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TERAPIA ADJUNTA COM AGENTES BIOLÓGICOS NA PERIODONTITE EXPERIMENTAL

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Bruna Silva de Menezes

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Dissertação de Mestrado (Acadêmico) submetida ao Programa de Pós-graduação em Odontologia (Área de Concentração: Periodontia) da Faculdade de Odontologia da Universidade Federal do Rio de Janeiro como parte dos requisitos para obtenção do título de Mestre em Odontologia (Área de Concentração: Periodontia).

Orientador(es): Dr. Prof. Rafael Scaf de Molon e Dr. Prof. Carmelo Sansone

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RESUMO

SILVA DE MENEZES, Bruna. Terapia adjunta com agentes biológicos na periodontite experimental. Rio de Janeiro, 2023. Dissertação (Mestrado Acadêmico em Odontologia – Área de concentração: Periodontia) – Faculdade de Odontologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2023.

A presente dissertação foi dividida em dois estudos. O primeiro estudo teve como objetivo desenvolver uma revisão de literatura de trabalhos pré-clínicos que resumisse o papel de diversos fármacos usados para prevenção e tratamento da doença periodontal experimental e destaca a ação direcionada de todos os fármacos com propriedades antirreabsortivas. Além disso, esta revisão fornece uma avaliação oportuna e crítica para o uso científico racional dos medicamentos antirreabsortivos e imunomoduladores em estudos pré-clínicos, o que ajuda a entender a base para sua aplicação clínica. Pode-se concluir a partir dos achados descritos, que fitoterápicos, suplementação com ômega 3 e probióticos tem ganhado cada vez mais atenção devido às suas atividades moduladoras e antirreabsortivas e a ausência de efeitos colaterais, sendo considerados alternativas promissoras como adjuvantes da raspagem subgengival e alisamento radicular em pacientes suscetíveis. O segundo estudo, objetivou-se realizar uma revisão sistemática sobre os potenciais benefícios de agentes biológicos como adjuvantes no tratamento da periodontite experimental em modelos animais, respondendo a seguinte questão: qual é o potencial protetor dos agentes biológicos contra a reabsorção óssea alveolar durante a progressão da periodontite experimental? Os critérios de elegibilidade dos estudos foram baseados na estratégia PI/ECOS, ou seja, P = periodontite; I/E agentes biológicos; C mesma exposição/intervenção; I = exceto sobre o uso de agentes biológicos; e S = resultados secundários (inflamação). Como resultado, 5236 estudos foram selecionados inicialmente por meio de buscas manuais nas bases de dados incluídas . Após a leitura dos resumos e dos textos e de acordo com os critérios de inclusão, 39 estudos que atenderam aos critérios de inclusão foram incluídos neste estudo. Vinte e três agentes biológicos foram avaliados nos estudos incluídos. A maioria dos estudos utilizou o modelo de ligadura de periodontite experimental (PE) para testar o uso de produtos biológicos como estratégias preventivas ou terapêuticas. As dosagens dos produtos biológicos e os períodos de indução da doença variaram com base no modelo de periodontite utilizado. Como principal resultado de todos os estudos, a perda óssea alveolar pôde ser significativamente inibida com a aplicação de produtos biológicos e a redução de mediadores pró-inflamatórios quando comparados aos controles tratados. Em conclusão, essa revisão sistemática demonstrou que os agentes biológicos possuem efeitos benéficos na redução da perda óssea e na diminuição da inflamação durante a progressão da periodontite em modelos animais.

Palavras-chave: Periodontite; reabsorção alveolar; doenças periodontais e modelos animais.

ABSTRACT

SILVA DE MENEZES, Bruna. Adjunctive therapy with biological agents in experimental periodontitis. Rio de Janeiro, 2023. Dissertação (Mestrado Acadêmico em Odontologia – Área de concentração: Periodontia) – Faculdade de Odontologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2023.

This MSc dissertation was divided into two studies. The first study aimed to develop a literature review including preclinical studies summarizing the role of various compounds used for the prevention and treatment of periodontitis and highlights the targeted action of all drugs with antiresorptive properties. In addition, this review provided a timely and critical assessment for the rational scientific use of antiresorptive and immunomodulatory drugs in preclinical studies, which helps to understand the basis for their clinical application. It can be concluded from the findings described, that phytotherapeutics, omega-3 supplementation, and probiotics have gained increasing attention due to their modulating and antiresorptive activities and the absence of side effects, and are considered promising alternatives as adjuvants to subgingival scaling and root planing in susceptible patients. In the second study, the aim was to perform a systematic review on the benefits of biological agents as adjuvants in the treatment of experimental periodontitis in animal models, answering the following question: What is the protective potential of biological agents against alveolar bone resorption during the course of experimental periodontitis progression? The criteria for eligibility of the studies were based in PI/ECOs strategy, as such (P) animal models with periodontitis; (I/E) biological agents with protective potential against bone resorption in periodontitis (prevention) or additional benefit as an adjunctive in the treatment of periodontitis; (C) same intervention/exposition as the intervention group (I), except for the use biological agents; (O) alveolar bone resorption (primary outcomes). Secondary outcomes: periodontal parameters, effectiveness of periodontitis-treatment, and adverse effects; (s) no restriction for study design on primary studies (exclusion criteria: randomized clinical trials, clinical studies, and different types of review studies). Study quality was assessed using Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) Risk of Bias tool. As a result, 5236 initial results from datasets, registries, and manual searches resulted in 39 suitable studies that met the inclusion criteria. Twenty-three biological agents were evaluated in the included studies. Most studies used the ligation model of experimental periodontitis (EP) to test the use of biologics as preventive or therapeutic strategies. The dosages of the biologics and periods of disease induction varied based on the PE model used. As the main outcome of all studies, alveolar bone loss could be significantly inhibited with the application of biologics and the reduction of pro-inflammatory mediators when compared to treated controls. Taken together, this systematic review demonstrated that biologics have beneficial effects in reducing bone loss and decreasing inflammation during the progression of periodontitis in animal models.

Key-words: Periodontitis; alveolar resorption; periodontal diseases and animal models.

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LISTA DE ABREVIATURAS E SIGLAS

AAV	Adenoassociated virus
ABR	Alveolar bone resorption
APN	Adiponectin
AAV2/1	Adenoassociated virus vector based on serotype 1
ABC	Alveolar bone crest
ABPs	Autologous bloodderived products
AdipoAI	Adiponectin receptor agonist
AIA-	Antigen-induced arthritis-
ALA	Alpha lipoic acid
BET	Bromodomain and extraterminal domain
C	Comparator
CAMARADES	Analysis and Review of Animal Experimental Studies
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CEJ	Cement-enamel junction
CENTRAL	Cochrane Central Register of Controlled Trials
chemR23	Chemerin receptor 23
CI	Confidence interval
CIA	Collagen-induced arthritis
CpG	Cytidine-phosphatase-guanosine
CTLA-4	Cytotoxic T lymphocyte-associated antigen 4
CtsK	Cathepsin K
D	Design
DeCS	Descritores em ciências da saúde
DP	Doença periodontal
EP	Experimental periodontitis
EPA	Eicosapentaenoic acid
et al.	E outros, do latim et alian
FACEPE	Fundação de Amparo a Ciência e Tecnologia de PE
GRADE	The Classification of Assessment, Development and Assessment Recommendations
I	Intervention

IHC	Immunohistochemistry
IL	Interleucina
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
Mesh	Medical Subject Headings
micro-CT	Microcomputed tomography
MMP	Matrix metaloproteinases
MMPs	Metaloproteinases de matriz
MOS	Mannan oligosaccharides
NF-kb	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	Nerve growth factor
NSPT	Non-surgical periodontal therapy
O	Outcome
OCN	Osteocalcin
ONJ	Osteonecrosis of the jaw
OPG	Osteoprotegerin
OVX	Ovariectomized
P	Population
PAMPs	Pathogen-associated molecular pattern molecules
PD	Periodontal disease
PGE2	Prostaglandina E2
PMN	Polimorfonucleares
PTH	Parathyroid hormone
PUFA	Polyunsaturated fatty acid
RA	Rheumatoid arthritis
RANKL	Nuclear factor-kappa B ligand
RANKL	Ativador do receptor do ligante do fator nuclear kappa-B
r OPG-Fc	The human recombinant OPG fusion protein
ROB	Risk of bias
RTqPCR	Real time polymerase chain reaction
RvE1	E-series resolvins
Scl-Ab	Sclerostin monoclonal antibody
SEM	Scanning electronic microscopy

she	Soluble epoxide hydrolase inhibitor
sIL-1R1	Soluble IL-1 receptor type 1
SINGLE	System for Information on Grey Literature in Europe
SPM	Specialized Proresolving Mediators
SR	Strontium Ranelate
SRP	Scaling and root planning
STAT	Signal transducer and activator of transcription
SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
SyRF	Collaborative Approach to Meta Systematic Review Facility
TCZ	Tocilizumab
TLR	Toll-like receptor
TNFR:Fc	TNF receptorimmunoglobulin Fc
TNF-α	Fator de necrose tumoral-α
TPNC	Terapia periodontal não-cirúrgica
UFRJ	Universidade Federal do Rio de Janeiro
UNESP	Universidade Estadual Paulista "Julio Mesquita Filho"
WOS	Web of Science

LISTA DE SÍMBOLOS

=	Igual
≥	Maior ou igual
≤	Menor ou igual

SUMÁRIO

1 INTRODUÇÃO

A doença periodontal (DP) é uma condição inflamatória crônica dos tecidos de suporte ao redor dos dentes que se desenvolve como resultado de uma interação complexa entre parasita e hospedeiro que afeta progressivamente a integridade dos tecidos periodontais (KINANE, STATHOPOULOU & PAPAPANOU, 2017; DE MOLON et al., 2022). Essa condição leva a uma perda irreversível das estruturas ao redor dos dentes e pode resultar na perda do elemento dentário se não for tratada (KINANE, STATHOPOULOU & PAPAPANOU, 2017; PIHLSTROM, MICHALOWICZ & JOHNSON, 2005). Sua etiologia é multifatorial, na qual um biofilme disbiótico em íntimo contato com o tecido gengival, inicia uma resposta inflamatória (TONETTI, GREENWELL & KORNMAN, 2018; HAJISHENGALLIS, CHAVAKIS & LAMBRIS, 2000; DE MOLON et al., 2022). A DP é modulada e mediada pelo sistema imune do hospedeiro, que tem um papel importante na progressão e severidade da doença (DE MOLON et al., 2019).As formas mais graves da periodontite (estádio III e IV) afeta 700 milhões de pessoas, a qual representa em torno de 11% da população mundial (EKE et al., 2012).

Na fase inicial da DP, há a ativação de resposta inflamatória, que é caracterizada por aumento do fluido gengival, e um influxo de células inflamatórias, especialmente neutrófilos polimorfonucleares (PMN), que diminui a agressão causada pelo biofilme disbiótico (PIHLSTROM, MICHALOWICZ & JOHNSON, 2005). Em indivíduos susceptíveis a periodontite, a resposta imune é exacerbada, desregulada e destrutiva levando a infiltração de células imune e inflamatórias (PAVANELLI et al., 2022). Inúmeras citocinas pró- inflamatórias como interleucina (IL)-1, IL-6, IL-17, prostaglandina E_2 (PGE_2), fator de necrose tumoral-α (TNF-α) e enzima degradadoras de matriz (catepsina e metaloproteinases de matriz -MMPs) produzidas por linfócitos, fibroblastos, leucócitos e células epiteliais. Essas citocinas tem sido identificadas como moléculas-chave induzindo destruição tecidual, e a expressão delas aumenta significantemente durante a progressão da doença (PAVANELLI et al., 2022). Além disso, elas permitem o aumento da ativação da via do ativador do receptor do ligante do fator nuclear kappa-B (RANKL) no osteoblasto, fibroblasto ou linfócitos, que resulta na diferenciação e ativação, e consequentemente a destruição de tecidos sustentação mineralizados (LEE et al., 2022).

A primeira opção de tratamento da DP é a terapia periodontal não-cirúrgica (TPNC), que consiste em raspagem supragengival, raspagem subgengival e alisamento radicular para remover o biofilme e cálculo aderido na superfície do dente e raiz. No entanto, nem sempre a remoção destes fatores retentivos faz com que haja a regeneração dos tecidos destruídos e assim retorne a homeostase (KRAYER, LEITE & KIRKWOOD, 2010; WANG et al., 2018). Nos últimos anos, há um número crescente de estudos investigando os efeitos benéficos de terapias adjuvantes em testes clínicos que objetivam a diminuição da inflamação e destruição do osso alveolar e então melhorando os resultados da TPNC (COSGAREA et al., 2022; ROCUZZO et al., 2022; RAMOS et al., 2022; KIANE, BIRANG & JAMSHIDIAN, 2022). Tem sido descrito o uso de drogas farmacológicas e compostos naturais que causam a diminuição da destruição óssea na DP em modelos animais (CHEN et al., 2016; KARAKAN et al., 2017; OZAKI et al., 2020). A inibição da perda óssea pode ser interrompida especificamente por inúmeras drogas farmacológicas com alendronato (STORRER et al., 2016), OPG-Fc (JIN et al., 2007), resolvina (MIZRAJI et al., 2018; GAO et al., 2013), ranelato de estrôncio (KARAKAN et al., 2017; MARINS et al., 2020), curcumina (WANG et al., 2018; ZAMBRANO et al., 2018) e inibidores de catepsina (STRALBERG et al., 2017; WEI et al., 2020).

Esta dissertação aborda dois artigos, sendo o primeiro uma revisão de estudos pré-clínicos sobre terapias farmacológicas que atuam no controle da inflamação e reabsorção óssea na DP; e o segundo é uma revisão sistemática que aborda os efeitos de agentes biológicos na periodontite experimental.

2 PROPOSIÇÃO

2.1 Objetivo Geral

Avaliar os efeitos de agentes antirreabsortivos e biológicos durante a progressão da periodontite experimental por meio de revisão narrativa da literatura e revisão sistemática.

2.2 Objetivos específicos

- Resumir de forma abrangente, por meio de uma revisão narrativa da literatura, os efeitos de várias abordagens terapêuticas durante a progressão da periodontite e descrever os principais achados de cada estudo incluído na revisão.
- Avaliar os efeitos dos agentes biológicos, por meio de uma revisão sistemática de estudos pré-clínicos, como adjuvantes na periodontite experimental.

3 DELINEAMENTO DA PESQUISA

A presente dissertação de mestrado é composta por duas revisões. A primeira é uma revisão narrativa de literatura de trabalhos pré-clínicos que resumem o papel de diversos fármacos usados para prevenção e tratamento da doença periodontal experimental correspondendo a um dos objetivos específicos propostos. A segunda é uma revisão sistemática de estudos pré-clínicos que avaliam os efeitos de agentes biológicos como adjuvantes no tratamento da periodontite experimental em modelos animais, respondendo a seguinte questão: qual é o potencial protetor dos agentes biológicos contra a reabsorção óssea alveolar durante a progressão da periodontite experimental?

4 DESENVOLVIMENTO DA PESQUISA

4.1 Artigo 1: Pharmacological therapies for the management of inflammatory boné resorption in periodontal disease: A review of preclinical studies

Artigo publicado no periódico BioMed Research International

4.1 Artigo 2: Beneficial effects of biological agents on experimental periodontitis: A systematic review

Artigo a ser submetido no periódico Clinical Oral Investigations

4.1 Artigo 1

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Review Article

Pharmacological Therapies for the Management of Inflammatory Bone Resorption in Periodontal Disease: A Review of Preclinical Studies

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Periodontitis, a highly prevalent multicausal chronic inflammatory and destructive disease, develops as a result of complex host-parasite interactions. Dysbiotic bacterial biofilm in contact with the gingival tissues initiates a cascade of inflammatory events, mediated and modulated by the host's immune response, which is characterized by increased expression of several inflammatory mediators such as cytokines and chemokines in the connective tissue. If periodontal disease (PD) is left untreated, it results in the destruction of the supporting tissues around the teeth, including periodontal ligament, cementum, and alveolar bone, which lead to a wide range of disabilities and poor quality of life, thus imposing significant burdens. This process depends on the differentiation and activity of osteoclasts, the cells responsible for reabsorbing the bone tissue. Therefore, the inhibition of differentiation or activity of these cells is a promising strategy for controlling bone resorption. Several pharmacological drugs that target osteoclasts and inflammatory cells with immunomodulatory and anti-inflammatory effects, such as bisphosphonates, anti-RANK-L antibody, strontium ranelate, cathepsin inhibitors, curcumin, flavonoids, specialized proresolving mediators, and probiotics, were already described to manage inflammatory bone resorption during experimental PD progression in preclinical studies. Meantime, a growing number of studies have described the beneficial effects of herbal products in inhibiting bone resorption in experimental PD. Therefore, this review summarizes the role of several pharmacological drugs used for PD prevention and treatment and highlights the targeted action of all those drugs with antiresorptive properties. In addition, our review provides a timely and critical appraisal for the scientific rationale use of the antiresorptive and immunomodulatory medications in preclinical studies, which will help to understand the basis for its clinical application.

1. Introduction

Periodontal disease (PD), a chronic inflammatory condition of the supporting tissues around the teeth, is characterized by the loss of supporting structures of the tooth, such as gingiva, periodontal ligament, alveolar bone, and cementum [1–3]. This condition leads to an irreversible loss of the dental structures and might result in tooth loss if left untreated [1, 4]. The etiology of PD is multifactorial in which the presence of a dysbiotic biofilm in intimate contact with the gingival margin initiates the inflammatory immune response [3, 5, 6]. Indeed, PD is the sixth most prevalent disease globally [7] and is considered the most important cause of tooth loss in the adult population [8].

PD is modulated and mediated by the immune host system, which plays an important role in disease severity and progression [6]. During the initiation and progression of PD, environmental conditions (smoking), systemic comorbidities (diabetes mellitus and rheumatoid arthritis), and genetic polymorphisms (IL-1ß) are important aspects that

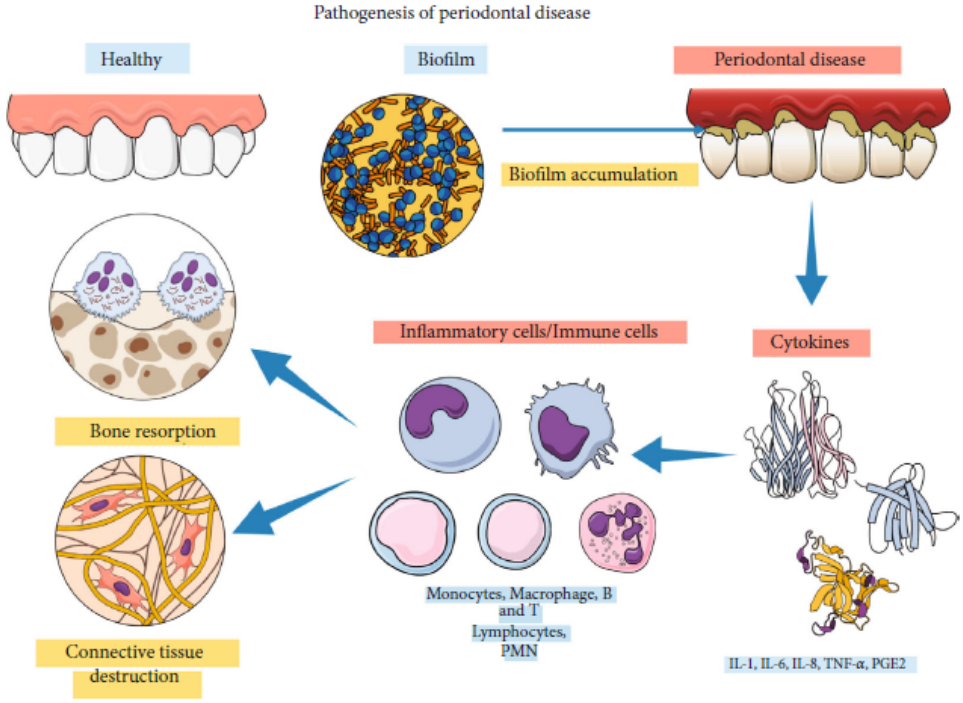

FIGURE 1: The pathogenesis of PD. The bacteria that compose the dental biofilm trigger the process of local inflammation generated by the increase of cytokines such as IL-1, IL-6, IL-8, TNF-alpha, and PGE2 by the immune cells and inflammatory cells, such as neutrophils and macrophages. Such inflammatory environment ultimately leads to the activation of osteoclasts, the cells responsible to resorb the bone tissue. Consequently, the signs and symptoms of PD (gingival inflammation, epithelial downgrowth, pocket formation, and alveolar bone destruction) occur.

dictate the disease progression [9–11]. In the initiation phase of PD, there is an activation of the inflammatory response, which is characterized by increased gingival crevicular fluid, and an influx of inflammatory cells (leukocytes), especially the polymorphonuclear neutrophils (PMN), that tends to diminish the insult caused by the dysbiotic biofilm [4]. All of these events are protective, and in most patients, the immune system is capable of controlling the disease progression. However, innate and adaptive responses in susceptible patients lead to the aggravation of periodontal tissue destruction. The activation of leucocytes and T cells in the connective tissue leads to the production of multiple inflammatory mediators, degrading enzymes such as matrix metalloproteinases (MMP), and the increased expression of the nuclear factor-kappa B ligand (RANKL), which is the primary activation factor for osteoclasts [12], leading to periodontal inflammation and finally causing the loss of bone supporting tissue (Figure 1) [13–15].

The primary treatment of PD is through scaling and root planning (SRP) to remove the attached biofilm from the root surface. However, removing bacterial biofilm does not imply a return to homeostasis and regeneration of lost tissues [16, 17], and SRP targeting only microorganisms does not accomplish favorable results in all patients [18]. Adjunctive treatments such as systemic local antibiotics, nonsteroidal anti-inflammatory drugs, and low doses of doxycycline have been used as host modulating agents in order to control the progression of PD [19–22]. Despite the clinical benefits of those approaches, their effects are limited in the context of inflammation-induced alveolar bone loss [23]. The major challenge for successfully treating PD is the difficulty in finding a target that can inhibit tissue inflammation and consequently alveolar bone destruction [24]. Therefore, the adjunct use of complementary therapies that are aimed at modulating the destructive events of the immune response has been proposed as a potential therapeutic strategy for PD treatment targeting inflammatory mediators and bone-resorbing osteoclasts.

In recent decades, the use of pharmacological drugs and natural compounds (herbal medicine) aiming to suppress bone destruction during experimental PD in animal models has been extensively reported [25–34]. Interestingly, several studies have shown that inhibition of bone loss can be targeted intervened by innumerous pharmacological drugs, such as alendronate [35–38], OPG-Fc [26], resolvin [39–42], strontium ranelate [27, 43], curcumin [17, 31, 44–47], and cathepsin inhibitors [29, 48]. Therefore, in this review, we comprehensively summarize the roles of several therapeutic drugs during the progression of PD and provide the main findings of each included study leading to the prevention of experimental PD.

In this review, the pharmacological products discussed below are examined through many experiments for their antiosteoclastic activity. The in vivo studies included in this

Table 1: Cathepsin K inhibitors.

Studies	Study design	Main outcomes
Yue et al. (2020) [72]	Animals: eighty male DBA/J1 mice (8 weeks old) Disease model: collagen-induced arthritis (CIA) model. Periodontal disease (PD) model with *P. gingivalis infection* Treatment: injections of adenoassociated virus (AAV) transfection in periodontal tissue and knee joint. AAVs 2.5×10^{-10} g/ml. Given once every 3 days for 65 days	Inhibition of articular tissue damage and alveolar bone loss, decreased number of macrophages, and expression of inflammatory cytokines in the synovia, due to inhibition of CtsK
Pan et al. (2019) [75]	Animals: twenty 6- to 7-week-old DBA/1 male wild-type mice Disease model: CIA mouse model and PD model with *P. gingivalis* infection Treatment: CtsK-specific inhibitor BML-244 (25.242 mg/kg per week) or dimethyl sulphoxide (DMSO; vehicle)	Reduced expression of inflammatory cytokines and infiltration by dendritic cells and T cells. Bone loss in PD and RA abrogated. Inhibition of CtsK decreased Toll-like receptor (TLR) 4 and TLR9 expression in vivo
Hao et al. (2015) [24]	Animals: seventy-five 8-week-old female wild-type BALB/cJ mice Disease model: bacterial-induced PD model; 100 μl (5×10^9 CFU/ml of *P. gingivalis*; 5×10^9 CFU/ml of *T. denticola* and *T. forsitia*) topical application eight consecutive times Treatment: orally with 3.606 or 0.7212 mg/kg per week (five times lower dose) of ODN in DMSO for 56 days	Decreased number of osteoclasts, T cells and macrophages, and toll-like receptors in vivo; inhibited the expression of TLRs 4, 5, and 9 and their downstream cytokine signaling in the gingival epithelial cell, indicating that the innate immune response was abrogated
Chen et al. (2016) [25]	Animals: twenty-one wild-type female BALB/cJ mice Disease model: PD induced by oral inoculation with *P. gingivalis* Treatment: gingival injections in the upper molar region of AAV-sh-CtsK or AAV-sh-luc-YFP (3 μl) daily for seven consecutive days	Less bone loss and inflammation in the gingival tissue due to CtsK inhibition
Da Ponte Leguizamon et al. (2022) [76]	Animals: twenty-four 8-week-old C57BL/6J male mice Disease model: ligature-induced periodontal disease Treatment: CsinCPI-2 (0.8 μg/g in PBS) for 15 days	Controlled the inflammatory process, inhibited osteoclastogenesis and alveolar bone loss

review are based on well-established experimental models of PD, such as ligature-induced bone loss [49–57], lipopolysaccharide (LPS) injections [58–61], and oral inoculation of periodontopathogenic bacteria into the animal mouth [50, 58–60]. Primary methods used to evaluate the inhibition of bone loss were assessed by microcomputed tomography (micro-CT) and histopathological analyses. Oral gavage, palatal injections, and intraperitoneal injections represent the main routes of drug administration in experimental models of periodontitis in rats and mice. Humanized mouse models, subcutaneous bacterial injections, or other animal models were not investigated in this review. We have described the objective, study design, main findings, and conclusions of all the included studies, according to Tables 1–9.

2. Cathepsin K Inhibitors

Cathepsin K (CtsK) is a member of the papain superfamily (C1 protein family) of cysteine protease that plays an important role in the innate immune response and osteoclast-mediated bone resorption [24, 62]. It was previously identified as an osteoclast selective protease CtsK [63] abundantly expressed in human osteoclasts, osteoblasts, periodontal ligament cells, osteocytes, and fibroblasts. In the bone tissue, CtsK can cleave the triple helix and the telopeptides from the type I collagen fibers that constitute 90% of the bone organic matrix [64]. In addition, this protease can also activate MMP-9 [65] and degrade type II collagen [66], osteonectin, and osteopontin, thus inhibiting the activity of osteoclasts [64]. It is important to mention that CtsK inhibitors are able to prevent bone resorption without affecting osteoblastic activity. Therefore, the crosstalk between osteoblast and osteoclast is maintained, which is beneficial during bone remodeling [67]. A summary of main study outcomes is described down below (Table 1).

Previous studies have described selective CtsK inhibitors that effectively reduce osteoclast resorption both in vitro and in vivo [68–70]. Furthermore, CtsK has been shown to be an efficient therapeutic strategy in preclinical studies, including inflammatory, metabolic, and autoimmune diseases, such as high fat acid-induced obese mice [71], experimental periodontitis [24, 25, 72], and collagen-induced arthritis (CIA) [72]. However, although CtsK inhibitor has potent inhibitory effects on osteoclast-mediated bone resorption, it has also been associated with some adverse side effects and undesired drug-drug interactions [67, 72, 73]. Odanacatib is an inhibitor of the family member of lysosomal cysteine proteases (cathepsin K inhibitor) involved in the degradation of the demineralized bone matrix; was tested in vitro, in animal models, and in humans; and reached phase III clinical trials [67]. The study was terminated due to an unforeseen increase in cerebrovascular events [74], but

Table 2: Bisphosphonates.

Studies	Study design	Main outcomes
Brunsvold et al. (1992) [94]	Animals: 27 adult cynomolgus monkeys with intact dentitions Disease model: PD induced by ligature placed around the lower premolars and molars, plus oral inoculation of *P. gingivalis* Treatment: alendronate (0.05 mg/kg) for 16 weeks	Decreased the progression of PD as measured by changes in bone density.
Moreira et al. (2014) [36]	Animals: thirty-six 3-month-old Wistar rats Disease model: ligature-induced PD around the upper right second molar Treatment: daily injections of 2.5 mg/kg body weight alendronate for 7 days before and 7, 14, and 21 days after PD induction.	Reduced the activity of osteoclasts and the resorption of the alveolar bone crest. After 21 days of treatment, some animals developed signs of ONJ due to reduced osteoclast activity
De Almeida et al. (2015) [35]	Animals: ninety 3-month-old Wistar rats Disease model: ligature-induced PD around the lower left first molar Treatment: scaling and root planning and/or administration of alendronate (irrigation with 1 ml of 10^{-5} M) for 7, 15, and 30 days	The combination of the two treatments showed less local inflammation and enhanced tissue repair

odanacatib antifracture efficacy encouraged further studies with new cathepsin inhibitors.

Researches have long held that inflammation and bone breakdown are the two major pathological features of periodontitis and rheumatoid arthritis (RA); consequently, prevention or reduction of these damaging events should be a main therapeutic objective. In this regard, Yue et al. [72] recently investigated the effect of CtsK inhibition on the course of a combined animal model of CIA and experimental PD through oral infection with *P. gingivalis*, a known periodontopathogenic bacterium. The results of this study have demonstrated that inhibition of CtsK by transfection of small interfering RNA (siRNA) resulted in diminished destruction of articular tissue and alveolar bone and decreased the macrophage number and inflammatory cytokine expression in the synovium, suggesting that CtsK inhibition might be implicated as a potential therapeutic strategy in experimental PD and RA [72].

Inhibition of CtsK effectively suppresses autoimmune inflammation of the joints as well as osteoclastic bone resorption in autoimmune arthritis [77]. Pan et al. [75] have used an experimental periodontitis model through oral bacterial inoculation combined with CIA in DBA/1J mice. One week before establishing the combined diseases, animals were treated with CtsK inhibitor BML-244. Alveolar bone resorption and paw swelling were more severe when these two comorbidities were present simultaneously. Furthermore, inhibition of CtsK reduced inflammatory cytokine production and infiltration by dendritic cells and T cells. Consequently, bone loss in PD and RA was abrogated as measured by bone erosion in periodontal lesions and cartilage destruction in knee joints. Inhibition of CtsK also decreased the expression of Toll-like receptor (TLR) 4 and TLR9 in vivo [75].

As previously stated, CtsK also has functions in dendritic cells through the TLR9, which plays a pivotal role in innate immunity recognition of microbial products and in the activation of immune host defense [24, 77]. In this context, Hao et al. [24] evaluated whether inhibition of CtsK would benefit both the immune system and bone system during the progression of bacterial-induced periodontitis in a mouse model. A small molecular inhibitor, odanacatib, was orally administered one week prior to experimental PD establishment. This study demonstrated that oral application of odanacatib decreased the number of osteoclasts, T cells and macrophages, and TLR, thus preventing bone loss and exacerbated immune response during the progression of PD [24]. Moreover, the same study evidenced that lack of cathepsin K inhibited the expression of toll-like receptors 4, 5, and 9 and their downstream cytokine signaling in the gingival epithelial cell, indicating that the innate immune response was abrogated in periodontitis.

Another study evaluated the inhibition of CtsK through adenoassociated virus (AAV) expressing CtsK small hairpin to silence CtsK [25]. Experimental PD was induced by oral gavage with *P. gingivalis*. AAV-sh-CtsK was administered locally into the palatal gingival tissue. The inhibition of CtsK drastically protected the mice from *P. gingivalis*-induced bone loss (>80%) and significantly reduced inflammation in the gingival tissue. The authors suggested that inhibition of CtsK could target both inflammation and bone resorption and efficiently protect against periodontal bone destruction.

Indeed, the use of CtsK inhibitors for the treatment of osteolytic diseases remains promising. However, in contrast to current antiresorptive agents, which target the osteoclast cells, CtsK inhibitors can cause effects in other tissues, as the enzyme not only is present in bone cells but also engages in several other metabolic processes and regulatory pathways. The challenge, then, is to develop more specific inhibitors, which act on the osteolytic activity of the CtsK, without affecting the activity of other enzyme catalytic sites, decreasing the chance of side effects [67, 78].

CtsK activity is regulated by endogenous cysteine proteinase inhibitors, such as cystatin C, which has a high binding affinity to cysteine proteinases [79]. These proteins are capable of inhibiting osteoclastogenesis and bone resorption in vitro and in an ex vivo model [80, 81]. Recently, our

Table 3: OPG-Fc and RANKL inhibitors.

Studies	Study design	Main outcomes
Teng et al. (2000) [96]	Animals: 8-9-week-old female mice Disease model: oral inoculation infection model with *A. actinomycetemcomitans* Treatment: intraperitoneal injections every other day with PBS or OPG-Fc (1 mg/kg) between weeks 4 and 8	Reduced alveolar bone loss, decrease in the number of osteoclasts
Mahamed et al. (2005) [97]	Animals: 200 NOD mice and 18 BALB/c mice 4-6-week-old female. Disease model: NOD mice were injected with STZ to induce hyperglycemia (40-50 mg/kg). Oral inoculation of *A. actinomycetemcomitans* (10 μg/ml) Treatment: intraperitoneal injections with 2.5 μg hu-OPG-Fc/100 μl PBS, 3 times a week for 8 weeks	Treatment of diabetic mice with OPG leads to the inhibition of bone resorption and reduced RANKL expression, and, therefore, OPG may hold therapeutic potential for treatment bone loss in inflammatory conditions
Jin et al. (2007) [26]	Animals: 32 male Sprague-Dawley rats Disease model: ligature-induced PD placed bilaterally between the lower first molars Treatment: human OPG-Fc (10 mg/kg) or vehicle by subcutaneous injection twice weekly for 6 weeks	OPG-Fc suppressed the number of osteoclasts in the alveolar crest. Preservation of alveolar bone volume
Kuritani et al. (2018) [98]	Animals: 8-week-old male C57BL/6j mice Disease model: LPS-induced calvarial bone destruction. Model of experimental PD using ligatures Treatment: administration of saline solution, anti-RANKL antibodies (3 mg/kg), or zoledronate (0.2 mg/kg).	Anti-RANKL antibodies significantly inhibited alveolar bone destruction and tooth root exposure. Zoledronate suppressed alveolar bone destruction

Table 4: Strontium ranelate (SR).

Studies	Study design	Main outcomes
Marie et al. (1993) [108]	Animals: 112 3-month-old Sprague-Dawley female rats Disease model: estrogen deficiency-induced bone loss Treatment: 17 beta-estradiol (10 μg/kg/day, sc) or divalent strontium by gavage at a dose of 77, 154, or 308 mg/kg/day or vehicle for 60 days	Prevented bone loss and increased trabecular bone volume
Karakan et al. (2017) [27]	Animals: 40 Wistar rats Disease model: ligature-induced experimental PD placed around the first molars in the right mandible Treatment: strontium in dosages: 300, 625, and 900 mg/kg. Administration by oral gavage for 11 days	Less alveolar bone loss, reduced number of osteoclasts, and increased number of osteoblast cells. Best results at a dosage of 900 mg/kg
Souza et al. (2018) [111]	Animals: 48 male Wistar rats Disease model: ligature-induced PD placed around the upper molars Treatment: oral administration of strontium ranelate (20 or 100 mg/kg) for 7 days	Prevented bone resorption and increased heme oxygenase-1 mRNA levels in gingival tissues
Marins et al. (2020) [43]	Animals: 96 female Wistar rats ovariectomized Disease model: ligature-induced PD in the mandibular first molar Treatment: oral gavage of 625 mg/kg/day strontium ranelate for 10, 20, and 30 days	Inhibited bone loss, increased the area of trabecular bone, affected the expression of bone markers

group demonstrated that natural inhibitors of cysteine peptidase derived from Citrus sinensis, named phytocystatin CsinCPI-2, was effective in decreasing the gene expression levels of cathepsin K, cathepsin B, IL-1β, and TNF-α. In addition, CsinCPI-2 significantly inhibited in vivo the activity of TNF-α in the blood of rats, previously stimulated by *E. coli* lipopolysaccharide (LPS). These data suggested that CsinCPI-2 has a potential anti-inflammatory effect during bacterial infection in rats [82]. Moreover, we have just showed the positive effects of phytocystatin CsinCPI-2 in the inhibition of bone loss in a mouse model of ligature-induced alveolar bone loss. In this study, it was demonstrated that systemic treatment with CsinCPI-2 significantly reduced inflammatory cell infiltrate, decreased the number of TRAP+ cells, and diminished alveolar bone destruction cause by PD. This treatment also showed downregulation of inflammatory cells expressing CD3, CD45, and MAC387 in the connective tissue. Furthermore, in vitro data demonstrated that CsinCPI-2 inhibited RANKL-induced TRAP+ osteoclast formation in BMM and abrogated RANKL-induced mRNA expression of *Acp5*, *Calcr*, *Ctsk*, and RANKL-induced upregulation of Nfatc1 [76].

Table 5: Anti-IL-6 and anti-TNF-α.

Studies	Study design	Main outcomes
Apolinario Vieira et al. (2021) [114]	Animals: 90 10- to 12-week-old male Wistar SPF rats Disease model: PD induced by cotton ligature placed on the right first molar in the mandible Treatment: systemic administration of tocilizumab (TCZ) intraperitoneally at concentration dosages (2 mg/kg, 4 mg/kg, and 8 mg/kg) for 7 and 14 days	Inhibited alveolar bone resorption and attachment loss, lower expression of inflammatory infiltrate and lower production of Th17 and RANKL-related cytokines.
Grauballe et al. (2015) [115]	Animals: 80 4-week-old obese diabetic male Zucker rats Disease model: PD induced by ligature placement around the maxillary second molars Treatment: anti-TNF-α Etanercept injections for 5 weeks	Blocking TNF-α improves metabolic state in obese rats with PD and diminishes periodontal tissue destruction associated with diabetes
Grauballe et al. (2017) [116]	Animals: 52 4-week-old male Zucker rats Disease model: 45 obese rats with type II diabetes and 17 lean rats as controls. PD induced by ligatures around the maxillary second molars Treatment: treatment with subcutaneous injections of 0.5 ml of 0.78 mg/ml Etanercept or RAGE (intraperitoneal injections of 0.8 ml of 1.25 g/l ARA) 3 times a week for 5 weeks	Anti-TNF-α treatment has a positive impact on the subgingival microbial profile in rats with diabetes and ligature-induced bone loss
Queiroz-Junior et al. (2013) [117]	Animals: 40 C57BL6 male mice of 6 weeks of age Disease model: mice underwent antigen-induced chronic arthritis (AIA) Treatment: intraperitoneal administration of pentoxifylline (50 mg/kg) daily for 14 days	Decreased expression of TNF-α and increased the expression of IL-10 in the maxilla of mice. It did not affect the expression of IFN-γ and IL-17. Decreased joint inflammation
Oates et al. (2002) [113]	Animals: 6 Macaca fascicularis from 3 to 7 years old Disease model: PD induced by silk ligatures inoculated with *P. gingivalis* in lower premolars, first and second molars Treatment: intrapapillary injections of soluble receptors (blockers), IL-1 and TNF-α (6.6 mg), 3 times a week for 6 weeks	Reduced radiographic bone loss

3. Bisphosphonates

Bisphosphonates, particularly nitrogen-containing ones, such as zoledronate and alendronate, are antiresorptive agents commonly used to treat bone metabolic diseases such as osteoporosis and bone neoplasia, Paget disease, and multiple myeloma. Bisphosphonates inhibit functioning osteoclasts by impairing differentiation, disrupting the cytoskeleton, decreasing intracellular transport, and inducing apoptosis and do so through the inhibition of farnesyl diphosphate synthase in the cholesterol biosynthesis pathway, which prevents prenylation of small guanosine triphosphatase signaling proteins [83–85].

Despite its beneficial effects in inhibiting bone resorption in osteolytic diseases, the use of bisphosphonates, especially intravenous administration of high doses of zoledronate, is associated with adverse side effects. The most significant effect associated with bisphosphonate administration is the osteonecrosis of the jaw (ONJ), a condition defined as an area of exposed bone in the maxillofacial region that does not heal after 8 weeks in patients receiving antiresorptive therapies [86, 87]. Furthermore, atypical fractures are also related to the long-term use of bisphosphonate due to its high maintenance of the drug into the bone tissue. On the other hand, the oral administration of alendronate to treat osteoporosis has shown to have a 0% to 0.4% chance of inducing ONJ [88]. Consequently, several studies have investigated the beneficial effects of alendronate administration to manage experimental periodontitis in rats [35, 37, 89, 90] and PD in clinical trials [91–93]. Some of the described studies are described in table 2.

One of the first studies that have used alendronate as adjunctive therapy to manage experimental PD was conducted by Brunsvold et al. in 1992 [94]. In this study, the authors have induced experimental PD in monkeys by placing a ligature around the mandibular premolars and molars followed by oral inoculation of *P. gingivalis* one week after alendronate administration. Alendronate was administered intravenously for 16 weeks, and clinical and radiographical analyses were performed. The authors demonstrated that 0.05 mg/kg alendronate treatment reduced the progression of PD, suggesting its use to treat PD. Similarly, Moreira et al. [36] have shown that 2.5 mg/kg alendronate administration in rats with experimental PD reduced the activity of osteoclasts and significantly decreased the resorption of the alveolar bone crest. However, after 21 days of treatment, some animals developed signs of ONJ due to the reduced activity of osteoclast. The authors pointed out that using alendronate to treat experimental PD in rats might increase the risk of ONJ development.

Table 6: Curcumin.

Studies	Study design	Main outcomes
Pimentel et al. (2020) [125]	Animals: 100 10-week-old male rats Disease model: diabetes was induced by streptozotocin. PD was induced by ligatures in the lower first molar and in the upper second molar Treatment: curcumin (100 mg/kg) and placebo solutions and insulin administration by gavage for 30 days	Decreased linear bone loss in the molar region. Reduced RANKL/OPG ratio
Zambrano et al. (2018) [31]	Animals: 16 Holtzman rats Disease model: PD induced by injections of 3 μl LPS (10 mg/ml from *E. coli*) into the maxillary tissues Treatment: 3 μl of nanocurcumin was injected contralaterally from the left side into the gingival tissues twice a week.	Inhibition of inflammatory bone resorption and decreased osteoclast count and inflammatory infiltrate; marked attenuation of p38 MAPK and NF-kB activation
Correa et al. (2017) [126]	Animals: 40 Wistar rats Disease model: Periodontitis induced by silk ligatures around the first molars Treatment: administration by gavage of placebo solution, 10 mg/kg resveratrol, 100 mg/kg curcumin, or 10 mg/kg resveratrol plus 100 mg/kg curcumin for 30 days	Diminished bone loss and inflammatory infiltrate for the resveratrol+curcumin group
de Almeida Brandao et al. (2019) [120]	Animals: 35 male albino rats Disease model: PD induced by LPS injections (*E. coli*) in gingival tissues Treatment: oral gavage of chemically modified curcumin (CMC2.24) at doses: 1, 3, 10, and 30 μM for 28 days	Inhibited alveolar bone resorption, osteoclastogenesis, and expression of TNF-α, regardless of dosages
Curylofo-Zotti et al. (2018) [46]	Animals: 50 male rats Disease model: PD induced by LPS injections into the gingival tissues in the maxilla three times a week Treatment: 2% CMC, CMC2.24 30 mg/ kg, curcumin 100 mg/kg. Administered by gavage for 15 days	CMC2.24 was able to reduce alveolar bone resorption
Elburki et al. (2017) [127]	Animals: 18 male Sprague-Dawley rats Disease model diabetes induced by intravenous injection of streptozotocin. PD induced by LPS injection into the maxilla Treatment: CMC 2.24 daily administered by oral gavage (30 mg/kg) for 3 weeks	It inhibited alveolar bone loss and local and systemic inflammation
Elburki et al. (2014) [121]	Animals: 11 male Holtzman rats Disease model: PD induced by LPS injection into the gingival tissue in the maxilla three times a week for 14 days. Treatment: daily oral administration of CMC 2.24 (30 mg/kg) for 14 days	Decreased alveolar bone loss, suppressed the inflammatory process, and decreased the expression of matrix metalloproteinases

The use of alendronate as adjunctive to scaling and root planning (SRP) in rats with induced PD was evaluated by De Almeida et al. [35]. Rats with ligature-induced PD received SRP after ligature removal associated with topical application of alendronate. The animals assigned to receive SRP plus alendronate showed less local inflammation and better tissue repair, associated with higher expression of osteoprotegerin (OPG) immunolabeling, suggesting that the treatment employed might be effective in the treatment of PD in rats. A recent systematic review investigated the potential use of bisphosphonate as an adjuvant to SRP in 13 clinical trials [95]. The results of this systematic literature review demonstrated that locally or systemically administered alendronate reduced probing pocket depth and resulted in a gain of clinical attachment level and improved radiographic assessment. Indeed, bisphosphonate as an adjuvant to SRP may result in clinical benefits in patients with PD. However, the risk to ONJ development after bisphosphonate administration limits their clinical use.

4. OPG-Fc and RANKL Inhibitors

The discovery of the RANK, RANK ligand (RANKL), and OPG axis has revealed its pivotal role in regulating bone metabolism and created a new field for the study of bone-related diseases [12]. Binding of RANKL to RANK results in the differentiation and maturation of osteoclast precursor cells to activated osteoclasts. Therefore, blocking the interaction between RANK and RANKL is accountable for inhibiting osteoclast differentiation, and it is considered an interesting alternative to inhibit bone loss in osteolytic lesions. Acting as a soluble decoy receptor for RANKL,

Table 7: Flavonoids.

Studies	Study design	Main outcomes
Lektemur Alpan et al. (2020) [142]	Animals: 32 male Wistar rats. Disease model: PD induced by ligatures in the lower first molars. Treatment: Administration by oral gavage of taxifolin at doses: 1 mg/kg and 10 mg/kg for 29 days.	Reduced alveolar bone loss. High BMP-2, OCN, ALP, and Col 1 expression and lower RANKL immunoexpression
Tominari et al. (2012) [145]	Animals: 6-week-old male mice Disease model: LPS-induced bone loss (25 μg) on days 0, 2, and 4 for 7 days Treatment: flavonoids—nobiletin or tangeretin (30 μM) for 7 days	Both flavonoids suppressed osteoclast formation and bone resorption. Decreased osteoclastogenesis in RAW264.7 macrophages
Gugliandolo et al. (2019) [140]	Animals: 40 male Sprague-Dawley rats Disease model: PD induced by LPS injection (10 μg/μl) in the gingival tissue between the first and second molars Treatment: bergamot juice flavonoids, 20 mg/kg administered by oral gavage for 14 days	Flavonoid improved the inflammatory process in the gingival tissues. Decreased NF-κB activation and proinflammatory cytokine levels
Huang et al. (2016) [141]	Animals: 24 8-week-old ovariectomized female C57BL/6 mice Disease model: ligature-induced PD in maxillary molars Treatment: intraperitoneal injections of low- or high-dose myricetin (2 or 5 mg) every other day for 30 days	In vivo, it suppressed bone loss and increased alveolar crest height In vitro, it inhibited osteoclast formation and bone resorption
Cheng et al. (2010) [138]	Animals: 6-week-old male Sprague-Dawley rats Disease model: ligature-induced PD in the molars of the maxilla and mandible Treatment: quercetin (75 mg/kg) for 5 days. LPS (5 mg/ml) and quercetin plus LPS	Decreased alveolar bone loss and reduced inflammatory cell infiltrate in connective tissue Decreased LPS-induced osteoclast formation in vitro
Carvalho et al. (2021) [137]	Animals: 60 BALB/c 4-week-old male mice Disease model: PD induced by microinjections of LPS on the palatal surface of both first molars Treatment: food supplement of eriocitrin and eriodictyol (25 and 50 mg) for 30 days	Inhibited periodontal inflammation
Kuo et al. (2019) [34]	Animals: 48 male rats Disease model: ligature-induced PD in the upper and lower first second molars Treatment: hesperidin at doses 75 or 150 mg/kg by intragastric gavage for 7 days	Inhibited alveolar bone loss and the production of proinflammatory mediators
Balci Yuce et al. (2019) [32]	Animals: 28 male Wistar rats Disease model: ligature-induced PD around the lower right first molars Treatment: luteolin 50 mg or 100 mg given by oral gavage for 11 days	Decreased bone loss in both groups. Greater number of osteoblast cells and decreased number of inflammatory cells
Taskan et al. (2019) [135]	Animals: 32 female Wistar rats Disease model: ligature-induced PD in the lower right first molar Treatment: administration by oral gavage of oleuropein 12 or 25 mg/kg for 14 days	Decreased alveolar bone loss due to decreased osteoclastic activity, inflammation, and apoptosis and increased osteoblastic activity

OPG binds to RANKL and inhibits osteoclast development preventing it from binding to RANK. OPG has been evaluated in preclinical studies of experimental PD as a therapeutic compound for counteracting bone loss (Figure 2).

The pioneering study that has used OPG to treat experimental PD was performed by Teng et al. [96]. Using an oral inoculation infection model with *A. actinomycetemcomitans* in mice, the authors demonstrated that in vivo inhibition of RANKL function with OPG treatment reduces alveolar bone loss and decreases the number of osteoclasts after microbial challenge. These data imply that OPG treatment may thus have therapeutic value to prevent alveolar bone and/or tooth loss in human periodontitis. In this context, Mahamed et al. [97] showed diminished alveolar bone resorption in diabetic mice treated with the RANKL antagonist OPG, which is in agreement with the study of Teng et al. [96]. Using an acute model of ligature-induced bone loss, Jin et al. [26] demonstrated protective effects of OPG-Fc during experimental PD with significant preservation of alveolar bone. Therefore, OPG revealed robust preventive effects on alveolar bone resorption in experimental PD, thus showing a promising therapeutic potential of OPG for PD treatment.

Moreover, an anti-RANKL monoclonal antibody denominated denosumab has been developed and used to treat bone metabolic diseases such as osteoporosis and metastatic bone cancers and other osteolytic bone conditions such as periodontitis and arthritis. Denosumab binds directly to the RANKL to prevent its interaction with RANK

Table 8: Specialized mediators in proresolution (SPM).

Studies	Study design	Main outcomes
Gao et al. (2013) [42]	Animals: chemR23tg mice Disease model: ligature-induced PD in the upper left second molar; 1 mm craniotomy defect Treatment: RvE1 (100 ng in 20 μl PBS) or vehicle was injected subcutaneously during craniotomy every 2 days	Less destruction of the alveolar bone after ligatures. It accelerated bone defect regeneration in a craniotomy model
Lee et al. (2016) [159]	Animals: 18 6-week-old male Wistar rats Disease model: ligature-induced PD placed on the upper right and left second molars Treatment: topical resolvin E1 at 0.28 mM or 1.4 mM, 3 times a week for 4 weeks	It reversed bone loss and inflammatory gene expression and reduced osteoclast number for both dosages
Hasturk et al. (2006) [40]	Animals: 21 male white rabbits Disease model: ligature-induced PD followed by *P. gingivalis* injection around the second mandibular premolars Treatment: RvE1, 4 μg applied every other day for 6 weeks	Less progression of PD, decreased proinflammatory mediators, and reduced inflammatory bone loss
Hasturk et al. (2007) [158]	Animals: 39 male white rabbits Disease model: ligature-induced PD followed by *P. gingivalis* infection around the second mandibular premolar Treatment: RvE1, 4 μg applied every other day for 6 weeks	Hard and soft tissue regeneration and decreased inflammation in the periodontal tissues

on osteoclasts. This binding inhibits osteoclast formation, differentiation, and function [85], thus inhibiting bone resorption. Denosumab does not bind to mouse RANKL; therefore, studies have used an anti-mouse monoclonal RANKL to investigate its potential effects on mice. In this context, Kuritani et al. [98] investigated the effects of systemic administration of anti-RANKL during the progression of ligature-induced bone loss in mice. The study findings showed that anti-RANKL antibody strongly suppressed alveolar bone loss associated with periodontitis. However, similar to bisphosphonates, the potential risk of development of medication-related osteonecrosis of the jaw [99–102] and the use of denosumab or RANKL inhibitors as an adjunctive treatment for PD are not indicated. Table 3 describes the main study outcomes with RANKL inhibitors.

5. Strontium Ranelate (SR)

SR, an antiresorptive compound mainly used for osteoporosis treatment, is a silver-white and soft metallic chemical element. It is placed primarily in areas where mineralization of new bone occurs, such as regions experiencing intramembranous or endochondral ossification [103]. SR is known as a divalent cation that has atomic and ionic properties related to calcium and is also considered as a dual-acting agent that diminishes bone resorption by decreasing osteoclastic activity and stimulating bone formation by proliferation of preosteoblast and secondarily increasing the activity of functional cells and synthesis of bone matrix [104, 105]. This dual-acting mechanism of SR (concomitant antiresorptive and osteoanabolic dual biological activity) represents an advantage over bisphosphonates. Thus, SR is able to increase biomechanical and structural properties of bone, such as mineral density [106]. There are two possible mechanisms of action presented in literature about SR: (1) activating calcium-sensing receptor or another cation-sensing receptor and (2) increasing expression of OPG in addition to decreasing RANKL expression by osteoblasts [107].

One of the first studies investigating the efficacy of SR in preventing bone resorption was made by Marie et al. [108]. This study tested low SR doses on bone loss induced by estrogen deficiency in female rats. Treatment for 60 days with SR resulted in a dose-dependent increase in plasma, urine, and bone strontium concentrations without any deleterious effect on total or skeletal growth. Furthermore, treatment of OVX rats with SR prevented bone loss and bone mineral content was restored to the values in sham rats. Moreover, SR treatment increased the trabecular bone volume up to 30%. On the other hand, two other studies showed that SR administration did not counteract the loss in bone architecture and bone strength in ovariectomized rats [109, 110]. These contradictory findings lead to a deeper investigation of the potential role of SR in other inflammatory diseases such as PD.

In this context, Karakan et al. [27] investigated the effects of SR administration in rats with ligature-induced PD. Three different dosages of SR were used: 300, 625, and 900 mg/kg, and the administration was performed daily by oral gavage. The rats were euthanized 11 days after ligature placement. The results indicated that SR leads to decreased bone loss and reduced osteoclast number. In addition, the number of osteoblast cells was significantly increased after SR treatment. Collectively, the findings of this study suggested that SR at 900 mg/kg might prevent alveolar bone loss in this animal model. Another study conducted by Souza et al. [111] has determined the effect of SR on ligature-induced bone loss in rats. The authors showed that SR prevented periodontal bone loss with concomitant upregulation of heme oxygenase 1 mRNA levels. A recent study also demonstrated the beneficial effects of SR on alveolar bone loss in rats with concomitant PD and estrogen deficiency [43]. The

Table 9: Probiotics.

Studies	Study design	Main outcomes
Moraes et al. (2020) [166]	Animals: 32 male rats Disease model: ligature-induced PD; ligature and live *L. reuteri*; ligature and dead *L. reuteri*, in the lower first molars Treatment: live or dead *L. reuteri* given orally 30 days before the disease and 14 days after	Increased alveolar bone volume and trabecular number
Cardoso et al. (2020) [167]	Animals: 32 male Wistar rats Disease model: ligature-induced PD and CIA arthritis model Treatment: probiotic (HN019) in deionized water was supplied to animals (1.5×10^9 CFU/ml) for 39 days	Reduced alveolar bone loss and TNF-α and IL-6 levels and increased IL-17 levels. Decreased levels of ACPA antibodies
Ricoldi et al. (2017) [168]	Animals: 32 adult male Wistar rats Disease model: ligature-induced PD around the lower right first molars Treatment: 10 ml of 10% skim milk with *B. lactis* HN019 once daily for 15 days	Reduced alveolar bone resorption and attachment loss. Increased expression of anti-inflammatory cytokines and reduced expression of proinflammatory cytokines
Oliveira et al. (2017) [169]	Animals: 32 adult male Wistar rats Disease model: PD induced by cotton ligatures around the lower first molars Treatment: Probiotic HN019 administered topically to the subgingival region of molars on days 0, 3, and 7	Less alveolar bone resorption and attachment loss
Gatej et al. (2018) [170]	Animals: 36 6-week old BALB/c mice Disease model: PD was induced by oral inoculation with *P. gingivalis* Treatment: probiotic Lactobacillus rhamnosus was given by oral gavage before and during disease induction	Reduced bone loss and gingival inflammation
Maekawa and Hajishengallis (2014) [171]	Animals: C57BL male mice Disease model: ligature-induced PD around the upper left second molar Treatment: *L. brevis* CD2 applied topically between the gingiva and the buccal mucosa	Decreased bone loss and lower expression of TNF, IL-1β, IL-6, and IL-17A
Kobayashi et al. (2017) [172]	Animals: 36 8-week-old BALB/c mice Disease model: PD induced by injection of *P. gingivalis* in the mandibular molars Treatment: *Lactobacillus gasseri* SBT2055 (LG2055) given by gavage daily for 5 weeks	Reduced alveolar bone loss and decreased TNF-α and IL-6 expression in the gingival tissue
Levi et al. (2018) [173]	Animals: 40 male Wistar rats Disease model: ligature-induced PD Treatment: *Mannanoligosaccharide* (MOS) added daily to the food for 30 days prior to PD	Decreased alveolar bone loss and increased bone mineral density. Decreased expression of IL-10 and IFN-γ and TNF-α genes

results indicated that SR prevented ligature-induced bone loss in an estrogen-deficiency condition and, to a certain extent, increased trabecular bone area in the presence and absence of periodontal collapse. Furthermore, SR also decreased the expression levels of bone markers, such as RANKL and osteocalcin, appearing to have acted predominantly as an antiresorptive agent. Taken together, the results of these investigations demonstrated that SR plays an important role in inhibiting bone loss in experimental PD (Table 4).

6. Biological Therapies

Biological therapies are a novel class of compounds mainly used to treat autoimmune diseases such as rheumatoid arthritis and other chronic inflammatory conditions, i.e., Crohn's disease, ankylosing spondylitis, and ulcerative colitis [18]. Biological therapies include a range of anticytokine agents, including anti-TNF-a, anti-IL-6, anti-IL-1, and T and B cells. These specific agents are monoclonal antibodies that act blocking the activity of cytokines and thus inhibiting the immune-inflammatory response of the host, functioning as an immune suppressant [18]. The use of biological agents to manage experimental PD in animal models has demonstrated potential efficacy for anticytokine therapies in ameliorating bone destruction and reducing inflammatory cell infiltrate [112–114], as described below (Table 5).

6.2. Anti-IL-6. A recent study has investigated the effects of systemic administration of anti-IL-6 monoclonal antibodies in the progression of experimental PD in rats [114]. Tocilizumab was intraperitoneally injected immediately after ligature placement, and the animals were sacrificed after 7 and 14 days postoperatively. The results indicated that tocilizumab diminished alveolar bone resorption and attachment loss. Moreover, inflammatory infiltrate was also

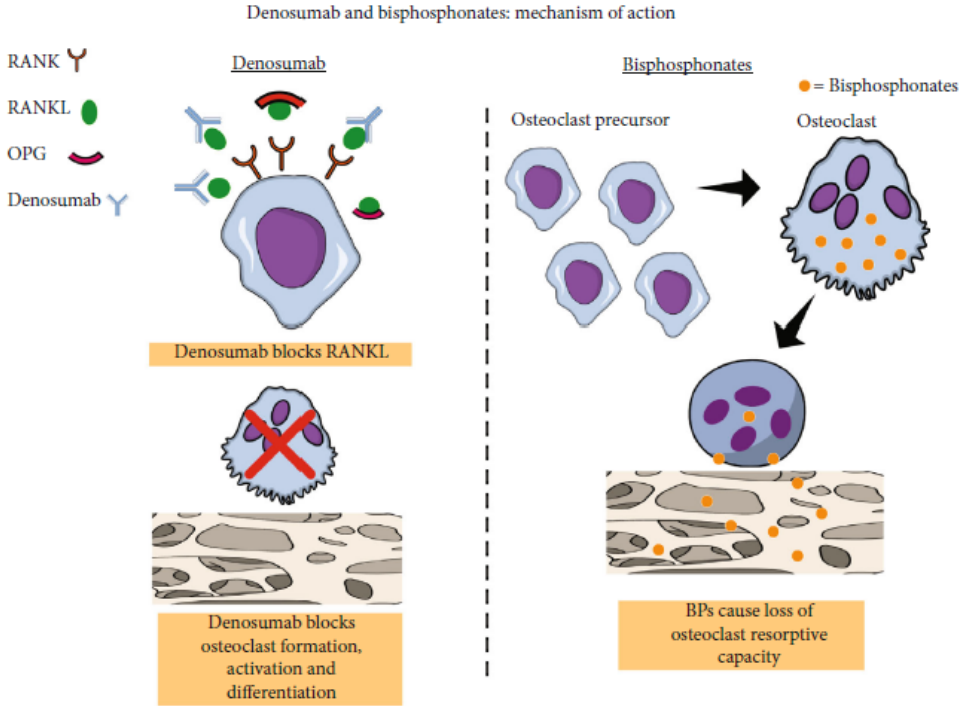

FIGURE 2: Denosumab acts similarly to OPG, which is RANKL's natural decoy receptor; denosumab binds to RANKL, preventing the binding of RANKL to its receptor, RANK, on the surface of osteoclasts and also on osteoclast precursors. Thus, the RANK signaling pathway is not activated, resulting in impaired osteoclast precursor differentiation and function and possibly osteoclast apoptosis. All these effects lead to inhibition of bone resorption. Bisphosphonates act on osteoclasts, but not on their precursors. Bisphosphonates are internalized into osteoclasts possibly by endocytosis. Subsequently, bisphosphonates inhibit FPP synthase, a key enzyme in the mevalonate signaling pathway. This leads to impaired intracellular protein prenylation impairing osteoclast function and apoptosis. Thus, bone resorption is inhibited.

decreased after treatment. The authors suggested that modulatory therapy with biological agents might be an interesting alternative to inhibit alveolar bone loss, and further studies are warranted to confirm the data.

6.3. Anti-TNF-α. Tumor necrosis factor-alpha is a key signaling modulator in the pathogenesis of PD, and its upregulation is associated with increased osteoclastogenesis. Thus, investigations targeting TNF-α have been evaluated to manage inflammatory bone resorption in animal models. In this context, a recent study evaluated the effects of systemic administration of Etanercept in mice with concomitant diabetes mellitus and periodontitis [115]. Obese diabetic Zucker rats were systemically administered with Etanercept and one week later received ligature to induce experimental PD. Animals were sacrificed after 5 weeks from the baseline. This study indicates that blocking TNF-α improves the metabolic status in obese rats with PD and decreases periodontal breakdown associated with diabetes. The same research group also confirmed that anti-TNF-α treatment positively impacts the subgingival microbial profile in rats with diabetes and ligature-induced bone loss [116]. Another study investigated anti-TNF-α effects with pentoxifylline in an experimental mouse model of chronic antigen-induced arthritis- (AIA-) associated PD [117]. The authors demonstrated that the treatment employed was able to diminish joint inflammation, reduce the levels of TNF-α and IL-17, and prevent signs of PD (decreased the number of osteoclasts and recruitment of neutrophils in the connective tissue). In addition, the treatment employed showed the anti-inflammatory and bone protective effects in mice with AIA and concomitant PD. Accordingly, a previous study also demonstrated the positive effects of anti-TNF-α on the progression of experimental PD induced by ligature placement by decreasing radiographical bone loss [113]. Finally, Cirelli et al. have used adenoassociated virus vector based on serotype 1 (AAV2/1) to deliver the TNF receptor-immunoglobulin Fc (TNFR:Fc) fusion gene to rats subjected to experimental periodontitis by means of *P. gingivalis* LPS-mediated bone loss [118]. The results showed that AAV2/1-TNFR:Fc administration diminished the levels of several pro-inflammatory cytokines and osteoclast-like cells in the connective tissue of rats. These data indicate that delivery of AAV2/1-TNFR:Fc might be a feasible approach to modulate PD progression.

7. Herbal Medicine

7.1. Curcumin. Curcumin is a bioactive compound of turmeric and derived from *Curcuma longa*, a tropical plant

native to Southeast Asia [119]. It is a yellow hydrophobic polyphenol composed of three curcuminoids, and it is largely used in dietary spice. It has been reported that curcumin has a variety of biological activities, including osteoimmune modulatory properties and anti-inflammatory, antioxidant, antiangiogenic, and antibacterial effects with the capacity to modulate the innate immune host response [31, 46, 120–123]. Due to the innumerous beneficial effects described in the literature with the use of curcumin to treat experimental PD, natural or chemically modified curcumin has been suggested as an interesting therapeutic approach to managing inflammatory bone resorption [30, 31, 45, 46, 120–123]. Nevertheless, different variables, such as diverse dosages (in vitro and in vivo), vehicle used, and administration route (intraperitoneally, intravenously, and orally), have also been described in the literature [30, 31, 46, 121, 123, 124].

Many investigations have been carried out to evaluate curcumin effects during the progression of experimental PD in murine [30, 31, 45, 46, 120–124] (Table 6). Recently, Pimentel et al. [125] assessed the impact of curcumin (100 mg/kg) on the progression of experimental PD in diabetic rats. The PD model was induced by placing cotton ligatures around the first mandibular molar and in the second maxillary molar. An injection of streptozotocin was intraperitoneally administered in the animals to induce experimental diabetes. Curcumin was administered daily by oral gavage for 30 days. The results indicated that natural curcumin reduces alveolar bone loss and favorably modulates the osteoimmune inflammatory process during disease progression. Interestingly, Zambrano et al. [31] investigate the local administration of curcumin-loaded nanoparticles in an experimental PD model. A model of *Escherichia coli* bacterial lipopolysaccharide (LPS) injection was used to induce PD. The curcumin nanoparticles were locally injected, 2 times per week for four weeks, in the palatal mucosa around the first maxillary molar. Radiographical analysis (micro-CT) showed significant reduction in the loss of alveolar bone caused by LPS in the animals treated with curcumin nanoparticles. A previous study [126] using the silk ligature model of PD in rats demonstrated the potent capacity of oral administration of curcumin (100 mg/kg/day) for 30 days to inhibit bone resorption, which is in agreement with the above-reported studies [31, 125].

Previous studies have used different strategies to enhance the clinical application of curcumin to treat experimental PD. Indeed, chemically modified compounds have been developed to increase their clinical efficacy, which resulted in greater bioavailability maintaining its biological and safety properties [46, 120, 121, 128]. de Almeida Brandao et al. [120] evaluated the effects of a modified curcumin so-called CMC22.4 that is a novel bis-dimethoxy-4-phenylaminocarbonyl curcuminoid. In this study, rats underwent experimental PD using direct microinjections of *Escherichia coli* bacterial LPS into the gingival tissue around the first maxillary molars three times per week. Curcumin was administered daily by oral gavage immediately after LPS injection and continued for the whole experimental period of 28 days. The outcomes showed that CMC2.24 inhibited bone loss, inflammation, and osteoclastogenesis in the LPS-induced periodontitis model even at a low dosage (1 mg/kg/day), suggesting that this compound is more effective than previously documented. Curylofo-Zotti et al. [46] also investigated the effects of CMC2.24 in a model of LPS-induced PD. Similar to the study mentioned above [120], the authors showed that oral administration of curcumin CMC2.24 (30 mg/kg/day) significantly inhibited inflammatory infiltrate in the gingival tissue, decreased the number of osteoclasts, and abrogates bone resorption, pointing to an interesting potential of CMC2.24 in preventing bone resorption in an inflammatory model of PD. Similarly, Elburki et al. [127] showed that oral gavage with CMC2.24 (30 mg/kg/day) also reduced inflammation-mediated connective tissue breakdown in rats with diabetes (induced by intravenous injection of streptozotocin) and PD (induced by *E. coli* LPS injections) and prevented hyperglycemia-induced tissue destruction. CMC2.24 was also able to attenuate the severity of inflammation and bone loss in the periodontal tissues, acting as a potential therapeutic inhibitor of bone resorption in inflammatory conditions. These findings parallel previous observations by the same research group [121] that demonstrated the positive effects of CMC2.24 in inhibiting bone resorption during LPS-induced experimental PD in rats.

Taken together, several studies have demonstrated the beneficial effects of natural curcumin or chemically modified curcumin to treat experimental PD without adverse side effects. Nevertheless, it is important to bear in mind that the differences in dosages used in the studies, the low absorption rate, reduced half-life, and rapid systemic elimination [129] might limit its clinical use to treat PD in humans.

7.2. Chalcones. Chalcone is a medicinal plant that has been conventionally used in Brazilian medicine to treat bleeding gums [130]. It is a phenolic compound extracted from the *Myracrodruon urundeuva* (Engl.). This compound presents analgesic and anti-inflammatory properties as evidenced by previous studies in experimental models of inflammation [130, 131]. Moreover, antioxidant, antimicrobial, and antiresorptive properties were previously described during inflammatory conditions, including RA and inflammatory bowel diseases [132, 133]. Therefore, based on the assumption that chalcone presents beneficial properties in inflammation, previous studies have investigated its potential therapeutic effects during experimental periodontitis in rats.

In a study of ligature-induced periodontal bone loss in rats, Botelho et al. [134] assessed the effects of a gel containing chalcones during the progression of PD. Rats underwent nylon ligature placement around the second maxillary molars and received immediately after its placement the chalcone gel (600 μg/g gel) topically applied to the gingival tissues three times per day during the entire experimental period (11 days). The results showed that chalcone gel prevents alveolar bone resorption in the conditions studied and presented with anti-inflammatory and antimicrobial effects during the course of PD.

More recently, Fernandes et al. [47] evaluated the effects of chalcone T4 during the progression of experimental PD.

In this study, PD was induced by placing a cotton ligature around the first mandibular molar. Chalcone T4 was systemically administered daily by intragastric gavage (5 and 50 mg/kg) starting on the same day of ligature placement. After 15 days of treatment, the animals were sacrificed, and measurements of radiographical, histological, and molecular analyses were performed. The data indicate that 5 mg/kg of chalcone T4 decreased bone resorption and cellular infiltrate in the connective tissue. Moreover, in vitro data demonstrated that this treatment resulted in a reduced number of osteoclasts and resorption area in raw 267.4 cells. As a proof-of-concept study, the data suggested the potential effect of chalcone T4 as an adjuvant for experimental PD treatment. More studies are warranted to investigate dose response, the effects in different inflammatory models, and the factors that might influence its bioavailability, to better comprehend the pharmacokinetics and pharmacodynamic behavior of chalcone T4 [47].

7.3. Flavonoids. In an attempt to pursue natural products with pharmacokinetic, anti-inflammatory, antioxidant, and immunomodulatory effects, growing attention has been dedicated to searching phenolic compounds that might have protective effects on bone and connective tissue [135]. Flavonoids, a group of polyphenolic compounds found in many plants (soybean, olive), fruits (orange peel), vegetables, seeds and beverages, have been suggested as a possible alternative to treat inflammatory bone resorption due to its wide range of biological properties and activities [136]. Therefore, the dietary intake of natural ingredients, including innumerous flavonoids, might be beneficial for bone tissues and can prevent PD progression and severity in different animal models of periodontitis. In this context, many studies have used different types of flavonoids to prevent and treat experimental periodontitis with beneficial effects on the alveolar bone tissue without adverse effects [32–34, 135, 137–142].

Genistein, an isoflavone found in soybean, attenuates alveolar bone loss in a rat model of ligature-induced periodontitis [139]. It has also been reported that genistein inhibits bone loss in ovariectomized (OVX) mice, pointing to an important role in preventing experimental postmenopausal osteoporosis [143]. Taxifolin is a flavanone with potent antioxidant properties that has been shown to stimulate osteoblast differentiation and suppress osteoclastogenesis in vitro [144]. Recently, Lektemur Alpan et al. [142] demonstrated that taxifolin attenuates inflammatory bone resorption in a model of ligature-induced bone loss in rats, decreases inflammatory infiltrate, and improves alveolar bone formation. In an experimental model of LPS-induced inflammatory bone loss, the administration of the flavonoids nobiletin and tangeretin was able to suppress LPS-induced osteoclast formation and bone loss. Furthermore, both flavonoids inhibited osteoclastogenesis in RAW264.7 macrophages [145]. Similarly, the effect of a flavonoid from the bergamot juice could inhibit bone loss and decrease gingival inflammation markers in a rat model of LPS-induced PD [140]. Huang et al. [141] evaluated the effects of myricetin, a naturally occurring flavonoid compound, in an experimental OVX mouse PD model. Systemic administration of myricetin prevented bone loss and enhanced alveolar crest height in vivo, and attenuated osteoclast formation and bone resorption in vitro [141] (Table 7).

Quercetin is an abundant flavonol-type flavonoid that has been associated with innumerous beneficial effects regarding the inflammatory process and immune functions [146–148]. The effects of quercetin on the progression of experimental PD were evaluated by Cheng et al. [138]. Utilizing a model of ligature-induced bone loss, the authors demonstrated decreased alveolar bone loss and reduced inflammatory cell infiltrate in the connective tissue of rats that have received systemic administration of quercetin. Moreover, in vitro data demonstrated that quercetin diminished LPS-induced osteoclast formation, suggesting that it might possess an ameliorative effect during PD progression [138]. Recently, it was demonstrated that a citrus flavonoid —eriocitrin and eriodictyol—diminished inflammatory cell infiltration in the connective tissue of rats with induced PD by means of LPS-injections suggesting that a diet supplemented with flavonoids might enhance local immunity and host defense [137]. Finally, other studies showed beneficial effects of hesperidin [34], luteolin [32], and oleuropein [135] on alveolar bone loss and inflammation in a rat model of ligature-induced PD indicating that flavonoids might be an interesting candidate for modulating inflammatory disease.

7.4. Colchicine. Colchicine, a natural compound extracted from *Colchicum autumnale*, possesses innumerous pharmacological properties, such as anti-inflammatory, antioxidant, antimitotic, and antiresorptive, that has been used to treat a variety of inflammatory diseases [149, 150]. The anti-inflammatory and antioxidant effects of colchicine rely on the inhibition of adhesion, mobilization, and chemotaxis of neutrophils and by the disruption of inflammasome activity (NALP3) and IL-1? secretion. A previous study has shown that colchicine inhibits bone resorption by preventing the release of lysosomal enzymes and blocking osteoclast activity. In this context, Aral et al. investigated the effects of colchicine on cytokine production, apoptosis, alveolar bone loss, and oxidative stress in rats with ligature-induced experimental periodontitis [151]. The animals received two different dosages of colchicine (30 and 100 μg/kg/day) immediately after ligature placement and were sacrificed 11 days after initial treatment. The results showed that colchicine treatment (both dosages) significantly decreased the expression of IL-1β, IL-8, and RANKL; RANKL/OPG ratio; total oxidative stress level; and bone volume ratio and increased total antioxidant suggesting that colchicine has prophylactic potential to prevent the progression of bone loss through anti-inflammatory and antiresorptive properties.

8. Specialized Proresolving Mediators (SPM)

Current key discoveries in the mechanisms of inflammation during PD initiation and progression encouraged the search for new treatment alternatives for PD using proresolving mediators. Resolution of inflammation comprises active biochemical programs that allow inflamed tissue to return to

homeostasis [152, 153]. SPMs are a novel family of oxylipids mediators, including resolvins, maresins, lipoxins, and protectins, derived from omega-3 polyunsaturated fatty acid (PUFA), which regulate the inflammatory process without immunosuppression [7]. The SPMs function in inflammation termination by activating specific mechanisms to restore tissue homeostasis [152, 153]. Briefly, they selectively inhibit leukocyte recruitment, activate macrophage phagocytosis of microorganisms, stimulate infiltration of monocytes, and stimulate the expression of molecules involved in antimicrobial defense [154]. Such SPMs promote tissue repair, eliminate bacteria, increase the host defense, and impact the responses of adaptive immune cells (Figure 3) [39]. The E-series resolvins (RvE1) are biosynthesized from the eicosapentaenoic acid (EPA), and it is considered a stereoselective agonist that interacts with two identified G protein-coupled receptors: BLT1 (expressed on neutrophils) and chemerin receptor 23 (chemR23) expressed on macrophages, monocytes, dendritic cells, and osteoblasts [155, 156]. RvE1 interacts with BLT1 or chemR23 to inhibit leukocyte infiltration and cytokine production, thus promoting the resolution of inflammation [154].

SPMs show significant effectiveness in treating inflammatory conditions including inflammatory pain [157], experimental PD [40, 158, 159], and bone preservation [42]. Furthermore, it has been reported that SPM attenuates atherosclerotic plaque formation in diet- and inflammation-induced atherogenesis [160]. Gao et al. [42] showed that transgenic mice overexpressing the human chemR23 were able to diminish the destruction of the alveolar bone induced by ligature placement. Moreover, local RvE1 treatment accelerated the regeneration of bone defects in a craniotomy model. Taken together, RvE1 modulates osteoclast differentiation and bone remodeling, rescuing OPG production and restoring a favorable RANKL/OPG ratio [42]. This data agrees with the previous report that evaluated the impact of RvE1 on bone remodeling in mice, using a calvaria osteolytic model with or without systemic administration of RvE1 [161]. The data demonstrated that RvE1 reduced bone resorption and osteoclastogenesis. RvE1 also negatively regulated osteoclast differentiation, which resulted in a reduction in inflammatory bone resorption, suggesting that RvE1 may be a therapeutic potential for treating inflammatory diseases [161]. Lee et al. [159] also demonstrated that topical application of RvE1 downregulated bone loss induced by ligature placement and decreased the inflammatory process and the number and size of osteoclasts in rats. In addition, RvE1 induced changes in the composition of the local microbiota suggesting the modulation of local inflammation has an important role in forming the subgingival microbiota composition [159].

Hasturk et al. demonstrated that topical application RvE1 was able to prevent initiation and progression of experimental PD and even induce the regeneration of periodontal tissues (alveolar bone, periodontal ligament, and cement) in a rabbit model of ligature-induced bone loss [40, 158]. RvE1 downregulated the progression of PD by decreasing proinflammatory mediators and reducing inflammatory bone loss. Furthermore, RvE1 is able to enhance the clearance of PD-associated bacteria [40, 158]. These outcomes suggest that PD-associated bacteria actively direct the protective bactericidal immune response into a dysfunctional state, which may be reversed by SPMs. The established protective action of SPM aiming in promoting the resolution of inflammation in innumerous animal models of PD makes them an interesting alternative to treat PD [7, 162]. Table 8 describes the primary findings of the selected studies.

9. Probiotics

The manipulation of the intestinal microbiota through probiotics has been proposed to alter bone remodeling during the course of PD both in preclinical studies and in randomized clinical trials. The rationale for this approach is based on the concept that bone health is affected by changes in the intestinal microbiota and therefore, strategies to induce beneficial effects through nutritional supplementation with probiotics have been evidenced. The term probiotics were introduced by Lilly and Stillwell in 1965 [163]. Probiotics are live microorganisms that, when administered in adequate amounts, confer beneficial effects on the host's health. They repopulate beneficial bacteria, which can help kill pathogenic bacteria and fight infection. Orally administered probiotics can benefit oral health by preventing microbiota growth or modulating mucosal immunity in the oral cavity [164]. Probiotics can help prevent and treat PD through several mechanisms, including direct interaction, competitive exclusion, and modulation of the host's immune response. Studies show that the treatment strategies conferred by probiotics against PD occur mainly by inhibiting specific pathogens or altering the host's immune response [165] (Table 9).

Several studies have been published using probiotics for the treatment of experimental PD. Moraes et al. investigated the effects of *L. reuteri* administration during the development of induced PD in rats [166]. The results showed that treatment with probiotics increased the percentage of bone volume and the thickness and number of trabeculae and decreased bone porosity and trabecular separation. Cardoso et al. evaluated the effects of systemic administration of the probiotic *Bifidobacterium animalis* HN019 on ligature-induced periodontitis in rats with experimental RA [167]. Probiotic treatment in animals with experimental arthritis and PD reduced alveolar bone loss, TNF-α, and IL-6 levels and increased IL-17 levels compared to those without probiotics. Furthermore, there was a decrease in the levels of anticitrullinated protein antibodies in animals with experimental RA. Ricoldi et al. [168] and Oliveira et al. [169] found similar results using HN019 to treat experimental PD, showing reductions in alveolar bone resorption and connective tissue attachment loss. These results were also observed using different strains of probiotics, including *Lactobacillus rhamnosus* [170], *Lactobacillus brevis* CD2 [171], and *Lactobacillus gasseri* SBT2055 [172].

Some limitations associated with the use of probiotic therapy (difficulty of exogenously administered bacteria in remaining in the oral environment) have stimulated the search for other strategies capable of manipulating the

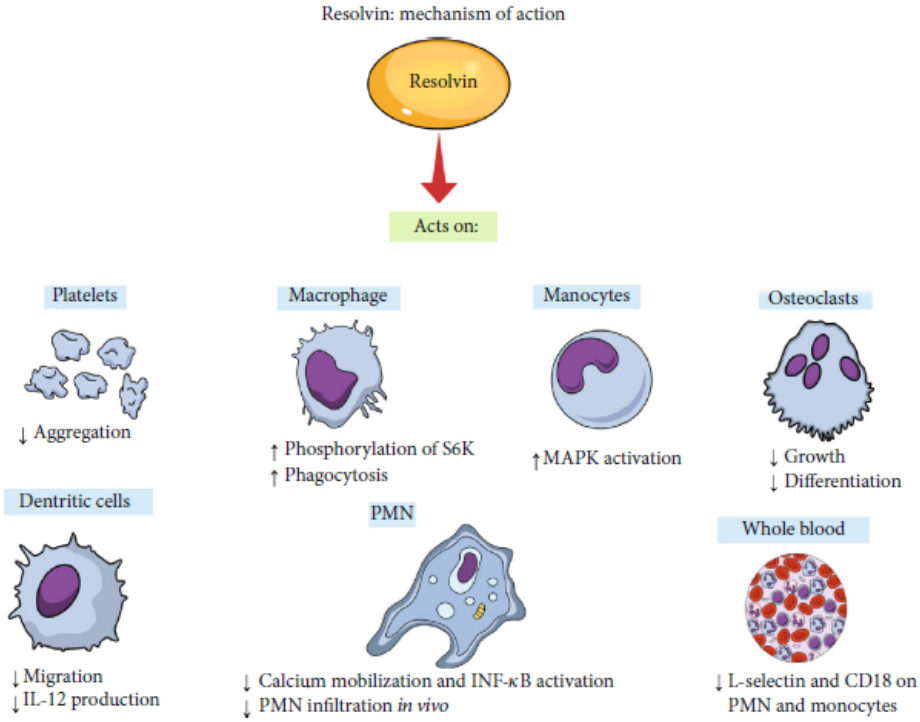

FIGURE 3: Resolvins (RvE1) act to reduce ADP-stimulated platelet aggregation. In macrophages, RvE1 increases S6K (ribosomal protein S6 kinase) phosphorylation and phagocytosis. In monocytes, MAPK (mitogen-activated protein kinase) activation occurs. RvE1 acts on osteoclasts decreasing their growth and differentiation. In dendritic cells, there is a decrease in their migration and in the production of IL-12. In vitro, RvE1 reduced calcium mobilization and activation of NF-kB, and in vivo, there was a lower infiltration of PMN (polymorphonuclear cell/neutrophil). In the blood, there is a decrease in L-selectin and CD18 in PMN and monocytes.

ecology of the oral biofilm [174]. An interesting approach concerns the nutritional stimulation of beneficial native bacteria to promote oral health. Prebiotics favor changes in microbial composition or activity, aiming to stimulate the growth of health-promoting bacteria in the resident intestinal microbiota, which provides local and systemic benefits for the host's health. By definition, prebiotics are selectively fermented ingredients that allow specific changes, either in the composition and/or activity of the gastrointestinal tract microflora, that confer benefits to the host, well-being, and health. They are substances not digested by enzymes, salts, and acids produced by the body. Currently, only oligosaccharides (fructooligosaccharides and galactooligosaccharides) can be called prebiotics. Their mechanism of action occurs through the following: (a) improvement in the growth of resident commensal intestinal bacteria, particularly *bifidobacteria* and *lactobacilli*; (b) they exert a direct effect on the host by stimulating the expression of IL-10 and INF-g, increased secretion of immunoglobulin (IgA), and modulation of inflammatory responses in pathogens [174].

Prebiotics and probiotics often work synergistically and, when combined in the same product, are known as symbiotics. Symbiotics contain both probiotic and prebiotic components. The rationale for such products is that the combination increases the survival of probiotic bacteria in the passage through the proximal region of the gastrointestinal tract, improving colonization of the probiotic in the large intestine, stimulating the effect on the growth of endogenous flora. The main prebiotics evaluated in humans are fructans and galactans. Mannan oligosaccharides (MOS) are also gaining importance. Levi et al. [173] performed a preclinical study in rats demonstrating that animals with ligature-induced PD showed changes in intestinal morphology compared to animals without the disease, confirming the possible relationship between oral and intestinal dysbiosis. When animals with experimental PD were treated with MOS, the intestinal morphology became more similar to that of animals without disease, demonstrating prebiotics' protective role in the intestinal environment under conditions of oral dysbiosis. Furthermore, animals with PD and MOS had less severe PD than those not treated with MOS. In fact, recent scientific evidence suggests that manipulating the microbiota through prebiotics and probiotics confers health benefits on the host through different mechanisms, improving periodontal health and other common skeletal diseases such as arthritis and osteoporosis.

10. Vitamins

10.1. Vitamin C. Vitamin C has powerful antioxidant properties and has been the focus of several investigations to manage inflammatory diseases, including PD [175]. Deficiency in the levels of systemic vitamin C might affect the gingival and connective tissue increasing the expression of

inflammatory cells and impairing collagen formation, thus worsening the severity of periodontitis [176, 177]. A study conducted by Akman et al. evaluated the therapeutic effect of vitamin C on alveolar bone loss in rats with ligature-induced experimental periodontitis [178]. The ligatures were maintained for 5 weeks to induce periodontal breakdown, and then, they were removed. Treatments with vitamin C or vitamin C plus alpha lipoic acid (ALA—50 mg/kg) were initiated immediately after ligature removal with a single intragastric dose for 15 days. Levels of bone alkaline phosphatase and myeloperoxidase activity were measured in the gingival tissues, and expressions of RANKL and bone density were determined histologically. The results indicated that vitamin C and ALA inhibit inflammatory bone resorption and osteoclast activation suggesting its beneficial improvements in osteoclast-mediated bone resorption [178].

10.2. Vitamin B. Previously published studies on the effects of food and nutrients with antioxidant and anti-inflammatory activities have constantly been linked to improvements in the periodontal status in animal models [179] and also in patients [180] when treated with vitamin B. Vitamin B complex, a class of water-soluble vitamins, play pivotal functions in cell metabolism [179]. The vitamin B complex includes eight different vitamins which differ in their chemical composition and pharmacological properties [181]. Studies have shown that vitamin B complex is important in soft wound healing and gingival health, and some studies have indicated that vitamin B12 [182], vitamin B9 [183], vitamins B1, B2, B3, B5, B6, and B7 reduced the periodontal destruction and tooth mobility [184]. Recently, Akpınar et al. investigated the effects of vitamin B complex supplementation on the progression of experimental periodontitis in rats. Daily systemic administration of vitamin B by oral gavage was initiated immediately after ligature placement and followed by 11 days. Then, animals were sacrificed and bone tissue samples were collected for histomorphometric evaluation. The authors showed that vitamin B administration increased osteoblast activity, diminished osteoclast numbers, and reduced alveolar bone loss in rat with experimental PD, suggesting beneficial effects of vitamin B complex on the bone tissue.

10.3. Vitamins D and K. Vitamin D receptor has been found on many immune cells, such as macrophages, dendritic cells, and T and B cells [185]. Additionally, it has been shown that vitamin D inhibits proinflammatory processes by suppressing the overactivity of CD4+ Th1, Th2, and Th17 cells and the production of their related cytokines such as IL-2, IFN-gamma, and TNF-alpha [186, 187]. Vitamin D has also regulatory effects on bone formation markers, such as osteocalcin and osteopontin, and acts as an immune modulator in inflammatory conditions [185]. Vitamin K plays important roles on bone protection, in the proliferation of bone marrow mesenchymal stem cells, in stimulating osteoblast differentiation and inhibiting adipocyte differentiation. In addition, it can protect osteoblasts and reduce apoptosis. Due to its anabolic effects on bone, the effect of vitamins B and K on gingival inflammation and alveolar bone destruction in rats was investigated by Aral et al. [188]. In this study, periodontitis was induced by placing cotton ligatures around the maxillary first molar for 7 days. Then, ligatures were removed, and tooth received scaling and root planning followed by oral gavage with vitamins D and K or a combination of vitamins D and K for 10 days. The results indicated that alveolar bone loss in rats administrated with vitamin D or K did not differ from rats without treatment, suggesting that this approach has no positive effects on alveolar bone and in gingival inflammatory markers.

11. Conclusion

This comprehensive review of the literature summarizes the main findings of studies that have used pharmacological drugs to manage experimental PD. The use of modulators of the immune host response or antiresorptive medications offers interesting alternatives to inhibit bone loss and decrease the inflammatory infiltrate in the connective tissue. All those treatments tested can help modulate the host inflammatory response and ameliorate the progression of the experimental disease. As stated earlier, the primary treatment of PD is through a mechanical approach, SRP, to remove the attached biofilm into the tooth and root surface. However, this local treatment does not respond equally well in susceptible patients. Thus, adjunctive therapies that decrease the inflammatory host response play an important role in achieving better clinical outcomes, especially in patients with associated comorbidities, such as diabetes mellitus and rheumatoid arthritis. It is important to bear in mind that some of the included drugs in this review, i.e., bisphosphonate, biological agents, and RANKL and CtsK inhibitors, possess some side effects that might limit their clinical use. Therefore, herbal medicine and supplementation with omega 3 and probiotics have gained growing attention due to its modulatory and antiresorptive activities and the lack of side effects being considered promising alternatives as adjunctive to SRP in susceptible patients.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest to report regarding the present study.

Authors' Contributions

The authors confirm contribution to the paper as follows: study conception and design: JAC and RSM; draft manuscript preparation: ALRP, BSM, EBBP, FASM, JAC, and RSM. All authors reviewed the article text and approved the final version of the manuscript.

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References

[1] D. F. Kinane, P. G. Stathopoulou, and P. N. Papapanou, "Periodontal diseases," *Nat Rev Dis Primers*, vol. 3, p. 17038, 2017.

[2] Z. S. Natto, R. H. Abu Ahmad, L. T. Alsharif et al., "Chronic periodontitis case definitions and confounders in periodontal research: a systematic assessment," *Biomed Res Int*, vol. 2018, p. 4578782, 2018.

[3] M. S. Tonetti, H. Greenwell, and K. S. Kornman, "Staging and grading of periodontitis: Framework and proposal of a new classification and case definition," *J Periodontol*, vol. 89, Suppl 1, pp. S159–S172, 2018.

[4] B. L. Pihlstrom, B. S. Michalowicz, and N. W. Johnson, "Periodontal diseases," *Lancet*, vol. 366, no. 9499, pp. 1809–1820, 2005.

[5] G. Hajishengallis, T. Chavakis, and J. D. Lambris, "Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy," *Periodontol*, vol. 84, no. 1, pp. 14–34, 2000.

[6] R. S. de Molon, E. D. de Avila, J. A. Cirelli, and J. P. Steffens, "Periodontal research contributions to basic sciences: from cell communication and host-parasite interactions to inflammation and bone biology," *Biocell*, vol. 46, no. 3, pp. 633–638, 2022.

[7] M. G. Balta, E. Papathanasiou, I. J. Blix, and T. E. Van Dyke, "Host modulation and treatment of periodontal disease," *J Dent Res*, vol. 100, no. 8, pp. 798–809, 2021.

[8] K. R. Phipps and V. J. Stevens, "Relative contribution of caries and periodontal disease in adult tooth loss for an HMO dental population," *J Public Health Dent*, vol. 55, no. 4, pp. 250–252, 1995.

[9] G. N. Belibasakis, D. Reddi, and N. Bostanci, "Porphyromonas gingivalis induces RANKL in T-cells," *Inflammation*, vol. 34, no. 2, pp. 133–138, 2011.

[10] T. Yucel-Lindberg and T. Bage, "Inflammatory mediators in the pathogenesis of periodontitis," *Expert Rev Mol Med*, vol. 15, article e7, 2013.

[11] R. S. de Molon, C. Rossa Jr., R. M. Thurlings, J. A. Cirelli, and M. I. Koenders, "Linkage of periodontitis and rheumatoid arthritis: current evidence and potential biological interactions," *International journal of molecular sciences*, vol. 20, no. 18, 2019.

[12] D. L. Lacey, W. J. Boyle, W. S. Simonet et al., "Bench to bedside: elucidation of the OPG-RANK-RANKL pathway and the development of denosumab," *Nat Rev Drug Discov*, vol. 11, no. 5, pp. 401–419, 2012.

[13] J. Bhuvaneswarri, B. Gita, and S. C. Chandrasekaran, "Detection of rankl positive cells in gingival tissue in healthy & chronic periodontal disease patients-a comparative study," *J Clin Diagn Res*, vol. 8, pp. 31–34, 2014.

[14] H. W. G. I. Birkedal-Hansen, W. G. I. Moore, M. K. Bodden et al., "Matrix metalloproteinases: a review," *Crit Rev Oral Biol Med*, vol. 4, no. 2, pp. 197–250, 1993.

[15] M. A. Taubman, P. Valverde, X. Han, and T. Kawai, "Immune response: the key to bone resorption in periodontal disease," *J Periodontol*, vol. 76, 11 Suppl, pp. 2033–2041, 2005.

[16] J. W. Krayer, R. S. Leite, and K. L. Kirkwood, "Non-surgical chemotherapeutic treatment strategies for the management of periodontal diseases," *Dent Clin North Am*, vol. 54, no. 1, pp. 13–33, 2010.

[17] X. Wang, Z. Jia, Y. Almoshari, S. M. Lele, R. A. Reinhardt, and D. Wang, "Local application of pyrophosphorylated simvastatin prevents experimental periodontitis," *Pharm Res*, vol. 35, no. 8, p. 164, 2018.

[18] P. M. Preshaw, "Host modulation therapy with anti-inflammatory agents," *Periodontol*, vol. 76, no. 1, pp. 131–149, 2000.

[19] L. M. Golub, H. M. Lee, M. E. Ryan, W. V. Giannobile, J. Payne, and T. Sorsa, "Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms," *Adv Dent Res*, vol. 12, no. 2, pp. 12–26, 1998.

[20] L. M. Golub, M. Wolff, S. Roberts, H. M. Lee, M. Leung, and G. S. Payonk, "Treating periodontal diseases by blocking tissue-destructive enzymes," *Journal of the American Dental Association*, vol. 125, no. 2, pp. 163–169, 1994.

[21] L. M. Golub, M. S. Elburki, C. Walker et al., "Non-antibacterial tetracycline formulations: host-modulators in the treatment of periodontitis and relevant systemic diseases," *Int Dent J*, vol. 66, no. 3, pp. 127–135, 2016.

[22] A. Yagan, S. Kesim, and N. Liman, "Effect of low-dose doxycycline on serum oxidative status, gingival antioxidant levels, and alveolar bone loss in experimental periodontitis in rats," *J Periodontol*, vol. 85, no. 3, pp. 478–489, 2014.

[23] G. Greenstein, "Local drug delivery in the treatment of periodontal diseases: assessing the clinical significance of the results," *J Periodontol*, vol. 77, no. 4, pp. 565–578, 2006.

[24] L. Hao, J. Chen, Z. Zhu et al., "Odanacatib, a cathepsin K-specific inhibitor, inhibits inflammation and bone loss caused by periodontal diseases," *J Periodontol*, vol. 86, no. 8, pp. 972–983, 2015.

[25] W. Chen, B. Gao, L. Hao et al., "The silencing of cathepsin K used in gene therapy for periodontal disease reveals the role of cathepsin K in chronic infection and inflammation," *J Periodontal Res*, vol. 51, no. 5, pp. 647–660, 2016.

[26] Q. Jin, J. A. Cirelli, C. H. Park et al., "RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodontitis," *J Periodontol*, vol. 78, no. 7, pp. 1300–1308, 2007.

[27] N. C. Karakan, A. Akpinar, F. Goze, and O. Poyraz, "Investigating the effects of systemically administered strontium ranelate on alveolar bone loss histomorphometrically and histopathologically on experimental periodontitis in rats," *J Periodontol*, vol. 88, no. 2, pp. e24–e31, 2017.

[28] Y. Ozaki, T. Morozumi, K. Watanabe et al., "Inhibitory effect of omega-3 fatty acids on alveolar bone resorption and osteoclast differentiation," *J Oral Sci*, vol. 62, no. 3, pp. 298–302, 2020.

[29] F. Strålberg, A. Kassem, F. Kasprzykowski et al., "Inhibition of lipopolysaccharide-induced osteoclast formation and bone resorption in vitro and in vivo by cysteine proteinase inhibitors," *J Leukoc Biol*, vol. 101, no. 5, pp. 1233–1243, 2017.

[30] H. H. Wang, H.-M. Lee, V. Raja et al., "Enhanced efficacy of chemically modified curcumin in experimental periodontitis:

systemic implications," *J Exp Pharmacol*, vol. 11, pp. 1–14, 2019.

[31] L. M. G. Zambrano, D. A. Brandao, F. R. G. Rocha et al., "Local administration of curcumin-loaded nanoparticles effectively inhibits inflammation and bone resorption associated with experimental periodontal disease," *Sci Rep*, vol. 8, no. 1, p. 6652, 2018.

[32] H. Balci Yuce, H. Toker, A. Yildirim, M. B. Tekin, F. Gevrek, and N. Altunbas, "The effect of luteolin in prevention of periodontal disease in Wistar rats," *J Periodontol*, vol. 90, no. 12, pp. 1481–1489, 2019.

[33] Y. H. Wu, R. Kuraji, Y. Taya, H. Ito, and Y. Numabe, "Effects of theaflavins on tissue inflammation and bone resorption on experimental periodontitis in rats," *J Periodontal Res*, vol. 53, no. 6, pp. 1009–1019, 2018.

[34] P.-. J. Kuo, E. Fu, C.-. Y. Lin et al., "Ameliorative effect of hesperidin on ligation-induced periodontitis in rats," *J Periodontol*, vol. 90, no. 3, pp. 271–280, 2019.

[35] J. De Almeida, E. Ervolino, L. H. Bonfietti et al., "Adjuvant therapy with sodium alendronate for the treatment of experimental periodontitis in rats," *J Periodontol*, vol. 86, no. 10, pp. 1166–1175, 2015.

[36] M. M. Moreira, V. Bradaschia-Correa, N. D. Marques, L. B. Ferreira, and V. E. Arana-Chavez, "Ultrastructural and immunohistochemical study of the effect of sodium alendronate in the progression of experimental periodontitis in rats," *Microsc Res Tech*, vol. 77, no. 11, pp. 902–909, 2014.

[37] C. L. M. Storrer, T. M. Deliberador, A. F. Giovanini, V. Crivellaro, J. C. Zielak, and G. A. Romito, "Effect of alendronate on the progression of periodontitis induced by Porphyromonas gingivalis and Fusobacterium nucleatum: a study in rats," *Clin Oral Investig*, vol. 20, no. 9, pp. 2565–2573, 2016.

[38] A. M. Menezes, F. A. Rocha, H. V. Chaves, C. B. Carvalho, R. A. Ribeiro, and G. A. Brito, "Effect of sodium alendronate on alveolar bone resorption in experimental periodontitis in rats," *J Periodontol*, vol. 76, no. 11, pp. 1901–1909, 2005.

[39] G. Mizraji, O. Heyman, T. E. Van Dyke, and A. Wilensky, "Resolvin D2 restrains Th1 immunity and prevents alveolar bone loss in murine periodontitis," *Front Immunol*, vol. 9, p. 785, 2018.

[40] H. Hasturk, A. Kantarci, T. Ohira et al., "RvE1 protects from local inflammation and osteoclast- mediated bone destruction in periodontitis," *FASEB J*, vol. 20, no. 2, pp. 401–403, 2006.

[41] B. S. Herrera, T. Ohira, L. Gao et al., "An endogenous regulator of inflammation, resolvin E1, modulates osteoclast differentiation and bone resorption," *Br J Pharmacol*, vol. 155, no. 8, pp. 1214–1223, 2008.

[42] L. Gao, D. Faibish, G. Fredman et al., "Resolvin E1 and chemokine-like receptor 1 mediate bone preservation," *J Immunol*, vol. 190, no. 2, pp. 689–694, 2013.

[43] L. M. Marins, M. H. Napimoga, F. de Souza Malta et al., "Effects of strontium ranelate on ligature-induced periodontitis in estrogen-deficient and estrogen-sufficient rats," *J Periodontal Res*, vol. 55, no. 1, pp. 141–151, 2020.

[44] T. Zhou, D. Chen, Q. Li, X. Sun, Y. Song, and C. Wang, "Curcumin inhibits inflammatory response and bone loss during experimental periodontitis in rats," *Acta Odontol Scand*, vol. 71, no. 2, pp. 349–356, 2013.

[45] M. R. Guimaraes-Stabili, S. G. de Aquino, F. de Almeida Curylofo et al., "Systemic administration of curcumin or piperine enhances the periodontal repair: a preliminary study in rats," *Clin Oral Investig*, vol. 23, no. 8, pp. 3297–3306, 2019.

[46] F. A. Curylofo-Zotti, M. S. Elburki, P. A. Oliveira et al., "Differential effects of natural curcumin and chemically modified curcumin on inflammation and bone resorption in model of experimental periodontitis," *Arch Oral Biol*, vol. 91, pp. 42–50, 2018.

[47] N. A. R. Fernandes, A. C. Camilli, L. A. G. Maldonado et al., "Chalcone T4, a novel chalconic compound, inhibits inflammatory bone resorption in vivo and suppresses osteoclastogenesis in vitro," *J Periodontal Res*, vol. 53, no. 3, pp. 569–578, 2021.

[48] W. Wei, J. Ren, W. Yin et al., "Inhibition of Ctsk modulates periodontitis with arthritis via downregulation of TLR9 and autophagy," *Cell Prolif*, vol. 53, no. 1, article e12722, 2020.

[49] R. S. de Molon, C. H. Park, Q. Jin, J. Sugai, and J. A. Cirelli, "Characterization of ligature-induced experimental periodontitis," *Microsc Res Tech*, vol. 81, no. 12, pp. 1412–1421, 2018.

[50] R. S. de Molon, E. D. de Avila, A. V. B. Nogueira et al., "Evaluation of the host response in various models of induced periodontal disease in mice," *J Periodontol*, vol. 85, no. 3, pp. 465–477, 2014.

[51] A. V. B. Nogueira, R. S. de Molon, M. Nokhbehsaim, J. Deschner, and J. A. Cirelli, "Contribution of biomechanical forces to inflammation-induced bone resorption," *J Clin Periodontol*, vol. 44, no. 1, pp. 31–41, 2017.

[52] R. S. de Molon, V. I. Mascarenhas, E. D. de Avila et al., "Long-term evaluation of oral gavage with periodontopathogens or ligature induction of experimental periodontal disease in mice," *Clin Oral Investig*, vol. 20, no. 6, pp. 1203–1216, 2016.

[53] J. Cavagni, I. C. de Macedo, E. J. Gaio et al., "Obesity and hyperlipidemia modulate alveolar bone loss in Wistar rats," *J Periodontol*, vol. 87, no. 2, pp. e9–17, 2016.

[54] M. E. S. Lopes, C. C. Marcantonio, R. S. de Molon et al., "Obesity influences the proteome of periodontal ligament tissues following periodontitis induction in rats," *J Periodontal Res*, 2022.

[55] B. Rath-Deschner, S. Memmert, A. Damanaki et al., "CXCL5, CXCL8, and CXCL10 regulation by bacteria and mechanical forces in periodontium," *Ann Anat*, vol. 234, article 151648, 2021.

[56] A. V. Nogueira, M. Nokhbehsaim, S. Tekin et al., "Resistin is increased in periodontal cells and tissues: in vitro and in vivo studies," *Mediators Inflamm*, vol. 2020, p. 9817095, 2020.

[57] M. M. Belluci, R. S. de Molon, S. T. Carlos Rossa Jr. et al., "Severe magnesium deficiency compromises systemic bone mineral density and aggravates inflammatory bone resorption," *J Nutr Biochem*, vol. 77, article 108301, 2020.

[58] R. S. de Molon, E. D. de Avila, and J. A. Cirelli, "Host responses induced by different animal models of periodontal disease: a literature review," *J Investig Clin Dent*, vol. 4, no. 4, pp. 211–218, 2013.

[59] D. T. Graves, J. Kang, O. Andriankaja, K. Wada, and C. Rossa Jr., "Animal models to study host-bacteria interactions involved in periodontitis," *Front Oral Biol*, vol. 15, pp. 117–132, 2012.

[60] D. T. Graves, D. Fine, Y. T. Teng, T. E. Van Dyke, and G. Hajishengallis, "The use of rodent models to investigate host-bacteria interactions related to periodontal diseases," *J Clin Periodontol*, vol. 35, no. 2, pp. 89–105, 2008.

[61] J. A. C. D. Souza, F. A. C. Magalhães, G. J. P. L. D. Oliveira, R. S. De Molon, J. A. Zuanon, and P. P. C. D. Souza, "Pam2CSK4 (TLR2 agonist) induces periodontal destruction in mice," *Braz Oral Res*, vol. 34, article e012, 2020.

[62] R. A. Dodds, I. E. James, D. Rieman et al., "Human osteoclast cathepsin K is processed intracellularly prior to attachment and bone resorption," *J Bone Miner Res*, vol. 16, no. 3, pp. 478–486, 2001.

[63] K.-I. Tezuka, Y. Tezuka, A. Maejima et al., "Molecular cloning of a possible cysteine proteinase predominantly expressed in osteoclasts," *J Biol Chem*, vol. 269, no. 2, pp. 1106–1109, 1994.

[64] P. Garnero, O. Borel, I. Byrjalsen et al., "The collagenolytic activity of cathepsin K is unique among mammalian proteinases," *J Biol Chem*, vol. 273, no. 48, pp. 32347–32352, 1998.

[65] J. Christensen and V. P. Shastri, "Matrix-metalloproteinase-9 is cleaved and activated by cathepsin K," *BMC Res Notes*, vol. 8, p. 322, 2015.

[66] W. Kafienah, D. Bromme, D. J. Buttle, L. J. Croucher, and A. P. Hollander, "Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix," *Biochem J*, vol. 331, no. 3, pp. 727–732, 1998.

[67] M. T. Drake, B. L. Clarke, M. J. Oursler, and S. Khosla, "Cathepsin K inhibitors for osteoporosis: biology, potential clinical utility, and lessons learned," *Endocr Rev*, vol. 38, no. 4, pp. 325–350, 2017.

[68] B. J. Votta, M. A. Levy, A. Badger et al., "Peptide aldehyde inhibitors of cathepsin K inhibit bone resorption both in vitro and in vivo," *J Bone Miner Res*, vol. 12, no. 9, pp. 1396–1406, 1997.

[69] S. K. Thompson, S. M. Halbert, M. J. Bossard et al., "Design of potent and selective human cathepsin K inhibitors that span the active site," *Proc Natl Acad Sci U S A*, vol. 94, no. 26, pp. 14249–14254, 1997.

[70] D. S. Yamashita and R. A. Dodds, "Cathepsin K and the design of inhibitors of cathepsin K," *Curr Pharm Des*, vol. 6, no. 1, pp. 1–24, 2000.

[71] J. Han, L. Wei, W. Xu et al., "CTSK inhibitor exert its anti-obesity effects through regulating adipocyte differentiation in high-fat diet induced obese mice," *Endocr J*, vol. 62, no. 4, pp. 309–317, 2015.

[72] Y. Yue, W. Yin, Q. Yang et al., "Inhibition of cathepsin K alleviates autophagy-related inflammation in periodontitis-aggravating arthritis," *Infect Immun*, vol. 88, no. 12, 2020.

[73] A. G. Costa, N. E. Cusano, B. C. Silva, S. Cremers, and J. P. Bilezikian, "Cathepsin K: its skeletal actions and role as a therapeutic target in osteoporosis," *Nat Rev Rheumatol*, vol. 7, no. 8, pp. 447–456, 2011.

[74] M. R. McClung, M. L. O'Donoghue, S. E. Papapoulos et al., "Odanacatib for the treatment of postmenopausal osteoporosis: results of the LOFT multicentre, randomised, double-blind, placebo-controlled trial and LOFT Extension study," *Lancet Diabetes Endocrinol*, vol. 7, no. 12, pp. 899–911, 2019.

[75] W. Pan, W. Yin, L. Yang et al., "Inhibition of Ctsk alleviates periodontitis and comorbid rheumatoid arthritis via down-regulation of the TLR9 signalling pathway," *J Clin Periodontol*, vol. 46, no. 3, pp. 286–296, 2019.

[76] N. Da Ponte Leguizamón, R. S. de Molon, G. Coletto-Nunes et al., "Phytocystatin CsinCPI-2 reduces osteoclastogenesis and alveolar bone loss," *J Dent Res*, vol. 101, no. 2, pp. 216–225, 2022.

[77] M. Asagiri, T. Hirai, T. Kunigami et al., "Cathepsin K-dependent toll-like receptor 9 signaling revealed in experimental arthritis," *Science*, vol. 319, no. 5863, pp. 624–627, 2008.

[78] R. Dai, Z. Wu, H. Y. Chu et al., "Cathepsin K: the action in and beyond bone," *Front Cell Dev Biol*, vol. 8, p. 433, 2020.

[79] D. Keppler, "Towards novel anti-cancer strategies based on cystatin function," *Cancer Lett*, vol. 235, no. 2, pp. 159–176, 2006.

[80] M. Brage, A. Lie, M. Ransjö et al., "Osteoclastogenesis is decreased by cysteine proteinase inhibitors," *Bone*, vol. 34, no. 3, pp. 412–424, 2004.

[81] U. H. Lerner and A. Grubb, "Human cystatin C, a cysteine proteinase inhibitor, inhibits bone resorption in vitro stimulated by parathyroid hormone and parathyroid hormone-related peptide of malignancy," *J Bone Miner Res*, vol. 7, no. 4, pp. 433–440, 1992.

[82] N. D. P. Leguizamon, E. M. Rodrigues, M. L. de Campos et al., "In vivo and in vitro anti-inflammatory and pro-osteogenic effects of citrus cystatin CsinCPI-2," *Cytokine*, vol. 123, article 154760, 2019.

[83] S. P. Luckman, D. E. Hughes, F. P. Coxon, R. Graham, G. Russell, and M. J. Rogers, "Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras," *J Bone Miner Res*, vol. 13, no. 4, pp. 581–589, 1998.

[84] D. B. Kimmel, "Mechanism of action, pharmacokinetic and pharmacodynamic profile, and clinical applications of nitrogen-containing bisphosphonates," *J Dent Res*, vol. 86, no. 11, pp. 1022–1033, 2007.

[85] R. Baron, S. Ferrari, and R. G. Russell, "Denosumab and bisphosphonates: different mechanisms of action and effects," *Bone*, vol. 48, no. 4, pp. 677–692, 2011.

[86] S. L. Ruggiero, T. B. Dodson, L. A. Assael, R. Landesberg, R. E. Marx, and B. Mehrotra, "American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws 2009 update," *J Oral Maxillofac Surg*, vol. 67, 5 Suppl, pp. 2–12, 2009.

[87] S. L. Ruggiero, T. B. Dodson, T. Aghaloo, E. R. Carlson, B. B. Ward, and D. Kademani, "American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw 2014 update," *J Oral Maxillofac Surg*, vol. 72, no. 10, pp. 1938–1956, 2014.

[88] S. L. Ruggiero and B. Mehrotra, "Bisphosphonate-related osteonecrosis of the jaw: diagnosis, prevention, and management," *Annu Rev Med*, vol. 60, pp. 85–96, 2009.

[89] S. Oktay, S. S. Chukkapalli, M. F. Rivera-Kweh, I. M. Velsko, L. S. Holliday, and L. Kesavalu, "Periodontitis in rats induces systemic oxidative stress that is controlled by bone-targeted antiresorptives," *J Periodontol*, vol. 86, no. 1, pp. 137–145, 2015.

[90] C. S. Santinoni, F. M. Silveira, M. L. Caldeira et al., "Topical sodium alendronate combined or not with photodynamic therapy as an adjunct to scaling and root planing: histochemical and immunohistochemical study in rats," *J Periodontal Res*, vol. 55, no. 6, pp. 850–858, 2020.

[91] B. Carvalho Dutra, A. Oliveira, P. A. D. Oliveira, L. O. Miranda Cota, J. O. Silveira, and F. O. Costa, "Effects of topical application of 1% sodium alendronate gel in the surgical treatment of periodontal intrabony defects: a 6-month

randomized controlled clinical trial," *J Periodontol*, vol. 90, no. 10, pp. 1079–1087, 2019.

[92] V. Sheokand, V. S. Chadha, and P. Palwankar, "The comparative evaluation of 1% alendronate gel as local drug delivery system in chronic periodontitis in smokers and non smokers: randomized clinical trial," *J Oral Biol Craniofac Res*, vol. 9, no. 2, pp. 198–203, 2019.

[93] D. Kanoriya, A. R. Pradeep, V. Garg, and S. Singhal, "Mandibular degree II furcation defects treatment with platelet-rich fibrin and 1% alendronate gel combination: a randomized controlled clinical trial," *J Periodontol*, vol. 88, no. 3, pp. 250–258, 2017.

[94] M. A. Brunsvold, E. S. Chaves, K. S. Kornman, T. B. Aufdemorte, and R. Wood, "Effects of a bisphosphonate on experimental periodontitis in monkeys," *J Periodontol*, vol. 63, no. 10, pp. 825–830, 1992.

[95] F. Muniz, B. F. D. Silva, C. R. Goulart, T. M. D. Silveira, and T. M. Martins, "Effect of adjuvant bisphosphonates on treatment of periodontitis: systematic review with meta-analyses," *J Oral Biol Craniofac Res*, vol. 11, no. 2, pp. 158–168, 2021.

[96] Y.-T. A. Teng, H. Nguyen, X. Gao et al., "Functional human T-cell immunity and osteoprotegerin ligand control alveolar bone destruction in periodontal infection," *J Clin Invest*, vol. 106, no. 6, pp. R59–R67, 2000.

[97] D. A. Mahamed, A. Marleau, M. Alnaeeli et al., "G(-) anaerobes-reactive CD4+ T-cells trigger RANKL-mediated enhanced alveolar bone loss in diabetic NOD mice," *Diabetes*, vol. 54, no. 5, pp. 1477–1486, 2005.

[98] M. Kuritani, N. Sakai, A. Karakawa et al., "Anti-mouse RANKL antibodies inhibit alveolar bone destruction in periodontitis model mice," *Biol Pharm Bull*, vol. 41, no. 4, pp. 637–643, 2018.

[99] A. Soundia, D. Hadaya, N. Esfandi et al., "Osteonecrosis of the jaws (ONJ) in mice after extraction of teeth with periradicular disease," *Bone*, vol. 90, pp. 133–141, 2016.

[100] R. S. de Molon, H. Shimamoto, O. Bezouglaia et al., "OPG-Fc but not zoledronic acid discontinuation reverses osteonecrosis of the jaws (ONJ) in mice," *J Bone Miner Res*, vol. 30, no. 9, pp. 1627–1640, 2015.

[101] R. S. de Molon, S. Cheong, O. Bezouglaia et al., "Spontaneous osteonecrosis of the jaws in the maxilla of mice on antiresorptive treatment: a novel ONJ mouse model," *Bone*, vol. 68, pp. 11–19, 2014.

[102] R. S. de Molon, C. Hsu, O. Bezouglaia et al., "Rheumatoid arthritis exacerbates the severity of osteonecrosis of the jaws (ONJ) in mice. a randomized, prospective, controlled animal study," *J Bone Miner Res*, vol. 31, no. 8, pp. 1596–1607, 2016.

[103] A. Panahifar, W. P. Maksymowych, and M. R. Doschak, "Potential mechanism of alendronate inhibition of osteophyte formation in the rat model of post-traumatic osteoarthritis: evaluation of elemental strontium as a molecular tracer of bone formation," *Osteoarthritis Cartilage*, vol. 20, no. 7, pp. 694–702, 2012.

[104] J. Rodriguez, N. D. Escudero, and P. M. Mandalunis, "Effect of strontium ranelate on bone remodeling," *Acta Odontol Latinoam*, vol. 25, no. 2, pp. 208–213, 2012.

[105] P. J. Marie, "Strontium ranelate: a physiological approach for optimizing bone formation and resorption," *Bone*, vol. 38, 2 Suppl 1, pp. S10–S14, 2006.

[106] P. Ammann, I. Badoud, S. Barraud, R. Dayer, and R. Rizzoli, "Strontium ranelate treatment improves trabecular and cortical intrinsic bone tissue quality, a determinant of bone strength," *J Bone Miner Res*, vol. 22, no. 9, pp. 1419–1425, 2007.

[107] N. Chattopadhyay, S. J. Quinn, O. Kifor, C. Ye, and E. M. Brown, "The calcium-sensing receptor (CaR) is involved in strontium ranelate-induced osteoblast proliferation," *Biochem Pharmacol*, vol. 74, no. 3, pp. 438–447, 2007.

[108] P. J. Marie, M. Hott, D. Modrowski et al., "An uncoupling agent containing strontium prevents bone loss by depressing bone resorption and maintaining bone formation in estrogen-deficient rats," *J Bone Miner Res*, vol. 8, no. 5, pp. 607–615, 1993.

[109] A. Bruel, J. B. Vegger, A. C. Raffalt, J. E. Andersen, and J. S. Thomsen, "PTH (1-34), but not strontium ranelate counteract loss of trabecular thickness and bone strength in disuse osteopenic rats," *Bone*, vol. 53, no. 1, pp. 51–58, 2013.

[110] Y. L. Ma, Q. Q. Zeng, L. L. Porras et al., "Teriparatide [rhPTH (1-34)], but not strontium ranelate, demonstrated bone anabolic efficacy in mature, osteopenic, ovariectomized rats," *Endocrinology*, vol. 152, no. 5, pp. 1767–1778, 2011.

[111] R. B. Souza, F. I. F. Gomes, K. M. A. Pereira et al., "Strontium ranelate elevates expression of heme oxygenase-1 and decreases alveolar bone loss in rats," *J Oral Maxillofac Res*, vol. 9, no. 4, article e4, 2018.

[112] R. Di Paola, E. Mazzon, C. Muia et al., "Effects of etanercept, a tumour necrosis factor-alpha antagonist, in an experimental model of periodontitis in rats," *Br J Pharmacol*, vol. 150, no. 3, pp. 286–297, 2007.

[113] T. W. Oates, D. T. Graves, and D. L. Cochran, "Clinical, radiographic and biochemical assessment of IL-1/TNF-alpha antagonist inhibition of bone loss in experimental periodontitis," *J Clin Periodontol*, vol. 29, no. 2, pp. 137–143, 2002.

[114] A. Vieira, H. Gustavo, A. C. A. Rivas et al., "Specific inhibition of IL-6 receptor attenuates inflammatory bone loss in experimental periodontitis," *J Periodontol*, vol. 92, no. 10, pp. 1460–1469, 2021.

[115] M. B. Grauballe, J. A. Ostergaard, S. Schou, A. Flyvbjerg, and P. Holmstrup, "Effects of TNF-alpha blocking on experimental periodontitis and type 2 diabetes in obese diabetic Zucker rats," *J Clin Periodontol*, vol. 42, no. 9, pp. 807–816, 2015.

[116] M. B. Grauballe, D. Belstrøm, J. A. Østergaard et al., "Ligature-associated bacterial profiles are linked to type 2 diabetes mellitus in a rat model and influenced by antibody treatment against TNF-alpha or RAGE," *Clin Exp Dent Res*, vol. 3, no. 1, pp. 25–31, 2017.

[117] C. M. Queiroz-Junior, R. L. Bessoni, V. V. Costa, D. G. Souza, M. M. Teixeira, and T. A. Silva, "Preventive and therapeutic anti-TNF-alpha therapy with pentoxifylline decreases arthritis and the associated periodontal co-morbidity in mice," *Life Sci*, vol. 93, no. 9-11, pp. 423–428, 2013.

[118] J. A. Cirelli, C. H. Park, K. MacKool et al., "AAV2/1-TNFR:Fc gene delivery prevents periodontal disease progression," *Gene Ther*, vol. 16, no. 3, pp. 426–436, 2009.

[119] S. Bisht, M. Mizuma, G. Feldmann et al., "Systemic administration of polymeric nanoparticle-encapsulated curcumin (NanoCurc) blocks tumor growth and metastases in preclinical models of pancreatic cancer," *Mol Cancer Ther*, vol. 9, no. 8, pp. 2255–2264, 2010.

[120] D. de Almeida Brandao, L. C. Spolidorio, F. Johnson, L. M. Golub, M. R. Guimaraes-Stabili, and C. Rossa Jr., "Dose-response assessment of chemically modified curcumin in

experimental periodontitis," *J Periodontol*, vol. 90, no. 5, pp. 535–545, 2019.

[121] M. S. Elburki, C. Rossa, M. R. Guimaraes et al., "A novel chemically modified curcumin reduces severity of experimental periodontal disease in rats: initial observations," *Mediators Inflamm*, vol. 2014, article 959471, 2014.

[122] A. M. Sha and B. T. Garib, "Antibacterial effect of curcumin against clinically isolated Porphyromonas gingivalis and connective tissue reactions to curcumin gel in the subcutaneous tissue of rats," *Biomed Res Int*, vol. 2019, p. 6810936, 2019.

[123] C. J. Xiao, X. J. Yu, J. L. Xie, S. Liu, and S. Li, "Protective effect and related mechanisms of curcumin in rat experimental periodontitis," *Head Face Med*, vol. 14, no. 1, p. 12, 2018.

[124] Y. Gu, H.-M. Lee, N. Napolitano et al., "4-Methoxycarbonyl curcumin: a unique inhibitor of both inflammatory mediators and periodontal inflammation," *Mediators Inflamm*, vol. 2013, article 329740, 2013.

[125] S. P. Pimentel, M. Z. Casati, F. V. Ribeiro et al., "Impact of natural curcumin on the progression of experimental periodontitis in diabetic rats," *J Periodontal Res*, vol. 55, no. 1, pp. 41–50, 2020.

[126] M. G. Corrêa, P. R. Pires, F. V. Ribeiro et al., "Systemic treatment with resveratrol and/or curcumin reduces the progression of experimental periodontitis in rats," *J Periodontal Res*, vol. 52, no. 2, pp. 201–209, 2017.

[127] M. S. Elburki, D. D. Moore, N. G. Terezakis et al., "A novel chemically modified curcumin reduces inflammation-mediated connective tissue breakdown in a rat model of diabetes: periodontal and systemic effects," *J Periodontal Res*, vol. 52, no. 2, pp. 186–200, 2017.

[128] Y. Zhang, Y. Gu, H.-M. Lee et al., "Design, synthesis and biological activity of new polyenolic inhibitors of matrix metalloproteinases: a focus on chemically-modified curcumins," *Curr Med Chem*, vol. 19, no. 25, pp. 4348–4358, 2012.

[129] B. Kumar, V. Singh, R. Shankar, K. Kumar, and R. K. Rawal, "Synthetic and medicinal prospective of structurally modified curcumins," *Curr Top Med Chem*, vol. 17, no. 2, pp. 148–161, 2017.

[130] G. S. Viana, M. A. Bandeira, and F. J. Matos, "Analgesic and antiinflammatory effects of chalcones isolated from Myracrodruon urundeuva allemao," *Phytomedicine*, vol. 10, no. 2-3, pp. 189–195, 2003.

[131] S. M. Souza, L. C. Aquino, A. C. Milach Jr., M. A. Bandeira, M. E. Nobre, and G. S. Viana, "Antiinflammatory and antiulcer properties of tannins from Myracrodruon urundeuva Allemao (Anacardiaceae) in rodents," *Phytother Res*, vol. 21, no. 3, pp. 220–225, 2007.

[132] E.-G. Jung, K.-I. Han, H.-J. Kwon et al., "Anti-inflammatory activity of sappanchalcone isolated from Caesalpinia sappan L. in a collagen-induced arthritis mouse model," *Arch Pharm Res*, vol. 38, no. 6, pp. 973–983, 2015.

[133] X. Chen, X. Cai, R. Le et al., "Isoliquiritigenin protects against sepsis-induced lung and liver injury by reducing inflammatory responses," *Biochem Biophys Res Commun*, vol. 496, no. 2, pp. 245–252, 2018.

[134] M. A. Botelho, V. S. Rao, D. Montenegro et al., "Effects of a herbal gel containing carvacrol and chalcones on alveolar bone resorption in rats on experimental periodontitis," *Phytother Res*, vol. 22, no. 4, pp. 442–449, 2008.

[135] M. M. Taskan, H. Balci Yuce, O. Karatas, F. Gevrek, and H. Toker, "Evaluation of the effect of oleuropein on alveolar bone loss, inflammation, and apoptosis in experimental periodontitis," *J Periodontal Res*, vol. 54, no. 6, pp. 624–632, 2019.

[136] E. Middleton Jr., C. Kandaswami, and T. C. Theoharides, "The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer," *Pharmacol Rev*, vol. 52, no. 4, pp. 673–751, 2000.

[137] J. de Souza Carvalho, D. Ramadan, V. de Paiva Gonçalves et al., "Impact of citrus flavonoid supplementation on inflammation in lipopolysaccharide-induced periodontal disease in mice," *Food Funct*, 2021.

[138] W. C. Cheng, R. Y. Huang, C. Y. Chiang et al., "Ameliorative effect of quercetin on the destruction caused by experimental periodontitis in rats," *J Periodontal Res*, vol. 45, no. 6, pp. 788–795, 2010.

[139] E.-Y. Choi, S. H. Bae, M. H. Ha et al., "Genistein suppresses Prevotella intermedia lipopolysaccharide-induced inflammatory response in macrophages and attenuates alveolar bone loss in ligature-induced periodontitis," *Arch Oral Biol*, vol. 62, pp. 70–79, 2016.

[140] E. Gugliandolo, R. Fusco, R. D'Amico et al., "Treatment with a flavonoid-rich fraction of bergamot juice improved lipopolysaccharide-induced periodontitis in rats," *Front Pharmacol*, vol. 9, p. 1563, 2018.

[141] J. Huang, C. Wu, B. Tian, X. Zhou, N. Ma, and Y. Qian, "Myricetin prevents alveolar bone loss in an experimental ovariectomized mouse model of periodontitis," *Int J Mol Sci*, vol. 17, no. 3, p. 422, 2016.

[142] A. Lektemur Alpan, A. Kizildag, M. Ozdede, N. C. Karakan, and O. Ozmen, "The effects of taxifolin on alveolar bone in experimental periodontitis in rats," *Arch Oral Biol*, vol. 117, article 104823, 2020.

[143] Y. Ishimi, C. Miyaura, M. Ohmura et al., "Selective effects of genistein, a soybean isoflavone, on B-lymphopoiesis and bone loss caused by estrogen deficiency," *Endocrinology*, vol. 140, no. 4, pp. 1893–1900, 1999.

[144] M. Satue, M. Arriero Mdel, M. Monjo, and J. M. Ramis, "Quercitrin and taxifolin stimulate osteoblast differentiation in MC3T3-E1 cells and inhibit osteoclastogenesis in RAW 264.7 cells," *Biochem Pharmacol*, vol. 86, no. 10, pp. 1476–1486, 2013.

[145] T. Tominari, M. Hirata, C. Matsumoto, M. Inada, and C. Miyaura, "Polymethoxy flavonoids, nobiletin and tangeretin, prevent lipopolysaccharide-induced inflammatory bone loss in an experimental model for periodontitis," *J Pharmacol Sci*, vol. 119, no. 4, pp. 390–394, 2012.

[146] A. W. Boots, G. R. Haenen, and A. Bast, "Health effects of quercetin: from antioxidant to nutraceutical," *Eur J Pharmacol*, vol. 585, no. 2-3, pp. 325–337, 2008.

[147] S. C. Bischoff, "Quercetin: potentials in the prevention and therapy of disease," *Curr Opin Clin Nutr Metab Care*, vol. 11, no. 6, pp. 733–740, 2008.

[148] Y.-D. Min, C.-H. Choi, H. Bark et al., "Quercetin inhibits expression of inflammatory cytokines through attenuation of NF-kappaB and p38 MAPK in HMC-1 human mast cell line," *Inflamm Res*, vol. 56, no. 5, pp. 210–215, 2007.

[149] T. Kallinich, D. Haffner, T. Niehues et al., "Colchicine use in children and adolescents with familial Mediterranean fever: literature review and consensus statement," *Pediatrics*, vol. 119, no. 2, pp. e474–e483, 2007.

[150] E. Ben-Chetrit, J. M. Scherrmann, E. Zylber-Katz, and M. Levy, "Colchicine disposition in patients with familial

Mediterranean fever with renal impairment," *J Rheumatol*, vol. 21, no. 4, pp. 710–713, 1994.

[151] C. A. Aral, K. Aral, A. Yay, O. Ozcoban, A. Berdeli, and R. Saraymen, "Effects of colchicine on gingival inflammation, apoptosis, and alveolar bone loss in experimental periodontitis," *J Periodontol*, vol. 89, no. 5, pp. 577–585, 2018.

[152] C. N. Serhan, "Pro-resolving lipid mediators are leads for resolution physiology," *Nature*, vol. 510, no. 7503, pp. 92–101, 2014.

[153] C. N. Serhan, N. Chiang, and T. E. Van Dyke, "Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators," *Nat Rev Immunol*, vol. 8, no. 5, pp. 349–361, 2008.

[154] R. S. De Molon, R. M. Thurlings, B. Walgreen et al., "Systemic resolvin E1 (RvE1) treatment does not ameliorate the severity of collagen-induced arthritis (CIA) in mice: a randomized, prospective, and controlled proof of concept study," *Mediators Inflamm*, vol. 2019, p. 5689465, 2019.

[155] M. Wan, C. Godson, P. J. Guiry, B. Agerberth, and J. Z. Haeggstrom, "Leukotriene B4/antimicrobial peptide LL-37 proinflammatory circuits are mediated by BLT1 and FPR2/ALX and are counterregulated by lipoxin A4 and resolvin E1," *FASEB J*, vol. 25, no. 5, pp. 1697–1705, 2011.

[156] S. Muruganandan, A. A. Roman, and C. J. Sinal, "Role of chemerin/CMKLR1 signaling in adipogenesis and osteoblastogenesis of bone marrow stem cells," *J Bone Miner Res*, vol. 25, no. 2, pp. 222–234, 2010.

[157] Z. Z. Xu, L. Zhang, T. Liu et al., "Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions," *Nat Med*, vol. 16, no. 5, pp. 592–597, 2010, 591p following 597.

[158] H. Hasturk, A. Kantarci, E. Goguet-Surmenian et al., "Resolvin E1 regulates inflammation at the cellular and tissue level and restores tissue homeostasis in vivo," *J Immunol*, vol. 179, no. 10, pp. 7021–7029, 2007.

[159] C.-T. Lee, R. Teles, A. Kantarci et al., "Resolvin E1 reverses experimental periodontitis and dysbiosis," *J Immunol*, vol. 197, no. 7, pp. 2796–2806, 2016.

[160] H. Hasturk, R. Abdallah, A. Kantarci et al., "Resolvin E1 (RvE1) attenuates atherosclerotic plaque formation in diet and inflammation-induced atherogenesis," *Arterioscler Thromb Vasc Biol*, vol. 35, no. 5, pp. 1123–1133, 2015.

[161] K. El Kholy, M. Freire, T. Chen, and T. E. Van Dyke, "Resolvin E1 promotes bone preservation under inflammatory conditions," *Front Immunol*, vol. 9, p. 1300, 2018.

[162] T. E. Van Dyke, "Shifting the paradigm from inhibitors of inflammation to resolvers of inflammation in periodontitis," *J Periodontol*, vol. 91, Suppl 1, pp. S19–S25, 2020.

[163] D. M. Lilly and R. H. Stillwell, "Probiotics: growth-promoting factors produced by microorganisms," *Science*, vol. 147, no. 3659, pp. 747-748, 1965.

[164] H. Shimauchi, G. Mayanagi, S. Nakaya et al., "Improvement of periodontal condition by probiotics with Lactobacillus salivarius WB21: a randomized, double-blind, placebo-controlled study," *J Clin Periodontol*, vol. 35, no. 10, pp. 897–905, 2008.

[165] I. Stamatova and J. H. Meurman, "Probiotics and periodontal disease," *Periodontol*, vol. 51, pp. 141–151, 2000.

[166] R. M. Moraes, C. M. Lescura, N. V. M. Milhan, J. L. Ribeiro, F. A. Silva, and A. L. Anbinder, "Live and heat-killed Lactobacillus reuteri reduce alveolar bone loss on induced periodontitis in rats," *Arch Oral Biol*, vol. 119, article 104894, 2020.

[167] R. S. Cardoso, M. R. Messora, P. H. F. Silva et al., "Effects of Bifidobacterium animalis subsp. lactis HN019 on ligature-induced periodontitis in rats with experimental rheumatoid arthritis," *Benef Microbes*, vol. 11, no. 1, pp. 33–46, 2020.

[168] M. S. Ricoldi, F. A. Furlaneto, L. F. Oliveira et al., "Effects of the probiotic Bifidobacterium animalis subsp. lactis on the non-surgical treatment of periodontitis. A histomorphometric, microtomographic and immunohistochemical study in rats," *PLoS One*, vol. 12, no. 6, article e0179946, 2017.

[169] L. F. Oliveira, S. L. Salvador, P. H. Silva et al., "Benefits of Bifidobacterium animalis subsp. lactis probiotic in experimental periodontitis," *J Periodontol*, vol. 88, no. 2, pp. 197–208, 2017.

[170] S. M. Gatej, V. Marino, R. Bright et al., "Probiotic Lactobacillus rhamnosus GG prevents alveolar bone loss in a mouse model of experimental periodontitis," *J Clin Periodontol*, vol. 45, no. 2, pp. 204–212, 2018.

[171] T. Maekawa and G. Hajishengallis, "Topical treatment with probiotic Lactobacillus brevis CD2 inhibits experimental periodontal inflammation and bone loss," *J Periodontal Res*, vol. 49, no. 6, pp. 785–791, 2014.

[172] R. Kobayashi, T. Kobayashi, F. Sakai, T. Hosoya, M. Yamamoto, and T. Kurita-Ochiai, "Oral administration of Lactobacillus gasseri SBT2055 is effective in preventing Porphyromonas gingivalis-accelerated periodontal disease," *Sci Rep*, vol. 7, no. 1, p. 545, 2017.

[173] Y. L. A. S. Levi, G. S. Novais, R. B. Dias et al., "Effects of the prebiotic mannan oligosaccharide on the experimental periodontitis in rats," *J Clin Periodontol*, vol. 45, no. 9, pp. 1078–1089, 2018.

[174] V. Slomka, E. Hernandez-Sanabria, E. R. Herrero et al., "Nutritional stimulation of commensal oral bacteria suppresses pathogens: the prebiotic concept," *J Clin Periodontol*, vol. 44, no. 4, pp. 344–352, 2017.

[175] T. Tomofuji, D. Ekuni, T. Sanbe et al., "Effects of vitamin C intake on gingival oxidative stress in rat periodontitis," *Free Radic Biol Med*, vol. 46, no. 2, pp. 163–168, 2009.

[176] D. E. Clark, J. M. Navia, L. R. Manson-Hing, and H. E. Duncan, "Evaluation of alveolar bone in relation to nutritional status during pregnancy," *J Dent Res*, vol. 69, no. 3, pp. 890–895, 1990.

[177] N. Amarasena, H. Ogawa, A. Yoshihara, N. Hanada, and H. Miyazaki, "Serum vitamin C-periodontal relationship in community-dwelling elderly Japanese," *J Clin Periodontol*, vol. 32, no. 1, pp. 93–97, 2005.

[178] S. Akman, V. Canakci, A. Kara, U. Tozoglu, T. Arabaci, and I. M. Dagsuyu, "Therapeutic effects of alpha lipoic acid and vitamin C on alveolar bone resorption after experimental periodontitis in rats: a biochemical, histochemical, and stereologic study," *J Periodontol*, vol. 84, no. 5, pp. 666–674, 2013.

[179] A. Akpinar, N. C. Karakan, A. L. Alpan, S. S. A. Dogan, F. Goze, and O. Poyraz, "Comparative effects of riboflavin, nicotinamide and folic acid on alveolar bone loss: a morphometric and histopathologic study in rats," *Srp Arh Celok Lek*, vol. 144, no. 5-6, pp. 273–279, 2016.

[180] R. F. Neiva, K. Al-Shammari, F. H. Nociti Jr., S. Soehren, and H. L. Wang, "Effects of vitamin-B complex supplementation on periodontal wound healing," *J Periodontol*, vol. 76, no. 7, pp. 1084–1091, 2005.

[181] B. Willershausen, A. Ross, M. Forsch, I. Willershausen, P. Mohaupt, and A. Callaway, "The influence of

micronutrients on oral and general health," *Eur J Med Res*, vol. 16, no. 11, pp. 514–518, 2011.

[182] G. Zong, B. Holtfreter, A. E. Scott et al., "Serum vitamin B12 is inversely associated with periodontal progression and risk of tooth loss: a prospective cohort study," *J Clin Periodontol*, vol. 43, no. 1, pp. 2–9, 2016.

[183] A. Mohammadi, L. Omrani, L. R. Omrani et al., "Protective effect of folic acid on cyclosporine-induced bone loss in rats," *Transpl Int*, vol. 25, no. 1, pp. 127–133, 2012.

[184] J. Lee, J.-C. Park, U.-W. Jung et al., "Improvement in periodontal healing after periodontal surgery supported by nutritional supplement drinks," *J Periodontal Implant Sci*, vol. 44, no. 3, pp. 109–117, 2014.

[185] E. Toubi and Y. Shoenfeld, "The role of vitamin D in regulating immune responses," *Isr Med Assoc J*, vol. 12, no. 3, pp. 174-175, 2010.

[186] J. Tang, R. U. Zhou, D. Luger et al., "Calcitriol suppresses antiretinal autoimmunity through inhibitory effects on the Th17 effector response," *J Immunol*, vol. 182, no. 8, pp. 4624–4632, 2009.

[187] Y. Arnson, H. Amital, and Y. Shoenfeld, "Vitamin D and autoimmunity: new aetiological and therapeutic considerations," *Ann Rheum Dis*, vol. 66, no. 9, pp. 1137–1142, 2007.

[188] K. Aral, B. A. Alkan, R. Saraymen, A. Yay, A. Sen, and G. O. Onder, "Therapeutic effects of systemic vitamin k2 and vitamin d3 on gingival inflammation and alveolar bone in rats with experimentally induced periodontitis," *J Periodontol*, vol. 86, no. 5, pp. 666–673, 2015.

4.2 Artigo 2

Beneficial effects of biological agents on experimental periodontitis: A systematic review

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Abstract

Aim: The aim of this systematic review was to answer the focused question: what is the protective potential of biological agents against alveolar bone resorption during the course of experimental periodontitis progression?

Material and Methods: A systematic literature search was performed in Pubmed, Web of Science, Cochrane Library, Scopus, and Embase electronic datasets, complemented by other sources and manual search until August 2022. The inclusion criteria comprised pre-clinical studies in animal models of experimental periodontitis on the effects of biological agents to protect against periodontal bone loss and tissue inflammation. The criteria for eligibility of the studies were based in PI/ECOs strategy, as such (P) animal models with periodontitis; (I/E) biological agents with protective potential against bone resorption in periodontitis (prevention) or additional benefit as an adjunctive in the treatment of periodontitis; (C) same intervention/exposition as the intervention group (I), except for the use biological agents; (O) alveolar bone resorption (primary outcomes). Secondary outcomes: periodontal parameters, effectiveness of periodontitis-treatment, and adverse effects; (s) no restriction for study design on primary studies (exclusion criteria: randomized clinical trials, clinical studies, and different types of review studies). Study quality was assessed using Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) Risk of Bias tool.

Results: Screening of the initially 5236 results from datasets, registers, other sources and hand-searches resulted in 39 suitable studies that met the inclusion criteria. Twenty-three biologicals were evaluated in the included studies. Most of the studies utilized the ligature-model of experimental periodontitis (EP) to test the use of biologicals as preventive or therapeutic strategies. The dosages of biologicals and the periods of disease induction varied based on the EP model utilized. As the main outcome of all studies, alveolar bone loss (the hallmark of EP) could be significantly inhibited applying biologicals, and reduced pro-inflammatory mediators when compared to treated controls.

Conclusion: Collectively, this systematic review demonstrated that biological agents possess beneficial effects in reducing bone loss and diminishing inflammation during periodontitis progression in animal models. Randomized clinical trials using

biologicals to treat periodontitis are warranted to corroborate the results achieved in these pre-clinical studies.

Keywords: Alveolar bone; animal model; bone resorption, periodontal disease; periodontitis

Introduction

Periodontitis, a chronic inflammatory condition of the supporting tissues around the teeth, develop as a result of complex host-parasite interaction that progressively affects the integrity of the periodontal tissues [1, 2]. It is described by bacterial-evoked inflammatory responses and periodontal tissue destruction, including periodontal ligament, cement and alveolar bone. The more severe forms of periodontitis (stage III and IV) affect over 700 million people, which represent around 11% of the worldwide population [3]. Indeed, periodontal disease (PD) is the sixth-most prevalent chronic condition globally, and it is considered the most important cause of teeth loss in the adult population [4, 5]. Hence, PD is a public health challenge due to its high prevalence and the significant burden caused by teeth loss and chewing disability negatively impacting quality of life. Meanwhile, there are a growing number of studies linking periodontitis with systemic diseases, such as diabetes mellitus, cardiovascular diseases, rheumatoid arthritis and others through the dissemination of pathogenic bacteria and their products into the bloodstream [6-9]. Consequently, the treatment of periodontitis plays pivotal roles in decreasing local and systemic inflammation.

PD is characterized by the unique property in that tissue damage results from the host innate and adaptive immune response to periodontal biofilm-associated multiple microorganisms [1, 4]. Imbalance between the resident commensal microbiota and the host immune response lead to the dysbiosis of the oral microbiome, and thus increased bacterial load and persistent inflammatory process are the hallmark of disease [10]. In individuals susceptible to periodontitis, the host immune response is usually exacerbated, dysregulated and destructive leading to infiltration of immune (B and T cells) and inflammatory cells (macrophages and neutrophils) [11]. Innumerous pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-17, prostaglandin E_2 (PGE_2), tumor necrosis

factor-α (TNF-α), and matrix-degrading enzymes (cathepsin and matrix metalloproteinases - MMPs) produced by lymphocytes, fibroblast, leukocytes and epithelial cells have been identified as key molecules inducing tissue destruction, and the expression of these molecules are significantly increased during disease progression [11]. More specifically, these cytokines enable the increased activation of receptor activator of nuclear factor kappa B ligand (RANKL) pathway in osteoblast, fibroblast or lymphocytes, which ultimately results in the differentiation and activation of osteoclasts, and consequently the destruction of mineralized connective tissue [12]. Therefore, the regulation of the immune response and the inhibition of osteoclastogenesis are key factors that should be considered when testing potential new treatment modalities for PD.

According to the clinical practice guideline from the European Federation of Periodontology, the recommendations for treatment of stage I-III periodontitis are based on four different interventions [13]. The first step of therapy is behavioral changes, and control of supragingival biofilm, gingival inflammation and associated risk factors. The second step of therapy is aimed at controlling the supra- and sub-gingival biofilm and calculus through subgingival instrumentation with the goal of controlling the infection and arresting the inflammatory process. Subgingival instrumentation may include adjunctive local or systemic medications. The third step consisted of treating unresponsive areas to the second step and include different types of periodontal surgical interventions. Finally, the supportive periodontal care should be implemented to extend benefits and to maintain periodontal stability over time [13].

The non-surgical periodontal therapy (NSPT), the term used to describe subgingival instrumentation, is accomplished through scaling and root planning (SRP), the gold standard treatment for stage I to III periodontitis [13]. The use of adjunctive therapies to treat periodontitis relies on the premise that not all the individuals responds well to NSPT especially in non-compliant and susceptible patients, and also in patients with systemic comorbidities (diabetes) associated with the presence of common risk factors for disease progression, such as local factors (deep periodontal pockets and complex root anatomy), environmental factors (smoking) and genetic background (polymorphisms) [11]. Therefore, the use of adjunctive therapies (biological) aiming at modulate the destructive events of the innate and adaptive immune host response have been proposed in pre-clinical

and clinical studies as a potential therapeutic strategy to treat periodontitis targeting inflammatory mediators and bone-resorbing osteoclasts [2, 11].

In the past years, a growing number of studies investigating the beneficial effects of adjunctive therapies in clinical trials aiming at decreasing inflammation and alveolar bone destruction and improving the outcomes of NSPT alone have been performed [14-17]. Moreover, different animal models of periodontitis have been used to test the efficacy of different compounds, and the molecular mechanisms involved in the inhibition of bone resorption [12, 18, 19]. In this context, pharmacological therapies, natural compounds, and biological agents have been studied to suppress the inflammatory process and bone destruction during periodontitis progression. Therefore, the current systematic review sought to investigate the evidence on the beneficial effects of biological agents against inflammation and bone resorption in experimental periodontitis.

Materials and Methods

Protocol and Registration

This study was conducted at the Systematic Review Facility (SyRF) (https://syrf.org.uk/projects) [20] as recommended by the Collaborative Approach to Meta Analysis and Review of Animal Experimental Studies (CAMARADES) (https://www.ed.ac.uk/clinical-brain-sciences/research/camarades/about-camarades). The structure of this paper was organized according to the PRISMA 2020 statement [21] (https://prisma-statement.org).

Focused question

Based on the PICOD principle.

- Population (P): Animal models with experimental periodontitis;

- Intervention (I): Biological factors used/tested for prevention/control of alveolar bone resorption in experimental periodontitis. Biological factors are defined as endogenously synthesized compounds that influence biological processes not otherwise classified under enzymes, hormones or hormone antagonists (i.e., antigens, antioxidants, biomarkers, blood coagulation factor inhibitors, blood coagulation factors, chemotactic

factors, host-derived cellular factors, immune checkpoint proteins, inflammation mediators, intercellular signaling peptides and proteins, pathogen-associated molecular pattern molecules [PAMPs], pheromones, phytochemicals, biological pigments, and biological toxins);

- Comparator (C): Sham or placebo group;

- Outcome of interest (O): Main outcomes – i) prevention/control of alveolar bone resorption by biological factors, ii) effect of biological factors on alveolar bone, including cellular, molecular, tissue, and functional assessment—Additional outcomes – i) harmful consequences, adverse events and systemic impact of biological factors used/tested for prevention/control of alveolar bone resorption in experimental periodontitis; ii) benefit-to-harm ratio of biological factors used/tested for prevention/control of alveolar bone resorption in experimental periodontitis; iii) mechanistic insights of biological factors used/tested for prevention/control of alveolar bone resorption in experimental periodontitis; and iv) hierarchy of biological factors with greater effectiveness (prevention/control of alveolar bone resorption), safety (harmful consequences, adverse events and systemic impact), evidence (study quality and risk of bias assessment) and lack of evidence from preclinical studies to support Phase I studies (clinical trial design) on the subject;

- Primary study Design (D): Preclinical study (*in vivo*) referring to tests, experiments, and procedures that researchers perform in or on a laboratory animal, this systematic review aimed to answer the focused question: What is the protective potential of biological factors against alveolar bone resorption in experimental periodontitis?

Study selection criteria

We included in this systematic review only preclinical studies (*in vivo*) on the protective potential of biological factors against alveolar bone resorption in experimental periodontitis. We excluded studies that: i) animal model of non-induced periodontitis; ii) antimicrobial agents; iii) prebiotics and probiotics; iv) inaccurate or unavailable information related to the intervention or outcomes; and v) inability to access the full text. No restrictions were placed on the language or date of publication when searching the electronic databases.

Information sources

An extensive literature search was performed among five electronic databases, namely MEDLINE via PubMed (http://www.ncbi.nlm.nih.gov/sites/pubmed), Web of Science – WOS (https://www.webofknowledge.com) accessed through the Clarivate Analytics (https://clarivate.com), Cochrane Central Register of Controlled Trials (CENTRAL) (https://www.cochranelibrary.com), Embase (https://www.embase.com) and Scopus (http://www.scopus.com) through Elsevier (https://www.elsevier.com). Other sources (grey literature) were consulted through Google Scholar (https://scholar.google.com.br) and System for Information on Grey Literature in Europe (SIGLE) through OpenGrey (https://easy.dans.knaw.nl/ui/datasets/id/easy-dataset:200362/tab/2) databases. Handsearch was performed in specialized periodicals and in reference lists of selected articles. Experts were identified using expertscape.com (https://expertscape.com) and contacted for other data sources.

Search strategy

Database search strategies included MeSH terms, entry terms and keywords to query in PubMed, Web of Science, Cochrane Library, other sources (gray literature), and protocol record. The search strategies for Embase and Scopus databases added Entree, Index and DeCS/MeSH terms, respectively. All terms were combined by the Boolean operators "OR" and "AND" connecting the key concepts in a “building blocks” strategy (Table 1). The electronic searches were performed in December 2022 and databases alerts were created to identify studies published after the time of the search, until the manuscript submission process.

Selection process

The retrieved articles were exported to rayyan reference manager (https://www.rayyan.ai) and duplicates were removed by the program (perfect match) and manually. The selection process was conducted in two phases: Phase 1, three researchers (BSM, MSF, and RSM) independently examined the titles and abstracts of all retrieved references, applying the inclusion criteria; and Phase 2, the same three reviewers independently applied the exclusion criteria in the full text screening. The full texts were evaluated and judged in the entire document. Inter-reviewer reliability in the

study selection process was determined by the Cohen κ test, assuming an acceptable threshold value of 0.80 [22]. The disagreement at any stage was resolved by discussion and mutual decision with a third reviewer (DSB). The final decision was always based on the full text reading—for more details on reasons for exclusion, see Figure 1.

Data collection process

Data were independently extracted by the three reviewers (BSM, MSF, and RSM) using a standardized form. The qualitative results were described in the article in a consensus meeting, in the order of the PICO contents: study, population, intervention, comparator, combination therapies, alveolar bone resorption, and main findings. Adverse effects were also assessed as secondary outcomes. Authors were contacted by email for five consecutive weeks as needed to obtain details on study design and data clarification.

Study risk of bias assessment

Three reviewers (BSM, MSF, and RSM) independently evaluated the quality of the included pre clinical studies using the SYRCLE's risk of bias tool for animal studies [23], as presented in Table 3. This tool is based on the Cochrane RoB tool and has been adjusted for aspects of bias that play a specific role in animal intervention studies. SYRCLE's is structured into a fixed set of 10 domains of bias: sequence generation, baseline characteristics, allocation concealment, random housing—blinding, random outcome assessment—blinding incomplete outcome data, selective outcome reporting, and other sources of bias. These entries are related to 6 types of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases. A "yes" judgment indicates a low risk of bias; a "no" judgment indicates high risk of bias; the judgment will be "unclear" if insufficient details have been reported to assess the risk of bias properly. Risk of bias analysis was conducted in an independent blinded process. In case of divergence, agreement was reached in a consensus meeting with a third reviewer (DSB).

Synthesis methods and effect measures

The study selection process, study characteristics, risk of bias in studies, results of individual studies, results of syntheses, reporting biases, and certainty of evidence, are described in the form of text, figure, and tables. Only studies with low risk of bias were

considered for the quantitative synthesis and certainty of the evidence. The synthesis of qualitative results followed the SWiM reporting guideline [24].

The effect estimation meta-analysis consisted of standard pairwise meta-analyses of direct comparisons using a fixed-effect model. The results were expressed as mean difference and relative 95% confidence interval (CI). Heterogeneity was assessed by Q-statistic method ($p \leq 0.1$) and inconsistency measurement ($I^2 \geq 75\%$ suggesting high heterogeneity) [25-27]. Meta-analyses with $p \leq 0.1$ in the χ^2 and $I^2 \geq 75$ % and primary studies with high risk of bias were not included in the data synthesis.

The protective potential of biological factors/agents against alveolar bone resorption in experimental periodontitis was consistent if the mean difference between comparator and intervention groups was ≥ 10%. Statistical tests were performed using RevMan 5.4 software (Review Manager (RevMan) [Computer program]. Version 5.4, The Cochrane Collaboration, 2020. Available at: https://training.cochrane.org/online-learning /core-software-cochrane-reviews/revman/revman-5-download).

The protective potential was considered the difference between the $\bar{X}(\sigma)$ of alveolar bone resorption (ABR) of intervention group and comparator group. The σ difference was calculated using the formula:

$$\sigma ABR = \sqrt[2]{(\sigma Comparator^2 + \sigma Intervention^2) - (2.R.\sigma Comparator.\sigma Intervention)}$$

Sensitivity analyses used to assess robustness of the synthesized results and the potential impact of confounding factors consisted of independent subgroup analysis for animal model, experimental protocol, biological factors, and method to assess alveolar bone resorption, cellular, molecular, tissue and functional outcomes.

Reporting bias assessment

Confounding factors such as animal model, experimental protocol, biological factors, and method to assess alveolar bone resorption, cellular, molecular, tissue and functional outcomes were considered in the result synthesis, including subgroup analysis. The presence of publication bias was investigated through visual detection/analysis of the funnel plot [27, 28].

Certainty assessment

The Classification of Assessment, Development and Assessment Recommendations (GRADE) was used to assess the quality of responses in this systematic review, based on five factors: serious risk of bias, serious inconsistence between studies, serious indirectness, serious imprecision, and likely publication bias [29, 30]. Thus, the evidence quality index was defined in four categories: high, moderate, low, and very low applied to the primary outcomes [31-33].

Results

Study selection

Figure 1 demonstrates a flowchart that summarizes the identification of study selection via databases and registers of studies that assessed the beneficial effects of biological agents on experimental periodontitis. Following databases screening, 5236 studies were identified by the authors, which were carried out into five databases (Pubmed, Scopus, Embase, Cochrane and Web of Science). After the removal of duplicates (969), the search strategies identified 4267 possible eligible articles, of which 4202 were immediately excluded after reading the titles and the abstracts. The full text of the 65 remaining manuscripts was assessed and 18 articles were then excluded, as they did not fulfill the eligibility criteria, totaling 49 studies. During the process for study eligibility, 10 studies were excluded after reading the full text due to wrong animal model and wrong medication used. Therefore, 39 manuscripts were included to serve as the basis for this systematic review.

Study characteristics

The thirty-nine included reports were published between 2000 and 2022 (Table 1). Different animal models were used in those studies, comprising mainly rodents. Ten studies have used Wistar rats as animal model [34-43], fourteen studies [44-57] have used C57BL/6 mice, eight studies [58-65] employed Sprague-Dawley rats, one study [66] have used Macaca fasciculares as an animal model, two studies [67, 68] used the F344 inbred rats, one study [69] used BALB/c as a mouse model, two studies [70, 71]

have used the DIO mouse model (C57BL/6 background), and finally one study [72] did not report the mice strain utilized.

Most of the included studies utilized a ligature-induced experimental periodontitis (EP) model, totalizing 23 articles [34-41, 43-46, 52, 56, 58, 59, 61, 63, 65, 67-70] varying in different thread materials, such as silk, cotton and dental floss. LPS injection as EP model was used in three studies [60, 62, 64], in which Cirelli et al. (2009) [60] used LPS from *P. gingivalis*, while Kirkwood et al. (2007) and Rogers et al. (2007) used LPS from *A. actinomycetemcomitans* [62, 64]; ligatures soaked with *P. gingivalis* as animal model was also used in four studies [51, 57, 66, 71]. Oral bacteria inoculation with live *P. gingivalis* was used in five studies [47-49, 54, 55], oral inoculation with *A. actinomycetemcomitans* was used in three studies [50, 53, 72], and one study [42] has used *P. gingivalis* plus *F. nucleatum* to induce EP via oral inoculation.

Studies using the ligature-induced bone loss have applied the therapeutic approach in six reports [35-37, 43, 63, 65] (treatment starting after the disease onset), while another thirty-three studies have used preventive strategies against alveolar bone destruction using the ligature model (treatment started immediately after ligature placement) or oral inoculation of periodontopathogenic bacteria or LPS injection. Additionally, eight studies have utilized local treatment (injection on the gingival tissue) [37, 43-45, 56, 65, 66, 69], and the other included reports used the systemic administration of biologicals to treat EP by means of intraperitoneal injection [34, 35, 41, 46-49, 51-53, 55, 63, 67], oral gavage [36, 62, 64, 70, 72], subcutaneous injection [38, 40, 50, 57-59, 61, 65, 68], intramuscular injection [60], intravenously injection [39], adoptive cell transfer [54], biological dissolved in the drinking water [42], and finally systemic infusion [43]. The follow-up period of evaluation in the included studies ranged from 5 days to eight weeks.

The key biological agents evaluated in these studies were: the humanized monoclonal anti-human interleukin (IL)-6 receptor (Tocilizumab) [34]; Melatonin, a hormone synthetized in the pineal gland and other organs, were tested in four studies [35, 41, 42, 63]; Vitamins K2 [36], and D3 [36, 48, 49, 55] tested in another 4 studies; the second generation concentrate named injectable platelet-rich fibrin (i-PRF) [37] and an autologous hemoderivate material obtained by the disruption of platelets; the platelet

lysate [43]; an immunoregulatory cytokine with anti-inflammatory properties, IL-35 [44]; a protein secreted primarily by osteocytes that regulates osteoblast mediated bone formation named sclerostin (sclerostin monoclonal antibody - Scl-Ab) [59, 65]; a major regulator of bone remodeling and calcium homeostasis, a protein composed of 84 amino acids, the parathyroid hormone (PTH) [40, 58, 68]; TNF-α blockers (TNFR:Fc - monoclonal anti-TNF-α antibodies or fusion proteins containing p75 TNFR linked to the Fc portion of human IgG1) [60]; The soluble IL-1 receptor type 1 (sIL-1R1) that function as a competitive inhibitor of IL-1 [66]; The nerve growth factor (NGF) neutralizing antibody [38]; the infliximab, a chimeric, human IgG1 TNF-α monoclonal antibody [39, 67]; CD40, a membrane-associated protein and a member of the TNF receptor superfamily [45]; the human recombinant OPG fusion protein (rhOPG-Fc) [57, 61]; a competitive inhibitor of p38α Mitogen-activated protein kinase (MAPK), the SD282 [62, 64]; a human monoclonal antibody that bind to RANKL, a TNF-super family cytokine produced by osteoblasts and stromal cells in bone tissues, (anti-mouse RANKL monoclonal antibody) [46, 57]; the sRAGE, an extracellular domain of the receptor, which binds ligand and blocks interaction with, and activation of, cell-surface RAGE [47]; the BET (bromodomain and extraterminal domain) inhibitor JQ1 (a cell-permeable small molecule) [51]; the Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) [52]; the soluble epoxide hydrolase inhibitor (she) TPPU [72]; the IL-17A neutralizing antibody [69]; the met-RANTES (specific antagonist of CCR1 and CCR5 receptors) [53]; B10 cells [54]; a novel adiponectin receptor agonist (AdipoAI) [70] and the adiponectin (APN) [71]; and finally, the TLR9 agonist cytidine-phosphatase-guanosine oligodeoxynucleotide (CpG), CD40 ligand [56].

Outcomes of interest

The majority of the included studies have utilized morphometric analysis [39, 45, 47, 48, 50, 51, 53-56, 71, 72] and microcomputed tomography (micro-CT) [34, 37, 43, 44, 46, 52, 58-62, 64, 65, 69, 70] to quantitate the bone level changes, an important parameter to assess periodontal status. Morphometric analyses were accomplished measuring the distance between the cement-enamel junction (CEJ) to the alveolar bone

crest (ABC) (2D linear measurements) after staining the jawbones with methylene blue, using specific software as reference to perform the measurements. A more accurate method of analysis, the micro-CT, is able to quantitate the tridimensional volumetric alterations and the architectural parameters of bone. One study employed scanning electronic microscopy (SEM) [49], two studies used conventional 2D radiography [35, 38], and seven studies utilized histological analysis to measure alveolar bone loss [36, 41, 57, 63, 66-68]. Histomorphological measurements using histologic images allow quantitation of bone destruction through linear measurements or by analyzing the total area of bone loss in the furcation or in the interproximal area. Two studies [40, 42] did not quantify the amount of bone loss inhibition after experimental periodontitis treatment. It is noteworthy to mention that almost all studies have demonstrated that biological agents protected the host from the progression of alveolar bone loss when compared to the control groups, except by one study [36] that used vitamin K2, D3 or combination of both. Taken together, based on the presented results, the biological agents are considered as a safe adjunctive treatment of EP (without side effects) to be used as a systemic or local compound. Furthermore, all of them showed encouraging data regarding prevention and treatment of EP.

Furthermore, gene expression analysis by real time polymerase chain reaction (RT-qPCR) was performed by fifteen studies do investigate the effects of biological agents on the expression levels of pro-inflammatory cytokine markers, such as IL-1β, TNF-α, IL-6, IL-17, IL-10, INF-γ, RANKL, and OPG [34, 38, 40, 43-45, 50, 51, 53, 54, 56, 60, 67, 69, 70]. Immunohistochemistry (IHC) analysis and TRAP staining were performed by twenty-one studies [37-40, 45-47, 49, 52, 54-56, 58, 60-64, 67, 68, 71] to investigate the effects of biologicals on protein levels, especially RANKL, OPG, MMP9, and in the number of positive-stained osteoclasts. Biochemical analysis of serum was performed by three studies [35, 36, 63] mainly to investigate the levels of TRAP-5b (marker for osteoclast), and bone formation markers, such as osteocalcin (OCN) and C-terminal telopeptide of type 1 collagen CTx-1). ELISA was made by another twelve studies [36, 39, 41, 44, 47, 48, 50, 53-55, 59, 65] to investigate protein levels in serum, and finally western blot analysis was accomplished by seven studies [39, 41, 48, 49, 55, 70, 72] to investigate the phosphorylation of signaling pathways involved in bone resorption, such as Janus family kinase 1 (JAK1), nuclear factor kappa-

light-chain-enhancer of activated B cells (NF-κB)- p65, and signal transducer and activator of transcription (STAT)-3. Collectively, the main outcomes of the selected studies regarding diminishing of bone loss, decreased expression of pro-inflammatory cytokines as well as inhibition of signaling pathways phosphorylation were achieved in all studies suggesting a promising alternative to use as adjuvant approach to treat EP.

Quality assessment of evidence

The assessment of evidence of the included studies was considered following the ARRIVE guidelines checklist containing a 21-itens, and the scores varied from 14 to 19 (Table 2a-c). None of the included articles reported information regarding animal inclusion or exclusion criteria. Only two studies reported data access (where study data are available) [52, 68]. Additionally, just four studies [34, 52, 64, 67] described the sample size calculation for study power, and information about conflict of interest was described in 11 studies [37, 42-44, 52, 54, 58, 59, 68-70]. Almost half of the included studies reported the randomization process of experimental groups [34-37, 41-44, 48, 49, 52, 55-58, 63, 71] and seventeen studies did not provide information about blinding process during the analysis [37, 39, 41, 43, 45-47, 51, 53, 54, 56, 58, 59, 63, 65, 68, 70, 71]. No information was found in twenty-four articles on animal care [38, 40, 41, 43, 45-47, 49-51, 53, 54, 56, 57, 61, 62, 64-66, 68-72]. Overall, all the articles were supported by strong study design, outcomes measures, statistical methods, interpretation of scientific evidence, generalizability translation, protocol registration, experimental animals and procedure, results, abstract, background, objectives and ethical statement.

Risk of bias within studies

To assess the risk of bias (ROB) of included studies, the SYRCLE checklist that is specific to pre-clinical studies was utilized. As reported in Table 3A and B, the included studies proceeded well in a majority of domains and checklist items, but with some deficiencies noted. It is important to mention that the domains “allocation concealment”, “random outcome assessment” and “attrition bias - incomplete outcome data” were all unclear in those included studies. Furthermore, the sequence generation (randomly animal allocation) when it comes to treatment assignments and random outcome assessment were not described in twenty-two studies [38-40, 45-47, 50, 51, 53, 54, 59-62, 64-70, 72]. Taken together, seventeen studies were classified as low risk of

bias [34-37, 41-44, 48, 49, 52, 55-58, 63, 71], fifteen reports were classified as to have moderate risk of bias [38, 40, 50, 53, 59-62, 64, 66-70, 72], and seven studies were considered to have high risk of bias [39, 45-47, 51, 54, 65].

Results of syntheses

Reporting biases

Discussion

A biological agent, according to the Food and Drug Administration, is defined as "a virus, therapeutic serum, toxin, antitoxin, vaccine, blood component or derivative, allergenic product, protein, or analogous product, applicable to the prevention, treatment, or cure of a disease or condition of human beings" [73]. In the field of periodontitis, the term "biologic" can be more closely defined as a therapeutic agent with biological activity that is administered to inhibit alveolar bone loss (the hallmark of periodontal disease). Biologics can be subclassified into stem cells, gene therapy agents, autologous blood-derived products (ABPs), and bioactive factors [74]. Therefore, this systematic review evaluated the beneficial effects of biologicals on experimental periodontitis. Based on the main outcomes of the included reports, biological agents have been recognized as a host-modulator of the inflammatory response and a potent inhibitor of alveolar bone resorption in the different animal models utilized in both preventive and therapeutic approaches. Their possible role in arresting the alveolar bone resorption turn them a promising therapeutic compound for periodontal disease treatment. Accordingly, a recent consensus statement on the use of biologicals in clinical practice [75] stated that the use of biologics in periodontal practice is usually safe and offers further benefits to periodontal conventional treatment.

Studies using animal models to mimic experimental conditions are widely used in various human-related diseases. The use of experimental models of PD permits the study of the molecular mechanisms involved in the immunopathogenesis of chronic inflammatory diseases [76, 77]. The ligature-induced bone loss, oral inoculation and LPS injection EP models, the main models utilized in the included studies, assist in the

understanding of events that lead to protection or tissue damage as consequence of bacterial dysbiosis and the dysregulation of the host immune response [76]. Animal models of EP stimulate an infection/inflammatory process similar to that seen in humans, while mimicking the natural disease pathogenesis. The advantages of rodent models of EP included: more cost-effective model for scientific research, easy to handle, and the cost are relatively low. Besides, the physiological systems and anatomic structures are well known [78]. Of importance, they can also contribute to the development of more successful therapeutic strategies, provide hypothesis validation and demonstrate the effectiveness of new treatment therapies supporting decisions about human clinical research [79, 80]. Systematic reviews of pre-clinical studies are recognized for their importance in identifying interventions with the best preventive or therapeutic potential for testing in randomized clinical studies since they might offer robust and comprehensive descriptions of those animal studies [81]. In making these decisions, the level of certainty in the evidence is fundamental. In this current systematic review, the ARRIVE checklist, comprising 21 items, was employed to evaluate the quality and reliability of the included manuscripts [82]. The 14-19 scores achieved in the quality assessment demonstrated that these reports were reliable and of high quality.

The included studies in this systematic review demonstrated innumerous methodological variations regarding the EP induction models (ligature, oral gavage, LPS inoculation), animals used (mouse and rat), inoculation of bacteria (*P. gingivalis*, *F. nucleatum*, *A. actinomycetemcomitans*), materials for ligature (cotton, silk and dental floss), number of days of for disease induction, and treatment duration with the biologicals (Table 1). After a comprehensive analysis of data on the characteristics of each included manuscript, it was observed that the most common used animal strain was de C57BL/6 mouse and the most common EP model was the ligature-induced bone loss placed in the first and second maxillary molar in most of the cases. The most common method for bone loss evaluation was the micro-CT and histological analyzes. Thus, despite the inconsistency of the models found in the literature, limited reports investigate and compare the temporal progression and the local and systemic results of EP through different biological regimens.

The hallmark of PD is the alveolar bone resorption that occurs in consequence of the imbalance between the bacterial insult and the host immune

response. Therefore, inhibition of bone loss was set as the main outcome parameter in this systematic review. Quantification of bone resorption in the included studies was performed by means of morphometric analysis [39, 45, 47, 48, 50, 51, 53-56, 71, 72], micro-CT [34, 37, 43, 44, 46, 52, 58-62, 64, 65, 69, 70], conventional radiograph [35, 38], SEM [49], and histomorphometric analysis [36, 41, 57, 63, 66-68]. Except by one study [36] that fail to show inhibition of bone loss after treatment, and by two studies that did not quantify the resorption of the alveolar bone [40, 42], all the other studies showed remarkable ameliorative effects regarding inhibition of bone loss. Osteoclasts, the cells responsible for resorbing the bone tissue, were evaluated in several studies [37-40, 45-47, 49, 52, 54-56, 58, 60-64, 67, 68, 71] utilizing the TRAP staining analysis. Decreased osteoclast activity and osteoclast number after biological treatment seems to significantly regulate alveolar bone resorption in those studies. Most of the biological investigated prevented alveolar bone loss by suppressing RANK-L mediated osteoclast development decreasing the ratio of RANKL/OPG.

In this current systematic review, the systemic administration of biologicals appeared to effectively inhibit alveolar bone destruction in the different animals models used [34-36, 38-43, 46-55, 57-65, 67, 68, 70, 72], as well as the local treatment [37, 43-45, 56, 65, 66, 69]. Both local and systemic administration of biologicals seems to control systemic and local inflammation induced by bacteria. However, the local treatment (injection directly into the gingival tissue) appeared to be more practical and reliable approach to treat periodontal disease in humans, as compared to the systemic administration, since the compound is applied directly into the periodontal pocket/sulcus enhancing the local effects against inflammation-induced bone loss. Of importance, some studies included showed that biologicals are effective in ameliorate systemic conditions associated with periodontitis, such as diabetes mellitus [47, 48, 55, 58, 63, 67, 68, 70], obesity [42, 71], and ovariectomized osteoporosis animal models [59] evidencing that these compounds have direct actions on the systemic inflammatory response of others inflammatory (diabetes and obesity) and non-inflammatory (osteoporosis) conditions. Therefore, further investigations are warranted to explore the influence of these biologicals in other systemic inflammatory conditions in clinical trials.

This systematic review included 39 studies that met the inclusion criteria, and these relatively high numbers of studies strengthen the achieved findings and support the use of biologicals in pre-clinical studies. When analyzing the data for risk of bias according to the SYRCLES's checklist specific for pre-clinical studies, most of the included reports demonstrated a low risk [34-37, 41-44, 48, 49, 52, 55-58, 63, 71] or moderate risk [38, 40, 50, 53, 59-62, 64, 66-70, 72] of bias. The studies classified as moderate and of high risk of bias were correlated with missing information regarding attrition bias, allocation concealment, and sequence generation. Consequently, we can infer that the studies included in this review are, most of them, of quality, reproducible, and reliable studies.

Conclusion

Collectively, the results of this systematic review, demonstrated that the use of biologicals agents are promising alternative to treat EP in pre-clinical studies when applied systemically or locally. The fact that these are biological molecules with the unique ability to both arrests the progression of bone loss as well as to decrease the inflammatory process, without any additional side effect, makes them attractive potential agents for preventing and treating periodontal disease. Although the results have showed promising results, randomized clinical trials are warranted to assess the effectiveness of biologicals in humans.

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Supplementary information None.

Data Availability Statement The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions DSB, and RSM performed the conception and design of the manuscript, and interpretation of the data. All authors performed the literature search, drafted the work, and revised it critically for important intellectual content. All authors agree to be accountable for all aspects of the study design and its content. All authors approved the final submitted version.

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Declarations

Conflict of interest None of the authors disclose any financial, consulting or personal relationships with other people or organizations that could influence the authors' work.

Ethical approval No ethical approval was required for this study since it was a systematic review.

Informed consent No informed consent was required.

References

1. Kinane DF, Stathopoulou PG and Papapanou PN (2017) Periodontal diseases. Nat Rev Dis Primers 3:17038. doi: 10.1038/nrdp.2017.38

2. de Molon RS, de Avila ED, Cirelli JA and Steffens JP (2022) Periodontal research contributions to basic sciences: from cell communication and host-parasite interactions to inflammation and bone biology. Biocell 46:633–638.

3. Eke PI, Thornton-Evans G, Dye B and Genco R (2012) Advances in surveillance of periodontitis: the Centers for Disease Control and Prevention periodontal disease surveillance project. J Periodontol 83:1337-42. doi: 10.1902/jop.2012.110676

4. Balta MG, Papathanasiou E, Blix IJ and Van Dyke TE (2021) Host Modulation and Treatment of Periodontal Disease. Journal of dental research 100:798-809. doi: 10.1177/0022034521995157

5. Phipps KR and Stevens VJ (1995) Relative contribution of caries and periodontal disease in adult tooth loss for an HMO dental population. J Public Health Dent 55:250-2. doi: 10.1111/j.1752-7325.1995.tb02377.x

6. Barutta F, Bellini S, Durazzo M and Gruden G (2022) Novel Insight into the Mechanisms of the Bidirectional Relationship between Diabetes and Periodontitis. Biomedicines 10. doi: 10.3390/biomedicines10010178

7. Beck JD, Papapanou PN, Philips KH and Offenbacher S (2019) Periodontal Medicine: 100 Years of Progress. Journal of dental research 98:1053-1062. doi: 10.1177/0022034519846113

8. de Molon RS, Rossa C, Jr., Thurlings RM, Cirelli JA and Koenders MI (2019) Linkage of Periodontitis and Rheumatoid Arthritis: Current Evidence and Potential Biological Interactions. Int J Mol Sci 20. doi: 10.3390/ijms20184541

9. Sedghi LM, Bacino M and Kapila YL (2021) Periodontal Disease: The Good, The Bad, and The Unknown. Front Cell Infect Microbiol 11:766944. doi: 10.3389/fcimb.2021.766944

10. Silva DNA, Casarin M, Monajemzadeh S, Bezerra BB, Lux R and Pirih FQ (2022) The Microbiome in Periodontitis and Diabetes. Front Oral Health 3:859209. doi: 10.3389/froh.2022.859209

11. Pavanelli ALR, de Menezes BS, Pereira EBB, de Souza Morais FA, Cirelli JA and de Molon RS (2022) Pharmacological Therapies for the Management of Inflammatory Bone Resorption in Periodontal Disease: A Review of Preclinical Studies. Biomed Res Int 2022:5832009. doi: 10.1155/2022/5832009

12. Lee J, Min HK, Park CY, Kang HK, Jung SY and Min BM (2022) A vitronectin-derived peptide prevents and restores alveolar bone loss by modulating bone re-modelling and expression of RANKL and IL-17A. J Clin Periodontol. doi: 10.1111/jcpe.13671

13. Haas AN, Furlaneto F, Gaio EJ, Gomes SC, Palioto DB, Castilho RM, Sanz M and Messora MR (2021) New tendencies in non-surgical periodontal therapy. Braz Oral Res 35:e095. doi: 10.1590/1807-3107bor-2021.vol35.0095

14. Cosgarea R, Ramseier CA, Jepsen S, Arweiler NB, Jervoe-Storm PM, Batori-Andronescu I, Rossler R, Conrad T, Eick S and Sculean A (2022) One-Year Clinical, Microbiological and Immunological Results of Local Doxycycline or Antimicrobial Photodynamic Therapy for Recurrent/Persisting Periodontal Pockets: A Randomized Clinical Trial. Antibiotics (Basel) 11. doi: 10.3390/antibiotics11060738

15. Roccuzzo A, Imber JC, Stahli A, Kloukos D, Salvi GE and Sculean A (2022) Enamel matrix derivative as adjunctive to non-surgical periodontal therapy: a systematic review and meta-analysis of randomized controlled trials. Clin Oral Investig 26:4263-4280. doi: 10.1007/s00784-022-04474-1

16. Ramos TCS, Boas MLV, Nunes CMM, Ferreira CL, Pannuti CM, Santamaria MP and Jardini MAN (2022) Effect of systemic antibiotic and probiotic therapies as adjuvant treatments of subgingival instrumentation for periodontitis: a randomized controlled clinical study. J Appl Oral Sci 30:e20210583. doi: 10.1590/1678-7757-2021-0583

17. Kiani S, Birang R and Jamshidian N (2022) Effect of Propolis mouthwash on clinical periodontal parameters in patients with gingivitis: A double-blinded randomized clinical trial. Int J Dent Hyg 20:434-440. doi: 10.1111/idh.12550

18. Da Ponte Leguizamon N, de Molon RS, Coletto-Nunes G, Nogueira AVB, Rocha SV, Neo-Justino DM, Soares-Costa A, Cerri PS, Lerner UH, Souza PPC and Cirelli JA (2022) Phytocystatin CsinCPI-2 Reduces Osteoclastogenesis and Alveolar Bone Loss. Journal of dental research 101:216-225. doi: 10.1177/00220345211027811

19. Fernandes NAR, Camilli AC, Maldonado LAG, Pacheco CGP, Silva AF, Molon RS, Spolidorio LC, Ribeiro de Assis L, Regasini LO, Rossa Junior C and Guimaraes-Stabili MR (2021) Chalcone T4, a novel chalconic compound, inhibits inflammatory bone resorption in vivo and suppresses osteoclastogenesis in vitro. J Periodontal Res 56:569-578. doi: 10.1111/jre.12857

20. Bahor Z, Liao J, Currie G, Ayder C, Macleod M, McCann SK, Bannach-Brown A, Wever K, Soliman N, Wang Q, Doran-Constant L, Young L, Sena ES and Sena C (2021) Development and uptake of an online systematic review platform: the early years of the CAMARADES Systematic Review Facility (SyRF). BMJ Open Sci 5:e100103. doi: 10.1136/bmjos-2020-100103

21. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hrobjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P and Moher D (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 372:n71. doi: 10.1136/bmj.n71

22. Landis JR and Koch GG (1977) The measurement of observer agreement for categorical data. Biometrics 33:159-74.

23. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M and Langendam MW (2014) SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol 14:43. doi: 10.1186/1471-2288-14-43

24. Campbell M, McKenzie JE, Sowden A, Katikireddi SV, Brennan SE, Ellis S, Hartmann-Boyce J, Ryan R, Shepperd S, Thomas J, Welch V and Thomson H (2020) Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline. BMJ 368:l6890. doi: 10.1136/bmj.l6890

25. Higgins J, Thompson S, Deeks J and Altman D (2002) Statistical heterogeneity in systematic reviews of clinical trials: a critical appraisal of guidelines and practice. J Health Serv Res Policy 7:51-61. doi: 10.1258/1355819021927674

26. Higgins JP, Thompson SG, Deeks JJ and Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327:557-60. doi: 10.1136/bmj.327.7414.557

27. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ and Welch VA (2021) Cochrane Handbook for Systematic Reviews of Interventions version 6.2 Book title.,

28. Sterne JA, Sutton AJ, Ioannidis JP, Terrin N, Jones DR, Lau J, Carpenter J, Rucker G, Harbord RM, Schmid CH, Tetzlaff J, Deeks JJ, Peters J, Macaskill P, Schwarzer G, Duval S, Altman DG, Moher D and Higgins JP (2011) Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ 343:d4002. doi: 10.1136/bmj.d4002

29. GDT G (2020) GRADEpro Guideline Development Tool [Software]. In: McMaster University dbEP, Inc.). Available from gradepro.org. (ed) Book title.,

30. Schünemann H, Brożek J, Guyatt G and A O (2013) GRADE handbook for grading quality of evidence and strength of recommendations. The GRADE Working Group Available from guidelinedevelopment.org/handbook.

31. Guyatt GH, Oxman AD, Schunemann HJ, Tugwell P and Knottnerus A (2011) GRADE guidelines: a new series of articles in the Journal of Clinical Epidemiology. J Clin Epidemiol 64:380-2. doi: 10.1016/j.jclinepi.2010.09.011

32. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, Schunemann HJ and Group GW (2008) GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 336:924-6. doi: 10.1136/bmj.39489.470347.AD

33. Santesso N, Glenton C, Dahm P, Garner P, Akl EA, Alper B, Brignardello-Petersen R, Carrasco-Labra A, De Beer H, Hultcrantz M, Kuijpers T, Meerpohl J, Morgan R, Mustafa R, Skoetz N, Sultan S, Wiysonge C, Guyatt G, Schunemann HJ and Group GW (2020) GRADE guidelines 26: informative statements to communicate the findings of systematic reviews of interventions. J Clin Epidemiol 119:126-135. doi: 10.1016/j.jclinepi.2019.10.014

34. Apolinario Vieira GH, Aparecida Rivas AC, Figueiredo Costa K, Ferreira Oliveira LF, Tanaka Suzuki K, Reis Messora M, Sprone Ricoldi M, Goncalves de Almeida AL and Taba M, Jr. (2021) Specific inhibition of IL-6 receptor attenuates inflammatory bone loss in experimental periodontitis. J Periodontol 92:1460-1469. doi: 10.1002/JPER.20-0455

35. Arabaci T, Kermen E, Ozkanlar S, Kose O, Kara A, Kizildag A, Duman SB and Ibisoglu E (2015) Therapeutic Effects of Melatonin on Alveolar Bone Resorption After Experimental Periodontitis in Rats: A Biochemical and Immunohistochemical Study. J Periodontol 86:874-81. doi: 10.1902/jop.2015.140599

36. Aral K, Alkan BA, Saraymen R, Yay A, Sen A and Onder GO (2015) Therapeutic effects of systemic vitamin k2 and vitamin d3 on gingival inflammation and alveolar bone in rats with experimentally induced periodontitis. J Periodontol 86:666-73. doi: 10.1902/jop.2015.140467

37. Aydinyurt HS, Sancak T, Taskin C, Basbugan Y and Akinci L (2021) Effects of injectable platelet-rich fibrin in experimental periodontitis in rats. Odontology 109:422-432. doi: 10.1007/s10266-020-00557-1

38. Gaspersic R, Kovacic U, Glisovic S, Cor A and Skaleric U (2010) Anti-NGF treatment reduces bone resorption in periodontitis. Journal of dental research 89:515-20. doi: 10.1177/0022034510363108

39. Goncalves DC, Evangelista RC, da Silva RR, Santos MJ, Silva FS, Jr., Aragao KS, Brito GA, Lucena HB, Leitao RC and Oria RB (2014) Infliximab attenuates inflammatory osteolysis in a model of periodontitis in Wistar rats. Exp Biol Med (Maywood) 239:442-53. doi: 10.1177/1535370213520114

40. Marques MR, dos Santos MC, da Silva AF, Nociti FH, Jr. and Barros SP (2009) Parathyroid hormone administration may modulate periodontal tissue levels of interleukin-6, matrix metalloproteinase-2 and matrix metalloproteinase-9 in experimental periodontitis. J Periodontal Res 44:744-50. doi: 10.1111/j.1600-0765.2008.01186.x

41. Renn TY, Huang YK, Feng SW, Wang HW, Lee WF, Lin CT, Burnouf T, Chen LY, Kao PF and Chang HM (2018) Prophylactic supplement with melatonin successfully suppresses the pathogenesis of periodontitis through normalizing RANKL/OPG ratio and depressing the TLR4/MyD88 signaling pathway. J Pineal Res 64. doi: 10.1111/jpi.12464

42. Virto L, Cano P, Jimenez-Ortega V, Fernandez-Mateos P, Gonzalez J, Haugen HJ, Esquifino AI and Sanz M (2018) Melatonin as adjunctive therapy in the treatment of periodontitis associated with obesity. J Clin Periodontol 45:1336-1346. doi: 10.1111/jcpe.13013

43. Zhang Y, Zhuang D, Zhang Y, Lu H, Zhang H, Li T and Bi L (2020) Super Activated Platelet Lysate, a Novel Autologous Platelet Lysate, Regulates the Expression of Inflammasome and Cytokine in the Experimental Periodontitis in Rats. Drug Des Devel Ther 14:5535-5543. doi: 10.2147/DDDT.S289753

44. Cafferata EA, Terraza-Aguirre C, Barrera R, Faundez N, Gonzalez N, Rojas C, Melgar-Rodriguez S, Hernandez M, Carvajal P, Cortez C, Gonzalez FE, Covarrubias C and Vernal R (2020) Interleukin-35 inhibits alveolar bone resorption by modulating the Th17/Treg imbalance during periodontitis. J Clin Periodontol 47:676-688. doi: 10.1111/jcpe.13282

45. Hu Y, Yu P, Yu X, Hu X, Kawai T and Han X (2017) IL-21/anti-Tim1/CD40 ligand promotes B10 activity in vitro and alleviates bone loss in experimental periodontitis in vivo. Biochim Biophys Acta Mol Basis Dis 1863:2149-2157. doi: 10.1016/j.bbadis.2017.06.001

46. Kuritani M, Sakai N, Karakawa A, Isawa M, Chatani M, Negishi-Koga T, Funatsu T and Takami M (2018) Anti-mouse RANKL Antibodies Inhibit Alveolar Bone Destruction in Periodontitis Model Mice. Biol Pharm Bull 41:637-643. doi: 10.1248/bpb.b18-00026

47. Lalla E, Lamster IB, Feit M, Huang L, Spessot A, Qu W, Kislinger T, Lu Y, Stern DM and Schmidt AM (2000) Blockade of RAGE suppresses periodontitis-associated bone loss in diabetic mice. J Clin Invest 105:1117-24. doi: 10.1172/JCI8942

48. Li H, Xie H, Fu M, Li W, Guo B, Ding Y and Wang Q (2013) 25-hydroxyvitamin D3 ameliorates periodontitis by modulating the expression of inflammation-associated factors in diabetic mice. Steroids 78:115-20. doi: 10.1016/j.steroids.2012.10.015

49. Li H, Zhong X, Li W and Wang Q (2019) Effects of 1,25-dihydroxyvitamin D3 on experimental periodontitis and AhR/NF-kappaB/NLRP3 inflammasome pathway in a mouse model. J Appl Oral Sci 27:e20180713. doi: 10.1590/1678-7757-2018-0713

50. Madeira MF, Queiroz-Junior CM, Montero-Melendez T, Werneck SM, Correa JD, Soriani FM, Garlet GP, Souza DG, Teixeira MM, Silva TA and Perretti M (2016) Melanocortin agonism as a viable strategy to control alveolar bone loss induced by oral infection. FASEB J 30:4033-4041. doi: 10.1096/fj.201600790R

51. Meng S, Zhang L, Tang Y, Tu Q, Zheng L, Yu L, Murray D, Cheng J, Kim SH, Zhou X and Chen J (2014) BET Inhibitor JQ1 Blocks Inflammation and Bone Destruction. Journal of dental research 93:657-62. doi: 10.1177/0022034514534261

52. Nakane S, Imamura K, Hisanaga R, Ishihara K and Saito A (2021) Systemic administration of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4)-Ig abrogates alveolar bone resorption in induced periodontitis through inhibition of osteoclast differentiation and activation: An experimental investigation. J Periodontal Res 56:972-981. doi: 10.1111/jre.12909

53. Repeke CE, Ferreira SB, Jr., Vieira AE, Silveira EM, Avila-Campos MJ, da Silva JS, Santos CF, Campanelli AP, Trombone AP and Garlet GP (2011) Dose-response met-RANTES treatment of experimental periodontitis: a narrow edge between the disease severity attenuation and infection control. PLoS One 6:e22526. doi: 10.1371/journal.pone.0022526

54. Shi T, Jin Y, Miao Y, Wang Y, Zhou Y and Lin X (2020) IL-10 secreting B cells regulate periodontal immune response during periodontitis. Odontology 108:350-357. doi: 10.1007/s10266-019-00470-2

55. Wang Q, Li H, Xie H, Fu M, Guo B, Ding Y, Li W and Yu H (2013) 25-Hydroxyvitamin D3 attenuates experimental periodontitis through downregulation of TLR4 and JAK1/STAT3 signaling in diabetic mice. J Steroid Biochem Mol Biol 135:43-50. doi: 10.1016/j.jsbmb.2013.01.008

56. Yu P, Hu Y, Liu Z, Kawai T, Taubman MA, Li W and Han X (2017) Local Induction of B Cell Interleukin-10 Competency Alleviates Inflammation and Bone Loss in Ligature-Induced Experimental Periodontitis in Mice. Infect Immun 85. doi: 10.1128/IAI.00645-16

57. Yuan H, Gupte R, Zelkha S and Amar S (2011) Receptor activator of nuclear factor kappa B ligand antagonists inhibit tissue inflammation and bone loss in experimental periodontitis. J Clin Periodontol 38:1029-36. doi: 10.1111/j.1600-051X.2011.01780.x

58. Chen H, Fu T, Ma Y, Wu X, Li X, Li X, Shen J and Wang H (2017) Intermittent administration of parathyroid hormone ameliorated alveolar bone loss in experimental

periodontitis in streptozotocin-induced diabetic rats. Arch Oral Biol 83:76-84. doi: 10.1016/j.archoralbio.2017.06.033

59. Chen H, Xu X, Liu M, Zhang W, Ke HZ, Qin A, Tang T and Lu E (2015) Sclerostin antibody treatment causes greater alveolar crest height and bone mass in an ovariectomized rat model of localized periodontitis. Bone 76:141-8. doi: 10.1016/j.bone.2015.04.002

60. Cirelli JA, Park CH, MacKool K, Taba M, Jr., Lustig KH, Burstein H and Giannobile WV (2009) AAV2/1-TNFR:Fc gene delivery prevents periodontal disease progression. Gene Ther 16:426-36. doi: 10.1038/gt.2008.174

61. Jin Q, Cirelli JA, Park CH, Sugai JV, Taba M, Jr., Kostenuik PJ and Giannobile WV (2007) RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodontitis. J Periodontol 78:1300-8. doi: 10.1902/jop.2007.070073

62. Kirkwood KL, Li F, Rogers JE, Otremba J, Coatney DD, Kreider JM, D'Silva NJ, Chakravarty S, Dugar S, Higgins LS, Protter AA and Medicherla S (2007) A p38alpha selective mitogen-activated protein kinase inhibitor prevents periodontal bone loss. J Pharmacol Exp Ther 320:56-63. doi: 10.1124/jpet.106.112466

63. Kose O, Arabaci T, Kara A, Yemenoglu H, Kermen E, Kizildag A, Gedikli S and Ozkanlar S (2016) Effects of Melatonin on Oxidative Stress Index and Alveolar Bone Loss in Diabetic Rats With Periodontitis. J Periodontol 87:e82-90. doi: 10.1902/jop.2016.150541

64. Rogers JE, Li F, Coatney DD, Otremba J, Kriegl JM, Protter TA, Higgins LS, Medicherla S and Kirkwood KL (2007) A p38 mitogen-activated protein kinase inhibitor arrests active alveolar bone loss in a rat periodontitis model. J Periodontol 78:1992-8. doi: 10.1902/jop.2007.070101

65. Taut AD, Jin Q, Chung JH, Galindo-Moreno P, Yi ES, Sugai JV, Ke HZ, Liu M and Giannobile WV (2013) Sclerostin antibody stimulates bone regeneration after experimental periodontitis. J Bone Miner Res 28:2347-56. doi: 10.1002/jbmr.1984

66. Delima AJ, Karatzas S, Amar S and Graves DT (2002) Inflammation and tissue loss caused by periodontal pathogens is reduced by interleukin-1 antagonists. J Infect Dis 186:511-6. doi: 10.1086/341778

67. Kim JH, Kim AR, Choi YH, Jang S, Woo GH, Cha JH, Bak EJ and Yoo YJ (2017) Tumor necrosis factor-alpha antagonist diminishes osteocytic RANKL and sclerostin expression in diabetes rats with periodontitis. PLoS One 12:e0189702. doi: 10.1371/journal.pone.0189702

68. Kim JH, Kim AR, Choi YH, Kim A, Sohn Y, Woo GH, Cha JH, Bak EJ and Yoo YJ (2018) Intermittent PTH administration improves alveolar bone formation in type 1 diabetic rats with periodontitis. J Transl Med 16:70. doi: 10.1186/s12967-018-1438-2

69. Pacheco CMF, Maltos KLM, Shehabeldin MS, Thomas LL, Zhuang Z, Yoshizawa S, Verdelis K, Gaffen SL, Garlet GP, Little SR and Sfeir C (2021) Local Sustained

Delivery of Anti-IL-17A Antibodies Limits Inflammatory Bone Loss in Murine Experimental Periodontitis. J Immunol 206:2386-2392. doi: 10.4049/jimmunol.2001432

70. Wu X, Sun Y, Cui R, Qiu W, Zhang J, Hu Z, Bi W, Yang F, Ma D, Van Dyke T, Tu Q, Yu Y and Chen J (2022) A novel adiponectin receptor agonist (AdipoAI) ameliorates type 2 diabetes-associated periodontitis by enhancing autophagy in osteoclasts. J Periodontal Res 57:381-391. doi: 10.1111/jre.12969

71. Zhang L, Meng S, Tu Q, Yu L, Tang Y, Dard MM, Kim SH, Valverde P, Zhou X and Chen J (2014) Adiponectin ameliorates experimental periodontitis in diet-induced obesity mice. PLoS One 9:e97824. doi: 10.1371/journal.pone.0097824

72. Napimoga MH, Rocha EP, Trindade-da-Silva CA, Demasi APD, Martinez EF, Macedo CG, Abdalla HB, Bettaieb A, Haj FG, Clemente-Napimoga JT, Inceoglu B and Hammock BD (2018) Soluble epoxide hydrolase inhibitor promotes immunomodulation to inhibit bone resorption. J Periodontal Res 53:743-749. doi: 10.1111/jre.12559

73. FDA Code of Federal Regulations Title 21 Book title. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=600.3,

74. Chambrone L and Avila-Ortiz G (2022) TISSUES: Critical Issues in Periodontal and Implant-Related Plastic and Reconstructive Surgery. . Quintessence Publishing,

75. Avila-Ortiz G, Ambruster J, Barootchi S, Chambrone L, Chen CY, Dixon DR, Geisinger ML, Giannobile WV, Goss K, Gunsolley JC, Heard RH, Kim DM, Mandelaris GA, Monje A, Nevins ML, Palaiologou-Gallis A, Rosen PS, Scheyer ET, Suarez-Lopez Del Amo F, Tavelli L, Velasquez D, Wang HL and Mealey BL (2022) American Academy of Periodontology best evidence consensus statement on the use of biologics in clinical practice. J Periodontol 93:1763-1770. doi: 10.1002/JPER.22-0361

76. de Molon RS, de Avila ED and Cirelli JA (2013) Host responses induced by different animal models of periodontal disease: a literature review. J Investig Clin Dent 4:211-8. doi: 10.1111/jicd.12018

77. de Molon RS, Park CH, Jin Q, Sugai J and Cirelli JA (2018) Characterization of ligature-induced experimental periodontitis. Microsc Res Tech 81:1412-1421. doi: 10.1002/jemt.23101

78. de Molon RS, Mascarenhas VI, de Avila ED, Finoti LS, Toffoli GB, Spolidorio DM, Scarel-Caminaga RM, Tetradis S and Cirelli JA (2016) Long-term evaluation of oral gavage with periodontopathogens or ligature induction of experimental periodontal disease in mice. Clin Oral Investig 20:1203-16. doi: 10.1007/s00784-015-1607-0

79. de Molon RS, de Avila ED, Boas Nogueira AV, Chaves de Souza JA, Avila-Campos MJ, de Andrade CR and Cirelli JA (2014) Evaluation of the host response in various models of induced periodontal disease in mice. J Periodontol 85:465-77. doi: 10.1902/jop.2013.130225

80. Hooijmans CR, de Vries RBM, Ritskes-Hoitinga M, Rovers MM, Leeflang MM, IntHout J, Wever KE, Hooft L, de Beer H, Kuijpers T, Macleod MR, Sena ES, Ter Riet G, Morgan RL, Thayer KA, Rooney AA, Guyatt GH, Schunemann HJ, Langendam MW and

Group GW (2018) Facilitating healthcare decisions by assessing the certainty in the evidence from preclinical animal studies. PLoS One 13:e0187271. doi: 10.1371/journal.pone.0187271

81. Alshibani N (2022) Resolvins as a Treatment Modality in Experimental Periodontitis: A Systematic Review of Preclinical Studies. Cureus 14:e21095. doi: 10.7759/cureus.21095

82. Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Hurst V, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T and Wurbel H (2020) Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol 18:e3000411. doi: 10.1371/journal.pbio.3000411

Figure legends

Fig. 1: Screening and enrolment according to the PRISMA flow diagram.

Table legends

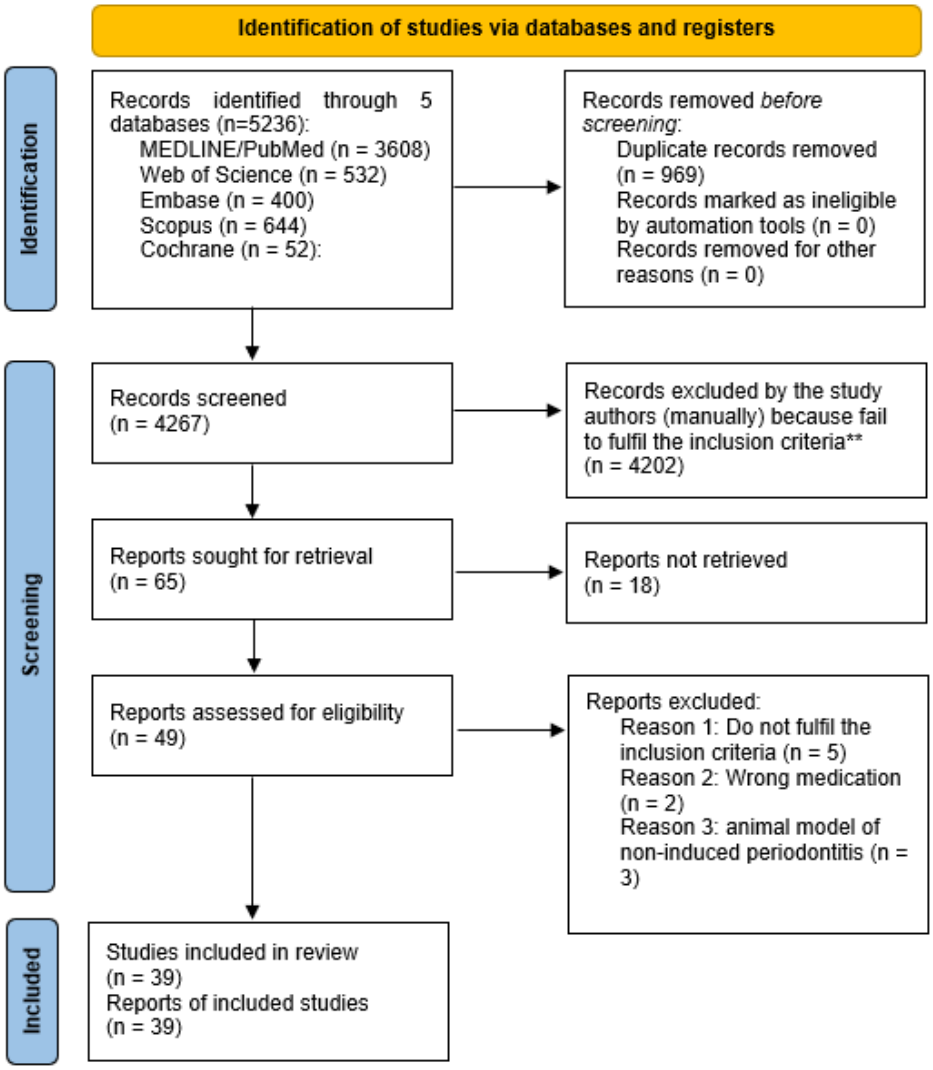
Table 1: Characteristics of the included studies.

Table 2A-C: The quality assessment of the included studies assessed by the ARRIVE guidelines containing a 21-item checklist.

Table 3A-B: Risk-of-bias assessment for animal studies using SYRCLE's tool.

Supplemental Table 1: Search strategies

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only



*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).

**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

Table 1 - Summary of the study characteristics

Author / Year	*Animal model*	*Intervention / comparison groups*	*Type of biological therapy*	*Follow-up time*	*Assessed outcomes*	*Summary of findings*
Apolinario-Vieira et al. (2021) [1]	90 male Wistar Hannover SPF rats 10-12 wk old	Experimental periodontitis (EP) was induced by placing Cotton ligature around the lower 1st molar a) Control b) Ligature c) Ligature + TCZ2 d) Ligature + TCZ4 e) Ligature + TCZ8	Tocilizumab (TCZ) - 2, 4 and 8 mg/kg. Intraperitoneal injection (daily)	7 and 14 days of TCZ treatment	Micro-CT Histology Gene expression (qPCR)	TCZ reduced alveolar bone loss and attachment loss; diminished inflammatory infiltrate and reduced pro-inflammatory cytokines
Arabaci et al. (2015) [2]	24 male Wistar rats 12 wk old (220-250g body weight)	EP was induced by placing 3-0 silk ligatures around the lower 1st molar for 4 wks. a) Control b) Ligature c) Ligature + MEL	Melatonin (MEL) 10 mg/kg Intraperitoneal injection (daily)	15 days of MEL treatment after ligature removal	Radiographic Histology Biochemical analyses	MEL treatment decreased alveolar bone resorption, MPO and osteoclastic activity
Aral et al. (2015) [3]	72 male Wistar rats (270-330g body weight)	EP was induced by placing 4-0 cotton ligature around the upper 1st molar for 1 wk. a) Control b) Periodontitis (P) c) P + SRP d) SRP + Vit D3 e) SRP + Vit K f) SRP + Vit D3 + Vit K	Vitamin K2 - menatetrenone 30 mg/kg Vitamin D3 - (2µg/kg) Oral gavage / daily	10 days of vitamin treatment after ligature removal	Histology Serum levels of ALP and TRAP-5b ELISA (IL-1β and IL-10)	Vit D3 and K2 alone or in combination did not influence the levels of alveolar bone neither IL-1β, IL-10, ALP and TRAP-5b levels
Aydinyurt et al (2021) [4]	30 Wistar albino rats (180-250g body weight)	EP was induced by placing 4-0 silk ligatures around lower molar for 3 wks. a) SRP b) SRP + iPRF c) i-PRF	Injectable platelet-rich fibrin (i-PRF) Subgingival injection (3 applications)	10 days after ligature removal	Micro-CT Histology IHC	No significant differences were observed among groups regarding bone resorption, inflammation and TNF-α, IL-1β, INF-γ and VEGF levels.

Cafferata et al. (2019) [5]	C57BL/6 mice 8 wk old	EP was induced by placing 5-0 silk ligature around the upper 2nd molar a) Control (sham) b) PD group c) PD + 2μg of IL-35 (ip) d) PD + 1ηg of IL-35 (ig) e) PD + 10ηg of IL-35 (ig) f) PD + 100ηg of IL-35 (ig)	IL-35 daily injections Intragingival (ig) injection of 1, 10 or 100ηg of IL-35 Intraperitoneal injection of 2μg of IL-35	15 days of IL-35 treatment	Micro-CT Histology ELISA (RANKL and OPG) mRNA expression levels Flow cytometry	IL-35 treatment inhibited alveolar bone loss, decreased osteoclast and downregulated RANKL expression. IL-35 upregulated Treg-related cytokines and downregulated Th17-related cytokines.
Chen et al. (2015) [6]	60 female Sprague-Dawley rats 4 month-old	Bilaterally ovariectomy (OVX) EP was induced by placing 3-0 silk ligature around the upper 1st and 2nd molars for 4 wks 30 days after OVX a) Control (Sham) b) Sham + ligature c) OVX + ligature d) OVX + ligature + Scl-Ab	Sclerostin antibody (Scl-Ab) 25mg/kg Twice weekly Subcutaneous injection	6 weeks Scl-Ab treatment	Micro-CT ELISA (OCN, TRAP5b and CTx-1) Histology	Treatment of OVX rats with Scl-Ab that underwent ligature-induced PD decreased alveolar bone loss (higher values of mineral apposition rate and mineralizing bone surface). Increased serum OCN and OPG and decreased TRAP and CTx-1 levels
Chen et al. (2017) [7]	50 male Sprague-Dawley rats 6-8 wks old 180-220g body weight	Diabetes induced (65 mg/kg streptozotocin - STZ) EP was induced by placing 4-0 silk ligature around the upper 1st molar one day after diabetes confirmation a) Control b) PTH c) Ligature (L) d) L + STZ e) L + STZ + PTH	Parathyroid hormone (PTH) Subcutaneous injection of 75 μg/kg PTH 4 times/wk for 4 wks one day after ligature placement	4 wks of PTH treatment	Micro-CT Histology IHC	Diabetes significantly aggravated alveolar bone destruction induced by ligature placement and PTH decreased alveolar bone loss, and tissue inflammation. PTH increased osteoblastic activity and decreased RANKL/OPG

						ratio.
Cirelli et al. (2009) [8]	45 male Sprague-Dawley rats 8-10 wks-old 200g body weight	Pg-LPS-induced periodontal disease 10 µL of Pg W83 LPS (4 palatal gingival sites - total of 40ul per animal); Injections 3 times/wk a) TNFR:Fc b) TNFR:Fc + LPS c) Pg-LPS d) Control	TNFR:Fc (100 µl of $1x10^{11}$ DRP) intramuscular administration; 4 wks before Pg-LPS injection	4 and 8 wks after the first injection	Micro-CT Histology qPCR IHC	TNFR:Fc protected against Pg-LPS-mediated alveolar bone loss and reduced the level of pro-inflammatory cytokines and osteoclasts cells in the periodontal tissues
Delima et al. (2002) [9]	9 Macaca fascicularis model 3-6 years old	EP was induced by placing silk ligatures inoculated with *P. gingivalis* (strain A7436) around the lower molar a) control b) Ligature + PBS c) Ligature + sIL-1R1	Soluble interleukin-1 receptor type I (sIL-1R1) - 6 µg per injection into the gingival tissue around the maxillary molars 3 times weekly	6 wks	Histology	Inhibition of IL-1 with soluble human IL-1R1 significantly reduced inflammation and bone resorption induced by ligature placement.
Gaspersic et al. (2010) [10]	18 female Wistar rats 200-250g body weight	EP was induced by placing ligature around the upper 2nd molar a) Control b) Ligature 1 wk c) Ligature 2 wks d) Ligature 2 wk + anti-NGF	anti-NGF (nerve growth factor) ab 30 µg Subcutaneous injection	1 and 2 wks	qPCR IHC X-ray measurement	Systemic anti-NGF treatment reduced IL-1β expression and alveolar bone resorption.
Goncalves et al. (2014) [11]	Wistar rats 200-250g body weight	EP was induced by placing 3-0 nylon ligature around the upper 2nd molar a) Control b) Ligature c) Ligature + infliximab	Infliximab (Remicade® 100mg) 5 mg/kg intravenously 1h before ligature placement	11 days after PD induction	Morphometric analysis Histology MPO Flow cytometry Western blot ELISA (IL-1 β and TNF-α) IHC	Infliximab reduced IL-1β, TNF-α and MPO; diminished MMP1-8, RANK and RANKL; and attenuated alveolar bone loss.

Hu et al. (2017) [12]	C57BL/6 mice 8-10 wks-age	EP was induced by placing 7-0 silk ligatures around the upper 2nd molar for 14 days	Combination of CD40L (1µg/ml) IL-21 (1µg/ml) anti-Tim1 (5µg/ml) Interdental papilla injection on days 3, 6 and 9	2 wks	qPCR Morphometric analysis TRAP	Combination of IL-21/anti-Tim1/CD40 increased IL-10 gingival mRNA and protein levels, decreased RANKL expression and alveolar bone loss.
Jin et al. (2007) [13]	32 male Sprague-Dawley rats 250-300g body weight	EP was induced by placing 3-0 cotton ligature around the lower 1st molar a) Control b) rhOPG-Fc c) Ligature + rhOPG-Fc d) Ligature + vehicle	Human recombinant OPG fusion protein (rhOPG-Fc) 10 mg/kg subcutaneously Twice a week	3 and 6 wks	Micro-CT Histology TRAP	OPG-Fc treatment decreased the levels of TRAP-5b, preserved alveolar bone volume, and suppressed osteoclast surface area.
Kim et al. (2017) [14]	56 Male inbred F344 rats 6 wk-old	Diabetes induced by iv STZ administration EP was induced by placing ligature (dental floss) around the lower 1st molar a) Control b) Periodontitis c) Diabetes + Periodontitis d) Diabetes + periodontitis + IFX	Infliximab (IFX) 5mg/kg Intraperitoneal; once for 3 days group (on day 0) and twice for the 20 days group (day 7 and 14)	3 days and 20 days after ligature placement	Histology IHC qPCR	IFX treatment demonstrated lower alveolar bone loss, decreased osteoclast formation and lower RANKL positive osteocytes.
Kim et al. (2018) [15]	Male F344 rats	Diabetes induced by iv STZ injection EP was induced by placing ligature (dental floss) around the lower 1st molars a) Control b) Periodontitis (PD) c) PD + PTH	Parathyroid hormone (PTH) administered after ligature placement subcutaneously 3 times per wk (40 µg/kg)	30 days	Histology Fluorescence IHC	Rats with diabetes and periodontitis treated with PTH presented with greater osteoid formation, more mineral deposition and lower percentage of

		d) Diabetes + PD e) Diabetes + PD + PTH				sclerostin-positive osteocytes; and diminished alveolar bone loss.
Kirkwood et al. (2007) [16]	40 female Sprague-Dawley rats 250g body weight	EP induced by *Aa* LPS (2µl of 10mg/ml solution) injected in the palatal mucosa 3x per wk for 8 wks; a) Aa LPS b) LPS + SD-282 (15mg/kg) c) LPS + SD-282 (45mg/kg) d) Control +vehicle e) Control + SD-282 (45 mg/kg)	ATP-competitive inhibitor of p38 MAPK SD-282 Twice daily by oral gavage	8 wk period	Micro-CT Histology IHC TRAP	Both doses of SD-282 showed significant protection from LPS induced bone loss; significantly fewer TRAP-positive osteoclasts and a significant decrease in IL-6, IL-1β and TNF-α expression.
Kose et al. (2016) [17]	70 male Sprague-Dawley rats 200-220g body weight	Diabetes (DM) induced by single dose of 120 mg/kg alloxan (ip); EP was induced by placing 3-0 silk ligature around the lower 1st molar kept for 4 wks. a) Control b) EP c) DM d) EP + DM e) EP + melatonin f) DM + melatonin g) EP + DM + melatonin	Melatonin (Ketalar®, Pfizer) 10mg/bw Administered by ip injection for 14 days after ligature removal	2 wks	Biochemical assay MPO activity Histology IHC	Melatonin treatment reduced serum oxidative stress index and alveolar bone loss and decreased MPO activity and osteoclast densities.
Kuritani et al (2018) [18]	C57BL/6 male mice 8 wk-old	LPS (E. coli 026:B6) injected into the calvarial bone; EP was induced by placing 5-0 silk ligature around the upper 2nd molar. a) LPS (25 mg/kg)	anti-RANKL Ab (3mg/kg) given at 0, 1 and 2 weeks after ligation via ip.	2 wks	Micro-CT TRAP	anti-RANKL administration inhibited osteoclast formation and bone resorption in calvaria; anti-RANKL also inhibited alveolar bone

		b) anti-RANKL c) Zolendronate d) Control				destruction in the EP mouse model.
Lalla et al. (2000) [19]	C57BL/6 male mice 6-7 wk-old	DM induced by 4 ip injection of STZ (55mg/kg) EP was induced by oral inoculation of *P. gingivalis* (0.2 m of $1.5x10^{12}$ cells/ml) every other day for a total of 4 days one month after DM induction; a) control (no DM) b) DM c) DM + sRAGE	sRAGE at dosage ranging from 3.5 to 100µg per day. Commencing the day after administration of *P. gingivalis* was completed and continuing for a total of 2 months; Daily ip injection	2 months	Morphometric analysis ELISA IHC Immunoblotting MMP protein and activity	sRAGE administration lead to reduced alveolar bone loss in a dose-dependent manner independently of the glycemic level. The levels of MMP-2, 3 and 9, TNF-α, and IL-6 were significantly reduced in sRAGE treated-mice
Li et al. (2013) [20]	50 male C57BL/6 WT mice 4 wk-old	DM was induced by ip injection of STZ (40 mg/kg/bw) EP was induced by oral inoculation of *P. gingivalis* (ATCC33277) with 100µl of 10^9 CFU of live P.g. a) Control b) Periodontitis (P) c) DM + P d) P + Vit D3 e) DM + P + Vit D3	Vitamin D3 hydroxylated to 25-hydroxyvitamin D3 - $25(OH)D_3$ 5µg/kg/bw ip injection every other day	8 wks	ELISA (TNF-α) Morphometric analysis Western blot	Administration of $25(OH)D_3$ (ip) reduced fasting glucose and TNF-α levels, decreased alveolar bone loss and attenuated the phosphorylation of Janus family kinase 1 (JAK1)
Li et al. (2019) [21]	30 male C57BL/6 WT mice 6 wk-old 20-228g body weight	EP was induced by oral inoculation with *P. gingivalis* (ATTC 33277); 3 times at 2-day intervals within 5 days (10^9 CFU) a) Control b) P c) P + VD3	Vitamin D3 was ip injected (2.5µg/kg/bw) every other day starting 5 wks after oral inoculation, and mice were injected for another 8 wks.	8 wks	Scanning electron microscopy (SEM) Western blot IHC	Ip injection of vitamin D3 for 8 wks significantly decreased alveolar bone loss. Vit D3 decreased NF-κB p65 phosphorylation and NLRP3, capase 1, IL-1β and IL-6 protein

						expression.
Madeira et al. (2016) [22]	15 C57BL/6 WT mice 8 wk-old	EP was induced by oral inoculation (3 times) with *Aa* ($1x10^9$CFU) a) Control b) Aa c) Aa + $DTrp^8$-MSH	Melanocortin agonist - $DTrp^8$-gMSH subcutaneously 10µg/mouse	30 days	Morphometric analysis Histology MPO ELISA Flow cytometry qPCR	Treatment with melanocortin agonist - $DTrp^8$-gMSH decreased alveolar bone loss and lowered the degree of neutrophil infiltration; and reduced levels of TNF-α, IFN-γ, and IL-17A.
Marques et al. (2009) [23]	76 male Wistar rats 4 wk-old 78 ± 7g	EP was induced by placing cotton ligature around the lower 1st molar a) Control b) PTH	Human parathyroid hormone (PTH) 40µg/kg 3x/wk subcutaneously 15 days of treatment	15 and 30 days	qPCR Zymography Immunoprecipitation TRAP IHC	PTH treatment decreased MMP-9 activity; decreased osteoclast numbers; and reduced the levels of mRNA for IL-6 and MMP-2.
Meng et al. (2014) [24]	31 male C57BL/6 mice 12 wk-old	EP was induced by placing 6-0 silk ligatures pre-soaked with *P. gingivalis* around the upper 2nd molar a) P b) P + JQ1 c) Control	Bromodomain and extraterminal domain (BET) inhibitor JQ1 (50 mg/kg) Daily ip injection	10 days after ligature placement	Histology qPCR Morphometric analysis	Systemic administration of JQ1 significantly inhibited inflammatory cytokine expression and alveolar bone loss.
Nakane et al. (2021) [25]	20 male C57BL/6 mice 8-10 wk-old	EP was induced by placing 6-0 silk ligature around the upper 2nd molar for 5 days. a) P b) P + CTLA-4	Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) 50 mg/kg ip injection at 1 and 3 days after ligature placement	5 days after ligature placement	Micro-CT Histology TRAP	Systemic administration of CTLA-4 significantly decrease the number of osteoclasts and reduced alveolar bone loss.

Napimoga et al. (2018) [26]	18 Male mice 20-25g body weight	EP was induced by oral inoculation with *Aa* (JP2 - 1x10^9 CFU) - 3 inoculations; a) Control b) P c) P + TPPU	Soluble epoxide hydrolase (sEH) inhibitor (1trifluoromethoxyphenyl-3-(1propionylpiperidin-4-yl) urea - TPPU 1mg/kg/day for 15 days by oral gavage	15 days	Morphometric analysis Western blott PCR array	Systemic treatment with TPPU showed inhibition of alveolar bone resorption; increased expression of sEH; and downregulation of cytokines and molecular markers in the gingival tissue.
Pacheco et al. (2021) [27]	15 male BALB/c mice	EP was induced by placing 6-0 silk ligature around the upper 2nd molar a) Ligature b) Ligature + anti-IL17 on day 0 c) Ligature + anti-IL17 on day 2	anti-IL-17A carried with microparticles (MP) locally delivered into four sites (buccal and palatal gingiva)	8 days after ligature placement	Micro-CT Histology qPCR	Local delivery of anti-IL-17A MP after periodontitis induction inhibited alveolar bone loss and osteoclastic activity and decreased the expression levels of IL-6, an IL-17A target genes.
Renn et al. (2018) [28]	56 male Wistar rats 250-300g body weight	EP was induced by placing 3-0 silk ligature around the upper 2nd molar a) PD + 10 mg melatonin (28 days) b) PD + 50 mg melatonin (28 days) c) PD + 100 mg melatonin (28 days) d) PD + 10 mg melatonin (14 days) e) PD + 50 mg melatonin (14 days) f) PD + 100 mg melatonin (14 days) g) PD h) Control	Melatonin ip injection in the dosage of 10, 50 and 100 mg/kg 14 and 28 days of treatment	4 wks	Histology ELISA Western blot	Melatonin treatment depressed the TLR4/MyD88-mediated ERK phosphorylation pathway, reduced pro-inflammatory cytokine levels, decreased the ratio of RANKL/OPG, decreased the extent of bone resorption, and preserved the micro-structure and BMD.

Repeke et al. (2011) [29]	Male C57BL/6 WT mice 8 wk-old (12 mice/ group)	EP was induced by oral inoculation with *Aa* JP2 (1x10^9 CFU) for 3 times. a) Control b) PD c) PD + metRANTES 0.05mg d) PD + metRANTES 0.1mg e) PD + metRANTES 0.5mg f) PD + metRANTES 1.5mg g) PD + metRANTES 5mg	Specific antagonist of CCR1 and 5 receptors - met-RANTES; ip injection of 0.05, 0.1, 0.5, 1.5 and 5mg/kg on alternative days initiated with PD induction until 30 days post-infection	30 days	Morphometric analysis Flow cytometry ELISA CRP measurement MPO qPCR	At 0.5 mg up to 5 mg doses, a strong reduction in the alveolar bone loss and inflammatory cell migration were observed. 5mg dose resulted in the maximum inhibition of inflammatory cell migration; Systemic treatment also downregulated the levels of inflammatory, Th1-type and osteoclastogenic cytokines, and CD3+ and F4/80+ cells.
Rogers et al. (2007) [30]	36 Female Sprague-Dawley rats	EP was induced by LPS injection of *Aa* (Y4) 2µl LPS into the palatal gingiva 3 times/wk for 8 wks. a) LPS (4 wks) + SD 282 b) LPS (4 wks) + vehicle c) LPS (8 wks) d) LPS (4 wks)	p38 MAPK inhibitor SD282 (45 mg/kg) administered via oral gavage twice daily starting in the fifth week	8 wks; 4 wks of treatment	Micro-CT IHC TRAP	Administration of SD282 significantly blocked alveolar bone destruction and significantly reduced IL-1β, TNF- α, and osteoclast formation
Shi et al. (2020) [31]	C57BL/6 WT mice 6-8 wk-old	EP was induced by oral inoculation with *P. gingivalis* (W83) 1x10^{10} CFU in 100µl performed twice a day for 1 wk. a) Control b) Periodontitis c) Adoptive transfer	Adoptive transfer of 10^6 B10 cells by tail vain injection	4 wks after last oral inoculation	Morphometric analysis TRAP Flow cytometry ELISA qPCR	Transfer of B10 cells alleviated alveolar bone resorption by reducing osteoclastogenesis; increased IL-10,decreased IL-17 and RANKL gene and protein expression; and reduced the

						proportion of Th17 cells in the gingival tissue.
Taut et al. (2013) [32]	Male Sprague-Dawley rats 9-10 wks-old 250-300g body weight	EP was induced by placing 3-0 silk ligatures around the upper 1st and 2nd molars for 4 wks and then was removed. a) Control + veh (PBS) 2 and 4 wks b) Control + Scl-Ab 2 and 4 wks c) EP + veh 3 and 6 wks d) EP + Scl-Ab 3 and 6 wks	Sclerostin (Scl-Ab) administered sc at dosage of 25 mg/kg twice weekly for therapeutic periods of 3 and 6 wks. Scl-Ab was also locally applied twice weekly for 3 and 6 wks into the palatal gingiva (5 µl of 35.6 mg/ml solution)	2 wks 3 wks 4 wks 6 wks	Micro-CT ELISA Histology Fluorescent calcein labeling	Scl-Ab treatment significantly improved maxillary bone healing, as measured by BVF, TMD and ABL; After 6 wks of treatment, BVF and TMD values in the Scl-Ab EP group were similar to those of healthy controls. Serum analysis demonstrated higher levels of osteocalcin and PINP.
Virto et al. (2018) [33]	42 Wistar rats 8 wk-old 180g body weight	Obesity induced by HFD. EP was induced by oral inoculation with *P. gingivalis* (W83) and *F. nucleatum* (DMSZ 20482). 1ml of bacterial suspension ($1x10^9$ CFU) inoculated for 4 consecutive days during 12 wks. a) HFD + EP b) Normal rats + EP Experimental treatment consisted of SRP + melatonin	Melatonin 25µg/ml dissolved in drinking water for 4 wks.	3 wks after treatment initiation	Clinical parameters Luminex Flow cytometry Micro-CT	Melatonin resulted in reduced gingival inflammation and BOP, with reductions in probing depth and enhanced bone repair in the HFD-EP group and significantly reduction in pro-inflammatory cytokines, IL-1β, IL-6, MCP-1 and TNF-α.

Wang et al. (2013) [34]	50 male C57BL/6 WT mice 4 wk-old	Diabetes induced by ip injection of STZ (40mg/kg) for 5 days. EP was induced by oral inoculation with *P. gingivalis* (ATCC 33277) 10^9 CFU dispersed in 100µl, 3 times every other day for 5 days. a) Normal control b) EP c) EP + DM c) EP + 25-OHD_3 d) DM + EP + 25-OHD_3	25-OHD_3 administered by ip injection at dose of 5µg/kg/bw at 2 day interval	8 wks	ELISA Morphometric analysis IHC Western blot	25-OHD_3 treatment attenuated DM-EP by reducing serum fasting blood glucose, glycosylated hemoglobin and TNF-α levels, which led to decreased alveolar bone loss. The expressions of Janus family kinase (JAK) 1 and signal transducer and activator of transcription (STAT) 3 as well as their phosphorylation were inhibited.
Wu et al. (2022) [35]	DIO (C57BL/6 background)	EP was induced by placing 5-0 silk ligature around the upper 2nd molar for 2 wks. a) EP b) EP + AdipoRon c) EP + AdipoAI	AdipoRon 50 mg/kg/bw AdipoAI 25 mg/kg/bw Oral gavage for 2 weeks concurrently with EP induction	2 wks	Micro-CT Histology qPCR Western blot Immunofluorescence	AdipoRon and AdipoAI decreased alveolar bone loss, osteoclast number, and inhibited the expression of inflammatory markers in the periodontium of DIO animals.
Yu et al. (2017) [36]	33 male C57BL/6 WT mice 8-10 wks-old	EP was induced by placing 7-0 silk ligatures around the upper 2nd molar and maintained for 2 wks. a) EP b) EP + CD40L + 1 µM CpG c) EP + CD40L + 10 µM CpG	CD40 ligand (CD40L) and TLR9 agonist cytidine-phosphatase-guanosine oligodeoxynucleotide (CpG) Palatal injections on days 3, 6, and 9.	2 wks	qPCR Morphometric analysis Histology IHC	CD40L and CpG treatment reduced the IL-10 mRNA expression and the number of IL-10^+ $CD45^+$. Alveolar bone loss was decreased and the gingival expression of IL-1β, TNF-

						α, and RANKL was reduced. The number of TRAP positive cells was also decreased after treatment.
Yuan et al. (2011) [37]	34 male C57BL/6 WT mice 12 wk-old	EP was induced by placing 5-0 silk ligature soaked with *P. gingivalis* (WT strain A7436) around the upper 2nd molar a) Vehicle b) EP c) EP + Hu-Fc d) EP + Kavain e) EP + OPG-Fc f) EP + RANK-Fc	- Hu-Fc (5 mg/kg, twice/week), - OPG-Fc (5 mg/kg, twice/ week) - RANK-Fc (5 mg/kg, twice/ week) subcutaneously delivered -Kavain (40 mg/kg, twice/week) intraperitoneal injection Injections were performed at day 0, 3, and 7.	10 days	Histology Cytokine measurement (BioPlex)	OPG-Fc, RANK-Fc and Kavain treatment showed significant bone loss reduction. Epithelial down-growth showed significant reduction in treatment groups with OPG-Fc performing better than Kavain or RANK-Fc. Kavain, OPG-Fc and RANK-Fc-treated mice displayed reduced inflammatory cell counts and cytokine expression.
Zhang et al. (2014) [38]	Male $APN^{-/-}$ (n=15), DIO (n=10) and WT (n=10) mice.	EP was induced by placing 5-0 silk ligature soaked with *P. gingivalis*. -$APN^{-/-}$ divided in 3 groups: a) EP b) EP + APN c) Control -WT divided in 2 groups: a) EP b) Control -DIO divided in 2 groups:	Adiponectin (APN) - APN administered by systemic infusion (pump delivering 2.5µg per day) with 1mg/ml concentration	10 days after EP induction	Morphometric analysis Histology TRAP	Systemic APN infusion reduced alveolar bone loss, osteoclast activity and infiltration of inflammatory cells in both EP mouse models. Furthermore, adiponectin treatment decreased the levels of pro

		a) EP b) EP + APN				inflammatory cytokines in white adipose tissue of diet-induced obesity mice with EP.
Zhang et al. (2020) [39]	36 female Wistar rats 8 wk-old 200-250g body weight	- EP was induced by placing 3-0 cotton ligature around the upper 2nd molar. - Ligatures were removed after 4wks a) Control b) EP c) EP + SPL	Super activated platelet lysate (SPL) - 50µl of SPL injected locally into the alveolar bone area, below the gingival margin every other day (8 times) for 2 wks.	16 days	Micro-CT Histology qPCR	SPL treatment diminished alveolar bone loss and reduced the gene expression levels of CCL2, CXCL2, IL-6, IL-18, IL-1α, IL-1β, CXCL10, CXCL16, CCL5. SPL treatment downregulated NLRP3, AIM2, and CASP1 inflammasome.

Table 2A: The quality assessment of the included studies assessed by the ARRIVE guidelines containing a 21-item checklist.

ARRIVE checklist itens	*Study design*	*Sample size*	*Inclusion exclusion criteria*	*Randomization*	*Blinding*	*Outcome measures*	*Statistical methods*
Apolinario-Vieira et al. (2021) [1]	1	1	0	1	1	1	1
Arabaci et al. (2015) [2]	1	0	0	1	1	1	1
Aral et al. (2015) [3]	1	0	0	1	1	1	1
Aydinyurt et al. (2021) [4]	1	0	0	1	0	1	1
Cafferata et al. (2019) [5]	1	0	0	1	1	1	1
Chen et al. (2015) [6]	1	0	0	0	0	1	1
Chen et al. (2017) [7]	1	0	0	1	0	1	1
Cirelli et al. (2009) [8]	1	0	0	0	1	1	1
Delima et al. (2002) [9]	1	0	0	0	1	1	1
Gaspersic et al. (2010) [10]	1	0	0	0	1	1	1
Goncalves et al. (2014) [11]	1	0	0	0	0	1	1
Hu et al. (2017) [12]	1	0	0	0	0	1	1
Jin et al. (2007) [13]	1	0	0	0	1	1	1
Kim et al. (2017) [14]	1	1	0	0	1	1	1
Kim et al. (2018) [15]	1	0	0	0	0	1	1
Kirkwood et al. (2007) [16]	1	0	0	0	1	1	1
Kose et al. (2016) [17]	1	0	0	1	0	1	1
Kuritani et al (2018) [18]	1	0	0	0	0	1	1
Lalla et al. (2000) [19]	1	0	0	0	0	1	1
Li et al. (2013) [20]	1	0	0	1	1	1	1
Li et al. (2019) [21]	1	0	0	1	1	1	1
Madeira et al.	1	0	0	0	1	1	1

(2016) [22]							
Marques et al. (2009) [23]	1	0	0	0	1	1	1
Meng et al. (2014) [24]	1	0	0	0	0	1	1
Nakane et al. (2021) [25]	1	1	0	1	1	1	1
Napimoga et al. (2018) [26]	1	0	0	0	1	1	1
Pacheco et al. (2021) [27]	1	0	0	0	1	1	1
Renn et al. (2018) [28]	1	0	0	1	0	1	1
Repeke et al. (2011) [29]	1	0	0	0	0	1	1
Rogers et al. (2007) [30]	1	1	0	0	1	1	1
Shi et al. (2020) [31]	1	0	0	0	0	1	1
Taut et al. (2013) [32]	1	0	0	0	0	1	1
Virto et al. (2018) [33]	1	0	0	1	1	1	1
Wang et al. (2013) [34]	1	0	0	1	1	1	1
Wu et al. (2022) [35]	1	0	0	0	0	1	1
Yu et al. (2017) [36]	1	0	0	1	0	1	1
Yuan et al. (2011) [37]	1	0	0	1	1	1	1
Zhang et al. (2014) [38]	1	0	0	1	0	1	1
Zhang et al. (2020) [39]	1	0	0	1	0	1	1

Table 2B: The quality assessment of the included studies assessed by the ARRIVE guidelines containing a 21-item checklist.

ARRIVE checklist items	*Experimental animals*	*Experimental procedure*	*Results*	*Abstract*	*Background*	*Objectives*	*Ethical statement*
Apolinario-Vieira et al. (2021) [1]	1	1	1	1	1	1	1
Arabaci et al. (2015) [2]	1	1	1	1	1	1	1
Aral et al. (2015) [3]	1	1	1	1	1	1	1
Aydinyurt et al. (2021) [4]	1	1	1	1	1	1	1
Cafferata et al. (2019) [5]	1	1	1	1	1	1	1
Chen et al. (2015) [6]	1	1	1	1	1	1	1
Chen et al. (2017) [7]	1	1	1	1	1	1	1
Cirelli et al. (2009) [8]	1	1	1	1	1	1	1
Delima et al. (2002) [9]	1	1	1	1	1	1	1
Gaspersic et al (2010) [10]	1	1	1	1	1	1	1
Goncalves et al. (2014) [11]	1	1	1	1	1	1	1
Hu et al. (2017) [12]	1	1	1	1	1	1	1
Jin et al. (2007) [13]	1	1	1	1	1	1	1
Kim et al. (2017) [14]	1	1	1	1	1	1	1
Kim et al. (2018) [15]	1	1	1	1	1	1	1
Kirkwood et al. (2007)[16]	1	1	1	1	1	1	1
Kose et al. (2016) [17]	1	1	1	1	1	1	1
Kuritani et al (2018) [18]	1	1	1	1	1	1	1
Lalla et al. (2000) [19]	1	1	1	1	1	1	1
Li et al. (2013) [20]	1	1	1	1	1	1	1
Li et al. (2019) [21]	1	1	1	1	1	1	1
Madeira et al.	1	1	1	1	1	1	1

(2016) [22]							
Marques et al. (2009) [23]	1	1	1	1	1	1	1
Meng et al. (2014) [24]	1	1	1	1	1	1	1
Nakane et al. (2021) [25]	1	1	1	1	1	1	1
Napimoga et al. (2018)[26]	1	1	1	1	1	1	1
Pacheco et al. (2021) [27]	1	1	1	1	1	1	1
Renn et al. (2018) [28]	1	1	1	1	1	1	1
Repeke et al. (2011) [29]	1	1	1	1	1	1	1
Rogers et al. (2007) [30]	1	1	1	1	1	1	1
Shi et al. (2020) [31]	1	1	1	1	1	1	1
Taut et al. (2013) [32]	1	1	1	1	1	1	1
Virto et al. (2018) [33]	1	1	1	1	1	1	1
Wang et al. (2013) [34]	1	1	1	1	1	1	1
Wu et al. (2022) [35]	1	1	1	1	1	1	1
Yu et al. (2017) [36]	1	1	1	1	1	1	1
Yuan et al. (2011) [37]	1	1	1	1	1	1	1
Zhang et al. (2014) [38]	1	1	1	1	1	1	1
Zhang et al. (2020) [39]	1	1	1	1	1	1	1

Table 2C: The quality assessment of the included studies assessed by the ARRIVE guidelines containing a 21-item checklist.

ARRIVE checklist itens	*Housing / husbandry*	*Animal care*	*Interpretation scientific implications*	*Generalizability translation*	*Protocol registration*	*Data access*	*Declaration of interest*
Apolinario-Vieira et al. (2021) [1]	1	1	1	1	1	0	0
Arabaci et al. (2015) [2]	1	1	1	1	1	0	0
Aral et al. (2015) [3]	1	1	1	1	1	0	0
Aydinyurt et al. (2021) [4]	1	1	1	1	1	0	1
Cafferata et al. (2019) [5]	1	1	1	1	1	0	1
Chen et al. (2015) [6]	1	1	1	1	1	0	1
Chen et al. (2017) [7]	1	1	1	1	1	0	1
Cirelli et al. (2009) [8]	1	1	1	1	1	0	0
Delima et al. (2002) [9]	0	0	1	1	1	0	0
Gaspersic et al. (2010)[10]	0	0	1	1	1	0	0
Goncalves et al. (2014)[11]	1	1	1	1	1	0	0
Hu et al. (2017) [12]	0	0	1	1	1	0	0
Jin et al. (2007) [13]	0	0	1	1	1	0	0
Kim et al. (2017) [14]	1	1	1	1	1	0	0
Kim et al. (2018) [15]	0	0	1	1	1	1	1
Kirkwood et al. (2007)[16]	0	0	1	1	1	0	0
Kose et al. (2016) [17]	1	1	1	1	1	0	0
Kuritani et al (2018) [18]	0	0	1	1	1	0	0
Lalla et al. (2000) [19]	0	0	1	1	1	0	0
Li et al. (2013) [20]	1	1	1	1	1	0	0
Li et al. (2019) [21]	1	0	1	1	1	0	0
Madeira et al.	0	0	1	1	1	0	0

(2016) [22]							
Marques et al. (2009)[23]	0	0	1	1	1	0	0
Meng et al. (2014) [24]	0	0	1	1	1	0	0
Nakane et al. (2021)[25]	1	1	1	1	1	1	1
Napimoga et al. (2018)[26]	1	0	1	1	1	0	0
Pacheco et al. (2021) [27]	1	0	1	1	1	0	1
Renn et al. (2018) [28]	0	0	1	1	1	0	0
Repeke et al. (2011) [29]	1	0	1	1	1	0	0
Rogers et al. (2007) [30]	1	0	1	1	1	0	0
Shi et al. (2020) [31]	1	0	1	1	1	0	1
Taut et al. (2013) [32]	1	0	1	1	1	0	0
Virto et al. (2018) [33]	1	1	1	1	1	0	1
Wang et al. (2013) [34]	1	1	1	1	1	0	0
Wu et al. (2022) [35]	1	0	1	1	1	0	1
Yu et al. (2017) [36]	1	0	1	1	1	0	0
Yuan et al. (2011) [37]	1	0	1	1	1	0	0
Zhang et al. (2014) [38]	1	0	1	1	1	0	0
Zhang et al. (2020) [39]	1	0	1	1	1	0	1

Table 3A: Risk-of-bias assessment for animal studies using SYRCLE's tool.

Table 3A: Summary of the risk of bias						
Authors, year reference	*Sequence generation*	*Baseline characteristics*	*Allocation concealment*	*Random housing*	*Performance bias blinding*	*Random outcome assessment*
	Randomly allocated	Were the groups similar at baseline or were they adjusted for confounders in the analysis?	Could the investigator allocating the animals to intervention or control group not foresee assignment?	Did the authors randomly place the cage or animals within the animal room?	Were the caregivers/investigators blinded from knowledge which intervention each animal received during the experiment?	Did the investigators randomly pick as animal during outcome assessment, or did they use a random component in the sequence generation for outcome assessment?
Apolinario Vieira et al. (2021) [1]	Yes	Yes	Unclear	Yes	Yes	Unclear
Arabaci et al. (2015) [2]	Yes	Yes	Unclear	Yes	Yes	Unclear
Aral et al. (2015) [3]	Yes	Yes	Unclear	Yes	Yes	Unclear
Aydinyurt et al. (2021) [4]	Yes	Yes	Unclear	Yes	No	Unclear
Cafferata et al. (2019) [5]	Yes	Yes	Unclear	Yes	Yes	Unclear
Chen et al. (2015) [6]	No	Yes	Unclear	No	No	Unclear
Chen et al. (2017) [7]	Yes	Yes	Unclear	Yes	No	Unclear
Cirelli et al. (2009) [8]	No	Yes	Unclear	No	Yes	Unclear
Delima et al. (2002) [9]	No	Yes	Unclear	No	Yes	Unclear
Gaspersic et al. (2010) [10]	No	Yes	Unclear	No	Yes	Unclear
Goncalves et al. (2014) [11]	No	Yes	Unclear	No	No	Unclear
Hu et al. (2017) [12]	No	Yes	Unclear	No	No	Unclear
Jin et al. (2007) [13]	No	Yes	Unclear	No	Yes	Unclear
Kim et al. (2017) [14]	No	Yes	Unclear	No	Yes	Unclear
Kim et al. (2018) [15]	No	Yes	Unclear	No	No	Unclear
Kirkwood et al. (2007)	No	Yes	Unclear	No	Yes	Unclear

[16]						
Kose et al. (2016) [17]	Yes	Yes	Unclear	Yes	No	Unclear
Kuritani et al (2018) [18]	No	Yes	Unclear	No	No	Unclear
Lalla et al. (2000) [19]	No	Yes	Unclear	No	No	Unclear
Li et al. (2013) [20]	Yes	Yes	Unclear	Yes	Yes	Unclear
Li et al. (2019) [21]	Yes	Yes	Unclear	Yes	Yes	Unclear
Madeira et al. (2016) [22]	No	Yes	Unclear	No	Yes	Unclear
Marques et al. (2009) [23]	No	Yes	Unclear	No	Yes	Unclear
Meng et al. (2014) [24]	No	Yes	Unclear	No	No	Unclear
Nakane et al. (2021) [25]	Yes	Yes	Unclear	Yes	Yes	Unclear
Napimoga et al. (2018) [26]	No	Yes	Unclear	No	Yes	Unclear
Pacheco et al. (2021) [27]	No	Yes	Unclear	No	Yes	Unclear
Renn et al. (2018) [28]	Yes	Yes	Unclear	Yes	No	Unclear
Repeke et al. (2011) [29]	No	Yes	Unclear	No	No	Unclear
Rogers et al. (2007) [30]	No	Yes	Unclear	No	Yes	Unclear
Shi et al. (2020) [31]	No	Yes	Unclear	No	No	Unclear
Taut et al. (2013) [32]	No	Yes	Unclear	No	No	Unclear
Virto et al. (2018) [33]	Yes	Yes	Unclear	Yes	Yes	Unclear
Wang et al. (2013) [34]	Yes	Yes	Unclear	Yes	Yes	Unclear
Wu et al. (2022) [35]	No	Yes	Unclear	No	No	Unclear
Yu et al. (2017) [36]	Yes	Yes	Unclear	Yes	No	Unclear
Yuan et al. (2011) [37]	Yes	Yes	Unclear	Yes	Yes	Unclear
Zhang et al. (2014) [38]	Yes	Yes	Unclear	Yes	No	Unclear
Zhang et al. (2020) [39]	Yes	Yes	Unclear	Yes	No	Unclear

Table 3B: Risk-of-bias assessment for animal studies using SYRCLE's tool.

Table 3B: Summary of the risk of bias (continued)					
Authors, year reference	*Detection bias Blinding*	*Attrition bias Incomplete outcome data*	*Selective outcome reporting*	*Other sources of bias*	*Risk of bias*
	Was the outcome assessor blinded	Were incomplete outcome data adequately addressed?	Are reports of the study free of selective outcome reporting?	Was the study apparently free of other problems that could result in high risk of bias?	Qualification
Apolinario Vieira et al. (2021) [1]	Yes	Unclear	Yes	Yes	Low
Arabaci et al. (2015) [2]	Yes	Unclear	Yes	Yes	Low
Aral et al. (2015) [3]	Yes	Unclear	Yes	Yes	Low
Aydinyurt et al. (2021) [4]	Yes	Unclear	Yes	Yes	Low
Cafferata et al. (2019) [5]	Yes	Unclear	Yes	Yes	Low
Chen et al. (2015) [6]	No	Unclear	Yes	Yes	Moderate
Chen et al. (2017) [7]	Yes	Unclear	Yes	Yes	Low
Cirelli et al. (2009) [8]	No	Unclear	Yes	Yes	Moderate
Delima et al. (2002) [9]	No	Unclear	Yes	Yes	Moderate
Gaspersic et al. (2010) [10]	No	Unclear	Yes	Yes	Moderate
Goncalves et al. (2014) [11]	No	Unclear	Yes	Yes	High
Hu et al. (2017) [12]	No	Unclear	Yes	Yes	High
Jin et al. (2007) [13]	No	Unclear	Yes	Yes	Moderate
Kim et al. (2017) [14]	No	Unclear	Yes	Yes	Moderate
Kim et al. (2018) [15]	No	Unclear	Yes	Yes	Moderate
Kirkwood et al. (2007) [16]	No	Unclear	Yes	Yes	Moderate
Kose et al. (2016) [17]	Yes	Unclear	Yes	Yes	Low
Kuritani et al (2018) [18]	No	Unclear	Yes	Yes	High
Lalla et al. (2000) [19]	No	Unclear	Yes	Yes	High
Li et al.	Yes	Unclear	Yes	Yes	Low

(2013) [20]					
Li et al. (2019) [21]	Yes	Unclear	Yes	Yes	Low
Madeira et al. (2016) [22]	No	Unclear	Yes	Yes	Moderate
Marques et al. (2009) [23]	No	Unclear	Yes	Yes	Moderate
Meng et al. (2014) [24]	No	Unclear	Yes	Yes	High
Nakane et al. (2021) [25]	Yes	Unclear	Yes	Yes	Low
Napimoga et al. (2018) [26]	No	Unclear	Yes	Yes	Moderate
Pacheco et al. (2021) [27]	No	Unclear	Yes	Yes	Moderate
Renn et al. (2018) [28]	Yes	Unclear	Yes	Yes	Low
Repeke et al. (2011) [29]	No	Unclear	Yes	Yes	Moderate
Rogers et al. (2007) [30]	No	Unclear	Yes	Yes	Moderate
Shi et al. (2020) [31]	No	Unclear	Yes	Yes	High
Taut et al. (2013) [32]	No	Unclear	Yes	Yes	High
Virto et al. (2018) [33]	Yes	Unclear	Yes	Yes	Low
Wang et al. (2013) [34]	Yes	Unclear	Yes	Yes	Low
Wu et al. (2022) [35]	No	Unclear	Yes	Yes	Moderate
Yu et al. (2017) [36]	Yes	Unclear	Yes	Yes	Low
Yuan et al. (2011) [37]	Yes	Unclear	Yes	Yes	Low
Zhang et al. (2014) [38]	Yes	Unclear	Yes	Yes	Low
Zhang et al. (2020) [39]	Yes	Unclear	Yes	Yes	Low

Table 1 Search strategies

Search Strategy	Electronic databases
("biological products" OR "biological factors" OR "products, biological" OR "biological product" OR "product, biological" OR "biologic product" OR "product, biologic" OR "biologic products" OR biopharmaceuticals OR biopharmaceutical OR biological OR biologic OR "biological drug" OR "drug, biological" OR "biologic drugs" OR "drugs, biologic" OR "biological medicine" OR "medicine, biological" OR "biological medicines" OR "medicines, biological" OR biologicals OR "biologic medicines" OR "medicines, biologic" OR "biologic pharmaceuticals" OR "pharmaceuticals, biologic" OR biologics OR "biologic drug" OR "drug, biologic" OR "biological drugs" OR "drugs, biological" OR "natural products" OR "natural product" OR "product, natural" OR "organic chemicals" OR "chemicals, organic" OR "organic chemical" OR "chemical, organic" OR "biological products therapeutic use" OR "biologic factors and agents acting on the immune system" OR "natural products and their synthetic derivatives") AND ("periodontal diseases" OR periodontitis OR "chronic periodontitis" OR "aggressive periodontitis" OR "periodontitis, aggressive, 2" OR periodont* OR parodont* OR "pyorrhea alveolaris" OR pericement* OR "experimental periodontitis") AND ("bone resorption" OR "bone resorptions" OR "resorption, bone" OR "resorptions, bone" OR "osteoclastic bone loss" OR "bone loss, osteoclastic" OR "bone losses, osteoclastic" OR "loss, osteoclastic bone" OR "losses,	MEDLINE|PubMed

osteoclastic bone" OR "osteoclastic bone losses" OR osteoclasts OR "alveolar bone loss" OR "alveolar bone losses" OR "alveolar process atrophy" OR "alveolar process atrophies" OR "alveolar resorption" OR "alveolar resorptions" OR "resorption, alveolar" OR "resorptions, alveolar" OR "bone loss, periodontal" OR "bone losses, periodontal" OR "periodontal bone losses" OR "periodontal bone loss" OR "periodontal resorption" OR "periodontal resorptions" OR "resorption, periodontal" OR "alveolar bone atrophy" OR "alveolar bone atrophies" OR "bone atrophies, alveolar" OR "bone atrophy, alveolar" OR "bone loss, alveolar" OR "experimental bone loss") NOT ("dental implants" OR "implant, dental" OR "implants, dental" OR "dental implant" OR "dental implants, mini" OR "dental implant, mini" OR "mini dental implant" OR "mini dental implants" OR "dental prostheses, surgical" OR "dental prosthesis, surgical" OR "surgical dental prostheses" OR "surgical dental prosthesis" OR "prostheses, surgical dental" OR "prosthesis, surgical dental" OR "dental implantation" OR "tooth, artificial") NOT (review OR reviews OR systematic review[Publication type] OR "systematic reviews as topic" OR "systematic reviews" OR "systematic review" OR meta-analysis[Publication type] OR "meta-analysis as topic" OR "meta-analysis" OR "meta-analyses" OR "meta analysis" OR "meta analyses" OR overview OR "umbrella review")	
("biological products" OR "biological factors" OR biopharmaceuticals OR biological OR biologic OR "biological drug" OR "biological medicine" OR "biologic medicines" OR "biologic pharmaceuticals"	Web of Science, Cochrane CENTRAL, LILACS|VHL, and other sources

OR “biologic drug” OR “biological drugs” OR “natural products” OR “natural product” OR “organic chemicals” OR “organic chemical” OR "biological products therapeutic use" OR “biologic factors and agents acting on the immune system” OR "natural products and their synthetic derivatives") AND (“periodontal diseases” OR periodontitis OR “chronic periodontitis” OR “aggressive periodontitis” OR “periodontitis, aggressive, 2” OR periodont* OR parodont* OR “pyorrhea alveolaris” OR pericement* OR “experimental periodontitis”) AND (“bone resorption” OR “bone resorption” OR “osteoclastic bone loss” OR osteoclasts OR “alveolar bone loss” OR “alveolar bone losses” OR “alveolar process atrophy” OR “alveolar process atrophies” OR “alveolar resorption” OR “alveolar resorption” OR “periodontal bone losses” OR “periodontal bone loss” OR “periodontal resorption” OR “periodontal resorption” OR “alveolar bone atrophy” OR “alveolar bone atrophies” OR “experimental bone loss”)	
(ALL ('biological AND products' OR 'biological AND factors' OR 'products, AND biological' OR 'biological AND product' OR 'product, AND biological' OR 'biologic AND product' OR 'product, AND biologic' OR 'biologic AND products' OR biopharmaceutical OR biopharmaceutical OR biological OR biologic OR 'biological AND drug' OR 'drug, AND biological' OR 'biologic AND drugs' OR 'drugs, AND biologic' OR 'biological AND medicine' OR 'medicine, AND biological' OR 'biological AND medicines' OR 'medicines, AND biological' OR biologicals OR 'biologic AND medicines' OR	Scopus

'medicines, AND biologic' OR 'biologic AND pharmaceuticals' OR 'pharmaceuticals, AND biologic' OR biologics OR 'biologic AND drug' OR 'drug, AND biologic' OR 'biological AND drugs' OR 'drugs, AND biological' OR 'natural AND products' OR 'natural AND product' OR 'product, AND natural' OR 'organic AND chemicals' OR 'chemicals, AND organic' OR 'organic AND chemical' OR 'chemical, AND organic' OR 'biological AND products AND therapeutic AND use' OR 'biologic AND factors AND agents AND acting AND on AND the AND immune AND system' OR 'natural AND products AND their AND synthetic AND derivatives')) AND (ALL ('periodontal AND diseases' OR periodontitis OR 'chronic AND periodontitis' OR 'aggressive AND periodontitis' OR 'periodontitis, AND aggressive, AND 2' OR periodont* OR parodont* OR 'pyorrhea AND alveolaris' OR pericement* OR 'experimental AND periodontitis')) AND (ALL ('bone AND resorption' OR 'bone AND resorption' OR 'resorption, AND bone' OR 'resorption, AND bone' OR 'osteoclastic AND bone AND loss' OR 'bone AND loss, AND osteoclastic' OR 'bone AND losses, AND osteoclastic' OR 'loss, AND osteoclastic AND bone' OR 'losses, AND osteoclastic AND bone' OR 'osteoclastic AND bone AND losses' OR osteoclasts OR 'alveolar AND bone AND loss' OR 'alveolar AND bone AND losses' OR 'alveolar AND process AND atrophy' OR 'alveolar AND process AND atrophies' OR 'alveolar AND resorption' OR 'alveolar AND resorption' OR 'resorption, AND alveolar' OR 'resorption, AND alveolar' OR 'bone AND loss,

AND periodontal' OR 'bone AND losses, AND periodontal' OR 'periodontal AND bone AND losses' OR 'periodontal AND bone AND loss' OR 'periodontal AND resorption' OR 'periodontal AND resorption' OR 'resorption, AND periodontal' OR 'alveolar AND bone AND atrophy' OR 'alveolar AND bone AND atrophies' OR 'bone AND atrophies, AND alveolar' OR 'bone AND atrophy, AND alveolar' OR 'bone AND loss, AND alveolar' OR 'experimental AND bone AND loss'))	
#1 'biological products' OR 'biological factors' OR 'products, biological' OR 'biological product' OR 'product, biological' OR 'biologic product' OR 'product, biologic' OR 'biologic products' OR biopharmaceuticals OR biopharmaceutical OR biological OR biologic OR 'biological drug' OR 'drug, biological' OR 'biologic drugs' OR 'drugs, biologic' OR 'biological medicine' OR 'medicine, biological' OR 'biological medicines' OR 'medicines, biological' OR biological OR 'biologic medicines' OR 'medicines, biologic' OR 'biologic pharmaceuticals' OR 'pharmaceuticals, biologic' OR biologics OR 'biologic drug' OR 'drug, biologic' OR 'biological drugs' OR 'drugs, biological' OR 'natural products' OR 'natural product' OR 'product, natural' OR 'organic chemicals' OR 'chemicals, organic' OR 'organic chemical' OR 'chemical, organic' OR 'biological products therapeutic use' OR 'biologic factors and agents acting on the immune system' OR 'natural products and their synthetic derivatives' #2 'periodontal diseases' OR periodontitis OR 'chronic periodontitis' OR 'aggressive periodontitis' OR	Embase

'periodontitis, aggressive, 2' OR periodont* OR parodont* OR 'pyorrhea alveolaris' OR pericement* OR 'experimental periodontitis'

#3 'bone resorption' OR 'bone resorption' OR 'resorption, bone' OR 'resorption, bone' OR 'osteoclastic bone loss' OR 'bone loss, osteoclastic' OR 'bone losses, osteoclastic' OR 'loss, osteoclastic bone' OR 'losses, osteoclastic bone' OR 'osteoclastic bone losses' OR osteoclasts OR 'alveolar bone loss' OR 'alveolar bone losses' OR 'alveolar process atrophy' OR 'alveolar process atrophies' OR 'alveolar resorption' OR 'alveolar resorption' OR 'resorption, alveolar' OR 'resorption, alveolar' OR 'bone loss, periodontal' OR 'bone losses, periodontal' OR 'periodontal bone losses' OR 'periodontal bone loss' OR 'periodontal resorption' OR 'periodontal resorption' OR 'resorption, periodontal' OR 'alveolar bone atrophy' OR 'alveolar bone atrophies' OR 'bone atrophies, alveolar' OR 'bone atrophy, alveolar' OR 'bone loss, alveolar' OR 'experimental bone loss'

#1 AND #2 AND #3

5 CONSIDERAÇÕES FINAIS

O uso de moduladores da resposta imune do hospedeiro ou de medicamentos antirreabsortivos oferece alternativas para inibir a perda óssea e diminuir o infiltrado inflamatório no tecido conjuntivo. Todos esses tratamentos testados podem ajudar a modular a resposta inflamatória do hospedeiro e melhorar a progressão da doença experimental. Como dito anteriormente, o tratamento primário da DP é por meio de uma abordagem mecânica, TPNC, para remover o biofilme aderido na superfície do dente e da raiz. Entretanto, esse tratamento local não responde da mesma maneira em todos os pacientes. Assim, as terapias adjuvantes que diminuem a resposta inflamatória do hospedeiro desempenham um papel importante na obtenção de melhores resultados clínicos, especialmente em pacientes com comorbidades associadas, como diabetes mellitus tipo 2 e artrite reumatoide. É importante ter em mente que alguns dos medicamentos incluídos na revisão de estudos pré-clínicos, ou seja, bisfosfonatos, agentes biológicos e inibidores de RANKL e CtsK possuem alguns efeitos colaterais que podem limitar seu uso clínico.

A medicina herbal e a suplementação com ômega 3 e probióticos têm ganhado cada vez mais atenção devido às suas atividades moduladoras e antirreabsortivas e a ausência de efeitos colaterais, sendo consideradas alternativas promissoras como adjuvantes da TPNC em pacientes suscetíveis. Na revisão sistemática, os resultados se mostraram promissores, porém ensaios clínicos randomizados são necessários para avaliar a eficácia dos agentes biológicos em humanos.

6 CONCLUSÕES

Os agentes biológicos se mostram seguros e eficazes quando testados em animais e testes em humanos podem ser bastante promissores.

- O uso de moduladores da resposta imune do hospedeiro ou de medicamentos antirreabsortivos que foram abordados no artigo 1, oferecem alternativas para inibir a perda óssea e diminuir o infiltrado inflamatório no tecido conjuntivo. Todos os tratamentos testados podem ajudar a modular a resposta inflamatória do hospedeiro e melhorar a progressão da doença experimental.
- Na revisão sistemática foi demonstrado que o uso de agentes biológicos é uma alternativa promissora para tratar a periodontite experimental em estudos pré-clínicos quando aplicados sistemicamente ou localmente. O fato de serem moléculas biológicas com a capacidade única de interromper a progressão da perda óssea e diminuir o processo inflamatório, sem nenhum efeito colateral adicional, torna-as agentes potenciais e atraentes para a prevenção e o tratamento da doença periodontal.

7 REFERÊNCIAS BIBLIOGRÁFICAS

CHEN, W.; GAO, B.; HAO, L.; et al**. The silencing of cathepsin K used in gene therapy for periodontal disease reveals the role of cathepsin K in chronic infection and inflammation**. *J Periodontal Res*, vol. 51, no. 5, pp. 647–660, 2016.

COSGAREA, R; RAMSEIER, C. A; JEPSEN, S; et, al. **One-Year Clinical, Microbiological and Immunological Results of Local Doxycycline or Antimicrobial Photodynamic Therapy for Recurrent/Persisting Periodontal Pockets: A Randomized Clinical Trial.** *Antibiotics* (Basel) 11, 2022

DE MOLON, R. S.; ROSSA JR, C.; THURLINGS, R. M.; CIRELLI , J. A.; KOENDERS, M. I. **Linkage of periodontitis and rheumatoid arthritis: current evidence and potential biological interactions**. *International journal of molecular sciences*, vol. 20, no. 18, 2019.

DE MOLON, R. S.; DE AVILA, E. D.; CIRELLI, J. A.; STEFFENS, J. P. **Periodontal research contributions to basic sciences: from cell communication and host-parasite interactions to inflammation and bone biology**. Biocell, vol. 46, no. 3, pp. 633–638, 2022.

EKE, P.I; THORNTON-EVANS, G.; DYE, B.; GENCO, R. **Advances in surveillance of periodontitis: the Centers for Disease Control and Prevention periodontal disease surveillance project.** *J Periodontol,* vol. 83, pp. 1337-1342, 2012.

GAO, L.; FAIBISH, D.; FREDMAN, G.; et al. **Resolvin E1 and chemokine-like receptor 1 mediate bone preservation.** *J Immunol*, vol. 190, no. 2, pp. 689–694, 2013.

HAJISHENGALLIS, G.; CHAVAKIS, T.; LAMBRIS, J. D. **Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy**. *Periodontol*, vol. 84, no. 1, pp. 14–34, 2000.

JIN, Q.; CIRELLI J. A.; PARK, C. H.; et al. **RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodontitis**. *J Periodontol*, vol. 78, no. 7, pp. 1300–1308, 2007.

KARAKAN, N. C.; AKPINAR, A.; GOZE, F.; POYRAZ, O**. Investigating the effects of systemically administered strontium ranelate on alveolar bone loss histomorphometrically and histopathologically on experimental periodontitis in rats**. *J Periodontol*, vol. 88, no. 2, pp. e24–e31, 2017.

KIANE, S.; BIRANG, R.; JAMSHIDIAN, N. **Effect of Propolis mouthwash on clinical periodontal parameters in patients with gingivitis: A double-blinded randomized clinical trial**. *Int J Dent Hyg*, vol. 20, p. 434-440, 2022

KINANE, D.F.; STATHOPOULOU, P. G;.PAPAPANOU, P. N. **Periodontal diseases**. *Nat Rev Dis Primers*, vol. 3, p. 17038, 2017.

KRAYER, J. W.; LEITE, R. S.; KIRKWOOD, K. L. **Non-surgical chemotherapeutic treatment strategies for the management of periodontal diseases.** *Dent Clin North Am*, vol. 54, no. 1, pp. 13–33, 2010.

LEE J.; MIN H. K.; PARK, C.Y; KANG, H.K; JUNG, S.Y.; MIN, B.M. **A vitronectinderived peptide prevents and restores alveolar bone loss by modulating bone re-modelling and expression of RANKL and IL-17A**. *J Clin Periodontol*, 2022

MARINS, L. M.; NAPIMOGA, M. H.; DE SOUZA MALTA, F.; et al. **Effects of strontium ranelate on ligature-induced periodontitis in estrogen-deficient and estrogen sufficient rats.** *J Periodontal Res*, vol. 55, no. 1, pp. 141–151, 2020.

MIZRAJI, G.; HEYMAN, O; VAN DYKE, T. E.; WILENSKY, A. **Resolvin D2 restrains Th1 immunity and prevents alveolar bone loss in murine periodontitis.** *Front Immunol*, vol. 9, p. 785, 2018.

OZAKI, Y.; MOROZUMI, T.; WATANABE, K.; et al**. Inhibitory effect of omega-3 fatty acids on alveolar bone resorption and osteoclast differentiation**. *J Oral Sci*, vol. 62, no. 3, pp. 298–302, 2020.

PAVANELLI, A.L.R.; DE MENEZES, B.S.; PEREIRA, E.B.B.; DE SOUZA MORAIS, F.A.; CIRELLI, J.A.; DE MOLON RS**. Pharmacological Therapies for the Management of Inflammatory Bone Resorption in Periodontal Disease: A Review of Preclinical Studies.** *Biomed Res Int* , vol. 2022, 2022.

PIHLSTROM, B. L.; MICHALOWICZ, B. S.; JOHNSON, N. W. **Periodontal diseases**. Lancet, vol. 366, no. 9499, pp. 1809–1820, 2005.

RAMOS, T.C.S.; BOAS, M.L.V.; NUNES, C.M.M.; FERREIRA, C.L.; PANNUTI C.M.; SANTAMARIA, M.P.; JARDINI, M.A.N. **Effect of systemic antibiotic and probiotic therapies as adjuvante treatments of subgingival instrumentation for periodontitis: a randomized controlled clinical study.** *J Appl Oral Sci*, vol. 30, 2022

ROCUZZO, A.; IMBER, J.C; STAHLI, A.; KLOUKOS, D.; SALVI, G.E.; SCULEAN, A. **Enamel matrix derivative as adjunctive to non-surgical periodontal therapy: a systematic review and meta-analysis of randomized controlled trials**. *Clin Oral Investig*, vol. 26, p. 4263-4280, 2022

STORRER C. L. M.; DELIBERADOR, T. M.; GIOVANINI A. F.; CRIVELLARO, V.; ZIELAK, J. C.; ROMITO G. A. **Effect of alendronate on the progression of periodontitis induced by Porphyromonas gingivalis and Fusobacterium nucleatum: a study in rats.** *Clin Oral Investig*, vol. 20, no. 9, pp. 2565– 2573, 2016.

STRALBERG, F.; KASSEM, A.; KASPRZYKOWSKI, F.; et al. **Inhibition of lipopolysaccharide-induced osteoclast formation and bone resorption in vitro and in vivo by cysteine proteinase inhibitors**. *J Leukoc Biol*, vol. 101, no. 5, pp. 1233–1243, 2017.

TONETTI, M. S; GREENWELL, H.; KORNMAN, K. S. **Staging and grading of periodontitis: Framework and proposal of a new classification and case definition**. *J Periodontol*, vol. 89, Suppl 1, pp. S159–S172, 2018.

WANG, X.; JIA, Z.; ALMOSHARI, Y.; LELE, S. M.; REINHARDT, R. A.; WANG D. **Local application of pyrophosphorylated simvastatin prevents experimental periodontitis**. *Pharm Res*, vol. 35, no. 8, p. 164, 2018.

WEI, W.; REN, J.; YIN, W.; et al. **Inhibition of Ctsk modulates periodontitis with arthritis via downregulation of TLR9 and autophagy**. *Cell Prolif*, vol. 53, no. 1, article e12722, 2020.

ZAMBRANO, L. M. G.; BRANDAO, D. A.; ROCHA , F. R. G.; et al. **Local administration of curcumin-loaded nanoparticles effectively inhibits inflammation and bone resorption associated with experimental periodontal disease.** *Sci Rep*, vol. 8, no. 1, p. 6652, 2018.