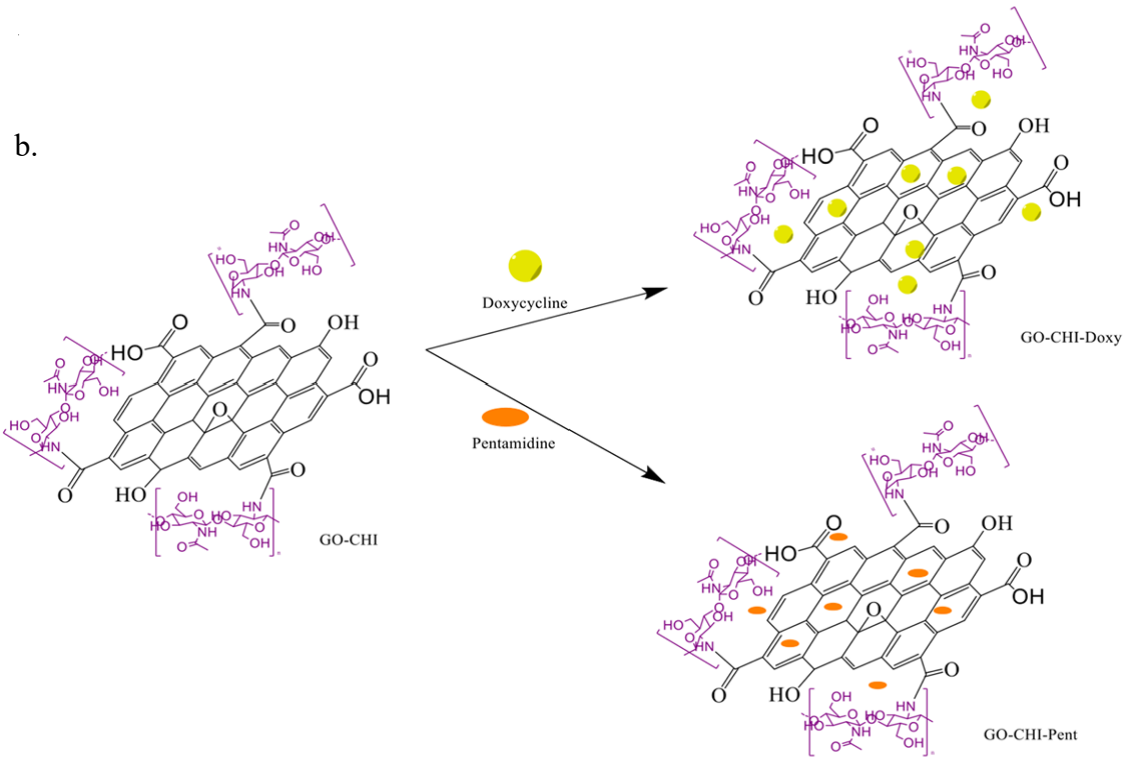

Figure 12: b- Drugs loaded GO-CHI (GO-CHI-Doxy and GO-CHI-Pent). Adapted from Jabri *et al*., (2024). No authorized.

The chitosan polymer encapsulated the GO–drug complex, enhancing mechanical strength and providing a diffusion barrier that protects the drugs and allows for gradual release. This polymeric nanocomposite showed improved drug uptake by the protozoan cells and reduced cytotoxicity to human cells, indicating enhanced therapeutic efficacy and safety [59]. Although the original study focused on anti-amoebic therapy, such GO–chitosan composites have promising potential for functionalization in dental materials, such as antimicrobial coatings or drug delivery systems embedded in polymeric dental devices, aligning with the broader goal of enhancing polymer-based biomaterials with graphene derivatives for clinical applications.

3.5. Biocompatibility, Enzymatic Interactions and Long-Term Safety of GO, rGO and GNPs

GO, rGO and GNPs have shown significant potential in various biomedical applications, including antimicrobial activity against a broad spectrum of bacteria, fungi, viruses and parasites. Their favourable biocompatibility and relatively low cytotoxicity in in vitro settings, combined with their physicochemical versatility (such as modifiable surface chemistry, tunable oxidation level, and high surface area) make them appealing candidates for integration into polymeric drug delivery systems, wound healing scaffolds and dental tissue engineering materials [60, 61].

GO is frequently incorporated into natural or synthetic polymeric matrices, such as PLGA (poly(lactic-co-glycolic acid)) and PVA (polyvinyl alcohol), to improve mechanical strength, regulate degradation rates, and achieve controlled drug release. In dental applications, GO–polymer composites are being explored for use in coatings, restorative materials and antimicrobial films, benefiting from the combined bioactivity of GO and the structural versatility of polymers [60].

rGO with its partially restored graphene structure and fewer oxygenated groups, offers higher electrical conductivity and mechanical robustness. It is also applied in polymeric systems, although it typically requires more careful functionalization or higher concentrations to achieve antimicrobial efficacy comparable to GO—raising the need for cytotoxicity evaluation. rGO is particularly relevant where conductivity or reinforcement is prioritized, such as in electrically active scaffolds or implant coatings.

GNPs, which consist of stacked graphene layers with larger lateral dimensions, are increasingly integrated into dental polymers to reinforce mechanical properties. Though less oxidized and not as inherently antimicrobial as GO, GNPs contribute to moderate antimicrobial effects, which can be improved via surface functionalization or combination with other bioactive agents. Their compatibility with various polymers and ability to improve thermal and structural stability support their growing role in dental nanocomposites.

3.5.1. Concerns with Enzyme Interactions

A particularly intriguing concern regarding GO, rGO and GNPs is their potential to interact with and compete against enzymes crucial for both microbial and human physiological processes. Enzymes are vital for maintaining cellular function, regulating metabolism, and synthesizing biomolecules. Due to their large surface areas and reactive chemistries, these graphene derivatives may adsorb or bind enzymes, potentially interfering with normal activity.

The antimicrobial effects mentioned may be due in part to such enzymatic interactions. For example, GO’s oxidative stress generation and rGO's membrane disruption, coupled with possible enzyme inhibition, can critically impair microbial metabolism and survival. GNPs, although less chemically reactive, may still interact sterically or through surface charges, affecting microbial enzymatic pathways when functionalized.

For human systems, interference with enzymes involved in detoxification, DNA repair, or cellular respiration could result in harmful biological responses. In particular GO, may adsorb key cofactors or compete for active sites, potentially blocking reactions vital for homeostasis. rGO and GNPs, depending on their surface chemistry and dose, may exhibit similar behaviour, though often to a lesser degree than GO due to lower surface oxygen content [62].

In polymeric composites, these effects may be mitigated by the encapsulating matrix. Polymers can modulate exposure to biological environments, and the surface functionalization of GO, rGO, or GNPs within the matrix can influence their interaction with enzymes. Depending on the polymer type and dispersion quality, such composites may enhance or reduce enzymatic interference. Therefore, understanding the interplay between graphene surface chemistry and polymer design is key to optimizing biosafety.

3.5.2. Excretion and Long-Term Health Effects

Another underexplored area is the excretion and long-term fate of GO, rGO and GNPs within the body. Many studies have yet to clarify how these particles are metabolized, cleared, or whether they accumulate in tissues over time. If not effectively excreted, they may provoke chronic inflammation, organ toxicity or interfere with immune responses.

Particularly in dental applications—where prolonged or repeated exposure (e.g., through implant coatings or resin-based restorations) is plausible—this raises concern. GO and rGO, due to their nanoscale size and surface reactivity, may induce oxidative stress or inflammation in adjacent tissues. GNPs, due to their larger size and fewer reactive sites, may pose lower risks of tissue penetration but still require evaluation for long-term safety. Additionally, hypersensitivity or allergic reactions remain possible, especially via mucosal or inhalation routes relevant to dental practice. Comparative studies are needed to assess the immunological profile of each derivative and their potential for accumulation in vital organs [60, 61].

3.5.3. Future Research

To ensure the safe clinical translation of GO, rGO and GNP–based materials, especially in polymeric dental systems, extensive in vivo studies are necessary. These should clarify the selectivity of these materials in targeting microbial processes while avoiding interference with human enzymes and tissues. Long-term safety, tissue integration, and immune responses must be evaluated under physiologically relevant conditions, including those specific to the oral cavity.

Given the unique properties of each graphene derivative—GO's high reactivity and functionalizability, rGO's conductivity and structural integrity and GNP's reinforcing capabilities—tailored material designs are essential. By leveraging polymer matrices to modulate their biological interfaces, researchers can optimize therapeutic potential while minimizing biosafety risks [60-62].

4. Conclusions

Graphene derivatives, particularly GO, and to a lesser extent rGO and GNPs, hold significant promise in antimicrobial applications, drug delivery, and regenerative medicine due to their unique structural and chemical properties. Among these, GO stands out for its high hydrophilicity, ease of surface functionalization, and relatively favourable biocompatibility, making it particularly attractive for biomedical use.

The integration of these graphene derivatives into natural and synthetic polymeric materials represents a growing area of interest. GO–polymer composites have been shown to enhance mechanical strength, control degradation, improve biological responses, and enable controlled drug release. Similarly, rGO and GNPs, especially when functionalized or incorporated as part of hybrid systems, demonstrate potential in enhancing structural and antimicrobial properties, although they may require higher concentrations or additional modification to achieve comparable effects to GO.

Despite these promising developments, several critical concerns remain insufficiently addressed. These include potential interference with enzymatic activity, long-term biocompatibility, immune system interactions and excretion or accumulation of these nanomaterials in biological systems. These challenges are particularly relevant in complex environments such as the oral cavity, where fluctuating pH and diverse microbiota may influence material performance.

Future research should aim to clarify the biological interactions of GO, rGO, and GNPs.Both in their pure forms and within composite systems. A deeper understanding of their behaviour in vivo is essential for ensuring the safe and effective clinical application of graphene-based materials, particularly in dentistry and regenerative medicine.

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