THE EFFECT OF ERYTHROPOIETIN IN THE NONSURGICAL TREATMENT OF PERIODONTITIS – A SYSTEMATIC REVIEW

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Mestrado Integrado em Medicina Dentária

Porto, 2023

U.PORTO
FACULDADE DE MEDICINA DENTÁRIA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

Unidade Curricular de Monografia ou Relatório de Estágio

REVISÃO BIBLIOGRÁFICA

"The Effect of Erythropoietin in the Nonsurgical Treatment of Periodontitis – a systematic review".

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ACKNOWLEDGMENTS

À minha Orientadora, Prof.ª Doutora Luzia Mendes, pelo rigor, exigência e profissionalismo durante todo o trabalho, e por todo o conhecimento partilhado.

Aos meus pais, Maria de Lurdes e Miguel, a quem dedico todas as minhas conquistas e esta dissertação, por terem acompanhado o meu percurso desde o início. Por toda a dedicação e apoio incondicionais, por serem o meu porto de abrigo, por me abrirem os horizontes, por acreditarem em mim e nas minhas capacidades, e por me ajudarem a alcançar os meus sonhos. Por tudo isto e muito mais, estou-vos eternamente grata e enche-me o coração ser vossa filha.

Ao meu irmão, Pedro, por toda a cumplicidade, amizade e coragem que me dá diariamente. Por toda a sabedoria transmitida, por toda a diversão proporcionada e por ser o irmão que todos sonhavam ter.

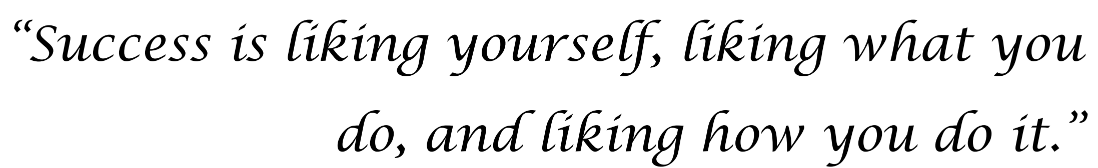
À minha avó, Inês, por todo o carinho e força, e por estar ao meu lado nos momentos importantes da minha vida.

Ao meu tio e padrinho, Rui, por todos os valores transmitidos e momentos partilhados, pelo orgulho demonstrado e por toda a ajuda e disponibilidade.

À minha querida amiga, Kika, por assistir a esta caminhada de perto desde o primeiro dia. Por todas as aventuras vividas e memórias criadas, por todos os risos partilhados e pela motivação e apoio transmitidos.

Às minhas amigas do colégio e da faculdade, que nos momentos chave me aconselharam e depositaram em mim e no meu trabalho a sua confiança, e por partilharem mais uma etapa comigo.

E a todos os que se cruzaram no meu caminho, que potenciaram a minha curiosidade e sentido crítico e que, de alguma maneira, deixaram a sua marca.



Maya Angelou

RESUMO

Introdução: A periodontite é uma doença inflamatória multifatorial crónica e irreversível que resulta na destruição do aparelho de suporte que envolve o dente. É considerada a doença inflamatória crónica oral mais comum na população humana, o que torna relevante a investigação das diferentes possibilidades terapêuticas viáveis para o seu tratamento. A utilização de farmacoterapias adjuvantes em combinação com o tratamento *gold standard* (instrumentação da superfície radicular) tem sido um constante objeto de estudo. A eritropoietina tem propriedades que atuam para além do sistema hematopoiético e que podem constituir uma vantagem para o tratamento da periodontite de uma forma minimamente invasiva.

Objetivos: A presente revisão sistemática do tipo *scoping* tem como objetivo identificar e mapear todos os efeitos documentados da eritropoietina nos tecidos periodontais num contexto de periodontite.

Materiais e métodos: Realizou-se uma pesquisa bibliográfica nas bases de dados MEDLINE (via PubMed), Cochrane (CENTRAL), ScienceDirect e Scopus (Elsevier), até dia 27 de fevereiro de 2023. Todos os estudos celulares, em animais e em humanos que demonstrassem os efeitos da eritropoietina no tratamento da periodontite foram recolhidos.

Resultados: Na pesquisa inicial, foram obtidos 119 artigos, dos quais 76 foram excluídos por duplicação. Após análise e leitura integral do texto, foram incluídos seis artigos nesta revisão, dos quais dois são estudos em linhagem celular, três em animais e um ensaio clínico desenvolvido em humanos.

Discussão: Os nossos resultados sugerem que a eritropoietina regula a resposta inflamatória, diminuindo a sua ativação exacerbada. A modulação ocorre por meio da inibição de citoquinas pro-inflamatórias e pela secreção de citoquinas anti-inflamatórias, o que reduz os níveis de produtos bacterianos e, consequentemente, assume o controlo do processo inflamatório. Recentemente, descobriu-se que os recetores de eritropoietina estão presentes em células fora

do sistema hematopoiético, possibilitando a ligação e a ativação de diferentes *pathways* pela eritropoietina, o que desencadeia diversas interações biológicas. A osteogénese é ativada desta forma. O mecanismo de formação de osteoblastos é estimulado e reabsorção osteoclástica inibida, favorecendo a formação óssea. Além disso, a angiogénese é promovida, conforme identificado nos nossos resultados, pela presença de novos vasos sanguíneos próximos a áreas com densa deposição de colagénio e cálcio. Estas associações proporcionam a atenuação dos sintomas da periodontite e a resolução dos sinais clínicos.

Conclusões: Nesta revisão, concluiu-se que a utilização da eritropoietina pode ser viável e clinicamente relevante, sendo uma potencial candidata para o tratamento da periodontite. Este método é biocompatível, não tóxico e permite a resolução da inflamação periodontal e da destruição óssea. No entanto, a evidência científica é escassa e existe uma grande variedade de metodologias aplicadas nos diferentes estudos, o que impossibilita a generalização dos resultados e acarreta maior risco de erro de viés.

Palavras-chave: “periodontite”, “doença periodontal”, “terapia periodontal”, “tratamento não-cirúrgico”, “eritropoietina”.

ABSTRACT

Introduction: Periodontitis is a chronic and irreversible multifactorial inflammatory disease that results in the destruction of the supporting apparatus surrounding the tooth. It is considered the most common chronic inflammatory oral disease in the human population, which makes it relevant to investigate the different therapeutic possibilities available for its treatment. The use of adjunctive pharmacotherapies in combination with the *gold standard* treatment (scaling and root planning) has been a constant object of study. Erythropoietin has properties that act beyond the hematopoietic system and may be an advantage for the treatment of periodontitis in a minimally invasive way.

Objectives: The present systematic scoping review aims to identify and map all documented effects of erythropoietin on periodontal tissues in a periodontitis context.

Materials and methods: A literature search was performed in MEDLINE (via PubMed), Cochrane (CENTRAL), ScienceDirect and Scopus (Elsevier) databases by 27 February 2023. All cellular, animal, and human studies demonstrating the effects of erythropoietin in the treatment of periodontitis were collected.

Results: In the initial search, 119 articles were obtained, of which 76 were excluded for duplication. After analysis and full-text reading, six articles were included in this review, of which two are cell lineage studies, three in animals and one clinical trial developed in humans.

Discussion: Our results suggest that erythropoietin regulates the inflammatory response, decreasing its exacerbated activation. Modulation occurs through inhibition of pro-inflammatory cytokines and by secretion of anti-inflammatory cytokines, which reduces the levels of bacterial products and, consequently, controls the inflammatory process. Recently, erythropoietin receptors have been found to be present in cells outside the hematopoietic system, enabling erythropoietin to bind and activate different pathways, which triggers various

biological interactions. Osteogenesis is activated in this way. The mechanism of osteoblast formation is stimulated, and osteoclastic resorption inhibited, favoring bone formation. In addition, angiogenesis is promoted, as identified in our results, by the presence of new blood vessels near areas with dense collagen and calcium deposition. These associations provide the attenuation of periodontitis symptoms and the resolution of clinical signs.

Conclusions: In this review, it was concluded that the use of erythropoietin may be feasible and clinically relevant, being a potential candidate for the treatment of periodontitis. This method is biocompatible, non-toxic and allows resolution of periodontal inflammation and bone destruction. However, scientific evidence is scarce and there is a wide variety of methodologies applied to different studies, which makes it impossible to generalise the results and carries a higher risk of bias error.

Keywords: “periodontitis”, “periodontal disease”, “periodontal therapy”, “non-surgical treatment”, “erythropoietin”.

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3-MA	3-methyladenine
AAB	apex of alveolar bone
ALP	alkaline phosphatase
Arg1	arginase 1
Bax	B-cell lymphoma associated X
Bcl2	B-cell lymphoma protein 2
bFGF	basic fibroblast growth factor
BI	bleeding index
BMD	bone mineral density
BMP-2	bone morphogenetic protein 2
BV/TV	bone volume/bone tissue fraction
CAL	clinical attachment loss
CD31	cluster of differentiation 31
CEJ	cementoenamel junction
CNP	ceria nanoparticle
COX	cyclooxygenase
CS	chitosan
DMEM	dulbecco’s modified eagle medium
DMSO	dimethyl sulfoxide
EphB4	ephrin type-B receptor 4
EPO	erythropoietin
EPOR	erythropoietin receptor
FBS	fetal bovine serum
FGF	fibroblast growth factor
GI	gingival index
H&E	hematoxylin and eosin
hPDLF	human periodontal ligament fibroblasts
hPDLSCs	human periodontal ligament mesenchymal stem cells
HUVECs	human umbilical vein endothelial cells
IGF-1	insulin-like growth factor-1
IHC	immunochemistry
IL	interleukin

ISP	Inês Sá Pereira
LC3B	light chain 3 beta
LM	Luzia Mendes
LPS	lipopolysaccharides
M1	macrophage 1
M2	macrophage 2
MAPK	mitogen-activated protein kinase
MMP-9	matrix metalloproteinase-9
MPO	myeloperoxidase
MSCs	mesenchymal stem cells
mRNA	messenger ribonucleic acid
MTC	Masson's trichrome
OCN	osteocalcin
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PD	probing depht
PI	plaque index
pPDLSCs	periodontitis mesenchymal stem cells
PDLSCs	periodontal ligament stem cells
qPCR	quantitative polymerase chain reaction
RANK-L	nuclear factor kappa B ligand
ROS	reactive oxide species
RT-qPCR	reverse transcription-quantitative polymerase chain reaction
RUNX2	runt-related transcription factor 2
Tb. Th	trabecular thickness
Tb.N	trabecular number
Tb.Sp	trabecular separation
TGF-β	transforming growth factor beta
TMD	mineral tissue density
TNF-α	tumor necrosis factor-α
TRAP	tartare-resistant and phosphatase
VEGF	vascular endothelial growth factor
α-MEM	α-minimum essential medium
β-GP	beta-sodium glycerophosphate

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CHAPTER I

INTRODUCTION

I. INTRODUCTION

Periodontitis is a chronic multifactorial inflammatory disease that affects the *periodontium*, resulting in the destruction of the supportive apparatus surrounding the tooth. The structures involved are the gingival tissue, alveolar bone, cementum, and periodontal ligament. (11) When the disease is activated, it produces very particular clinical signs that unmask its presence. Commonly observed are clinical attachment loss, deep periodontal pockets, tooth mobility, and gingival bleeding. Radiographically, there is evidence of alveolar bone loss, which is indicative of loss of supporting tissue. (17, 32) Thus, periodontal disease appears as the progression from a reactive, reversible inflammatory state of the gingiva to a chronic, destructive, and irreversible inflammatory state that, when not properly treated, results in tooth loss. (11)

The etiopathogenesis is related to a complex interaction of commensal oral bacteria and host response that can create a process of dysbiosis. (23) These bacteria and their released products (*biofilm*) have direct and indirect implications on the *periodontium*. Directly, bacteria lead to activation of the inflammatory response, which causes edema and increased gingival bleeding. Additionally, indirect pathogenic effects cause mobilization of host defenses and the production of cytokines and inflammatory mediators that result in the release of tissue enzymes, which destroy the extracellular matrix and bone. (13) The severity of periodontal disease is directly proportional to modifiable and non-modifiable risk factors. The most significant include smoking and diabetes mellitus. Nonetheless, other situations such as occlusal trauma, open contact points and over-contoured restorations also enhance its progression. (15, 17)

Nowadays, periodontitis is considered the most common chronic inflammatory oral disease among the human population, affecting an estimated 20-50% in its milder form and an estimated 7,4% in a more severe stage. (30, 32) Taking into consideration these statistics, it becomes relevant to investigate further feasible therapeutic possibilities for the treatment of this periodontal disease.

The treatment of periodontitis has been divided into three incremental pre-established steps, designed to restore the lost balance in the oral cavity. The first step is intended to instruct and motivate the patients to make a change in their habits and control modifiable risk factors, so that they learn how to effectively remove supragingival dental *biofilm*. Therefore, this step should be reinforced in any phase of therapy. The second step focuses on the elimination of the supra and subgingival *biofilm* and calculus by professional subgingival instrumentation (nonsurgical therapy). The third step is considered when the previous stages were unable to restore the periodontal health. This represents the need of continued treatment, and a surgical approach can be chosen to repair or deep periodontal pockets or bone defects, increasing the complexity of the treatment. (32)

Scaling and root planning are considered the *gold standard* approaches of the nonsurgical therapy. However, in some sites and/or patients, the response to treatment does not achieve the expected results, remaining in a dysbiotic infectious state, either due to microbial factors or the inability to solve the inflammatory response. (12, 17) Thus, has been encouraged the search for new and potentially effective adjuvant therapies that, in addiction to nonsurgical therapy, can achieve balance and tissue repair and regeneration. (10)

Erythropoietin (EPO) is classified as a glycoprotein hormone and cytokine of the hematopoietic superfamily class I, and it is secreted by the kidneys. This growth hormone is usually identified for its role in stimulating neovascularization and circulating erythrocyte mass. However, the biological functions of EPO go far beyond the hematopoietic system. (3, 49) Several studies showed that EPO is involved in bone formation, through the deposition of bone extracellular matrix that induces rapid regeneration of the alveolar ridge. Simultaneously, it inactivates osteoclast activity and inhibits bone resorption. (20) It has also been shown that in situations of excessive or chronic inflammation, such as periodontitis, EPO modulates pro-inflammatory cytokines, facilitating healing and restoring function by increasing vascular proliferation, maturation of extracellular matrix, angiogenesis, and vascular density. (2) This cytokine-dependent action

ensures tissue reconstruction. (14) In the following figure, we see the functions of the EPO schematized (**FIGURE 1**).

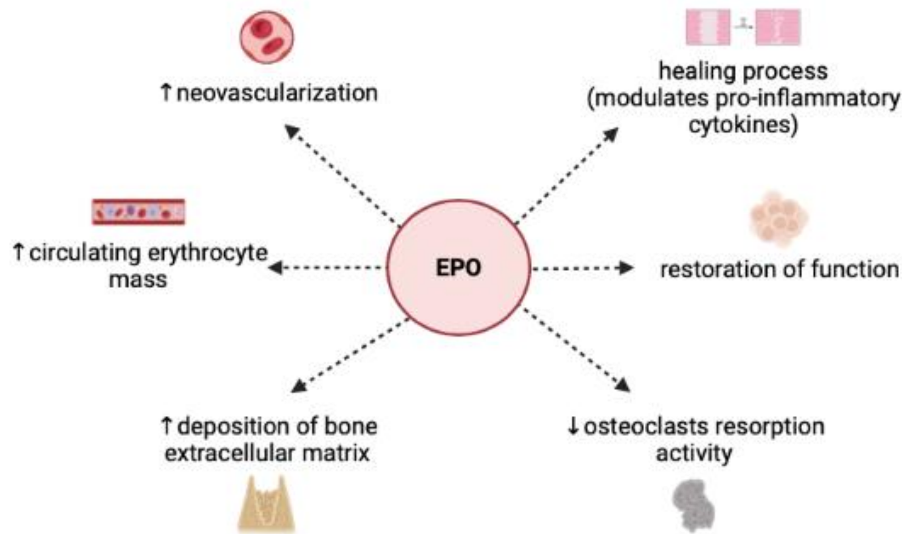

FIGURE 1. Described interactions of EPO in the human organism. Original diagram made by ISP.

In chronic inflammatory conditions, erythropoiesis is reduced, and EPO gene expression inhibited. (49) Thereby, if in this condition the hormone is found in decreased concentrations and is known to have properties that stimulate healing and bone repair, it becomes interesting to ascertain whether its adjuvant application will be advantageous for the treatment of periodontal disease and whether it could serve as a potential candidate for periodontal treatment.

1.1. Objectives

The aim of this scoping review is to understand the potential benefit of erythropoietin use in the treatment of periodontitis, in an unstable active phase. In this way, by choosing to conduct a scoping review, it is intended to identify ongoing research and map the existing literature on this topic in order to recognize its limitations and whether it is beneficial to engage in further research.

CHAPTER II

MATERIALS & METHODS

II. MATERIALS AND METHODS

2.1. Focused question

The following scoping review was structed based on the guidelines of Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) (27, 31, 40) to answer the focused question (Population, Concept, Context): “Is the adjuvant use of erythropoietin in the nonsurgical periodontal treatment clinically effective and feasible?”.

2.2. Search strategy and information sources

In the initial stage, a search for suitable articles was conducted in four electronic sources of evidence that corresponded to the aim of this review: MEDLINE (via PubMed), Cochrane (CENTRAL), ScienceDirect and Scopus (Elsevier) online databases. The databases previously referred were consulted up to 27th of February 2023.

During the research, were used the following keywords: “Periodontitis”, “Periodontal Disease”, “Periodontal Therapy”, “Periodontal Treatment”, “Periodontal Regeneration”, “Nonsurgical Treatment” and “Erythropoietin” and combined with the Boolean operators “AND” or “OR”, used appropriately in order to limit the search and exclude irrelevant articles.

The following table (**TABLE 1**) represents the search strategy used in the online databases. The electronic search was complemented by handsearching the references of all included papers.

TABLE 1. Search Strategy.

Erythropoietin	
AND	(periodontitis OR periodontal disease)
	(periodontal therapy OR periodontal treatment)
	periodontal regeneration
	nonsurgical treatment

2.3. Eligibility criteria

Eligible studies were required to broadly describe the effects of using erythropoietin to treat periodontitis in both animals and humans. Studies *in vitro* were also included to a broader understanding of erythropoietin in different environments.

The exclusion criteria applied in this scoping review, implied the removal of articles whose main objective was not the role of erythropoietin in the treatment of periodontitis. The type of article was also considered and, therefore, letters, reviews and opinion articles were excluded. Moreover, papers published in languages other than Portuguese, English or Spanish weren't included in this scoping review.

2.4. Screening and selection of sources of evidence

The first screening phase consisted of reading and analysing the titles and abstracts of the selected articles, after duplicates had been removed. Two independent researchers (ISP, LM) participated in the process and the decision was made by consensus. The collected papers were then read in full and removed if they didn't meet the inclusion criteria, or if they contained any of the exclusion parameters described above.

2.5. Data extraction and analysis

In a second step, the selected papers were analysed. Information on the author, the year of publication, type of study, aim of the study, results, and conclusions were collected.

Paper found during the initial literature search that were deemed inappropriate or removed after full-text reading were used later in the introduction and discussion, if relevant.

2.6. Synthesis of results

Based on the selected articles, the information was organized in a table, categorizing the collected data.

CHAPTER III

RESULTS

III. RESULTS

3.1. Selection of sources of evidence

The initial electronic search resulted in 119 studies: 37 in PubMed, 19 in Cochrane (CENTRAL), 8 in Science Direct and 55 in Scopus (Elsevier). After removing 76 duplicate studies, the title and abstract of the remaining articles were analysed, and 64 articles were removed. Thus, 12 articles were obtained for full-text reading. Of the articles analysed by full-text reading, 6 were excluded based on the application of the defined inclusion and exclusion criteria. No additional studies were identified by handsearching the reference lists of the selected articles. As such, 6 papers were included in the present scoping review. The selection process was clearly demonstrated by the PRISMA-ScR flow diagram (**FIGURE 2**).

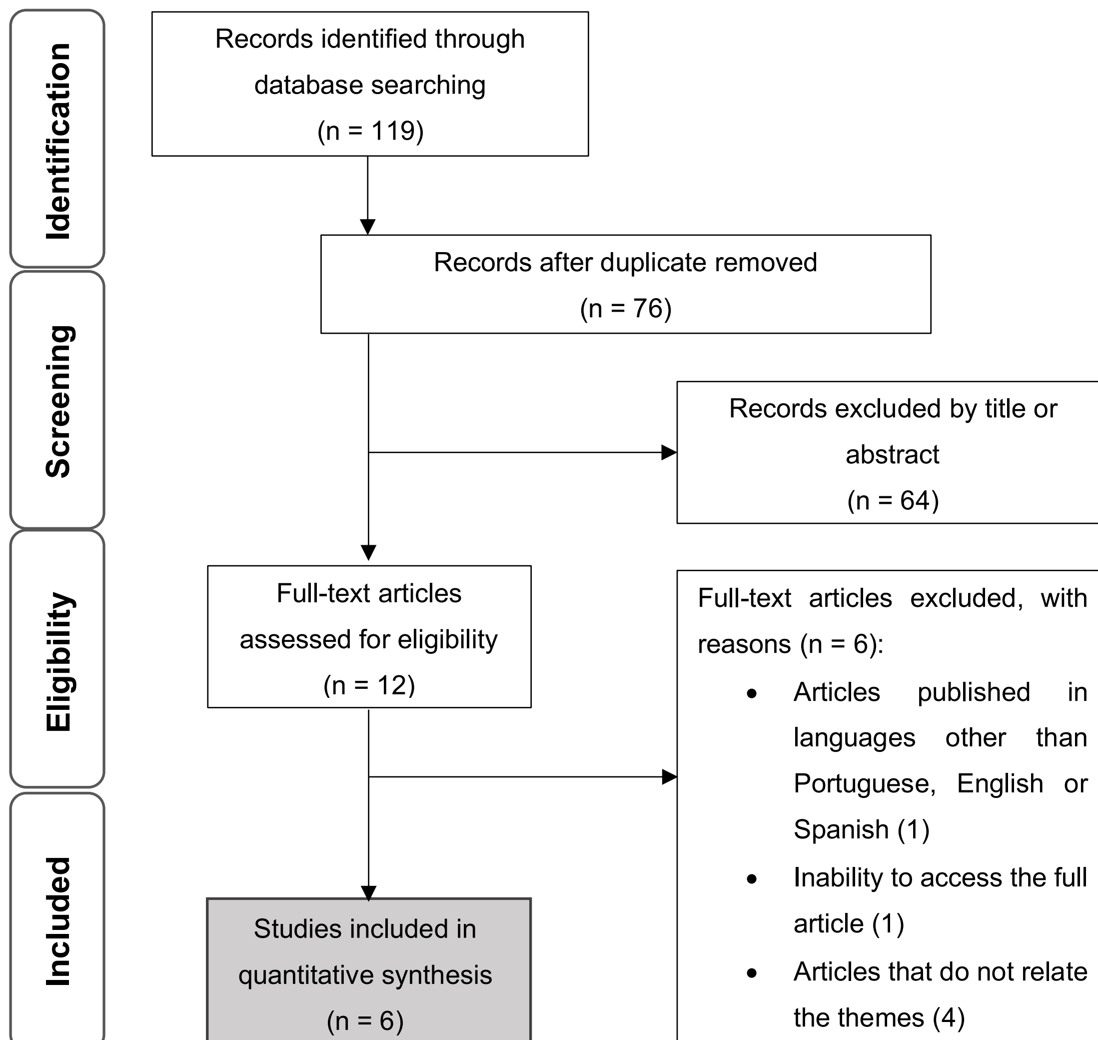

FIGURE 2. Flowchart of the selection process of the included articles.

3.2. Results of individual sources of evidence

ART. #1 | **Wang et al. (2018)**

In this clinical trial, the osteogenic effects of EPO in human periodontal ligament mesenchymal stem cells (hPDLSCs) and periodontitis mesenchymal stem cells (pPDLSCs) were investigated.

The hPDLSCs were obtained from 10 individuals aged 35-45 years, from premolars extracted for orthodontic reasons and from third molars. pPDLSCs were taken from 7 volunteers, aged 27 to 52 years, and diagnosed with reduced periodontal health, with two-thirds bone destruction or with at least one periodontal pocket (probing depth > 5 mm). Individuals with a recent diagnosis of periodontitis or systemic disease, history of smoking or history of maxillofacial surgery, radiotherapy or chemotherapy were excluded.

hPDLSCs and pPDLSCs cells were introduced in an α-minimum essential medium (α-MEM), with 10% fetal bovine serum (FBS) and at then were isolated and transported to a medium containing EPO. To study the osteogenic capacity of the cells after two days of contact with EPO, they were transferred to an osteogenic-inducing medium for 7 or 21 days.

The proliferative capacity of these cells was measured by means of a flow cytometer, which determined the proliferation index. All cells that were in G2 or S phases were considered to be in proliferation phase. In EPO-treated medium, the proliferation index of both cells largely exceeded the index of cells in normal medium, as indicated in **TABLE 2**. The osteogenic effects of EPO were analysed by alkaline phosphatase (ALP) staining and ALP activity in an optical microscope. It was found that on the 7th post-treatment day, the medium with EPO presented a more accentuated staining, greater cellular proximity and the presence of the ALP gene was detected. The expression of mRNA transcription levels of runt-related transcription factor 2 (RUNX2), ALP and osteocalcin (OCN) genes, involved in bone formation regulation, was measured by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). All of them showed superior results when the cells were in a medium with EPO. Finally, to demonstrate the role of the mitogen-activated protein kinase (MAPK) pathway in osteogenesis, the phosphorylation of p38 was assessed by western blot analysis and cells were

transferred to an α-MEM with the pathway inhibitor SB203580. Mineralized nodule formation and EPO-induced ALP activity were significantly decreased when the inhibitor was applied, as well as RUNX2, OCN and ALP gene expression.

ART. #2 | **H. Huang, L. Jie, L. Xingrui et al. (2022)**

This paper aims to investigate the anti-apoptotic, autophagic and angiogenic effects of EPO in an inflammatory microenvironment, as well as the triggering pathway in order to find a new technique that allows the treatment of periodontitis.

Periodontal ligament cells were collected from orthodontic teeth of 50 healthy patients aged 12-20 years and then cultured in an α-MEM, which contained 10% FBS and 1% penicillin-streptomycin solution, allowing periodontal ligament stem cells (PDLSCs) to be obtained. To determine whether the PDLSCs were able to redirect differentiation, they were transferred to an osteogenesis-inducing medium and the results were observed with Alizarin Red solution. The process was repeated but in a lipogenesis-inducing medium and the results were observed using oil red O solution.

The PDLSCs were inoculated in 96-well plates with a density of 2000 cells per well and divided into different treatment groups: (**1**) concentration gradient of tumour necrosis factor-α (TNF-α); (**2**) concentration gradient of EPO under inflammatory environment, induced by 20ng/ml of TNF-α; (**3**) the Akt/ERK1/2/BAD pathway, which is divided into (**3a**) TNF-α, (**3b**) TNF-α, EPO and the pathway inhibitor LY294002, (**3c**) TNF-α and EPO; and (**4**) to investigated the role of autophagy, also dividing into (**4a**) TNF-α, (**4b**) TNF-α, EPO and the pathway inhibitor 3-methyladenine (3-MA), (**4c**) TNF-α and EPO.

The apoptotic capacity was detected by flow cytometry, through the detection of the B-cell lymphoma associated X (Bax) and B-cell lymphoma protein 2 (Bcl2) ratio, which was strongly detected in group 1 and when EPO activity was repressed by the use of an inhibitor of the Akt/Erk1/2/BAD pathway (group 3b) and by the autophagy inhibitor (group 4b). The expression of target genes was measured by qPCR and protein concentration by western blot. These techniques

analysed the expression of vascularization-related cytokines, such as vascular endothelial growth factor α (VEGF-α), insulin-like growth factor-1 (IGF-1) and fibroblast growth factor 2 (FGF-2), of autophagy-related genes, Beclin1 and light chain 3 beta (LC3B), of apoptotic proteins, Bax and Bcl2, and of inflammatory mediators, interleukin-1 beta (IL-1β) and interleukin-8 (IL-8). According to the results obtained, when EPO was introduced, the values of the cytokines VEGF-α, IGF-1 and FGF-2 and of the genes Beclin1 and LC3B increased, in comparison with the group without treatment (group 1), and the Bax/Bcl2 ratio decreased significantly.

Finally, EPO regulatory pathways were measured with RNA-Sequencing Assay. To determine the role of the Akt/Erk1/2/BAD pathway, a specific inhibitor (LY294002) was introduced, which demonstrated the ability of EPO to regulate the processes of anti-apoptosis, autophagy and angiogenesis. Autophagy was further investigated with the application of the specific inhibitor, 3-MA. The results were further confirmed by immunohistochemistry (IHC) and confocal laser microscopy.

ART. #3| **Y. Li, H. Peng, W. Tang et al. (2023)**

This study aims to develop a new strategy to improve the damaged *periodontium* as an alternative to traditional therapies. This consists in making a thermosensitive pre-gel activated at body temperature that turns into a hydrogel (FPH) and contains ceria nanoparticle (CNP) and EPO. The research was divided in two phases: one *in vitro* and the other *in vivo*, with the second only developed after confirmation of the results in a cellular environment.

In vivo, healthy male rats were used and randomly divided into six groups: (**1**) control group, without periodontitis, (**2**) periodontitis without treatment, (**3**) periodontitis treated with hydrogel alone, (**4**) periodontitis treated with CNP hydrogel, (**5**) periodontitis treated with EPO hydrogel, and (**6**) periodontitis treated with CNP and EPO hydrogel. To mimic a periodontitis environment, anesthesia was administered with 10% chloral hydrate and ligature wire was tied around the maxillary 2nd molar. After one week, 25 μg of saline was introduced into the

periodontal pocket in the control and periodontitis groups, and in the treatment groups, 25 µg of pre-gel was injected, with different concentrations of CNP and EPO, depending on the group. At the fourth week post-treatment, the rats were sacrificed, and the effects of the treatment were evaluated.

First, the distance between the cementoenamel junction (CEJ) and the apex of the alveolar bone (AAB) was measured, and the bone quality was measured by micro-CT analysis of a three-dimensional image. As expected, the untreated periodontitis group showed a greater distance than the control group ($p<0.01$). However, in the test group, an improvement of the results was identified in the CNP and EPO hydrogel group, showing a higher recovery ($p<0.01$). Bone quality was obtained by measuring the parameters of bone volume/bone tissue fraction (BV/TV), trabecular thickness (Tb. Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp). In comparison to the control group, the periodontitis group had a reduction of all indices except Tb.Sp which increased its value ($p<0.01$). However, in the EPO hydrogel, CNP hydrogel, and CNP and EPO hydrogel groups, the indices BV/TV, Tb.Th and Tb. N reached higher results than in the periodontitis group ($p<0.05$), and there was a decrease in Tb.Sp ($p<0.05$), as indicated in **TABLE 3**.

Hematoxylin and eosin (H&E) staining was performed to confirm the results previously found. In the group with periodontitis, an accentuated level of inflammation was observed, with cellular infiltration and bone uptake, an apical migration of the junctional epithelium and a destruction of the bone structure were also detected. Remarkably, in the groups treated with EPO and/or CNP, the inflammation disappeared and there was clear bone regeneration, with alveolar height, similar to the control group. Furthermore, through Masson's trichrome staining (MTC), it was possible to observe that, at the 4th week of treatment, these treatment groups showed a repair and reorganisation of collagen fibres, which had been lost in the group with periodontitis. Finally, the expression of the osteogenesis marker OCN was also evaluated by immunochemistry. The introduction of hydrogel containing EPO and/or CNP induced the expression of OCN, compared to the periodontitis group. A stronger effect was observed in the group containing EPO and CNP.

In this study, a new minimally invasive therapeutic strategy is proposed for the treatment of periodontitis. The combination of chitosan (CS), beta-sodium glycerophosphate (β-GP) and gelatine allowed the formation of an injectable and thermo-sensitive pre-gel, converted into a hydrogel at body temperature, and that allows the continuous release of aspirin and EPO.

An *in vivo* experiment was conducted in Winstar male rats randomly divided into six groups: (**1**) control group, without periodontits, (**2**) periodontits without treatment, (**3**) periodontitis treated with CS/β-GP/ gelatin hydrogel alone, (**4**) periodontitis treated with CS/β-GP/ gelatin hydrogel loaded with aspirin, (**5**) periodontitis treated with CS/β-GP/ gelatin hydrogel loaded with EPO, and (**6**) periodontitis treated with CS/β-GP/ gelatin hydrogel loaded with aspirin and EPO. To promote a periodontitis environment, the rats were anesthetised with 10% chloral hydrate and a ligature wire was placed in the interproximal zone of the first and second maxillary molars. In test groups, 25 µg of pre-gel was introduced into the submucoperiosteous tissue at the middle of the buccal and palatal surfaces of the first maxillary molars five times every three days for a two-week period. In the control group, 25 µg of phosphate buffered saline (PBS) was used.

Two weeks later, data were extracted by immunochemistry (IHC) and micro-CT analysis, which allowed the identification of the capacities of aspirin and EPO in the inflamed and damaged *periodontium*, and later verified by histological observations.

Through cell count of cyclooxygenase-2 (COX-2), an enzyme responsible for inflammation, and of matrix metalloproteinase-9 (MMP-9), a matrix-degrading protein, the response to inflammation was studied and, in the group treated with aspirin and EPO hydrogel, the results were significantly low ($p<0.01$). The process of osteogenesis was detected by measuring the distance between CEJ and AAB and by the values of bone mass and mineralisation given by bone mineral density (BMD), mineral tissue density (TMD), BV/TV, Tb. Th, Tb.N and Tb.Sp. In the group with aspirin and EPO hydrogel, the CEJ-AAB distance was practically recovered and the parameters BMD, TMD, BV/TV and Tb.Th

decreased compared to the control group, while Tb.Sp increased in value, as indicated in the following **TABLE 3**. In the clinical criteria Tb.N, there was a decrease relatively to the control, but the result was not statistically significant (p>0.05). Finally, an analysis was performed with H&E staining and the amount of osteoclasts was measured through the tartrate-resistance-phosphatase (TRAP) that these cells release. In the periodontitis group, a high quantity of inflammatory cells and osteoclasts was detected inside the bone gaps, indicating a clear bone resorption. In the experimental group of aspirin and EPO hydrogel, the number of osteoclasts detected was minimal.

ART. #5 | **Bae et al. (2022)**

The aim of this study was to analyse the precise effects of EPO at the cellular level, in the condition of periodontitis, as well as its ability in bone healing *in vivo*. To determine the effects at the cellular level, EPO was applied *in vitro* to osteoblast line cells (MC3T3-E1) and human periodontal ligament fibroblasts (hPDLF) in order to verify which cellular events occur and gene expression patterns. The hPDLF cells were taken from premolars extracted for orthodontic reasons from 3 patients aged between 10 and 20 years. MC3T3-E1 cells were isolated from the calvaria of C57BL/6 mice.

Both cells were cultured at a density of $5x10^3$ cells/well in 96-well plates. The hPDLF were placed in a dulbecco's modified eagle (DMEM) low-glucose medium, supplemented with 10% FBS. MC3T3-E1 were placed in an α-MEM, supplemented with 10% FBS. After 24h, they were transferred to a medium with 2% FBS and EPO at various concentrations. 24h and 48h after this transition, the results were again evaluated to determine which had a higher proliferation and differentiation rate. The most satisfactory variation was in the medium with 10 IU/ml EPO. Compared to the medium without EPO, at 24h it showed 100.0 ± 6.9 vs 130.9 ± 5.3, (p<0.01) and at 48h it obtained 100.0 ± 7.1 vs 142.7 ± 5.6, (p<0.01). The measurements were measured by a microtiter plate reader at 450 nm.

Then, hPDLF cells were again placed in DMEM and MC3T3-E1 cells in an α-MEM, both supplemented with 10% FBS and at a cell density of $1x10^3$ cells/well in 6-well plates. After 24h, the percentage of FBS was reduced to 2% and EPO at 0 or 10 IU/ml was introduced. The results were extracted at days 1, 3, 5, 7 and 14 and analysed by real-time PCR. Through this technique, the expression of osteogenic markers ALP and OCN and of bone morphogenetic protein 2 (BMP-2) was observed. In the EPO treated mediums, there was a significant increase ($p<0.05$) in the proliferation of both cell types, as indicated in **TABLE 3**.

In the *in vivo* experiment, six-week-old male C57BL/6 mice were analysed and randomly divided into two groups: (**1**) control group, and (**2**) experimental group, treated with EPO. To simulate a periodontitis environment under anesthesia, in both groups, the left maxillary second molar was sutured and after 5 days extracted. The right maxillary second molar was used as a control. In the alveolus of the control group, a mixture of surfactant and 0.01% dimethyl sulfoxide was deposited. In the experimental group, EPO was added to these two components. The results were extracted on the 1st, 5th and 14th days and analysed by histological technique, H&E and MTC staining, and IHC technique. In the group treated with EPO, after 14 days, new bone formation and collagen bonds were identified, close to blood vessel-forming regions, assessed by observation of inflammatory (myeloperoxidase – MPO), blood vessel (cluster of differentiation 31 – CD31, and vascular endothelial growth factor – VEGF) and osteogenic (RUNX2 and OCN) markers. The results were more successful in the experimental group ($p<0.05$), as shown in **TABLE 3**.

ART. #6 | Aslroosta et al. (2021)

In this study, the effect of topical application of EPO as an adjuvant for non-surgical treatment of periodontitis was evaluated. This was done by enrolling adults with a diagnosis of Periodontitis, Stage III, Grade B/C, who had not received periodontal treatment in the last 6 months, with a clinical attachment loss of 5 mm or more and a probing depth of 6 mm or more, with bleeding on probing around 2 or more non-adjacent teeth. Individuals diagnosed with systemic diseases, on medication that may influence the immune response,

affecting periodontal inflammation and bone turnover, with paraphysiological conditions, and who have undergone surgical periodontal therapy in the last year were excluded.

The patients were randomly divided into two groups: (**1**) control group, in which was only performed scaling and root planning, and (**2**) test group, which in addition to receiving the non-surgical treatment, received EPO. In the test group, a 28G syringe was introduced along the entire length of the periodontal pocket containing EPO mixed with carboxymethylcellulose gel. On the control group, a mixture of distilled water and carboxymethylcellulose gel, with the same volume as the one applied on the test group, was injected. The baseline values of plaque index (PI), gingival index (GI), bleeding index (BI), probing depth (PD) and clinical adherence level (CAL) were recorded at the beginning of treatment, before the introduction of EPO, and three months after treatment, in both groups.

At the beginning of the therapy, there were no significant difference in the results of the clinical parameters established between test and control groups ($p>0.05$). A new evaluation of the clinical parameters was performed after three months: the GI values decreased, especially in the test group, and the difference between groups was significant ($p=0.002$). The BI scores also decreased in both groups and their difference was statistically relevant ($p=0.000$). Regarding the PI levels, was observed a reduction in both groups without a significant difference between them ($p=0.705$). In addition, there was a considerable improvement in CAL values, which was statistically higher in the EPO group ($p=0.002$), as well as in PD values ($p=0.004$). The results are presented in **TABLE 3**.

3.3. Synthesis of the results

The results were divided into two tables according to the type of study. **TABLE 2** contains the results of cell culture studies and **TABLE 3** the randomised trials. For all the studies analysed in this scoping review, *p*-values < 0.05 were considered statistically significant.

TABLE 2. Synthesis of the results of the cell culture studies. **hPDLSCs**: human periodontal ligament tissue-derived mesenchymal stem cells; **pPDLSCs**: periodontitis mesenchymal stem cells; **ALP**: alkaline phosphatase; **OCN**: osteocalcin; **RUNX2**: runt-related transcription factor 2; **MAPK**: mitogen-activated protein kinase; **TNF-α**: tumor necrosis factor-α; **3-MA**: 3-methyladenine; **Bax**: B-cell lymphoma associated X; **Bcl2**: B-cell lymphoma protein 2; **LC3B**: light chain 3 beta; **VEGF-α**: vascular endothelial growth factor α; **FGF-2**: fibroblast growth factor 2; **IGF-1**: insulin-like growth factor-1; **IL-1β**: interleukin-1 beta; **IL-8**: interleukin-8; **PDLSC**: periodontal ligament stem cells.

#	Cases		EPO concentration	Administration vehicle	Endpoints and Results	Conclusions	Follow-up
	Groups	n					
1	**G1**: hPDLSCs in normal culture	1×10^5 cells/well	-	-	**I. Proliferation Index** **G1**:10.58%; **G2**: 20.60%; **G3**: 16.61%; **G4**: 29.34%, *p*<0.01 *ALP staining and ALP activity assay* At day 7, the osteogenic differentiation was improved in **G2** and **G4**, *p*<0.01 and fold upregulation of ALP activity in **G2** and **G4**, *p*<0.05. *RT-qPCR* **II. Expression level of ALP** **G2**>**G1**, *p*<0.01; **G4**>**G3**, *p*<0.01 **III. Expression level of OCN** **G2**>**G1**, *p*<0.05; **G4**>**G3**, *p*<0.01	Expression of osteogenic genes (ALP, OCN and RUNX2) and the increased proliferation of hPDLSCs and pPDLSCs in **G2** and **G4** demonstrated that EPO is able to regenerate new bone. The p38 phosphorylation was promoted in **G2** and **G4**, demonstrating that the MAPK pathway can be	7 and 21 days
	G2: hPDLSCs in EPO-induced culture		20 IU/ml	Medium containing EPO			
	G3: pPDLSCs in normal culture		-	-			
	G4: pPDLSCs in EPO-induced culture		20 IU/ml	Medium containing EPO			

					IV. Expression level of RUNX2 **G2**>**G1**, $p<0.01$; **G4**>**G3**, $p<0.01$ *Alizarin Red* At day 21, after inducing the osteogenic differentiation, the nodules presented a stronger staining in **G2** and **G4**. The calcium concentration was also measured: **G2**>**G1**, $p<0.01$ and **G4**>**G3**, $p<0.01$. *Western blot* **V. Phosphorylation of p38** **G2**>**G1**, $p<0.01$; **G4**>**G3**, $p<0.01$	activated by EPO in both cell types. When the pathway inhibitor was introduced, the effects of EPO on the cells were attenuated. This supports the hypothesis that EPO induces osteogenesis in hPDLSCs and pPDLSCs cells through activation of the p38 MAPK pathway.	
2	**G1**: Concentration gradient of TNF-α (at 0, 5, 10, 20, 50 and 100 ng/ml)	2000 cells/well	-	-	*qPCR and Western blot* **I. Results in G1** • ↑ mRNA and protein expression of **Bax/Bcl2**, especially in 100 ng/ml, $p<0.01$, • ↓ mRNA and protein expression levels of **Beclin1** ($p<0.05$), **LC3B** ($p<0.05$), **VEGF-α** ($p<0.001$), **IGF-1** ($p<0.05$), and **FGF-2** ($p<0.01$), particularly in 20, 50 and 100 ng/ml. • ↑ **IL-1β** and **IL-8**, in 50 and 100 ng/ml ($p<0.0001$). **II. Results in G2** • ↓ mRNA expression of **Bax/Bcl2**, mostly in 20 and 50 IU/ml ($p<0.01$). • ↑ mRNA and protein expression levels of **VEGF-α**, **IGF-1**, and **FGF-2**, especially in 10, 20 and 50 IU/ml ($p<0.05$). • ↑mRNA and protein expression of **Beclin1**, and **LC3B**, in 20 IU/ml ($p<0.01$). • ↓ **IL-1β** and **IL-8**, in 10 IU/ml, ($p<0.0001$).	In **G1**, the inflammatory environment promoted the enhancement of apoptosis, which compromised the cells viability. The autophagy and angiogenic capacities of PDLSCs were suppressed. In **G2**, the inflammation was mitigated, and the cell apoptosis was soothed. Angiogenic and autophagy markers were upregulated. In **G3**, the group containing the pathway-inhibitor (**G3b**) revealed a downregulation of autophagic and angiogenic factors, and	7 days
	G2: Concentration gradient of EPO + TNF-α (20 ng/mL)		0, 5, 10, 20 and 50 IU/ml	Matrix gel containing EPO			
	G3: Role of Akt/ERK1/2/BAD signaling pathway (**a**): TNF-α (50 ng/ml) (**b**): TNF-α (50 ng/ml) + LY294002 (10 μM) + EPO		(b) and (c): 20 IU/ml	Matrix gel containing EPO			

	(**c**): TNF-α (50 ng/ml) + EPO				**III. Results in G3** • Expression of p-Akt/Akt protein: **G3c**>**G3a**>**G3b**, $p<0.01$. • Expression of p-Erk1/2/Erk1/2 protein: **G3c**>**G3b**>**G3a**, $p<0.01$ • Expression of p-BAD/BAD protein: **G3c**>**G3a**>**G3b**, $p<0.01$. • VEGF-α: **G3c**>**G3a**>**G3b**, $p<0.05$. • FGF-2: **G3c**>**G3a**>**G3b**, $p<0.01$. • IGF-1: **G3c**>**G3a**>**G3b**, $p<0.05$. • Bax/Bcl2: **G3b**>**G3a**>**G3c**, $p<0.01$. • Beclin1: **G3c**>**G3a**>**G3b**, $p<0.01$. • LC3B: **G3c**>**G3a**>**G3b**, $p<0.01$.	a positive regulation of apoptotic marker. This demonstrates that EPO activates the Akt/ERK1/2/BAD pathway to operate.	
	G4: Role of autophagy (**a**): TNF-α (50 ng/ml) (**b**): TNF-α (50 ng/ml) + 3-MA + EPO (**c**): TNF-α (50 ng/ml) + EPO		(b) and (c): 20 IU/ml		**IV. Results in G4** • VEGF-α: **G4c**>**G4a**>**G4b**, $p<0.01$. • FGF-2: **G4c**>**G4a**>**G4b**, $p<0.01$. • IGF-1: **G4c**>**G4a**>**G4b**, $p<0.05$. • Bax/Bcl2: **G4b**>**G4a**>**G4c**, $p<0.05$. • Beclin1: **G4c**>**G4a**>**G4b**, $p<0.05$. • LC3B: **G4c**>**G4a**>**G4b**, $p<0.05$.	In **G4**, by introducing an autophagy inhibitor, we could investigate the ability of EPO to regulate cellular autophagy during apoptosis and angiogenesis (**G4b**). In **G4c**, the EPO-regulated autophagic and angiogenic factors, reduced their expression, and the ratio of apoptotic factors increased.	

TABLE 3. Synthesis of the results of the randomised trials in animals and in humans. **FPH**: hydrogel; **CNP**: ceria nanoparticle; **CEJ**: cementoenamel junction; **AAB**: apex of the alveolar bone; **BV/TV**: bone volume/bone tissue; **Tb.Th**: trabecular thickness; **Tb.N**: trabecular number; **Tb.Sp**: trabecular separation; **OCN**: osteocalcin; **COX-2**: cyclooxygenase-2; **MMP-9**: matrix metalloproteinase-9; **BMD**: bone mineral density; **TMD**: mineral tissue density; **DMSO**: dimethyl sulfoxide; **hPDLF**: human periodontal ligament fibroblasts; **ALP**: alkaline phosphatase; **BMP-2**: bone morphogenic protein-2; **VEGF**: vascular endothelial growth factor; **CD31**: cluster of differentiation 31; **RUNX2**: runt-related transcription factor 2; **MPO**: myeloperoxidase; **OC**: osteoclasts; **PI**: plaque index; **GI**: gingival index; **BI**: bleeding index; **PD**: probing depth; **CAL**: clinical adherence level.

#	Cases		EPO concentration	Administration vehicle	Endpoints and Results	Conclusions	Follow-up
	Groups	**n**					
3	**G1**: Control (Healthy rats without periodontitis)	30 animals (6 each group)	-	-	*Micro-CT analysis* **I. ΔCEJ-AAB** **G1** vs **G2**: Δ1.15 mm, $p<0.01$; **G2** vs **G4**: Δ26.7%, $p<0.05$; **G2** vs **G5**: Δ27.5%, $p<0.05$; **G2** vs **G6**: Δ32.3%, $p<0.05$ **II. ΔBV/TV** **G1** vs **G2**: -Δ41.1%, $p<0.01$ **G1**>**G6**>**G2**, $p<0.05$ **III. ΔTb.Th** **G1** vs **G2**: -Δ52.0%, $p<0.01$ **G1**>**G6**>**G2**, $p<0.05$ **IV. ΔTb.N** **G1** vs **G2**: -Δ22.2%, $p<0.01$ **G1**>**G6**>**G2**, $p<0.05$ **V. ΔTb.Sp** **G1** vs **G2**: Δ60.4%, $p<0.01$ **G2**>**G6**>**G1**, $p<0.05$	The results obtained in **G2** demonstrated that the periodontitis model was well established, allowing a comparison between this group and the treatment groups. The inflammation was suppressed, allowing EPO to act in the sense of regenerating the *periodontium*, as observed mainly in the **G6**.	4 weeks
	G2: Periodontitis group		-	-			
	G3: Periodontitis treated with FPH		-	-			
	G4: Periodontitis treated with CNP@FPH		-	-			
	G5: Periodontitis treated with EPO@FPH		2000 IU/ml	Plastically injectable hydrogel			
	G6: Periodontitis treated with EPO/CNP@FPH		2000 IU/ml				

					H&E staining In **G6**, the destruction was suppressed, and the inflammation dissolved. The bone regeneration was similar to the tridimensional image obtained with micro-CT. *MTC staining* The collagenous fibers were strongly visible and in a well-organized manner in **G6**. *IHC technique* The expression of the osteogenesis marker OCN was highly detected in **G6**, in comparison to **G2**.		
4	**G1**: Control	40 animals (8 each group)	-	-	*IHC technique* **I. Number of COX-2** **G1**: 0; **G2**: 87.5 ± 12.4; **G3**: 80 ± 6.2; **G4**: 19.5 ± 2.9; **G5**: 42.5 ± 11.3; **G6**: 1.5 ± 1.6, (p<0.01) **II. Number of MPP-9** **G1**: 0; **G2**: 58.5 ± 13.7; **G3**: 37.5 ± 5.2; **G4**: 8.5 ± 2.2; **G5**: 32 ± 6.7; **G6**: 15 ± 3.6, (p<0.01) *Micro-CT Analysis* **III. ΔCEJ-AAB** **G1** vs **G2**: Δ1.1-fold greater, p<0.01; **G1** vs **G3**: Δ57.1%, p<0.01; **G1** vs **G4**: Δ36.9%, p<0.05; **G1** vs **G5**: Δ0, p>0.05; **G1** vs **G6**: Δ0, p>0.05 **IV. ΔBMD** **G1** vs **G2**: -Δ9.3%, p<0.01; **G2** vs **G6**: Δ10.3%, p<0.01 **G6**>**G4**>**G5**>**G3**, p<0.05	The inflammation and bone loss values in the untreated periodontitis group (**G2**) indicate that the periodontitis model was successfully achieved. Regarding the anti-inflammatory effects of hydrogel, the most satisfactory values were obtained in **G4** and **G6** in which aspirin was incorporated. In **G6**, periodontal inflammation was controlled. The osteogenic effects of EPO were mostly verified in **G6**, that presented similar results to the control group	2 weeks
	G2: Periodontitis		-	-			
	G3: Hydrogel		-	Hydrogel injected with an insulin syringe			
	G4: Hydrogel + Aspirin		-				
	G5: Hydrogel + EPO		20 units				
	G6: Hydrogel + Aspirin + EPO		20 units				

					V. ΔTMD **G1** vs **G2**: -Δ54.6%, $p<0.01$; **G2** vs **G6**: Δ1.42-fold greater, $p<0.01$ **G6**>**G4**>**G5** and **G3**, $p<0.05$ **VI. ΔBV/TV** **G1** vs **G2**: -Δ68.7%, $p<0.01$; **G2** vs **G6**: Δ3.4-fold greater, $p<0.01$ **G6**>**G4**>**G3**>**G5**, $p<0.01$ **VII. ΔTb.Th.** **G1** vs **G2**: -Δ41.5%, $p<0.05$; **G2** vs **G6**: Δ82.4%, $p<0.05$ **G6**>**G5**>**G3**>**G4**, $p<0.05$ **VIII. ΔTb.N** **G1** vs **G2**: -Δ25.2%, $p<0.05$; **G2** vs **G6**: Δ45.9%, $p<0.01$ **G6**>**G4**>**G3** and **G5**, $p>0.05$ **IX. ΔTb.Sp.** **G1** vs **G2**: Δ1.23 mm, $p<0.01$; **G2** vs **G6**: Δ0, $p>0.05$ **G4**>**G5**>**G3** and **G6**, $p<0.05$ *TRAP staining* **X. Number of osteoclasts** **G1**: 0.7 ± 0.5; **G2**: 16.3 ± 3.3; **G3**: 11.7 ± 2.9; **G4**: 6.3 ± 1.7; **G5**: 6.7 ± 1.2; **G6**: 3 ± 0.8, $p<0.01$	(**G1**), indicating a complete recovery of the periodontium. However, the results were not statistically significant ($p>0.05$). The group treated only with EPO (**G5**) obtained lower results than **G6**, because there wasn´t an effective control of inflammation. The resorptive action common in the periodontitis condition is also diminished in **G6**, since the number of osteoclasts is significantly reduced in relation to **G2**, and even approaching the values of **G1**. Thus, it was demonstrated that the hydrogel loaded with EPO and aspirin also inhibited the resorption function of osteoclasts.	

5	**G1**: Control group	30 animals (15 each group)	-	-	*Real-time PCR* **I. Level of ALP (baseline vs final)** In **hPDLF** cells **G1**: 1 ± 0.18 vs 1 ± 0.1, *p*<0.05; **G2**: 0.90 ± 0.15 vs 2.37 ± 0.79, *p*<0.05 In **MC3T3-E1** cells **G1**: 1 ± 0.26 vs 1 ± 0.21, *p*<0.05; **G2**: 0.70 ± 0.09 vs 2.75 ± 1.01, *p*<0.05 **II. Level of OCN (baseline vs final)** In **hPDLF** cells **G1**: 1 ± 0.06 vs 1 ± 0.06, *p*<0.05; **G2**: 0.92 ± 0.01 vs 2.06 ± 0.36, *p*<0.05 In **MC3T3-E1** cells **G1**: 1 ± 0.13 vs 1 ± 0.19, *p*<0.05; **G2:** 1.01 ± 0.13 vs 1.37 ± 0.10, *p*<0.05 **III. Level of BMP-2 (baseline vs final)** In **hPDLF** cells **G1**: 1 ± 0.01 vs 1 ± 0.06, *p*<0.05; **G2**: 0.96 ± 0.10 vs 1.91 ± 0.32, *p*<0.05 In **MC3T3-E1** cells **G1**: 1 ± 0.08 vs 1 ± 0.27, *p*<0.05; **G2:** 0.95 ± 0.10 vs 3.37 ± 0.65, *p*<0.05 *MTC staining* At day 1, both groups presented residual hPDLF cells, and the sockets were filled with the coagulum. **G2** presented more vascular structures. At day 5, both a new bone formation was identified in both groups. **G2** also revealed spindle-shaped fibroblasts cells, blood vessel-like cells and denser collagen in the newly formed bone. At day 14, both groups presented calcification of bone tissue, but **G2** exhibited a higher sign of bone formation.	Cell proliferation and differentiation rates in the experimental group (**G2**) exceeded those of the control group (**G1**), mainly in osteoblastic lineage cells MC3T3-E1. The processes of osteogenesis and vascular tissue formation were also more significant in **G2**, being again more evident in MC3T3-E1 cells. The bone tissue was mainly located in the central part of the extraction socket, with areas of new collagen formed near vascularised zones and with proliferation and differentiation of osteoblasts. EPO was shown to be able to decrease healing time under inflammatory conditions, maintain cell integrity, increase osteoblast proliferation and differentiation, and promote bone formation by regulating inflammation and angiogenesis. MC3T3-	14 days
	G2: Experimental group		10 IU/ml	Mixture comprising surfactant, EPO and DMSO introduced directly into the socket using a gas-tight Hamilton syringe			

					Immunostaing Between days 1 and 5, **G2** revealed a stronger staining of CD31 and VEGF, and of RUNX2 with newly synthesized collagen bundles. In **G2**, the anti-MPO area was similar or less that in **G1** and was observed a higher number of OC-positive cells. At day 14, new bone was formed in both groups, but only in **G2** was observed an extensive anti-OC immunostained area.	E1 cells responded with greater sensitivity to EPO.	
6	**G1**: Control group	30 ind. (15 each group)	-	-	**I. Level of PI (baseline vs final)** **G1**: 2.71 ± 0.46 vs 1.1 ± 0,68, p=0.705; **G2**: 2.53 ± 0.89 vs 1.1 ± 0,79, p=0.705 **II. Level of GI (baseline vs final)** **G1**: 2.6 ± 0.72 vs 0.98 ± 0.58, p=0.02; **G2**: 2.75 ± 0.75 vs 1.33 ± 0.07, p=0.02 **III. Level of BI (baseline vs final)** **G1**: 2.71 ± 0.65 vs 0.84 ± 0.48, p=0.00; **G2**: 2.53 ± 0.89 vs 1.3 ± 0.79, p=0.00 **IV. Level of PD (baseline vs final)** **G1**: 3.72 ± 0.72 vs 1.95 ± 0.76, p=0.04; **G2**: 3.94 ± 0.78 vs 2.55 ± 0.86, p=0.04 **V. Level of CAL (baseline vs final)** **G1**: 5.1 ± 4.1 vs 3.40 ± 2.71, p=0.02; **G2**: 35.67 ± 4.32 vs 4.33 ± 3.19, p=0.02	In the **G2** group there was complete epithelialization. BI and GI values decreased in both groups, as well as PD and CAL, but especially in **G2**. The Δbaseline-final was statistically significant between groups. The application of EPO allowed a decrease in periodontal inflammation and EPO to achieve more satisfactory results.	3 months
	G2: Test group		4000 units	Gel solution injected into the depth of the pocket using a 28G needle			

CHAPTER IV

DISCUSSION

IV. DISCUSSION

The combination of chemical agents with periodontal treatment has been investigated in order to assist non-surgical treatment and reduce the need to move towards more invasive treatment, as this therapeutic option entails long-term side effects. Some advances have been made but none have yet managed to surpass the results of non-surgical treatment *per se*. Thus, this review aimed to explore whether EPO is effective and reliable to be used adjunctively to periodontal treatment.

4.1. Summary of evidence

This scoping review summarises the scientific evidence from 4 randomised trials and 2 cell culture studies on the advantages of the clinical use of erythropoietin in the treatment of periodontitis, at the cellular, animal, and human levels.

Our findings indicate a very recent line of research with a small number of studies. However, the typical research pathway from cellular studies to human studies can be found. In our review the interactions of EPO with biological processes, such as with immune response, osteogenesis, angiogenesis, and cell proliferation are presented, and the toxicity associated with its use was analysed. Furthermore, a comparison with the conventional scaling and root planning treatment was made, in one clinical study.

Our results suggest that EPO has an anti-inflammatory effect by inhibiting pro-inflammatory cytokines and secreting anti-inflammatory agents, which balances the microenvironment and stops the constant activation of the immune response. It is also suggested that EPO influences the mechanism of osteogenesis by achieving homeostasis of the bone formation, and EPO also acts to regulate angiogenesis, which is a key step in preventing bone necrosis.

The small number of studies and samples and the great variety of methodologies limits the generalisation of results. Despite these challenges, the clinical benefits that EPO brings offer a new perspective for periodontal treatment.

4.1.1. Control of the inflammatory environment

Periodontal inflammation begins with a slow and steady bacterial colonisation of the tooth surface and below the gingival margin, which is exacerbated by the host inflammatory response. Cell permeability increases, which triggers microbial dysbiosis. In the innate immune response, bacteria are able to stimulate leukocytes to produce pro-inflammatory mediators, such as cytokines and chemokines, including the tumour necrosis factor (TNF-α), interleukins (IL-1β, IL-6, IL-8, IL-12, IL-17), and the receptor activator of the nuclear factor kappa B ligand (RANK-L). The release of these factors contributes to the progression of the condition and activation of the acquired immune response. This uncontrolled activation causes the irreversible destruction of the hard and soft periodontal tissues, leading to the appearance of periodontal pockets, attachment loss, gingival recession, mobility, tooth migration and tooth loss. (25) Therefore, the first step to treat periodontitis is to control the inflammation.

When bacterial colonisation occurs, lipopolysaccharides (LPS) are released and cause a disruption of homeostasis by the exaggerated accumulation of reactive oxide species (ROS). An oxidative stress is formed, triggering a sustained inflammatory response and molecular damage. (37) ROS are capable to desregulate the polarization between proinflammatory (M1) and anti-inflammatory macrophages (M2), used to analyse the inflammatory condition. (20) The pathogenic activity of periodontitis described so far has been summarised in the following diagram (**FIGURE 3**).

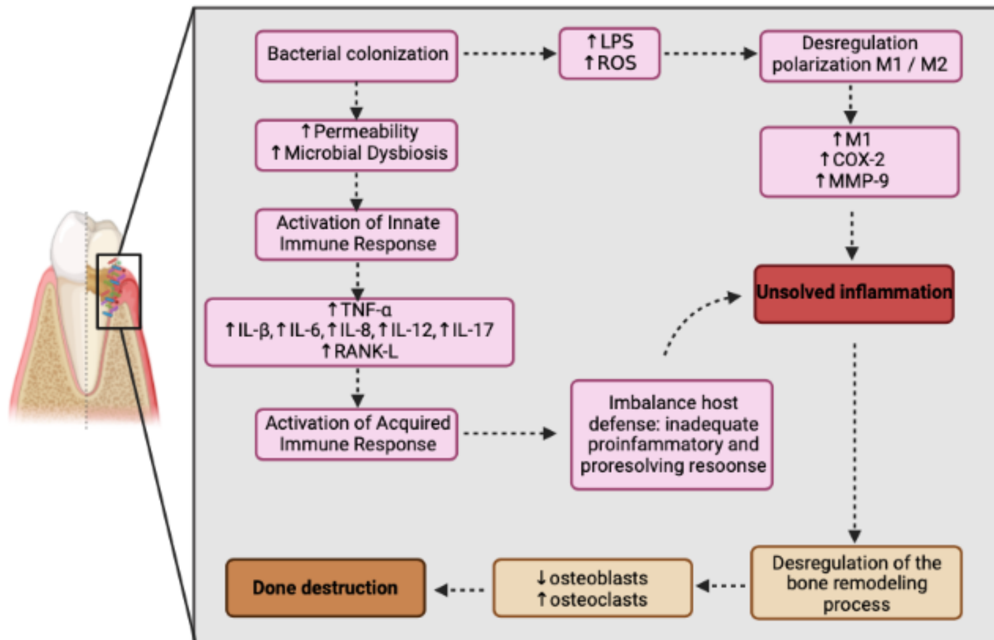

FIGURE 3. Overview of the etiopathogenesis of periodontitis. Microbial dysbiosis and maintenance of pro-inflammatory mechanisms, which complicates the monitoring of disease activity. Diagram adapted by ISP from Martínez-García & Herández-Lemus, 2021 (25).

Our results suggest that erythropoietin (EPO) presents an anti-inflammatory effect. In EPO-treated cases, there is a downregulation of pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8 and TNF-α, and a secretion of the transforming growth factor beta (TGF-β), IL-10 and arginase 1 (Arg-1), anti-inflammatory cytokines that can help in the recovery of the *periodontium*. (14, 20) Thus, EPO is able to restore homeostasis and to shift the macrophage polarization from M1 to M2 by controlling the level of ROS and supressing the proliferation of LPS. (29) The number of cyclooxgygense-2 (COX-2) and matrix metalloproteinase 9 (MMP-9) positive cells, two inflammatory markers, (22, 43) also decreased after EPO application.

Moreover, in the study developed by **Wang *et al.* (2018)**, the runt-related transcription factor (RUNX2), an osteogenic marker involved in promoting bone morphogenic protein levels and expressed at the end of osteoblast differentiation, was highly detected. Previous studies have shown that proinflammatory T cells stimulate the production of TNF-α which negatively regulates expression of RUNX2. The inflammatory factor inhibits osteoblastic function and stimulates the osteoclasts resorption, impairing bone formation at the cellular level mediated by

MSCs. (23) In a medium containing EPO, RUNX2 values increased compared to a medium without the hormone, suggesting that EPO has the capacity to supress the expression of TNF-α and promote the proliferation of osteoblasts. (46)

4.1.2. Effects on the osteogenesis

The first known function of EPO was its regulatory power in the erythropoiesis process. Years later, it was discovered that its application in clinical practice to treat anaemic conditions allowed these patients to be treated. (26) More recently, EPO receptors (EPOR) have been detected in several cells and tissues, in addition to erythroid progenitors in bone marrow, and their effects have been investigated. The presence of EPOR in non-erythroid cells justifies the implications of EPO beyond erythropoiesis. (38) Among these cells, we find dental mesenchymal stem cells (MSCs). (41) These are multipotent cells, which have no maximum limit of mitotic divisions, and can originate and regulate skeletal tissues, including the formation of osteoblasts and osteoclasts, two important cells in the osteogenesis. (33)

In periodontitis, there is a deregulation of the bone formation and destruction process, and osteoclasts are overexpressed. The space left by them is occupied by connective tissue and gingival epithelium, hindering the bone formation activity by osteoblasts. (42)

Studies *in vitro* have investigated the behaviour of EPO in bone remodelling in human periodontal ligament tissue-derived mesenchymal stem cells (hPDLSCs). The reason for their research is related to the fact that these are a type of MSCs, which allows them to be isolated and expanded from the periodontal ligament and differentiate into various types of cells, according to the stimulus received. Another property of hPDLSCs, demonstrated *in vitro* and *in vivo,* is their ability to differentiate into a periodontal ligament and cementum-like tissues, to which collagen fibres are attached, similar to Sharpey's fibres. In this way, connections are created between these tissues, suggesting a strong possibility of obtaining true regeneration of the *periodontium* and treating periodontal diseases. (34)

Wang *et al.* (2018) sought to demonstrate that the addition of EPO to a biomaterial could increase the osteogenic capacities of these cells and be used as a therapeutic option for periodontitis. Therefore, hPDLSCs under normal conditions and periodontitis mesenchymal stem cells (pPDLSCs) were cultured. When hPDLSCs were exposed to long periods of inflammation, osteogenesis was negatively compromised. However, their association with EPO increased the proliferation rate, especially the pPDLSCs, as well as upregulated osteogenesis by inducing the expression of osteogenic genes in both cells, proving it promotes bone remodelling under inflammatory conditions. (46)

Similar results were obtained in **Y. Li, H. Peng, W. Tang *et al.* (2023)** and **Bae *et al.* (2022)** trials. Under inflammatory conditions *in vitro*, the expression of osteogenic markers, such as the alkaline phosphatase (ALP), osteocalcin (OCN), bone morphogenic protein 2 (BMP-2) and RUNX2, was strongly detected in the groups treated with EPO. Furthermore, was found the presence of cell mineralized nodule, an important osteoblastic maturation marker, a significant calcium deposition and bone tissue with dense collagen. These results support the hypothesis that EPO can reverse the differentiational capacity of PDLSCs, favouring bone formation. Besides, spindle-shaped fibroblasts cells were also identified, which supports the theory that there is simultaneous bone and tissue regeneration. Nevertheless, *in vivo* experiments, as the ones conducted by **Y. Li, H. Peng, W. Tang *et al.* (2023)** and **X. Xu *et al.* (2019)**, bone regeneration presented a more favourable increase when EPO was combined with an anti-inflammatory agent. (3, 20, 47)

CNP has recently received attention in clearing inflammation due to its ability to eliminate ROS through the conversion, by catalase and superoxide dismutase, of Ce^{3+} into Ce^{4+} electrons and the reversible binding of oxygen atoms. (48) Furthermore, CNP has been shown to have the ability to regulate macrophage activation by converting M1, which are activated by ROS and accelerate periodontal destruction, into M2. Therefore, **Y. Li, H. Peng, W. Tang *et al.* (2023)** suggested the combined use of CNP and EPO to simultaneously supress inflammation and stimulate osteogenic effects. The evaluation of bone quality parameters revealed more promising results when EPO and CNP were

associated than when they were used alone. There was a remarkable recovery of bone height and no sign of inflammatory cells, implying that the effects of EPO are potentiated when inflammation is controlled. New collagen bridges were formed, increasing in number and with a well-organized arrangement. There was also deposition of extracellular matrix and a raised expression of the marker OCN. These results are promising for the selection of this method for the treatment of periodontitis. (20)

On the other hand, **X. Xu *et al.* (2019)** opted for the incorporation of aspirin as the anti-inflammatory component. Aspirin has been required for several treatments due to its anti-inflammatory and analgesic properties. Its mechanism of action involves the inhibition of the metabolism of arachidonic acid through the acetylation of cyclooxygenase-1 (COX-1) and COX-2. This action causes the inhibition of prostaglandin E2 and the inactivation of MMP-9, which consequently prevents the formation of thromboxane. This vasoconstrictor is produced by platelets for their activation and aggregation. However, the lack of it prevents a clot from forming, which demonstrates that aspirin also exerts an anti-coagulant effect. (18, 47) Since EPO and aspirin have a short half-life, administration must be controlled and maintained over time until the objectives are achieved. (6, 9) Therefore, they were mixed with a hydrogel, which has a high biological compatibility and adapts to the irregularities of the bone wall.

The realising curves of aspirin and EPO were measured, reaching respectively 86.6% and 69.4% in the first three days, and 94.2% and 83.4% at day 8. The small molecular structure of aspirin is the reason for this discrepancy. However, the faster release of aspirin becomes advantageous for the promotion of osteogenesis by EPO for the following reasons: (i) inhibits inflammatory cytokines, (ii) presents a chemotactic capacity that accelerates tissue regeneration, and (iii) local drug concentration remains unaltered due to localised and sustained deposition. (4, 21) After achieving a controlled periodontal microenvironment, a synergic action with EPO was created and the bone recovery was reached. The proof of this interaction was that the results obtained in the group treated with EPO were similar to the group that only contained hydrogel, while the group treated with aspirin and EPO had similar outcomes to the control group. Newly formed bone and vascular structures, residual presence

of osteoclasts and a recovery of the alveolar bone in the furcation zone and between the first and second maxillary molars were detected. (47)

The osteogenic capacity of EPO is explained through the activation of the ephirinB2/EphB4 signalling pathway that regulates bone homeostasis. Studies revealed that EPO directly stimulates osteoblastic differentiation and the expression of osteoblastic phenotypes, and indirectly softens osteoclasts resorption, leading to an EPO-mediated bone remodelling. (19) This function is also enhanced through the activation of the p-38 MAPK pathway by EPO. (28) The interaction was tested by **Wang *et al.* (2018)**, when a pathway-specific inhibitor was introduced, and the osteogenic effects of EPO were attenuated. (46)

4.1.3. Capacity to modulate angiogenesis

To ensure bone regeneration, blood vessels must be formed to secure cell nutrition and avoid bone necrosis. However, its activation depends on hypoxia and pro-angiogenic stimuli, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), transforming growth factor-beta (TGF-β), insulin like growth factor (IGF-1) and EPO. (45)

During a periodontal infection, bacteria release their acids and products, which causes a decrease in oxygen concentration. (7) This allows the proliferation of anaerobic species, that are essential to sustain the infection and cause cellular and vascular changes. (39) In a situation of hypoxia, EPO expression is stimulated, which causes a greater release of red blood cells into the bloodstream. Thus, EPO is responsible for the activation of the angiogenesis process and helps bone reconstruction by promoting the transport and supply of oxygen to proliferating and differentiating cells. (50)

In the study of **Y. Li, H. Peng, W. Tang *et al.* (2023)**, the angiogenic capacity of EPO in an inflammatory situation was assessed in human umbilical vein endothelial cells (HUVECs), in which an EPOR can be found. In the EPO-induced groups, increased tube formation was observed in HUVECs, as well as an up-regulation of gene expression of the blood vessel markers VEGF, basic

fibroblast growth factor (bFGF) and ephrin type-B receptor 4 (EphB4). VEGF is also involved in reducing the apoptosis process, which contributes to the proliferation of these cells. Increased vascularisation of the area and suppression of apoptosis, which are necessary steps for osteogenesis, were promoted due to the presence of EPO. (20)

In another study *in* vitro carried out by **H. Huang, L. Jie, L. Xingrui *et al.* (2022)**, an inflammatory environment was created by promoting a high concentration of TNF-α levels, and the effects of EPO on PDLSCs were studied. Cell proliferation was up-regulated, as well as the expression of VEGF, FGF-2 and IGF-1, indicating that angiogenesis had been triggered. In addition, the ratio between B-cell lymphoma associated X (Bax) and B-cell lymphoma protein 2 (Bcl2), proteins that activate the apoptotic cascade (16), decreased. Enhanced gene expression of Beclin1 and light chain 3 beta (LC3B), two proteins regulated by autophagy (8), was also detected. Thus, it is suggested that EPO stimulates PDLSCs angiogenesis and autophagy, as well as suppresses apoptosis, which is very promising for periodontitis control. To find the pathway involved in these biological changes and which receives activation by EPO, the Akt pathway was studied, since it had already been pointed out as being responsible by other researchers. (35) Upon EPO treatment, there was significant activation of this pathway, which was determined on RNA-sequencing, as well as Erk1/2 and BAD. While Akt mainly targeted Beclin1 and LC3B, Erk1/2 was able to regulate VEGF, FGF-2 and IGF-1, and BAD was responsible for the Bax/Bcl2 ratio. Therefore, all three processes were activated by the Akt/Erk1/2/BAD pathway. (14)

Angiogenesis was also measured in vivo by counting the number of CD31 and VEGF blood vessels markers, in **Bae *et al.* (2022)** study. CD31 is a transmembrane glycoprotein used for vascular differentiation, due to its sensitivity and specificity, and is expressed by endothelial cells. (44) These factors were identified mainly in the EPO-treated groups in the central zone of the tooth root socket, located in the vicinity of newly synthesised collagen bundles. Once again, is suggested the ability of EPO to increase microcirculation, which promotes angiogenesis and, consequently, osteogenesis, two important steps for a more effective periodontal treatment. (3)

The following image summarises the interactions of EPO discussed and presented in the topics above that help clear inflammation and enable more effective periodontal treatment (**FIGURE 4**).

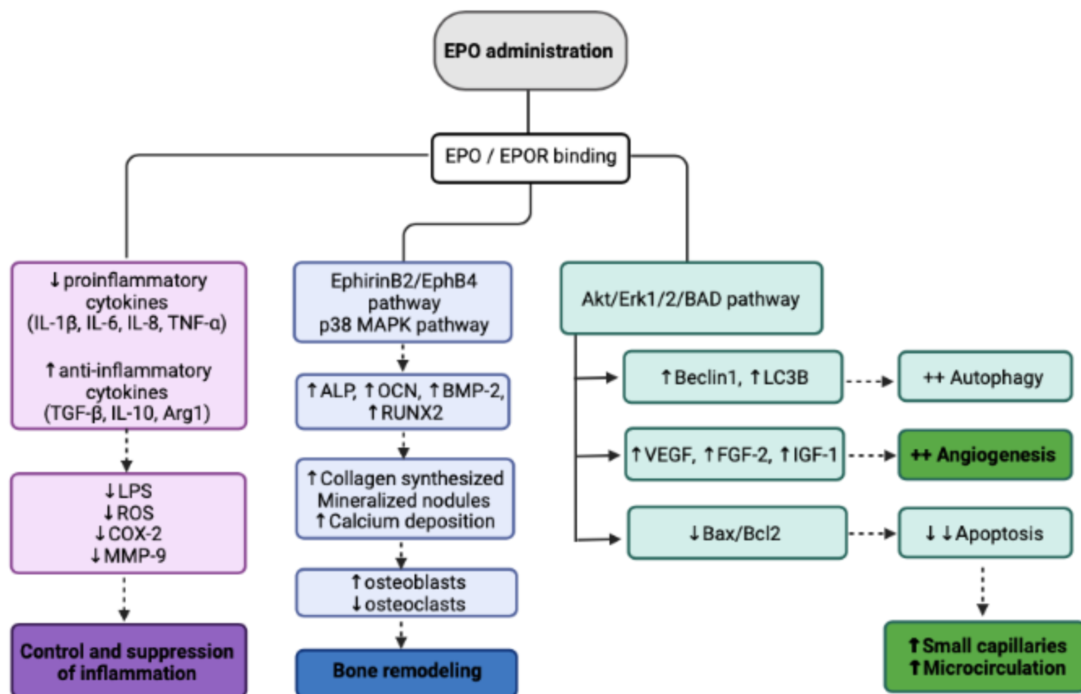

FIGURE 4. Biological mechanisms of erythropoietin when in a periodontitis setting, in order to decrease inflammation and contribute to bone and periodontal restitution. Original diagram made by ISP.

4.1.4. Nonsurgical management of periodontitis with and without EPO administration

Non-surgical treatment emerges with the main objective of eliminating the existing bacterial *biofilm* and calculus in order to control the microbial periodontal infection. (1) This arises as a therapeutic option to improve periodontal parameters, in a second phase of treatment, after a first attempt, keeping the patient in phage directed towards the cause.

At this point in the treatment, scaling and root planning are repeated, and oral hygiene instructions reinforced. In addition, the application of an adjuvant agent to the treatment may be chosen. This approach is considered necessary

when at periodontal re-evaluation, the patient cannot achieve periodontal pockets ≤4 mm, with bleeding on probing, or pockets <6 mm deep. (32)

In the systematic review conducted by **Silvestre *et al.* (2016)**, it was suggested that the treatment of periodontal disease could help reduce periodontal inflammation through preventive measures and the application of conservative treatment. At the end of the review, they concluded that after non-surgical periodontal treatment of scaling and root planning, the evaluated periodontal parameters – bleeding on probing, pocket depth and attachment loss – clearly improved and reduced systemic inflammation values in patients with rheumatoid arthritis. (36)

Aslroosta *et al.* (2021) tested the efficacy of combining non-surgical treatment with EPO, assessing gingival inflammation and periodontal disease factors. There was a significant recovery, compared to the group that used conventional treatment, of the bleeding index (BI), gingivitis index (GI), clinical attachment loss (CAL) and probing depth (PD) factors. These findings are the result of the effects of EPO explained above. The resolution of inflammation allowed cell proliferation and differentiation, which led to the synthesis of new collagen bundles, and periodontal and bone tissues. This enabled BI and GI, indices of gingival inflammation, to recover and, in addition, the level of CAL to decrease. Furthermore, by having a bone recovery at the site of EPO application in the periodontal pocket, as well as a controlled inflammatory response and gingival recession resulting from periodontitis, PD also decreases.

However, the plaque index was not statistically significant, indicating that EPO is not involved in the destruction of microbial plaque, requiring an auxiliary medium to carry out this part. Thus, by associating a non-surgical treatment that reduces the inflammation produced and removes the bacterial population with a hormone with anti-inflammatory, osteogenic and angiogenic capacities, offers promising results. The need for surgical treatment was reduced from 52% in the control group, to 48% in the group treated with EPO. (2)

4.1.5. Analysis of toxicity *in vivo* associated with the use of erythropoietin

The toxicity and inflammatory reactions of EPO *in vivo* were investigated in **X. Xu *et al.* (2019)** study. The researchers injected the pre-gel solution into the dorsal subcutaneous area of Wistar rats. On contact with body temperature, the solution changed to the hydrogel state, adapting to the irregularities of the surface. Macroscopically, there were no signs of local redness, swelling, abscess or necrosis. By H&E technique, pre-gel was detected in a well delineated area and there was no leakage to neighbouring structures before its transformation into hydrogel. Only remnants of COX-2 positive cells were found around the marked area, indicating that there was no associated inflammatory response.

Major organs such as heart, liver, spleen, and kidneys were collected and analysed. No abnormalities or pathological changes were found, suggesting good biocompatibility. Furthermore, by comparing macroscopic photographs of the application *in situ* volume and histological images, it was demonstrated that the hydrogel was able to be effectively degraded. (47)

Some studies have reported effective recovery after ischaemic neuronal damage through systemic administration of EPO. However, these trials were conducted in animal models, not ensuring the safety of systemic application of EPO. (5) Therefore, until more research is done in this direction, a topical and controlled application is the most appropriate option.

4.2. Limitations

Our results show that the available evidence is based on a limited number of studies. In addition, some limitations of the studies should be highlighted: the use of a wide variety of methods and materials, as well as their concentrations, measurement techniques, assessment time and short follow-up period, create some gaps and variations in the studies, as there is a lack of a pre-established protocol to guide the researchers. The limited number of samples also limits the generalisability of the results. Assuming that these observed effects of EPO in the *periodontium* are somehow indicative of clinical benefit, targeting the host response offers an exciting new perspective in periodontal treatment that needs confirmation in large-scale studies.

An important drawback is the local concentration of EPO. Some studies (2, 3) have failed to find a system that allows slow and controlled release of EPO at the site of action, and the effect is lost more quickly. Even with slow-release systems (20, 47), the continuous flow of crevicular fluid could flush the product out at a rate too fast to achieve significant clinical benefits. Repeated administration can lead to EPO passage into the bloodstream, and the increased concentration of circulating EPO can have harmful side effects for the patient, including cardiovascular problems. (24)

It is also important to emphasise that cutting-edge research is more susceptible to publication bias. Studies with positive results are more likely to be published and published more quickly than studies with negative results.

4.3. Conclusions and future perspectives

Over the past few years, the study of periodontal disease activity has led to the introduction of topical and systemic adjuvant pharmacotherapies to modulate the host response. However, to date, no alternative has been able to overcome the results of non-surgical periodontal treatment alone. More recently, EPO has shown promising results in epithelial regeneration and healing, due to its molecular and biological interactions, which becomes very interesting for the treatment of periodontitis.

In conclusion, the best available evidence suggests that the use of EPO in periodontal treatment can be feasible and clinically relevant. Its anti-inflammatory effect can be achieved by the use of the hormone alone, or in combination with anti-inflammatory methods such as chemical agents released in a sustained and controlled manner, or with conventional non-surgical treatment itself. After the reduction of pro-inflammatory stimuli, the cytoprotective capabilities of EPO began to be verified, developing a favourable environment for bone formation. Another particularity identified was the increase in microcirculation and in the number of small capillaries, suggesting that EPO also participates in angiogenesis, nourishing the newly formed structures. Knowing that osteogenesis and angiogenesis go hand in hand, it was found that the presence of the hormone creates an opportunity for new regeneration, reducing the signs and symptoms of periodontitis. Furthermore, when placed directly into the periodontal pocket, it resists the hypoxic conditions created by the released bacterial acids, reaching the deeper areas, which allows the regeneration of bone defects.

Large-scale studies, particularly randomised clinical trials, are encouraged as this method is biocompatible, non-toxic and allows resolution of inflammation and bone destruction. It also has the potential to reduce the percentage of severe disease, thereby improving patients' quality of life.

REFERENCES

REFERENCES

1. Aimetti M. Nonsurgical periodontal treatment. Int J Esthet Dent. 2014 Summer;9(2):251-67. PMID: 24765632.
2. Aslroosta H, Yaghobee S, Akbari S, Kanounisabet N. The effects of topical erythropoietin on non-surgical treatment of periodontitis: a preliminary study. BMC Oral Health. 2021 May 6;21(1):240. doi: 10.1186/s12903-021-01607-y. PMID: 33957902; PMCID: PMC8101234.
3. Bae JE, Hwang SM, Aryal YP, Kim TY, Sohn WJ, An SY, Kim JY, An CH, Lee Y, Kim YG, Park JW, Lee JM, Kim JY, Suh JY. Effects of erythropoietin on osteoblast in the tooth extraction socket in mice periodontitis model. Front Physiol. 2022 Oct 6;13:987625. doi: 10.3389/fphys.2022.987625. PMID: 36277197; PMCID: PMC9582603.
4. Berk M, Dean O, Drexhage H, McNeil JJ, Moylan S, O'Neil A, Davey CG, Sanna L, Maes M. Aspirin: a review of its neurobiological properties and therapeutic potential for mental illness. BMC Med. 2013 Mar 18;11:74. doi: 10.1186/1741-7015-11-74. PMID: 23506529; PMCID: PMC3751197.
5. Brines ML, Ghezzi P, Keenan S, Agnello D, de Lanerolle NC, Cerami C, Itri LM, Cerami A. Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. Proc Natl Acad Sci U S A. 2000 Sep 12;97(19):10526-31. doi: 10.1073/pnas.97.19.10526. PMID: 10984541; PMCID: PMC27058.
6. Cao X, Chen Z, Yu Z, Ge Y, Zeng X. Pharmacokinetics of PEGylated Recombinant Human Erythropoietin in Rats. J Anal Methods Chem. 2014;2014:918686. doi: 10.1155/2014/918686. Epub 2014 Jan 9. PMID: 24575314; PMCID: PMC3913096.
7. Celik D, Kantarci A. Vascular Changes and Hypoxia in Periodontal Disease as a Link to Systemic Complications. Pathogens. 2021 Oct 5;10(10):1280. doi: 10.3390/pathogens10101280. PMID: 34684229; PMCID: PMC8541389.
8. Chifenti B, Locci MT, Lazzeri G, Guagnozzi M, Dinucci D, Chiellini F, Filice ME, Salerno MG, Battini L. Autophagy-related protein LC3 and Beclin-1 in the first trimester of pregnancy. Clin Exp Reprod Med. 2013 Mar;40(1):33-7. doi: 10.5653/cerm.2013.40.1.33. Epub 2013 Mar 31. PMID: 23614114; PMCID: PMC3630291.

9. Dovizio M, Tacconelli S, Sostres C, Ricciotti E, Patrignani P. Mechanistic and pharmacological issues of aspirin as an anticancer agent. Pharmaceuticals (Basel). 2012 Dec 5;5(12):1346-71. doi: 10.3390/ph5121346. PMID: 24281340; PMCID: PMC3816673.
10. Figuero E, Roldán S, Serrano J, Escribano M, Martín C, Preshaw PM. Efficacy of adjunctive therapies in patients with gingival inflammation: A systematic review and meta-analysis. J Clin Periodontol. 2020 Jul;47 Suppl 22:125-143. doi: 10.1111/jcpe.13244. PMID: 31869441.
11. Gasner NS, Schure RS. Periodontal Disease. 2022 Aug 16. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 32119477.
12. Haas AN, Furlaneto F, Gaio EJ, Gomes SC, Palioto DB, Castilho RM, Sanz M, Messora MR. New tendencies in non-surgical periodontal therapy. Braz Oral Res. 2021 Sep 24;35(Supp 2):e095. doi: 10.1590/1807-3107bor-2021.vol35.0095. PMID: 34586209.
13. Hegde R, Awan KH. Effects of periodontal disease on systemic health. Dis Mon. 2019 Jun;65(6):185-192. doi: 10.1016/j.disamonth.2018.09.011. Epub 2018 Oct 29. PMID: 30384973.
14. Huang D, Lei J, Li X, Jiang Z, Luo M, Xiao Y. Erythropoietin Activates Autophagy to Regulate Apoptosis and Angiogenesis of Periodontal Ligament Stem Cells via the Akt/ERK1/2/BAD Signaling Pathway under Inflammatory Microenvironment. Stem Cells Int. 2022 Sep 20;2022:9806887. doi: 10.1155/2022/9806887. PMID: 36199627; PMCID: PMC9527112.
15. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers. 2017 Jun 22;3:17038. doi: 10.1038/nrdp.2017.38. PMID: 28805207.
16. Kulsoom B, Shamsi TS, Afsar NA, Memon Z, Ahmed N, Hasnain SN. Bax, Bcl-2, and Bax/Bcl-2 as prognostic markers in acute myeloid leukemia: are we ready for Bcl-2-directed therapy? Cancer Manag Res. 2018 Mar 2;10:403-416. doi: 10.2147/CMAR.S154608. PMID: 29535553; PMCID: PMC5841349.
17. Kwon T, Lamster IB, Levin L. Current Concepts in the Management of Periodontitis. Int Dent J. 2021 Dec;71(6):462-476. doi: 10.1111/idj.12630. Epub 2021 Feb 19. PMID: 34839889; PMCID: PMC9275292.

18. Levine LD, Holland TL, Kim K, Sjaarda LA, Mumford SL, Schisterman EF. The role of aspirin and inflammation on reproduction: the EAGeR trial [1]. Can J Physiol Pharmacol. 2019 Mar;97(3):187-192. doi: 10.1139/cjpp-2018-0368. Epub 2018 Dec 18. PMID: 30562044; PMCID: PMC8330243.
19. Li C, Shi C, Kim J, Chen Y, Ni S, Jiang L, Zheng C, Li D, Hou J, Taichman RS, Sun H. Erythropoietin promotes bone formation through EphrinB2/EphB4 signaling. J Dent Res. 2015 Mar;94(3):455-63. doi: 10.1177/0022034514566431. Epub 2015 Jan 13. PMID: 25586589; PMCID: PMC4336159.
20. Li Y, Peng H, Tang W, Gu D, Ren S, Yu Y, et al. Accelerating periodontal regeneration through injectable hydrogel-enabled sequential delivery of nanoceria and erythropoietin Yuy. Materials & Design. 2023;225.
21. Liu H, Li W, Liu Y, Zhang X, Zhou Y. Co-administration of aspirin and allogeneic adipose-derived stromal cells attenuates bone loss in ovariectomized rats through the anti-inflammatory and chemotactic abilities of aspirin. Stem Cell Res Ther. 2015 Oct 16;6:200. doi: 10.1186/s13287-015-0195-x. PMID: 26474767; PMCID: PMC4609080.
22. López DE, Ballaz SJ. The Role of Brain Cyclooxygenase-2 (Cox-2) Beyond Neuroinflammation: Neuronal Homeostasis in Memory and Anxiety. Mol Neurobiol. 2020 Dec;57(12):5167-5176. doi: 10.1007/s12035-020-02087-x. Epub 2020 Aug 29. PMID: 32860157.
23. Ma J, Wang Z, Zhao J, Miao W, Ye T, Chen A. Resveratrol Attenuates Lipopolysaccharides (LPS)-Induced Inhibition of Osteoblast Differentiation in MC3T3-E1 Cells. Med Sci Monit. 2018 Apr 6;24:2045-2052. doi: 10.12659/msm.905703. PMID: 29624568; PMCID: PMC5903312.
24. Ma Y, Zhou Z, Yang GY, Ding J, Wang X. The Effect of Erythropoietin and Its Derivatives on Ischemic Stroke Therapy: A Comprehensive Review. Front Pharmacol. 2022 Feb 17;13:743926. doi: 10.3389/fphar.2022.743926. PMID: 35250554; PMCID: PMC8892214.
25. Martínez-García M, Hernández-Lemus E. Periodontal Inflammation and Systemic Diseases: An Overview. Front Physiol. 2021 Oct 27;12:709438. doi: 10.3389/fphys.2021.709438. PMID: 34776994; PMCID: PMC8578868.
26. Mudalagiri NR, Mocanu MM, Di Salvo C, Kolvekar S, Hayward M, Yap J, Keogh B, Yellon DM. Erythropoietin protects the human myocardium against

hypoxia/reoxygenation injury via phosphatidylinositol-3 kinase and ERK1/2 activation. Br J Pharmacol. 2008 Jan;153(1):50-6. doi: 10.1038/sj.bjp.0707461. Epub 2007 Oct 22. PMID: 17952111; PMCID: PMC2199395.

27. Munn Z, Peters MDJ, Stern C, Tufanaru C, McArthur A, Aromataris E. Systematic review or scoping review? Guidance for authors when choosing between a systematic or scoping review approach. BMC Med Res Methodol. 2018 Nov 19;18(1):143. doi: 10.1186/s12874-018-0611-x. PMID: 30453902; PMCID: PMC6245623.
28. Nagata Y, Moriguchi T, Nishida E, Todokoro K. Activation of p38 MAP kinase pathway by erythropoietin and interleukin-3. Blood. 1997 Aug 1;90(3):929-34. PMID: 9242520.
29. Nairz M, Sonnweber T, Schroll A, Theurl I, Weiss G. The pleiotropic effects of erythropoietin in infection and inflammation. Microbes Infect. 2012 Mar;14(3):238-46. doi: 10.1016/j.micinf.2011.10.005. Epub 2011 Nov 4. PMID: 22094132; PMCID: PMC3278592.
30. Nazir M, Al-Ansari A, Al-Khalifa K, Alhareky M, Gaffar B, Almas K. Global Prevalence of Periodontal Disease and Lack of Its Surveillance. ScientificWorldJournal. 2020 May 28;2020:2146160. doi: 10.1155/2020/2146160. PMID: 32549797; PMCID: PMC7275199.
31. Peters MD, Godfrey CM, Khalil H, McInerney P, Parker D, Soares CB. Guidance for conducting systematic scoping reviews. Int J Evid Based Healthc. 2015 Sep;13(3):141-6. doi: 10.1097/XEB.0000000000000050. PMID: 26134548.
32. Sanz M, Herrera D, Kebschull M, Chapple I, Jepsen S, Beglundh T, Sculean A, Tonetti MS; EFP Workshop Participants and Methodological Consultants. Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. J Clin Periodontol. 2020 Jul;47 Suppl 22(Suppl 22):4-60. doi: 10.1111/jcpe.13290. Erratum in: J Clin Periodontol. 2021 Jan;48(1):163. PMID: 32383274; PMCID: PMC7891343.
33. Sasaki R, Masuda S, Nagao M. Erythropoietin: multiple physiological functions and regulation of biosynthesis. Biosci Biotechnol Biochem. 2000 Sep;64(9):1775-93. doi: 10.1271/bbb.64.1775. PMID: 11055378.

34. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 2004 Jul 10-16;364(9429):149-55. doi: 10.1016/S0140-6736(04)16627-0. PMID: 15246727.
35. Shi L, Li B, Zhang B, Zhen C, Zhou J, Tang S. Mouse embryonic palatal mesenchymal cells maintain stemness through the PTEN-Akt-mTOR autophagic pathway. Stem Cell Res Ther. 2019 Jul 29;10(1):217. doi: 10.1186/s13287-019-1340-8. PMID: 31358051; PMCID: PMC6664599.
36. Silvestre FJ, Silvestre-Rangil J, Bagán L, Bagán JV. Effect of nonsurgical periodontal treatment in patients with periodontitis and rheumatoid arthritis: A systematic review. Med Oral Patol Oral Cir Bucal. 2016 May 1;21(3):e349-54. doi: 10.4317/medoral.20974. PMID: 26946202; PMCID: PMC4867209.
37. Sui L, Wang J, Xiao Z, Yang Y, Yang Z, Ai K. ROS-Scavenging Nanomaterials to Treat Periodontitis. Front Chem. 2020 Nov 4;8:595530. doi: 10.3389/fchem.2020.595530. PMID: 33330384; PMCID: PMC7672210.
38. Suresh, S., de Castro, L.F., Dey, S. *et al.* Erythropoietin modulates bone marrow stromal cell differentiation. *Bone Res* **7**, 21 (2019). https://doi.org/10.1038/s41413-019-0060-0
39. Tanner AC. Anaerobic culture to detect periodontal and caries pathogens. J Oral Biosci. 2015 Feb;57(1):18-26. doi: 10.1016/j.job.2014.08.001. PMID: 25678835; PMCID: PMC4321760.
40. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, Moher D, Peters MDJ, Horsley T, Weeks L, Hempel S, Akl EA, Chang C, McGowan J, Stewart L, Hartling L, Aldcroft A, Wilson MG, Garritty C, Lewin S, Godfrey CM, Macdonald MT, Langlois EV, Soares-Weiser K, Moriarty J, Clifford T, Tunçalp Ö, Straus SE. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. Ann Intern Med. 2018 Oct 2;169(7):467-473. doi: 10.7326/M18-0850. Epub 2018 Sep 4. PMID: 30178033.
41. Tsiftsoglou AS. Erythropoietin (EPO) as a Key Regulator of Erythropoiesis, Bone Remodeling and Endothelial Transdifferentiation of Multipotent Mesenchymal Stem Cells (MSCs): Implications in Regenerative Medicine. Cells. 2021 Aug 20;10(8):2140. doi: 10.3390/cells10082140. PMID: 34440909; PMCID: PMC8391952.

42. Usui M, Onizuka S, Sato T, Kokabu S, Ariyoshi W, Nakashima K. Mechanism of alveolar bone destruction in periodontitis - Periodontal bacteria and inflammation. Jpn Dent Sci Rev. 2021 Nov;57:201-208. doi: 10.1016/j.jdsr.2021.09.005. Epub 2021 Oct 13. PMID: 34703508; PMCID: PMC8524191.
43. Vafadari B, Salamian A, Kaczmarek L. MMP-9 in translation: from molecule to brain physiology, pathology, and therapy. J Neurochem. 2016 Oct;139 Suppl 2:91-114. doi: 10.1111/jnc.13415. Epub 2016 Mar 21. PMID: 26525923.
44. Vanchinathan V, Mirzamani N, Kantipudi R, Schwartz EJ, Sundram UN. The vascular marker CD31 also highlights histiocytes and histiocyte-like cells within cutaneous tumors. Am J Clin Pathol. 2015 Feb;143(2):177-85; quiz 305. doi: 10.1309/AJCPRHM8CZH5EMFD. Erratum in: Am J Clin Pathol. 2015 Jun;143(6):911. Mizramani, Neda [corrected to Mirzamani, Neda]. PMID: 25596243.
45. Wan L, Zhang F, He Q, Tsang WP, Lu L, Li Q, Wu Z, Qiu G, Zhou G, Wan C. EPO promotes bone repair through enhanced cartilaginous callus formation and angiogenesis. PLoS One. 2014 Jul 8;9(7):e102010. doi: 10.1371/journal.pone.0102010. Erratum in: PLoS One. 2014;9(10):e111830. PMID: 25003898; PMCID: PMC4087003.
46. Wang L, Wu F, Song Y, Duan Y, Jin Z. Erythropoietin induces the osteogenesis of periodontal mesenchymal stem cells from healthy and periodontitis sources via activation of the p38 MAPK pathway. Int J Mol Med. 2018 Feb;41(2):829-835. doi: 10.3892/ijmm.2017.3294. Epub 2017 Nov 28. PMID: 29207066; PMCID: PMC5752238.
47. Xu X, Gu Z, Chen X, Shi C, Liu C, Liu M, Wang L, Sun M, Zhang K, Liu Q, Shen Y, Lin C, Yang B, Sun H. An injectable and thermosensitive hydrogel: Promoting periodontal regeneration by controlled-release of aspirin and erythropoietin. Acta Biomater. 2019 Mar 1;86:235-246. doi: 10.1016/j.actbio.2019.01.001. Epub 2019 Jan 3. PMID: 30611793.
48. Yu H, Jin F, Liu D, Shu G, Wang X, Qi J, Sun M, Yang P, Jiang S, Ying X, Du Y. ROS-responsive nano-drug delivery system combining mitochondria-targeting ceria nanoparticles with atorvastatin for acute kidney injury.

Theranostics. 2020 Jan 16;10(5):2342-2357. doi: 10.7150/thno.40395. PMID: 32104507; PMCID: PMC7019163.

49. Zheng DH, Wang XX, Ma D, Zhang LN, Qiao QF, Zhang J. Erythropoietin enhances osteogenic differentiation of human periodontal ligament stem cells via Wnt/β-catenin signaling pathway. Drug Des Devel Ther. 2019 Jul 26;13:2543-2552. doi: 10.2147/DDDT.S214116. PMID: 31440036; PMCID: PMC6666380.

50. Zhou Y, Zhang T, Zhang YY, Xu J, Li M, Zhang Q, Qiao Z, Yang K. Expression and distribution of EPO, VEGF and VEGFR-2 in small intestine of yaks at different ages. Folia Morphol (Warsz). 2022 Jun 13. doi: 10.5603/FM.a2022.0058. Epub ahead of print. PMID: 35692112.

APPENDIX

APPENDIX I

SUPPLEMENTAL TABLES

SUPPLEMENTAL TABLE I. Reporting items for the methodology of this scoping review. Adapted from the "Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist" (40).

SECTION	ITEM	PRISMA-ScR CHECKLIST ITEM	REPORTED ON PAGE
TITLE			
Title	1	Identify the report as a scoping review.	III
ABSTRACT			
Structed summary	2	Provide a structed summary that includes (as applicable): background, objectives, eligibility criteria, sources of evidence, charting methods, results, and conclusions that relate to the review questions and objectives.	IX-XII
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known. Explain why the review questions/objectives lend themselves to a scoping review approach.	3-5
Objectives	4	Provide an explicit statement of the questions and objectives being addressed with reference to their key elements (e.g., population or participants, concepts, and context) or other relevant key elements used to conceptualize the review questions and/or objectives.	5
METHODS			
Eligibility criteria	5	Specify characteristics of the sources of evidence used as eligibility criteria (e.g., years considered, language, and publication status), and provide a rationale.	10
Information sources	6	Describe all information sources in the search (e.g., databases with dates of coverage and contact with authors to identify additional sources), as well as the date the most recent search was executed.	9
Search	7	Present the full electronic search strategy for at least 1 database, including any limits used, such that it could be repeated.	9, 62
Selection of sources of evidence	8	State the process for selecting sources of evidence (i.e., screening and eligibility) included in the scoping review.	9

Data charting process	9	Describe the methods of charting data from the included sources of evidence (e.g., calibrated forms or forms that have been tested by the team before their use, and whether data charting was done independently or in duplicate) and any processes for obtaining and confirming data from investigators.	10
Data items	10	List and define all variables for which data were sought and any assumptions and simplifications made.	10
Synthesis of results	11	Describe the methods of handling and summarizing the data that were charted.	11
RESULTS			
Selection of sources of evidence	12	Give numbers of sources of evidence screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally using a flow diagram.	15
Results of individual sources of evidence	13	For each included source of evidence, present the relevant data that were charted that relate to the review questions and objectives.	16-23
Synthesis of results	14	Summarize and/or present the charting results as they relate to the review questions and objectives.	24-31
DISCUSSION			
Summary of evidence	15	Summarize the main results (including an overview of concepts, themes, and types of evidence available), link to the review questions and objectives, and consider the relevance to key groups.	35-45
Limitations	16	Discuss the limitations of the scoping review process.	46
Conclusions	17	Provide a general interpretation of the results with respect to the review questions and objectives, as well as potential implications and/or next steps.	47

SUPPLEMENTAL TABLE II. Database-specific search strategy equation.

DATABASE	SEARCH STRATEGY EQUATION
MEDLINE (via PubMed)	(“Periodontitis” [All Fields] OR “Periodontal disease” [All Fields] OR “Periodontal Therapy” [All Fields] OR “Nonsurgical Treatment” [All Fields] OR “Periodontal Treatment” [All Fields] OR “Periodontal Regeneration” [All Fields]) AND (“Erythropoietin” [All Fields])
Cochrane (CENTRAL)	Tittle Abstract Keyword: (“Periodontitis” AND “Erythropoietin”); (“Periodontal Disease” AND “Erythropoietin”); (“Periodontal Therapy” AND “Erythropoietin”); (“Periodontal Treatment” AND “Erythropoietin”); (“Nonsurgical Treatment” AND “Erythropoietin”); (“Periodontal Regeneration” AND “Erythropoietin”
Science Direct	Title, abstract or author-specified keywords: (“Periodontitis” OR “Periodontal Disease” AND “Erythropoietin”); (“Periodontal Therapy” OR “Periodontal Treatment”) AND “Erythropoietin”; “Nonsurgical Treatment” AND “Erythropoietin”; “Periodontal Regeneration” AND “Erythropoietin”
Scopus (Elsevier)	TITLE-ABS-KEY (“Periodontitis” OR “Periodontal disease” OR “Periodontal Therapy” OR “Nonsurgical Treatment” OR “Periodontal Treatment” OR “Periodontal Regeneration”) AND “Erythropoietin”

APPENDIX II

DECLARAÇÕES

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PARECER DA ORIENTADORA

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Informo que o Trabalho de Monografia/Relatório de Estágio desenvolvido pela estudante **Inês Olímpia Nascimento de Sá Pereira** com o título **"The Effect of Erythropoietin in the Nonsurgical Treatment of Periodontitis – a systematic review"**, está de acordo com as regras estipuladas pela FMDUP, foi por mim conferido e encontra-se em condições de ser apresentado em provas públicas.

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Porto, 22 de maio de 2023

Inês Olímpia Nascimento de Sá Pereira

Inês Olímpia Nascimento de Sá Pereira
(A estudante)

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Identificação da publicação

Dissertação de Mestrado Integrado (Monografia) ☒ Relatório de Estágio ☐

Título Completo: "The Effect of Erythropoietin in the Nonsurgical Treatment of Periodontitis – a systematic review"

Orientadora: Prof.ª Doutora Luzia da Conceição Martins Mendes Gonçalves

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Faculdade de Medicina Dentária da Universidade do Porto