DOI: 10.1111/prd.12342

REVIEW ARTICLE

Periodontology 2000 WILEY

# Periodontitis is an inflammatory disease of oxidative stress: We should treat it that way

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## 1 | OXIDATIVE STRESS AND ITS RELATIONSHIP TO CHRONIC PERIODONTITIS

In recent years, there has been an increasing body of evidence pointing to the role of reactive oxygen species in establishing an oxidatively stressed environment that underlies the pathogenesis of a wide range of chronic inflammatory conditions, such as type 2 diabetes,[1] atherosclerosis, rheumatoid arthritis,[2] cancer,[3] inflammatory lung disease, and periodontitis.[4] Periodontitis is an inflammatory condition that affects 10%-15% of the adult population[5], and when left untreated or not treated adequately, leads to chronic pain and to the loss of teeth and tooth-supporting tissues.[4,6,7] The persistence of plaque (a microbial film) on the dental surface and the migration of this plaque to the surrounding periodontal pockets leads to the recruitment of leukocytes, predominantly polymorphonuclear neutrophils, from the bloodstream to the site of infection.

This polymorphonuclear neutrophil infiltrate (which represents 50%-70% of the overall leukocytic infiltrate) is the first line of defense against the bacterial pathogens contained within dental plaque.[8] Indeed, polymorphonuclear neutrophils play an essential role in periodontal health and in the innate immune system as first-responder cells, acting through several unique defense mechanisms, including degranulation, chemotaxis, phagocytosis, NETosis, and the release of reactive oxygen species. Interestingly, a "hyperactivated" polymorphonuclear neutrophil phenotype appears to be associated with periodontal disease. This "hyperactivated" polymorphonuclear neutrophil phenotype is characterized by overproduction of reactive oxygen species and proteases, making the subset of patients with higher levels of this particular phenotype of polymorphonuclear

neutrophil more susceptible to the development of periodontitis.[9-12] We suggest that a complex interplay between the subgingival biofilm and the robustness of the host immune response to this biofilm is key to establishing a dysbiotic environment and the pathogenesis of periodontitis.[13-15]

Primarily acting in an antimicrobial capacity, the generation of reactive oxygen species can be considered as a "double-edged" sword because reactive oxygen species can help to kill invading pathogenic microorganisms during health but can become cytotoxic to host cells when overactivated. Reactive oxygen species play an important role in cell signaling, gene regulation, and antimicrobial defense,[16] but an overabundance of reactive oxygen species leads to increased oxidant load together with either unaltered or reduced antioxidant capacity, resulting in oxidative stress within the affected tissues, which then leads to pathological changes and consequently the destruction of host tissues (and hence loss of teeth as their supporting structures become destroyed).[3,17] Intracellularly, reactive oxygen species damage biomolecules and cell membranes.[18]

During phagocytosis, free radicals (especially $O_2^-$, $H_2O_2$, and $OH^{\bullet}$[18]), which are end-products of the mitochondrial respiratory burst in polymorphonuclear neutrophils, act mainly through lipid peroxidation[19] but may also act through damage of protein[20] and DNA.[21] This leads to an oxidative imbalance that triggers proinflammatory mechanisms, and importantly osteoclastogenesis, which then leads to the bone loss that is observed in patients with periodontitis.[22-25] Additionally, reactive oxygen species affect the master antioxidant regulator, nuclear factor erythroid 2-related factor 2[12,26], with downregulation of nuclear factor erythroid 2-related factor 2 being correlated with some of the inflammatory diseases previously mentioned, especially periodontitis and rheumatoid arthritis, and their progression.[22] Finally, through direct damage to the extracellular connective tissue (aside from bone alone), reactive oxygen species production is responsible for the attachment loss that leads to periodontal destruction.[18,27-29]

The destructive pattern that characterizes chronic periodontitis clinically as a disease affecting the tooth-supporting tissues is a consequence of active connective tissue destruction and progressive bone resorption arising through the presence of different stimulatory factors in plasma; these factors stem from the increased respiratory burst of polymorphonuclear neutrophils (and reactive oxygen species production) and may also alter or cause neutrophil priming and lengthen the lifespan of neutrophils in patients with chronic periodontitis.[10,30] Accordingly, the production of reactive oxygen species has been associated with enhanced expression of proinflammatory cytokines that are responsible, both directly and indirectly, for connective tissue destruction and bone resorption. Although the destruction of mineralized and nonmineralized tissues occurs concomitantly, specific proinflammatory products are associated with damage either to one or to both types of tissue (ie, mineralized and nonmineralized). As an example, bone remodeling is uncoupled as a result of the reactive oxygen species-mediated increase in the RANKL/osteoprotegerin ratio; in this process, the homeostatic relationship between bone formation and bone resorption is broken, with bone loss, one of the major features of periodontitis, being favored.[31-33] More specifically, this relates to the differentiation of macrophage/monocyte precursor cells into osteoclasts, which is caused by the increased level of *RANKL* mRNA that disrupts the balance between RANKL and osteoprotegerin. The RANKL/osteoprotegerin axis plays a key role in relation to bone resorption, not only in periodontal diseases but also in several other chronic inflammatory diseases in which bone remodeling occurs (eg, rheumatoid arthritis, osteoarthritis, osteoporosis).[22] Moreover, the reduced levels of collagen produced by reactive oxygen species-affected fibroblasts and the wide range of different matrix metalloproteinases released at excessive levels into the site of infection during the immune response also promote degradation of connective tissue and bone matrix.[34-36] Following the release of increased levels of matrix metalloproteinases, an imbalance between them and their endogenous inhibitors (tissue inhibitors of matrix metalloproteinases) also plays an important role in tissue destruction. Together, this cascade of events leads to continual degradation of the mineral and organic matrixes by which chronic inflammatory diseases are characterized.[37]

Along the same lines, a literature review evaluating the groups of cytokines that influence bone uncoupling showed that different groups of proinflammatory cytokines (such as interleukin-1, interleukin-6, and tumor necrosis factor-alpha) have been reported, through induction of RANKL during periodontal destruction, to stimulate osteoclast activity, stimulate osteoblast death, or influence bone remodeling.[33,37] Most interestingly, others have demonstrated release of increased levels of cytokines (interleukin-8, interleukin-6, tumor necrosis factor-alpha, and interleukin-1beta) from hyperreactive peripheral blood polymorphonuclear neutrophils in patients with periodontitis and reported that this hyperreactivity was not altered significantly, even after nonsurgical periodontal therapy.[38] This might suggest a genetic predisposition for certain patients to create a 'dysregulated' polymorphonuclear neutrophil phenotype, specifically a hyperactive phenotype that displays hyperreactivity even after microbial challenges have been neutralized. Similarly, higher amounts of interleukin-8, interleukin-1beta, and tumor necrosis factor-alpha are released by unstimulated peripheral blood neutrophils from patients with type 2 diabetes than from healthy controls, indicating an increased susceptibility to colonization with microorganisms,[39] which could explain the increased susceptibility of diabetic patients to periodontitis, as well as the development of more severe periodontitis in these patients than in nondiabetic patients.

Reports from different studies reveal increased levels of markers of oxidative stress in saliva, gingival crevicular fluid, and plasma from patients with periodontitis, further strengthening the association between oxidative stress and periodontal inflammation. It has been shown that the levels of oxidant-induced DNA damage, as measured by the biomarker 8-hydroxy-2′-deoxyguanosine, are higher in patients with chronic periodontitis than in healthy controls.[21] These findings agree with previous reports.[17] Similarly, when 58 patients with periodontal disease were compared with 234 healthy controls it was demonstrated (using ELISA) that there were significantly higher levels of total protein carbonyls in the patients with periodontal

disease and that this correlated with increased loss of periodontal attachment.[20] Along similar lines, the levels of malondialdehyde were found to be increased in serum, saliva, and gingival crevicular fluid samples from patients presenting chronic periodontitis in comparison with their periodontally healthy counterparts.[17,40] In the same experiment, nonsurgical therapy significantly modified the levels of malondialdehyde to levels that were comparable with those found in periodontally healthy patients, highlighting the interplay between the expression of oxidative biomarkers and periodontitis. Others have demonstrated that nonsurgical therapy for periodontitis reduces salivary levels of the antioxidant glutathione peroxidase.[40]

In order to combat excess levels of reactive oxygen species, antioxidant enzymes, such as superoxide dismutases, catalases, and glutathione peroxidases, are released into the oral cavity in an attempt to balance and reestablish a normal oxidative status and prevent tissue destruction.[41,42] Along with these enzymatic antioxidants, endogenous albumin and uric acid also play fundamental roles in maintaining the redox state in favor of a positive oxidative balance.[43] There are a significant number of case-control and longitudinal studies in which the levels of different antioxidant markers in health and periodontitis, or before and after nonsurgical treatment of periodontitis, have been compared; all demonstrate a direct correlation between elevated oxidative stress or high levels of reactive oxygen species and either the presence or severity of periodontitis or clinical improvements in periodontitis following treatment.[19,40,44] In analyzing salivary markers of oxidative stress in patients with periodontitis, Banasova et al.[19] found a tendency toward reduced DNA integrity and significantly reduced (54% reduction) antioxidant status in subjects with periodontal disease compared with periodontally healthy controls. Moreover, the levels of the antioxidants glutathione peroxidase, albumin, and uric acid, as well as the total antioxidant capacity, were measured before and after nonsurgical therapy.[40,42] It was concluded that conventional periodontal treatment had a beneficial influence on the levels of all the antioxidant markers that were measured, as judged by comparison with the levels of these markers before intervention. By contrast, when studying longitudinal changes in the total antioxidant capacity of gingival crevicular fluid and plasma using the total antioxidant capacity method, no statistical differences between the plasma samples of periodontally compromised and periodontally healthy subjects could be found.[9] Interestingly, after nonsurgical periodontal treatment of periodontitis, the total antioxidant capacity levels in gingival crevicular fluid samples reached levels similar to those from control/periodontally healthy patients, leading the authors to suggest that decreased total antioxidant capacity is more likely to be a consequence of inflammation rather than a cause for periodontal diseases. However, there is no consensus established thus far when comparing other, similar, studies.[45-47] Suffice it to say that oxidative stress induces inflammation, which leads to tissue damage, and that the inflammation either is related directly to ongoing and persistent microbial activation or is triggered by microbes, meaning that ongoing infection is no longer required for destruction to take place in certain cases. Although opinions vary regarding the function of increased levels of reactive oxygen species, specifically in terms of creating the precondition allowing development of periodontal disease, excess of free radicals in conjunction with reduced host antioxidant status play a central role in the pathogenesis and progression of chronic periodontitis.[9,48,49] This imbalance extends to a broader range of other inflammatory conditions and behaviors that stimulate oxidative stress and present a correlation with oral manifestations, such as type 2 diabetes, smoking, obesity, and rheumatoid arthritis. Thus, the aim of the next section is to demonstrate that the aforementioned are not only associated with periodontitis of greater severity but that they probably demonstrate such effects on account of their ability to upregulate oxidative stress.

## 2 | CONDITIONS AND BEHAVIORS THAT STIMULATE OXIDATIVE STRESS

Establishing an environment characterized by oxidative stress can exaggerate proinflammatory conditions, which may create a wide range of metabolic disorders that are typified by tissue destruction.[50] It is known that several other complex multifactorial disorders, including (but not limited to) type 2 diabetes, obesity, and rheumatoid arthritis,[51-55] share characteristics with periodontitis, including upregulated production of reactive oxygen species and the development of oxidative stress that (as with periodontitis) play a central role in the development and progression of these conditions.[56,57] Thus, establishing a connection between this group of diseases, their correlation with severe periodontal disease, and their proinflammatory similarities is of great value.

### 2.1 | Diabetes

Diabetes mellitus is a chronic disorder that affects over 340 million people worldwide.[58] In North America, 9% of the population has been diagnosed with this chronic metabolic disorder. Type 1 diabetes is characterized by the autoimmune destruction of pancreatic beta cells and the complete lack of insulin production; it represents 5%-10% of all cases of diabetes and has a relatively early age at onset. Hyperglycemia and hypoglycemia are 2 possible conditions that might be faced by patients presenting type 1 diabetes.[50] Type 2 diabetes is the most common subtype of diabetes, being present in 85%-90% of patients with a diagnosis of diabetes. With a more prolonged onset than type 1 diabetes, type 2 diabetes presents different degrees of pancreatic beta cell dysfunction and insulin resistance, and is usually accompanied by overweight, obesity, a sedentary lifestyle, or the development of gestational diabetes mellitus during pregnancy.[58] In type 2 diabetes, chronic dysregulation of glucose metabolism and lipid metabolism are present. Pancreatic beta cells, responsible for secretion of insulin, fail to compensate for insulin resistance by peripheral cells. This leads to hyperglycemia, which disturbs the physiological activity of blood vessels, and enhanced production of reactive oxygen species, which

results in oxidative stress.[1,50,59,60] As a consequence of insulin resistance, the pancreas ineffectively starts secreting higher loads of insulin in order to compensate for the failure of peripheral cells in the muscles, adipose tissues, and liver to absorb glucose. The liver then starts releasing glucose into the blood, increasing blood sugar levels even more.[61,62] Secondary complications of uncontrolled type 2 diabetes can include microangiopathies (nephropathy, neuropathy, retinopathy, cardiomyopathy, and periodontal disease), macrovascular diseases (cardiovascular diseases, hypertension, ischemic heart disease, stroke, infertility, and necrosis), as well as an impaired immune response.[58,63-65]

Within the broad scope of end-stage complications related to type 2 diabetes, chronic periodontal disease is considered the sixth most common diabetic complication, playing a bidirectional role with this chronic metabolic disorder.[66] The relationship between impaired blood glucose levels and periodontitis has been widely established in the literature.[50,67,68] Both are chronic inflammatory diseases that share common risk factors and interact mutually with one another, presenting increased oxidative stress and exacerbated release of proinflammatory mediators.[68,69] There is a consistent body of literature reporting on how increased levels of diabetic parameters, such as glycated hemoglobin, correlate positively with oral inflammatory biomarkers and polymorphonuclear activity, disease progression, and the probability of developing periodontitis.[1,16,58,69-72] The immune defense mechanisms of patients affected by type 2 diabetes struggle to respond to the microbial challenge in periodontitis (in particular because of the higher level of subgingival pathogenic microbes in such subjects), which leads to tissue destruction.[68] Moreover, periodontal disease parameters, such as probing depth, clinical attachment loss, bleeding on probing, and gingival index, are worsened by the presence of chronic hyperglycemia and pancreatic beta cell dysfunction.[1,67] It has been shown in a systematic review of the literature that nonsurgical periodontal treatment of patients with type 1 and type 2 diabetes leads to significant improvements in clinical attachment loss and reduced probing depth levels,[73] suggesting a bidirectional relationship between periodontitis and type 2 diabetes. Similarly, it has been shown that periodontal treatment can improve clinical measures of type 2 diabetes, including reductions in the levels of glucose and glycated hemoglobin, as well as in the overall measures of oxidative stress.[67,71] It has also been shown that insulin resistance and altered pancreatic beta cell function may predict the progression and severity of chronic periodontal disease.[72,74]

As one of the main disease mechanisms, advanced glycation end products are a major link between type 2 diabetes and its complications. Advanced glycation end products originate from the irreversible nonenzymatic glycation and oxidation of proteins, lipids, and nucleic acids by the addition of sugars to their polypeptide chain, which alters their structure and functionality.[75] Advanced glycation end products are produced in general in conjunction with aging, with the end product, carboxymethyl lysine, being the most common product.[76] Elevated levels of advanced glycation end products are a result of chronic hyperglycemia and promote a proinflammatory state by increasing the production of specific cytokines, such as tumor necrosis factor-alpha, interleukin-6, interleukin-1beta, and prostaglandin $E_2$, which alter oxygen diffusion by changing membrane structure and permeability, and are associated with a state of enhanced oxidant stress.[50,58,59,75,77,78] An increase in binding of advanced glycation end products to their cognate receptors (receptors for advanced glycation end products), and the consequent activation of such receptors, is thought to explain the increased inflammatory environment observed in patients with poorly controlled diabetes plus chronic periodontal disease.[59] There are increases in the levels of highly cross-linked collagen, which leads to thickening of blood vessel membranes. Understandably, this thickening has a deleterious effect on the transport of molecules between the endothelial membrane and tissues. This also leads to increased production of vascular endothelial growth factor, further exacerbating problems with both micro- and macrovascular structures.[59] In a review of over 200 articles evaluating the relationship between type 2 diabetes and periodontal disease, it was concluded that interactions, on monocytes, between advanced glycation end products and their receptors increases cellular oxidative stress and activates the transcription factor, nuclear factor kappa-light-chain-enhancer of activated B cells.[59] It has also been reported, in a recent consensus report focused on periodontitis and systemic diseases (which included both clinical and animal studies), that interactions between advanced glycation end products and their receptors leads to the exaggerated inflammatory response and periodontal tissue destruction found in patients with type 2 diabetes.[58]

As mentioned previously, a representative group of oxidative stress-stimulating mechanisms and antioxidant markers have been analyzed and proposed to play important roles in the pathogenesis of type 2 diabetes and in its interplay with periodontal disease. Through literature evaluating protein, DNA, or lipid oxidation end-products, antioxidant markers, or enzymatic antioxidant mechanisms, and utilizing different methods of analysis, a consistent link has been established between type 2 diabetes and periodontal disease in terms of overproduction of reactive oxygen species and their downstream effects.[79-85]

In an environment characterized by the overproduction of free radicals, various molecules release enzymatic antioxidants in an attempt to prevent oxidative damage. One of the most prominent enzyme families in this regard is that of the superoxide dismutases. Individuals with type 2 diabetes and periodontal disease have decreased antioxidant capacity demonstrated by low levels of superoxide dismutase activity.[79] In a case-control study with a sample size of 150, plasma analyses revealed that superoxide dismutase activity is decreased in periodontally compromised patients but increased in patients with periodontitis who also have type 2 diabetes.[80] Interestingly, others have also demonstrated that superoxide dismutase activity in patients with type 2 diabetes plus periodontitis is higher than in systemically healthy patients presenting with periodontal disease, suggesting that type 2 diabetes increases gingival activity as an adaptive mechanism, while patients with periodontitis have diminished antioxidant defenses.[81] However, use of gingival tissue samples for qPCR evaluation of

*superoxide dismutase-2* mRNA demonstrated that expression of superoxide dismutase-2 genes was only slightly increased in patients with periodontal disease, whereas in individuals with poorly controlled type 2 diabetes, expression of these genes was substantially increased.[82]

Another key antioxidant enzyme that acts together with superoxide dismutase in the conversion of superoxide to hydrogen peroxide is glutathione peroxidase.[83] These enzymatic antioxidants are associated with the glycation of hemoglobin in such a way that when the level of glycated hemoglobin increases, glutathione peroxidase activity decreases.[84] Similar results were reported for salivary glutathione peroxidase levels before and after periodontal treatment.[40] Using gingival biopsies harvested from patients with poorly and well-controlled type 2 diabetes, others demonstrated, by mRNA analyses, that glutathione peroxidase levels are upregulated by periodontal disease and independent of the diabetic status of the individual.[82] Yet, different observational studies have concluded that poor glycemic control and untreated periodontal disease are directly correlated to the decreasing of oxidative stress markers for both diseases and that a similarly decreased pattern is found for the levels of C-reactive protein and protein carbonyl groups.[1,50,60,74,79,85]

Along the same lines, the levels of malondialdehyde, a marker for lipid peroxidation, have been reported to be increased significantly in periodontal tissues derived from patients presenting with type 2 diabetes, indicating the presence of excessive free radical activity.[60,79] Similarly, the same pattern of increased malondialdehyde is found in systemically healthy patients with chronic periodontitis.[17,38] Most interestingly, malondialdehyde parameters were found to be present at significantly lower levels in serum samples after subjects with type 2 diabetes underwent periodontal treatment and lycopene administration, demonstrating how both diseases relate.[86] Additionally, the DNA damage biomarker 8-hydroxy-2′-deoxyguanosine, the level of which is increased in patients presenting with periodontal disease in comparison with their healthy counterparts, is decreased in patients with type 2 diabetes after scaling and root planing.[21,87] Following analysis of gingival crevicular fluid from 48 individuals, the authors concluded that the group presenting the most prominent reduction in an oxidative stress parameter (represented by 8-hydroxy-2′-deoxyguanosine), as well as in clinical parameters, was that of patients affected by both type 2 diabetes and periodontitis.[87]

Total antioxidant capacity was increased in both peripheral blood samples and gingival crevicular fluid samples from patients with type 2 diabetes and periodontitis.[9,45-47,79,80,88] As such, the generation of oxidative stress may be an underlying systemic condition directly related to alveolar bone loss in periodontitis, in patients with type 2 diabetes.[89] However, these results must be interpreted with caution, given that most total antioxidant capacity assays have not been characterized fully.[90] In relation to this, a model for measuring total antioxidant capacity has been developed that has a predictability rate of 86%.[91] Using data from the Third National Health and Nutrition Examination Survey, this tool was used to quantify the total antioxidant capacity in serum, which was actually found to be inversely associated with periodontitis.[5]

Aryl hydrocarbon receptor ligands are environmental contaminants found in a wide range of pollutants (such as pesticides and herbicides) used in agriculture, by burning garbage, as by-products of combustion processes, and also in cigarette smoke (see below).[92] Tetrachlorodibenzo-para-dioxin, or dioxin, is a prototypical aryl hydrocarbon, which, as with other dioxin-like compounds, binds to aryl hydrocarbon receptor and mediates a variety of toxic effects, such as increased risk of cancer and stroke, suppression of the immune system, hormonal imbalances, and type 2 diabetes.[93] Different studies have reported that aryl hydrocarbon receptor ligands might provoke the development of type 2 diabetes through dioxin-induced impaired metabolism of glucose,[92] impaired metabolism of glucose by pancreatic beta-cells,[94] and reduced insulin sensitivity.[95] The majority of studies relating aryl hydrocarbon receptor ligands to type 2 diabetes have been performed using animal models, such as an experimental study in which dioxin was repeatedly administered to mice to assess its influence on insulin sensitivity.[95] The authors found that activation of aryl hydrocarbon receptor by dioxin caused insulin resistance and an elevated concentration of plasma insulin.[95] Their findings agree with the results of a case-control study in which serum aryl hydrocarbon receptor activity was measured in subjects with type 2 diabetes. There was significantly higher aryl hydrocarbon receptor activity in patients with type 2 diabetes than in age-, sex-, and body mass index-matched subjects presenting impaired glucose tolerance and in healthy controls. The authors suggest that insulin resistance might be a possible link between aryl hydrocarbon receptor and development of type 2 diabetes.[92] Additionally, there is evidence that the levels of aryl hydrocarbon receptor nuclear translocator (also known as hypoxia-inducible factor 1-beta), a regulator of beta-cell function required to keep pancreatic beta-cells in a glucose-responsive state, are profoundly reduced in pancreatic islet cells obtained from patients with type 2 diabetes.[96] Most interestingly, it has been demonstrated that even in patients with no history of type 2 diabetes, a low concentration of serum aryl hydrocarbon receptor is associated with reduced beta-cell function.[94] Altogether, these findings suggest that aryl hydrocarbon receptor may be involved in the pathogenesis of abnormal glucose tolerance and consequently in the development of type 2 diabetes. It is also worthy of note to point out that bacteria metabolize aryl hydrocarbons (not dioxin) into even more toxic compounds, which could conceivably exacerbate even further the pathogenic relationships between type 2 diabetes and periodontitis.[97]

Using an in vitro chick periosteal osteogenesis model, it was shown that dioxin mediated significant reductions in bone formation. This was established, in part, by a reduction, of approximately 80%, in alkaline phosphatase activity.[93] It was suggested that dioxin could therefore further predispose smokers to osteoporosis, as well as periodontal bone loss, through this mechanism and also demonstrated that treatment with resveratrol (an antioxidant and aryl hydrocarbon receptor antagonist) blocked those deleterious effects on osteogenesis.[93] Additionally, as colonization with gram-negative bacteria plays a fundamental role in the pathogenesis of periodontitis, the putative synergistic or additive effects of lipopolysaccharide

derived from *Porphyromonas gingivalis* and aryl hydrocarbons (benzo[a]pyrene, an aryl hydrocarbon found in cigarette smoke) on bone formation were totally abrogated in an in vitro bone marrow cell osteogenesis model. Alone, the agents caused marked reductions in osteogenesis. Most interestingly, the effects of the combination of lipopolysaccharide and benzo[a]pyrene were considered as additive in terms of inhibiting bone nodule formation (by 9-fold), compared with administration individually, and their effects could be reversed by treatment with resveratrol but at higher levels than otherwise used (for example, in the periosteal model).[98] Similar results were found in human periodontal ligament cells. In this recent study, the addition of benzo[a]pyrene decreased the levels of mRNA expressed by osteogenic genes and also reduced alkaline phosphatase activity.[99] In the current literature, there is evidence demonstrating the inhibitory effects of aryl hydrocarbon receptor and RANKL signaling pathways on bone metabolism, more specifically, osteoclastogenesis.[100,101] It can be postulated that the destructive effects on the structure of tooth-supporting tissues are enhanced by long-term exposure to aryl hydrocarbons in a dose-dependent manner, and a representative group of experimental in vitro and in vivo studies have tested the ability of different aryl hydrocarbon receptor antagonists to counteract these deleterious effects,[93,98-101] which will be discussed in more detail below. The data also suggest, quite strongly, that the deleterious effects of smoking on inflammatory disease, and in this case, periodontitis, are probably mediated, at least in part, by activation of the aryl hydrocarbon receptor, as will also be discussed below.

## 2.2 | Smoking

Tobacco smoking is one of the greatest health concerns in human history.[102] It is estimated that more than $200 billion is spent annually to address smoking-related consequences of disease in the United States alone. It has also been estimated that, globally, 5 million people die annually from cigarette smoking and its deadly consequences, with almost 1 in every 2 victims of smoking dying from the effects of secondary smoke inhalation.[102,103] Fortunately, cigarette smoking has been decreasing in developed countries, but is still practiced throughout the rest of the world, especially in low-income countries.[102] The negative health effects of smoking include ischemic heart disease, cerebrovascular disease, chronic obstructive pulmonary disease, multiple types of cancer, and for our purposes, periodontal diseases.[70,102-104]

Although different mechanisms through which smoking affects the progression of periodontitis have been described, a definitive theory explaining this process remains unclear, but is probably related to activation of the aryl hydrocarbon receptor and downstream effects on inflammation as well as oxidative stress. Some have suggested that one of the mechanisms involves a smoking-induced shift in the microbial composition of the oral biofilm so that more periodontopathogenic microorganisms are favored, both in number and in pathogenicity, thereby leading to increased risk for periodontitis in smokers, as well as development of a more severe periodontal disease in smokers than in nonsmokers. Smoking has also been shown to reduce polymorphonuclear neutrophil migration and to cause problems with chemotaxis, leading to a deficient immune response to the microbial threat. Accordingly, we must differentiate periodontal destruction that occurs in the presence of a deficient immune system from periodontal destruction that occurs when the immune system is upregulated pathologically. In the former, we anticipate that tissue destruction is mediated largely by pathogenic and highly virulent bacteria and is therefore not actually representative of chronic periodontitis,[17,19,28] while, in the latter, upregulation of immune system sensitivity leads to increased tissue destruction that is more independent of actual microbial action and is therefore considered a form of chronic periodontitis. In relation to chronic periodontitis, tobacco smoking may lead to upregulation of polymorphonuclear neutrophil activity (ie, the development of a hyperactive polymorphonuclear neutrophil state), which increases the release of proinflammatory cytokines and, of course, the overproduction of reactive oxygen species through a respiratory burst, followed by gingival tissue destruction associated with oxidative stress.[103,105,106]

The negative effects of smoking on periodontal health have been reported extensively in the literature in a vast number of clinical trials, systematic reviews, meta-analyses, and epidemiological studies.[106-112] The highly significant positive association between heavy smoking and periodontal disease incidence, progression, and severity, as well as the low success rates for periodontal treatment, makes cigarette smoking the most preventable risk factor for periodontal disease.[106] A national cross-sectional survey in the US has estimated that current smokers are at 4 times higher risk of developing periodontitis than nonsmokers. Similarly, a higher risk of tooth loss has been found in smokers, as an increased number of missing teeth and a progressive pattern of tooth loss was observed as smoking intensity increased in a sample of health professionals. The authors have also shown that smokers had twice the risk of tooth loss when compared with never smokers.[107] Later on, the same group updated the odds ratio to 3.6.[108] Their findings are consistent with the current literature.[109-112] In a systematic review and meta-regression, the pooled adjusted odds ratios have estimated that smoking habits increased the risk for periodontitis in 85% (odds ratio = 1.85, 95% CI: 1.5-2.2) of smokers, for clinical studies with follow-up periods ranging from 2 to 37 years. Studies with similar designs have presented comparable results, with one reporting a 30% improvement in bone level, assessed radiographically, among quitters.[113,114] Smokers are also at higher risk for clinical attachment loss and recession, deeper probing depths, lower tooth retention, and severity of periodontal destruction.[106,115,116] Interestingly, the literature seems consistent with lower gingival index and bleeding on probing parameters in smokers as a result of the vasoconstrictor effect of smoking on periodontal blood vessels.[117,118]

The literature is still controversial regarding the gingival crevicular fluid biomarker profile of smokers who present with periodontal disease. While the majority of studies report a depressive effect of smoking on the expression of proinflammatory cytokines, others

report no significant difference, or even an increased cytokine profile in smokers.[119] Evaluation of gingival crevicular fluid found the proinflammatory cytokine (interleukin-1beta, interleukin-6) and chemokine profiles to be decreased.[119] Similarly, no statistically significant difference in expression of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in peri-implant sulcus fluid was shown between smokers and nonsmokers.[120] This may reflect the immunosuppressant effects of smoking, which, in turn, may increase susceptibility to periodontal destruction. Even though tumor necrosis factor-alpha and interleukin-1beta are secreted through similar mechanisms, interleukin-1beta did not seem to be influenced by cigarette smoking in patients with periodontitis.[117] These findings are contradicted by other results.[121] In an experimental study, nonsmokers were given nicotine supplements after they quit smoking: the interleukin-1beta levels in gingival crevicular fluid were higher at the final follow-up in comparison with 1 month after quitting (baseline).[121] Interleukin-1beta stimulates bone resorption, inhibits bone formation, and is considered even more potent than tumor necrosis factor-alpha in terms of its effects on bone metabolism. A multiple linear regression analysis showed significant correlations between gingival crevicular fluid cytokine levels and smoking.[122] The authors of this study analyzed samples of gingival crevicular fluid and observed associations between smoking and the total amounts of interleukin-6 and interleukin-8 but not with the levels of interleukin-1beta.[122] These findings are in accordance with those reported from another study.[123]

Periodontal disease progression is mainly caused by bone resorption and tissue destruction. One of the main discoveries in bone biology is the RANK/RANKL/osteoprotegerin system, a fundamental mechanism for bone health which, if disrupted, causes several bone diseases, such as chronic periodontal disease.[24] Bone remodeling is a dynamic mechanism that is mostly regulated by osteoblasts, which mediate osteoclastogenesis through different signaling pathways, such as balancing the ratio between RANKL and osteoprotegerin. In general, RANKL needs to become attached onto the surface of osteoclast precursors in order to stimulate differentiation of these precursors into osteoclasts (ie, through RANKL-induced osteoclast activation). Osteoprotegerin, a bone protector expressed by osteoblasts, is a natural inhibitor of osteoclast differentiation that binds to the RANKL surface and stops osteoclast precursors from differentiating into osteoclasts, thus preventing bone resorption. An imbalance in the RANKL/osteoprotegerin ratio has been positively associated with bone loss in smoking-related periodontitis patients.[24,123-125] Gingival crevicular fluid samples from 149 patients with periodontitis, divided into 3 groups (never smokers, former smokers, and current smokers), were evaluated using ELISA. In the current smoker plus periodontitis group, the level of osteoprotegerin was significantly reduced and consequently the RANKL/osteoprotegerin ratio was significantly increased.[126] The authors concluded that the suppression of osteoprotegerin production may have led to bone loss. These results agree with similar studies in which both gingival biopsies and serum were analyzed.[123,124,127] Additionally, the osteoprotegerin concentration in whole saliva samples was compared with full-mouth clinical periodontal measurements; a positive correlation with probing depth, clinical attachment loss, and bleeding on probing was demonstrated.[125] Finally, the RANKL/osteoprotegerin ratio may act as an indicator of the extent of periodontal breakdown, and thus treatment modalities capable of "switching off" this mechanism should be considered as an adjunctive tool for periodontal disease management.[24,128] Interestingly, a recent study compared the gingival crevicular fluid total oxidant status with the level of RANKL and the RANKL/osteoprotegerin ratio.[25] A significant association between increased total oxidant status, RANKL, and RANKL/osteoprotegerin values in both local and systemic samples was shown, suggesting that oxidative stress might be a common link between bone resorption markers and periodontitis severity.[25] However, assays for total antioxidant capacity andtotal oxidant status are very obscure regarding what they measure in vivo and therefore the results have to be interpreted with caution.

The mechanisms through which cigarette smoking influences periodontal destruction are complex. As mentioned above, the clinical effects of smoking are singular and unique in their own manner as smoke-induced disease progression is likely to present low bleeding on probing measurements, but significant changes in clinical attachment loss and probing depth. Additionally, nicotine is not the only chemical compound with a negative effect on periodontal tissues as the role played by aryl hydrocarbon receptor ligands in bone destruction has been confirmed in different studies and as discussed above.[93,97,98]

In addition to evidence of RANKL-induced destruction of tooth-supporting tissue, there is evidence of smoke-induced oxidative stress caused mainly by increased generation of reactive oxygen species within gingival tissues.[129] The results of screening tests for markers of DNA (8-hydroxy-2′-deoxyguanosine) and protein (C-reactive protein) oxidation have been compared with the smoking status of periodontally diseased patients, as well as the formation of antioxidant compounds, such as superoxide dismutase, catalase, and glutathione peroxidase. As already confirmed in previous studies,[129-133] both smoking and periodontal disease affect C-reactive protein levels in a separate manner. Nonetheless, a small number of studies evaluated the effects of the combination of smoking and periodontal disease on the level of the referred protein damage marker. A recent cohort study in which data were collected retrospetively demonstrated that the effect of periodontal status on C-reactive protein is significantly influenced by the pack year values (the pack year value is calculated by multiplying the number of packs of cigarettes smoked per day by the number of years for which the person has smoked): in this study, a pack year value of >30 was shown to be significantly associated with higher levels of C-reactive protein in patients with periodontitis.[134] These findings are in accordance with another publication from the same group, which observed that oxidative stress is higher in smokers with chronic periodontitis than in nonsmokers with chronic periodontitis.[133,134] Even though

the 8-hydroxy-2′-deoxyguanosine levels are significantly higher in patients with chronic periodontitis than in healthy controls, smoking status does not seem to play a role in terms of increasing DNA damage in whole saliva samples.[21,135,136] Robust evidence on the combined effects of smoking and periodontal disease on 8-hydroxy-2′-deoxyguanosine levels are still needed as a recent Korean study reported a higher odds ratio between periodontitis and this marker of DNA damage in whole saliva from patients with periodontal disease who smoke.[136]

Some studies imply that smoking increases gingival tissue antioxidant activities through the development of a protective and adaptive mechanism in the gingival tissue, but that this increase is not capable of reversing smoked-related periodontal destruction; by contrast, it was suggested in another study that this mechanism relates smoking habits to a decrease in local and systemic antioxidant defenses which, in turn, results in progressive tissue destruction. As an example, the superoxide dismutase levels of smokers and nonsmokers presenting with periodontal disease were compared.[130] The authors reported insignificant changes in the superoxide dismutase levels in blood but significantly higher superoxide dismutase levels in gingival tissue. Similar findings were found by other authors.[129,137] However, in a recent observational study in which the effects of periodontal treatment on oxidative biomarkers were evaluated, a significant interaction between smoking status and salivary superoxide dismutase levels at baseline and after treatment was reported. Smokers had significantly lower reductions in superoxide dismutase levels after treatment in comparison with nonsmokers and former smokers. The authors implied that cigarette smoking influences redox homeostasis and alters antioxidant levels in favor of reactive oxygen species.[138] Their findings agree with other studies.[132,139] In both reports, superoxide dismutase levels were found to be significantly lower in smokers than in nonsmokers and, most interestingly, the antioxidant levels of heavy smokers differed from those of light smokers, leading the authors to imply that tobacco consumption influences superoxide dismutase levels in a dose-dependent manner.[132,139] The same pattern is reported by other groups using blood and saliva samples.[140] Similar discrepancies in the literature are shared for other antioxidant markers,[129,131,132,138] but the evidence currently available seems to confirm a significant reduction of total antioxidant capacity in patients with periodontal disease who also smoke.[21] Additionally, cigarette smoke directly induces an increased neutrophil respiratory burst, suggesting periodontal disease progression through a reduced innate immune response in smokers.[141] Thus, cigarette smoking has been proven to affect neutrophil function, which stimulates reactive oxygen species release and oxidative stress-mediated tissue damage.[131] Consequently, with the capacity of protection being diminished in smokers, it is plausible that the use of antioxidant compounds which are capable of acting against the overproduction of reactive oxygen species within this setting should be addressed.

## 2.3 | Obesity

Obesity is considered as one of the main global public health concerns. Approximately 600 million people suffer from the disease worldwide, and 31% of North American adults are affected. Obesity is usually caused by excessive food intake, lack of physical activity, genetic susceptibility, or a combination of those and other factors, such as endocrine and mental disorders.[142] Obesity is characterized by the deposition of excessive or abnormal fat in adipose tissues and is diagnosed according to the World Health Organization criteria by using primarily the body mass index (ie, the ratio between body weight and body height). Overweight/obese is divided into the following body mass index categories: pre-obesity (25.00-29.99 kg/m$^2$), obesity class I (30.00-34.9 kg/m$^2$), obesity class II (35.00-39.99 kg/m$^2$), and obesity class III (≥40.00 kg/m$^2$). Additional measures, such as waist circumference, waist/hip ratio, and skin fold thickness (as a measurement of subcutaneous fat) are used for complementary screening.[88,143,144] Obesity is a chronic metabolic disease associated with a subclinical inflammatory response in adipocytes and the release of adipose tissue–derived hormones and cytokines (adipokines), which leads to altered hormonal activity, a proinflammatory state, and subsequently to secondary consequences, such as hypertension, increased cholesterol and triglyceride levels, increased insulin resistance, and continued oxidative stress.[143] Interestingly, some proinflammatory functions of peripheral blood neutrophils, such as production of reactive oxygen species and cytokine release, have been reported to be reduced following bariatric (gastric band) surgery.[145] Obesity is also strongly associated with other chronic diseases, such as type 2 diabetes, cardiovascular diseases, osteoarthritis, respiratory disorders, and periodontitis.[143,146]

There is a strong association between body fat measurements and chronic periodontal disease.[147] One group analyzed longitudinal and experimental studies and concluded that, especially in longitudinal studies (with a follow up of > 20 years), overweight, obesity, weight gain, and increased weight gain may be risk factors for development of periodontitis.[148] Additionally, a systematic review and meta-analysis delineated the profile of subjects with a high body mass index as more likely to present with greater mean attachment loss.[149] A similar pattern of association is also found for obese patients: the high levels of serum triglycerides plus low levels of high-density lipoprotein cholesterol in such patients are associated with deepened periodontal pockets.[150] In terms of clinical periodontal parameters, there are several clinical trials and comparative studies relating different levels of periodontal disease severity with high body mass index.[142,151-154] Obese patients with periodontal disease are described as presenting higher gingival index and gingival bleeding index levels than nonobese patients with periodontal disease.[146] There are also significantly higher probing depth and clinical attachment loss values in obese subjects ($P < .05$) and a tendency for a positive correlation between body mass index and clinical attachment loss.[151] Moreover, a cohort study with over 1000 participants in Brazil identified a higher risk for unfavorable periodontal outcomes, represented by bleeding on probing and clinical attachment loss, in

obese patients (riks ratio: 1.45).[152] These results are in accordance with results published in previous studies.[153,154]

Evidence demonstrating the beneficial effects of scaling and root planing on obese and normal-weight individuals presenting chronic periodontal disease, in terms of comparisons performed before and after treatment, shows that clinical parameters (plaque index, bleeding on probing, probing depth, and clinical attachment loss) are significantly reduced in both groups after treatment, as is the expression of proinflammatory cytokines (interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha), implying that improvement does not seem to be modified by obesity.[155,156] In both studies, the sample comprised mainly individuals with obesity class I and obesity class II. These findings agree with a meta-review of the literature in which inconsistent evidence on the response to nonsurgical periodontal therapy in obese patients is shown.[157] However, studies on the effects of obesity on periodontal disease identify clinical parameters that improve less in obese individuals with periodontitis than in normal-weight individuals with periodontitis and thus indicate high body mass index as a significant predictor of periodontal treatment success.[153,158-160] A systematic review of the literature on the effects of obesity on nonsurgical periodontal therapy outcomes concluded that this subject is still controversial and that there may be a negative association between obesity and periodontal treatment outcomes. The authors, however, indicate that, based on their pathophysiological inflammatory models, there is a potentially inferior healing response for individuals with a high body mass index.[161] The results from that study agree with the findings from Goncalves et al.[162] who reported that patients with obesity present lower reductions in periodontal disease after scaling and root planing than do nonobese patients with chronic periodontal disease. In another systematic review, obese individuals showed no statistical differences in clinical periodontal measures after scaling and root planing, but significant differences in inflammatory and metabolic parameters were found before and after treatment between obese individuals and periodontally healthy subjects.[163] Results along similar lines were reported in another recent comparative study.[164]

Obesity is considered to be a modifying factor for periodontal disease through the promotion of a more proinflammatory state, which may increase the susceptibility of obese subjects to pathogenic bacteria and favor a shift toward progression of periodontitis.[85,146] Tumor necrosis factor-alpha is considered to be the main candidate connecting both conditions.[165] One proposed model linking obesity to periodontitis describes the secretion of increased levels of proinflammatory cytokines, especially tumor necrosis factor-alpha: this cytokine inhibits insulin signaling, causing insulin resistance and the development of type 2 diabetes, which leads to a hyperinflammatory state, priming of periodontal tissues, an exaggerated response to microbial colonization, and finally periodontal disease destruction.[166] Additionally, a positive association was found between the levels of gingival crevicular fluid tumor necrosis factor-alpha and high body mass index in periodontally healthy subjects, suggesting that this specific cytokine might originate from a tissue other than the periodontium and might affect structures other than just the adipose tissue.[167] Most interestingly, increased expression of tumor necrosis factor-alpha was detected in gingival crevicular fluid samples of obese children before the development of periodontitis was diagnosed.[168] Conversely, another group suggested that, rather than tumor necrosis factor-alpha, interleukin-6 might mediate the connection between body weight and deepened periodontal pockets, mainly because it stimulates expression of C-reactive protein.[169] Their findings are contradicted by different authors who suggest that interleukin-6 might act as a contributory factor instead of playing a major role.[165,170] Nevertheless, even though the underlying mechanisms relating obesity to periodontitis remain unclear and their relationship is considered bidirectional, high body mass index is a significant risk factor for periodontal disease, which suggests that obese subjects have a 35% increased chance of developing periodontitis and that chronic oxidative stress might be the common link between both conditions.[52,153,170]

Oxidative stress is characterized as a persistent imbalance between antioxidant responses and the release of reactive oxygen species and reactive nitrogen species.[50] As such, the role of obesity in overproduction of reactive oxygen species is consistent in the literature.[171,172] When facing pathogenic oral microbes, obese individuals develop an exacerbated inflammatory response that leads to exaggerated production of reactive oxygen species, confirmed by a significant, positive correlation between oxidative markers and gingival index, probing depth, and clinical attachment loss.[88,128,154,159] Most interestingly, increased levels of circulating reactive oxygen species may induce gingival oxidative stress and potentiate the onset and/or progression of obesity-induced gingival inflammation.[159] Studies tracking the most relevant oxidative markers in obese subjects presenting different levels of severity of periodontal disease show that the levels of myeloperoxidase, protein carbonyl, and 8-hydroxy-2′-deoxyguanosine are significantly increased both systemically (ie, in serum) and locally (ie, in gingival crevicular fluid).[88,154] Local markers for protein carbonylation are found to be higher in obese individuals than in normal-weight individuals, regardless of both periodontal status, whereas the total antioxidant capacity is found to be diminished in obese individulas, indicating that increased body mass index might act as a periodontitis modifying factor.[88] Interestingly, a prospective clinical study evaluating tooth alignment in obese and normal-weight orthodontic patients found that markers for oral inflammation and hormone activity (myeloperoxidase, resistin, and leptin levels in gingival crevicular fluid samples) were upregulated in obese subjects.[173] Their findings agree with others' conclusions.[174] The presence of these markers in periodontal samples, even in the absence of periodontal infection, could be interpreted as indicative of a bidirectional intimate connection between obesity and periodontitis. On the one hand, adipokines that are secreted at excessive levels into the bloodstream of obese patients help to establish an inflammatory state, causing overproduction of oxide end-products within periodontal tissues. On the other hand, periodontal infection releases a wide range of proinflammatory cytokines, contributing to the manifestation of other chronic diseases, such as obesity.[143]

Therefore, obese individuals are statistically more susceptible to the development of periodontal disease through a continuous inflammatory state and a hyperoxidative environment that negatively influences the immune response facing periodontal pathogens. Once destruction of the tooth-supporting tissues begins, a wide range of proinflammatory cytokines is released into the bloodstream, contributing to the expansion of both inflammatory conditions.

## 2.4 | Rheumatoid arthritis

Rheumatoid arthritis is a chronic inflammatory autoimmune disease that leads to joint swelling, joint tenderness, and synovial inflammation, and subsequently to the destruction of cartilage and bone, leading to severe disability and premature mortality.[175] The combination of genetic and environmental factors, such as smoking and alcohol intake, can increase the likelihood of its development.[51,176,177] Even though some authors state that the etiology and pathogenesis of rheumatoid arthritis still remain unknown,[178] there is some evidence suggesting that genetic risk factors associated with environmental triggers can induce molecular changes to host proteins, leading to loss of immune tolerance through protein citrullination.[177,179]

Rheumatoid arthritis and periodontitis display various pathogenic similarities. These include parallels in relation to dysregulation of the host immune response, leading to soft tissue inflammation with subsequent hard tissue destruction. There are even shared risk factors, including smoking and overweight or obesity.[2] Additionally, early studies indicate that patients with rheumatoid arthritis may have a higher incidence of periodontal disease, and vice versa, and the possibility exists that both conditions result from common underlying pathologic features, resulting in a strong association between both.[2,36] Moreover, both diseases have common aspects in terms of the pattern of destruction of soft and hard tissue. While rheumatoid arthritis is responsible for inflammation of the synovial fluid and destruction of the joints, periodontitis causes inflammation of the periodontal tissues and bone loss.[180]

Interestingly, although rheumatoid arthritis is not considered an infectious disease, it has been shown that oral bacteria strongly associated with periodontitis, such as *P. gingivalis* and *Aggregatibacter actinomycetemcomitans*, can be found in the serum of patients with rheumatoid arthritis. It has been postulated that these microbes could contribute to chronic and more generalized inflammation, including the generation of autoantibodies that might then trigger rheumatoid arthritis. Also, the production of deamination enzymes by *P. gingivalis* causes the citrullination of proteins, further inducing autoantibody formation, which is reported as a link between periodontal infection and the development or progression of rheumatoid arthritis.[36]

The periodontal status of patients presenting rheumatoid arthritis has been assessed relative to that of patients without rheumatoid arthritis. Clinical attachment loss and probing depth are the periodontal measures most commonly used for this comparison, with reports of patients with rheumatoid arthritis presenting significantly deeper probing depths and a 4.28-fold increased likelihood of having periodontitis.[180] A systematic review of the literature identified 10 studies that made this comparison. Seven studies showed a statistical difference in clinical attachment loss between patients who had periodontal disease but not rheumatoid arthritis and patients with both periodontal disease and rheumatoid arthritis.[36] The analyses also indicated increased tooth loss associated with patients with rheumatoid arthritis compared with their counterparts without rheumatoid arthritis. Some studies included in this review showed not just a statistically significant difference between these 2 groups of patients but also that patients with rheumatoid arthritis presented with clinical attachment loss 2-fold greater, and almost double the possibility of showing clinical attachment loss of > 5 mm, than patients without rheumatoid arthritis.[181-184] To highlight this relationship, experimental studies assessing the effects of periodontal treatment on biochemical markers for rheumatoid arthritis have shown a statistically significant improvement of erythrocyte sedimentation rate, C-reactive protein, and the DAS28 (a measure of disease activity in rheumatoid arthritis) in patients with rheumatoid arthritis.[185,186] Their findings are in accordance with another-two studies and a systematic review.[175,180,187] Even though some of those studies had relatively small sample sizes, they may represent a significant indicatory trend in terms of a possible rheumatoid arthritis–periodontitis relationship and, most importantly, additional treatment tools for rheumatoid arthritis.

The profile of inflammatory cytokines seen in periodontitis is quite similar to that found in rheumatoid arthritis. Specifically, there are persistently high levels of proinflammatory cytokines (including interleukin-1beta, matrix metalloproteinase-8, and tumor necrosis factor-alpha) and low levels of cytokines (such as interleukin-10 and transforming growth factor-alpha) that suppress the immunoinflammatory response. Both periodontitis and rheumatoid arthritis manifest as a result of an imbalance between proinflammatory and anti-inflammatory cytokines.[22] Additionally, there is strong evidence for a correlation between increased levels of interleukin-1beta and the presence of periodontal disease and rheumatoid arthritis,[36] and evidence also shows that salivary levels of interleukin-1beta, tumor necrosis factor-alpha, and matrix metalloproteinase-8 are increased in rheumatoid arthritis;[188] these factors represent the various stages of progression of an inflammatory response in both periodontal disease and rheumatoid arthritis.[189]

Furthermore, rheumatoid arthritis and periodontal disease share common molecular pathways within the RANK/osteoprotegerin/tumor necrosis factor-related apoptosis-inducing ligand axis, leading to osteoclast differentiation and bone resorption.[36] When secreted by activated T cells within the inflamed joints, RANKL is responsible for mediating the joint destruction observed in patients with rheumatoid arthritis.[11,23,190] Similarly, in periodontitis, periodontal bone loss is highly dependent on the existence and stimulation of osteoclasts, which are regulated by the balance between RANKL and osteoprotegerin,[12] with evidence showing a negative correlation between the RANKL/osteoprotegerin ratio and periodontal disease.[191]

Despite the proinflammatory similarities between rheumatoid arthritis and periodontitis, both diseases also share comparable oxidative stress parameters, even though the literature on local and systemic oxidant levels in patients with both rheumatoid arthritis and periodontitis is still scarce.[51,192] While hyperactive peripheral blood polymorphonuclear neutrophils in periodontal patients produce higher amounts of reactive oxygen species systemically and locally, the neutrophil respiratory burst also occurs in the joints and synovial fluids of patients with rheumatoid arthritis and accounts for excessive production of a wide range of reactive oxygen species.[193] As an example, the plasma concentrations of myeloperoxidase and the levels of lipid and protein peroxidation markers are found to be significantly lower in control subjects (ie, those without rheumatoid arthritis) than in patients with rheumatoid arthritis, in whom they are reported to play important roles in the pathogenesis of the disease.[193,194] Similarly, oxidative damage accounts for destruction of periodontal tissues and progressionof pediodontal disease through lipid, protein, and DNA damage, as well as reduction of the physiological antioxidant mechanisms.[17,46] Interestingly, lipid peroxidation has been implicated in several interconnected conditions, such as rheumatoid arthritis and periodontal diseases.[195] Along similar lines, the oxidative stress index is reported as being significantly higher for periodontally compromised patients with rheumatoid arthritis than for patients presenting chronic periodontal disease only, suggesting that the combined effect of rheumatoid arthritis and periodontitis significantly increases oxidative stress and its destructive consequences.[192]

In spite of a lack of well-controlled and representative clinical studies evaluating the periodontal consequences of rheumatoid arthritis, and vice versa, in terms of their oxidative patterns, the role played by the imbalance between the increased presence of free radicals and the host's incapability to protect against peroxidation mechanisms is well described in the current literature for the 2 conditions separately. As 2 destructive chronic inflammatory conditions that raise questions of whether one is the consequence or trigger of the other, it seems quite possible that novel techniques and methods of comparison between rheumatoid arthritis and periodontitis will enable clarification of the mechanisms that link their pathogenesis, not just to separate entities but to a common condition with oxidative damage in its central axis.

### 2.5 | Concluding remarks regarding oxidative stress and periodontitis

As discussed above, chronic periodontal disease is highly prevalent in patients presenting type 2 diabetes, cigarette smoking habits, obesity, and/or rheumatoid arthritis. The literature is consistent in reports on how the diseases/conditions aforementioned are not only associated with more severe periodontitis but also how they probably achieve their effects on account of their ability to upregulate oxidative stress both locally and systemically, which results in a sustained redox imbalance that favors disease progression. As such, oxidative stress as a therapeutic target for management of periodontitis will be discussed in the following section, in which 3 antioxidant compounds (resveratrol, resveratrol derivative–rich Melinjo seed extract, and curcumin) are examined as adjunctive tools for periodontal treatment.

## 3 | OXIDATIVE STRESS AS A THERAPEUTIC TARGET FOR MANAGEMENT OF PERIODONTITIS

### 3.1 | Resveratrol and Melinjo seed extract

Resveratrol (trans-3,40,-5-trihydroxystilbene), a plant-derived polyphenolic compound found in the skin of dark-colored grapes, red wine, berries, and peanuts,[196,197] is a natural compound with anti-inflammatory properties.[198] There are reports also suggesting its anticancer, cardioprotective, and vasoprotective effects,[199] as well as its ability to improve control of type 2 diabetes and to treat rheumatoid arthritis.[11,200] Additionally, the plant polyphenol has been described as an antioxidant itself, acting directly against reactive oxygen species overproduction and in reestablishing the redox balance.[12] Resveratrol, which is found in 2 isoforms, *trans*-resveratrol and *cis*-resveratrol,[201] is composed of 2 phenolic rings that are connected by a double bond and, as a natural compound, has been considered as an alternative to synthetic drugs because of the virtual absence of side effects associated with its use.[199] Melinjo seed extract is one source of resveratrol and contains variants of this compound, such as *trans*-resveratrol, gnetin C, and gnemonosides A and D.[12] In the following section, the effects of resveratrol and resveratrol derivative–rich Melinjo seed extract on chronic periodontal disease and its destructive consequences will be addressed. In addition, the main mechanisms through which resveratrol acts to prevent and control periodontal tissue destruction and to heal periodontal tissue already destroyed, will be discussed.

#### 3.1.1 | Host–response modulation

There are many different mechanisms through which resveratrol acts to control, prevent, and reverse the destructive progression of inflammatory conditions, such as periodontal disease. One of these is modulation of the host response when the host is faced with exacerbated inflammation.[202] An oxidative stress environment was created by exposing human gingival fibroblast cultures to hydrogen peroxide, and this environment was used to evaluate the effects of resveratrol on the control of reactive oxygen species production, mitochondrial respiratory capacity, and type 1 collagen synthesis. Resveratrol inhibited most effectively free radicals with a longer incubation period in comparison with the other two tested antioxidants. In addition, mitochondrial respiratory modulation induced by the polyphenol was more pronounced. Most interestingly, expression of *type 1 collagen* mRNA was significantly upregulated when resveratrol was administered.[203] Conversely,

when human gingival fibroblasts were used to analyze the protective role of resveratrol in rats, the induction of different inflammatory factors, such as matrix metalloproteinases 2 and 9, was strongly reduced in the presence of resveratrol, even when the human gingival fibroblasts were also treated with lipopolysaccharide.[199] These findings agree with other reports in which it is also suggested that antioxidants may play a role in biological functions and in both soft and hard tissue turnover during periodontitis-induced oxidative stress.[204,205] Along the same lines, human periodontal ligament cells from teeth extracted for orthodontic purposes were stimulated with *P. gingivalis* lipopolysaccharide in order to simulate periodontal infection. The cultures were treated with different concentrations (25, 50, and 100 μM) of resveratrol, and the levels of nitric oxide and proinflammatory cytokines after treatment were assessed. Nitric oxide production in these stimulated human periodontal ligament cells was inhibited by resveratrol in a resveratrol concentration–dependent manner. Most interestingly, secretion of interleukin-1beta, interleukin-6, interleukin-8, interleukin-12, and tumor necrosis factor-alpha was significantly decreased in comparison with the control group, irrespective of the concentration of resveratrol used.[196] Conversely, others have demonstrated a similar disease-control pattern using a resveratrol derivative,[205,206] and a compatible trend was observed for experimental periodontitis in diabetic rats.[207] In addition, resveratrol prevented RANKL-induced osteoclastogenesis through the inhibition of reactive oxygen species.[208] Another experimental study found that resveratrol promoted a significant reduction of interleukin-17 levels. A ligature-induced periodontitis model in rats was used in which resveratrol, at a dose of 10 mg/kg diluted in water, was administered daily for 30 days.[209] Microbiological analysis of the ligatures used in this study was employed to evaluate the impact of resveratrol on the bacterial load of species related to the periodontium (*P. gingivalis*, *Tannerella forsythia*, and *A. actinomycetemcomitans*). It has been demonstrated that resveratrol does not promote benefits for microbiological outcomes of an experimental model of periodontitis, which reinforces its role in modulating the host response.[210] Using a similar experimental model and the same duration of daily administration, others combined resveratrol with another plant-derived antioxidant, curcumin, to assess their possible effects on gingival tissue cytokine levels and bone loss. The ligated and unligated sides showed significant reductions in interleukin-1alpha levels when the plant-derived antioxidants were administered.[11] These results are in accordance with other reports.[12,97] Thus, modulation of cytokine levels and reactive oxygen species within periodontal tissues may represent possible mechanisms by which resveratrol acts on the host response, thereby leading to control of initiation and advancement of periodontal disease.[11,209]

### 3.1.2 | Nuclear factor erythroid 2-related factor 2-related pathway

Another important antioxidant mechanism triggered by resveratrol is activation of the master regulator of antioxidants, nuclear factor erythroid 2-related factor 2, which attenuates osteoclastogenesis,[211] modulates intracellular reactive oxygen species,[212] inhibits periodontal ligament cell apoptosis,[213] and is downregulated in polymorphonuclear neutrophils derived from patients with chronic periodontitis.[214] Nuclear factor erythroid 2-related factor 2 is directly responsible for antioxidant defenses and resistance to oxidative stress,[12,199] thus demonstrating a protective role.[26] There is evidence demonstrating that the dysregulation (ie, hyperactivity and redox disturbances) of neutrophils from patients with chronic periodontitis is associated with a defect in binding of *nuclear factor erythroid 2-related factor 2* DNA, suggesting that a disruption in the nuclear factor erythroid 2-related factor 2 pathway is a potential mechanism leading to the cellular redox imbalance observed in this disease.[215] The role of different levels of nuclear factor erythroid 2-related factor 2 in periodontal disease has been investigated over the years,[26,122,216] and the positive effects of resveratrol on the levels of nuclear factor erythroid 2-related factor 2 in different tissues[217-219] have also been investigated. However, the effects of resveratrol on the nuclear factor erythroid 2-related factor 2 pathway, specifically with respect to periodontitis, have yet to be addressed in detail. Along these lines, immunohistochemistry was used to assess the effects of Melinjo seed extract–derived resveratrol (mainly a source of the resveratrol dimer, gnetin-c) on the levels of nuclear factor erythroid 2-related factor 2 protein in an experimental periodontitis model in rats. Higher levels of immunostaining for nuclear factor erythroid 2-related factor 2 were demonstrated clearly in the tissue samples taken from the rats treated with Melinjo seed extract. This suggests that Melinjo seed extract (and, by extension, resveratrol and/or the resveratrol dimer) activates the nuclear factor erythroid 2-related factor 2 pathway, leading to downregulation of oxidative stress. Moreover, the authors also suggest that through interaction with the aryl hydrocarbon receptor (for which resveratrol is an antagonist), not only was the production of reactive oxygen species reduced, but any reactive oxygen species that were produced were subsequently neutralized by resveratrol or the resveratrol dimer as well as by nuclear factor erythroid 2-related factor 2 protein-mediated reduction of 8-hydroxy-2′-deoxyguanosine.[12] These findings agree with the results reported by others using similar methodological approaches.[219] Specifically, in both studies, in addition to nuclear factor erythroid 2-related factor 2 activation, the levels of 8-hydroxy-2′-deoxyguanosine were significantly reduced, both locally[12] and systemically,[219] in the presence of resveratrol. Different reports also show that the sirtuin 1/adenosine monophosphate-activated protein kinase pathway was triggered by resveratrol.[205,219] This pathway has important anti-inflammatory effects, modulates the activity of nuclear factor kappa-light-chain-enhancer of activated B cells, and suppresses oxidative stress, and might represent another defense pathway induced by the administration of resveratrol or resveratrol dimer.[220-222]

The roles played by the nuclear factor erythroid 2-related factor 2 pathway, such as the inhibition of fibroblast apoptosis and osteoclastogenesis, as well as the scavenging of reactive oxygen species, have unprecedented and beneficial clinical implications in relation to diseases that are mediated by oxidative stress: in this

case, periodontitis. Therefore, the effects of resveratrol imply that it, and similar derivatives, can be utilized as novel adjunctive tools in the management of chronic periodontitis, given their demonstrated capacity to protect against periodontitis-mediated damage and periodontal disease progression.[199]

### 3.1.3 | Osteoclastogenesis, osteoblast proliferation, aryl hydrocarbon receptor, RANKL, and bone loss

Given that one of the major structures of the periodontium is bone, anything that has a beneficial effect on bone homeostasis and bone-cell (osteoblast) health should also be beneficial in the management of periodontitis. In relation to this, various protective effects of resveratrol on bone metabolism have been reported in the literature,[198,223] as well as in studies performed in vitro (see below).[90,93] Studies have investigated the positive effects of resveratrol in a ligature-induced periodontitis model, an experimental model effective for inducing alveolar bone loss in rats.[220] Using sutures placed around molars, periodontal disease is induced during a specific period of time, usually ranging from 15 to 30 days, and then the suture is either removed, which is considered "conventional treatment", or left in place in the presence or absence of the drug aimed to be tested. Usually, the contralateral teeth are used as controls in a split-mouth design.

Eighteen male Wistar rats were divided into 3 groups, as described earlier.[90,93,220] The rats were given resveratrol solution at a dose of 10 mg/kg body weight and were sacrificed after 20 days of ligature-induced periodontitis (the ligatures were not removed, meaning that the causative factors were still present; tantamount to untreated disease). Using micro-computed tomography analyses, it was demonstrated that there was decreased periodontal bone loss in the periodontitis plus resveratrol group compared with the periodontitis-only group (not treated with resveratrol).[220] Thus, even in the absence of "treatment", resveratrol *prevented* periodontal disease progression. Similar results were also reported by others using morphometric measurements of alveolar bone loss from standardized photographs.[11,209] In addition, resveratrol modulated the production of osteoclastogenesis-related factors, as shown by significantly decreased interleukin-17 levels in rat gingival tissues.[209] Most interestingly, the antioxidant capacity of resveratrol was demonstrated by the finding of significant reduction of the levels of 8-hydroxy-2′-deoxyguanosine in urine in the periodontitis plus resveratrol group compared with the group with periodontitis not treated with resveratrol, probably through activation of the nuclear factor erythroid 2-related factor 2/antioxidant defense pathway.[219] These findings agree with those published by other authors.[12,216,217] A similar study design, with a daily subcutaneous injection of resveratrol (5 mg/kg), combined with administration of lipopolysaccharide (1 mL/mg) 3 times per week, was performed using male Sprague-Dawley rats. The rats were then randomly divided into four groups: no periodontitis, ligatures with placebo injection, ligatures with lipopolyssacharide injection, and ligatures with lipopolyssacharide injection and resveratrol administration. Micro-computed tomography scans showed that the decreased bone mineral density and bone volume found in the lipopolysaccharide-treated group were restored in the presence of resveratrol, which also significantly reduced alveolar bone loss ($P < .05$) and inhibited osteoclastogenesis in comparable levels to the controls.[199] In addition to osteoclastogenesis inhibition, others reported that resveratrol increased osteoblast cell differentiation, as demonstrated by increased levels of alkaline phosphatase and osteoprotegerin (both of which are used as biomarkers for osteoblast cell differentiation in mesenchymal stem cells), even in the presence of lipopolysaccharide.[198] This agrees with the results from another study, whereby oxidative stress parameters were also positively influenced (ie, reduced) by resveratrol as serum antioxidant superoxide dismutase activity was found to be increased when resveratrol was applied.[199] These findings suggest that resveratrol may modulate the host response by controlling the redox state in periodontitis.

A recent study also demonstrated that not only does resveratrol appear to prevent the initiation and progression of alveolar bone loss caused by periodontitis but also has the capacity to actually *reverse* the loss of alveolar bone once the disease has been established, and that this occurs even when the triggering factors (eg, the silk ligatures) have not been removed.[12] That healing of bone loss or regeneration of periodontal tissue occurs even when the triggering ligatures are still in place, suggests a rather powerful effect as leaving the ligatures in place essentially represents the equivalent of periodontitis having not been treated from the perspective of debridement.[220] To reiterate, even with the ligatures still in place, periodontal bone regeneration was observed.[12] This finding, although in accordance with previous findings suggesting that resveratrol positively affects periodontal tissues, might be considered as the first report showing that resveratrol can mediate actual regeneration and healing of periodontal lesions as opposed to solely inhibiting initiation and progression of periodontal lesion formation. Again, this might relate to the fact that resveratrol has been reported to upregulate expression of proteins that induce osteodifferentiation and osteoblast cell activity, such as bone morphogenetic protein-2, bone morphogenetic protein-7, and osteopontin. Having said this, other studies have not necessarily replicated the aforementioned findings as regards bone morphogenetic protein-2.[197] Nonetheless, in the study by Casarin and colleagues, [202] calvarial defects were created for assessing bone remodeling in rats and for investigating the effects of resveratrol on the biomechanical retention of implants placed in tibia. They suggested that resveratrol stimulated the early phases of ossification and bone maturation as the treatment group showed higher removal torque force in comparison with the control group.[202] In another study, occlusal trauma was induced on maxillary first molars of mice by overlaying composite resin onto their occlusal surfaces. Inhibited expression of RANKL was demonstrated in mice treated with resveratrol, leading to decreased loss of bone compared with the control group.[224]

Using a chick periosteal osteogenesis model and the rat bone marrow stromal cell model, others demonstrated that dioxin, a prototypical aryl hydrocarbon receptor ligand and agonist that is analogous to aryl hydrocarbons found in cigarette smoke, inhibited osteodifferentiation and therefore bone formation in vitro.[93] Using these model systems, different concentrations of resveratrol were added to the cultures treated with dioxin; the results showed that the negative effects of dioxin could be blocked by resveratrol. This was demonstrated by assessing the levels of biomarkers for osteogenesis biomarkers, such as alkaline phosphatase, osteopontin, and bone sialoprotein, in the presence and absence of dioxin. As expected, dioxin-mediated inhibition of osteodifferentiation was reversed by resveratrol in both in vitro model systems. Moreover, because the model systems relied on cells derived from wholly unrelated species, the findings observed with resveratrol and dioxin (as well as other smoke-related hydrocarbons studied in other experiments) could be given significant credence as representing generally expected biological effects.[93] Along the same lines, a rat bone marrow cell osteogenesis model was used to test another aryl hydrocarbon receptor ligand (benzo[a]pyrene; this aryl hydrocarbon actually being a component of cigarette smoke) in combination with lipopolysaccharide derived from *P. gingivalis*, on osteogenesis, also with and without resveratrol.[98] In this case, osteogenesis was assessed using several approaches, including enzymatic, molecular, and electrophoretic methods, as well as by the formation of bone nodules that stain red when using Alizarin Red. The additive inhibitory effects of benzo[a]pyrene plus lipopolysaccharide on nodule bone formation were confirmed. Furthermore, it was also demonstrated that, depending on the concentration of the lipopolysaccharide, the deleterious effects of benzo[a]pyrene plus lipopolysaccharide were attenuated partially or completely by the addition of resveratrol. The authors concluded that in addition to antagonizing aryl hydrocarbon receptor activation, resveratrol also demonstrates direct anti-inflammatory effects.[98]

Another study performed in male Wistar rats assessed the effects of resveratrol in combination with smoke inhalation on the repair of critical-sized bone defects in calvarial bone.[202] Histomorphometric analyses showed no statistically significant difference between the resveratrol plus smoke inhalation group and control groups. These findings could fit with the notion that the effects of resveratrol, as shown in the periosteal osteogenesis model, are most profound during osteodifferentiation. Hence, if osteodifferentiation is not a requirement of healing, the effects of resveratrol would be limited. This said, it was still demonstrated that the levels of *RANKL* and *osteoprotegerin* mRNA were significantly lower in the resveratrol plus smoke inhalation group than in smoke inhalation and placebo groups ($P = .017$).[202] These results agree with the findings from another recent study that used a similar model and showed upregulated levels of sirtuin 1 and superoxide dismutase activity, as well as reduced alveolar bone loss and NADPH oxidase levels in the group exposed to daily cigarette smoke inhalation and resveratrol administration.[225] The authors suggest that resveratrol might be an additional tool in periodontal treatment, especially in smokers. The same group showed reduction in the progression of both periodontitis and rheumatoid arthritis using a rat model.[176] Periodontitis was ligature-induced, and rheumatoid arthritis was induced by immunizations through injections in the tail, fist, paw, knee joint, and subcutaneously. The findings suggest that resveratrol modulates the levels of rheumatoid factor and antibody to citrullinated protein in serum, which may give resveratrol the ability to induce beneficial effects on disease severity and progression. Morphometric analyses of oral tissues showed significantly higher bone loss in the placebo group in comparison with the groups in which 2 drugs (resveratrol and ibuprofen) were tested. Interestingly, resveratrol presented no difference in bone loss when compared with ibuprofen. These findings indicate that resveratrol might be used to modulate periodontal destruction and articular damage with no related side effects.

There are still no human studies in which the effects of resveratrol on periodontal disease have been tested. However, in a randomized double-blind, placebo-controlled, clinical trial, patients with type 2 diabetes plus chronic periodontitis were divided into intervention (480 mg/day of resveratrol for 4 weeks) and control (placebo) groups. Nonsurgical periodontal therapy was performed in both groups. The mean serum levels of fasting insulin and insulin resistance were significantly lower in the intervention group than in the control group, as was the mean pocket depth ($2.35 \pm 0.6$ mm in the intervention group vs $3.38 \pm 0.5$ mm in the control group).[200] The authors suggest that resveratrol supplementation might improve insulin resistance and control periodontal disease activity. As systemic resveratrol presents poor oral bioavailability, resveratrol-containing microbeads have been developed for local treatment of periodontitis. The formula presented by this research group showed strong mucoadhesion and a slow rate of resveratrol release. The authors indicated these microbeads as candidates for a locally adjunctive treatment modality as a result of the high intrasulcular drug concentration and absence of systemic side effects,[226] in accordance with consistent data showing no side effects with systemically administered resveratrol.[199]

## 3.2 | Curcumin

Curcumin, a plant-derived compound isolated from dried rhizomes of *Curcuma longa*,[227] is usually used as dietary spice. As the major active compound present in the roots of the turmeric plant, natural curcumin is an extended pseudosymmetric polyphenol (diferuloylmethane) composed of a mixture of 3 curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin),[228] and has been increasingly investigated as a result of its potent anti-inflammatory, antimicrobial, anti-cancer, and (most importantly) antioxidant properties.[229] Curcumin administration has also been associated with beneficial effects on different tissues, such as skin,[230] lungs,[231] and liver[232] with, as yet, few to no side effects being reported.[233]

Therefore, as for the antioxidant resveratrol, experimental periodontitis models (in which development of periodontal disease and periodontal destruction is effective) have been used to assess the effects of curcumin on periodontal tissues.[234] The experimental ligature-induced periodontitis model in rats, discussed in section 3.1.3, has been induced/modified by the addition of lipopolysaccharide injections or induction of type 2 diabetes (or, more accurately, hyperglycemia). Natural curcumin presents relatively poor pharmacological properties, such as poor bioavailability, high insolubility in water, and a short half-life in plasma; therefore, several studies have compared natural curcumin with chemically modified analogs or chemically modified curcumins, which present better chemical characteristics.[235] In addition, studies have been carried out to investigate whether curcumin or chemically modified curcumins might also alter the progression and/or initiation of periodontal disease.

### 3.2.1 | Inhibition of the nuclear factor kappa-light-chain-enhancer of activated B cells activation pathway and host modulation

Activation of the transcription factor, nuclear factor kappa-light-chain-enhancer of activated B cells, is associated with a hyperinflammatory state and expression of proinflammatory cytokines (such as interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha),[235] osteoclastogenesis markers (such as RANKL),[233] matrix metalloproteinase activity,[236] and reactive oxygen species overproduction.[237] Even though there are other signaling pathways that can be used by curcumin,[229] inhibiting activation of the nuclear factor kappa-light-chain-enhancer of activated B cells pathway is considered as one of the main mechanisms through which curcumin acts to prevent and control activity of matrix-degrading enzymes,[235] RANKL-mediated bone resorption,[233] and exacerbated release of free radicals,[236] all of which play a role in periodontal disease destruction.

Modulation of the immune response through the reduction of proinflammatory cytokines was reported in a lipopolysaccharide-induced experimental periodontitis study in rats given daily doses of natural curcumin at 2 concentrations (30 and 100 mg/kg) via oral gavage. Stereometric analyses showed significant reductions in inflammatory infiltrate, as increased collagen content was observed in rats given either concentration of curcumin.[238] Along a similar line, others stimulated human gingival fibroblasts with *P. gingivalis* lipopolysaccharide and treated the cultures with curcumin. Pretreatment with curcumin resulted in inhibition of the nuclear factor kappa-light-chain-enhancer of activated B cells pathway and consequently in the downregulation of tissue-destructive mediators through attenuated expression of lipopolysaccharide-stimulated cycloxygenase-2.[239] Brandao et al. reported positive effects of chemically modified curcumins on bone resorption, osteoclastogenesis, and tumor necrosis factor-alpha but found that these effects were not curcumin dose-dependent.[237] By contrast, the results from Hu et al. showed curcumin dose-dependent effects on human gingival fibroblasts[239], which agrees with the findings from other research groups in which curcumin produced a marked, dose-dependent inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells.[229,235] Along the same lines, 2 recent studies also illustrated an inhibitory effect of curcumin on the nuclear factor kappa-light-chain-enhancer of activated B cells, considering it the main mechanism used by curcumin in tissue healing.[11,238]

The effects of curcumin administration on lipopolysaccharide-induced periodontitis (in which periodontitis is induced by repeated injections of lipopolysaccharide from *Escherichia coli*) and diabetes-associated periodontitis (in which diabetes is induced in mice by intravenous injections of streptozotocin into the tail) were tested in mice in another study.[235] Parenthetically, it should be emphasized that although the induction of hyperglycemia by injection of streptozotocin does not actually produce a model for type 2 diabetes, the term type 2 diabetes will be used for the sake of convenience from time to time. Chemically modified curcumin-2.24 was administered orally to hyperglycemic rats. Significant reduction in bone loss was observed in both lipopolysaccharide-induced and diabetes-associated periodontitis models, of 22.3% and 24.4%, respectively, as a result of administration of the curcumin analog. Additionally, marked reductions in the levels of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha, of 50%, 50%, and 70%, respectively, were recorded in the lipopolysaccharide-induced periodontitis model, "normal" interleukin-1beta levels were achieved in the diabetes-associated periodontitis model, and inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells activation was comparable with that found in controls in both models after administration of chemically modified curcumin-2.24. The authors concluded that in chemically modified curcumin-2.24 controls, periodontal disease was both locally induced and systemically modified.[227,235] Conversely, the combination of lipopolysaccharide-induced and diabetes-associated periodontitis was positively influenced by chemically modified curcumin-2.24 in terms of reduction of the levels of interleukin-1beta, interleukin-6, and matrix metalloproteinases 2, 8, and 9, and of the bone loss levels. Even though chemically modified curcumins did not induce significant effects on connective tissue turnover, the authors suggested that they might present beneficial effects on the breakdown of collagen and probably also of bone, thereby having the potential for use in the treatment of periodontal disease.[235]

### 3.2.2 | Prevention of alveolar bone loss, and inhibition of osteoclastogenesis

The effect of natural curcumin was compared with that od chemically modified curcumin-2.24 on osteoclast-mediated bone resorption, apoptosis, and inflammation in a lipopolysaccharide-induced periodontitis model in rats. Both curcumin compounds showed a benefit for modulation of inflammation, as a significant reduction of inflammatory cell infiltrate was observed. Interestingly, chemically modified curcumin-2.24, but not natural curcumin, reduced alveolar bone loss (demonstrated using micro-computed tomography analyses)

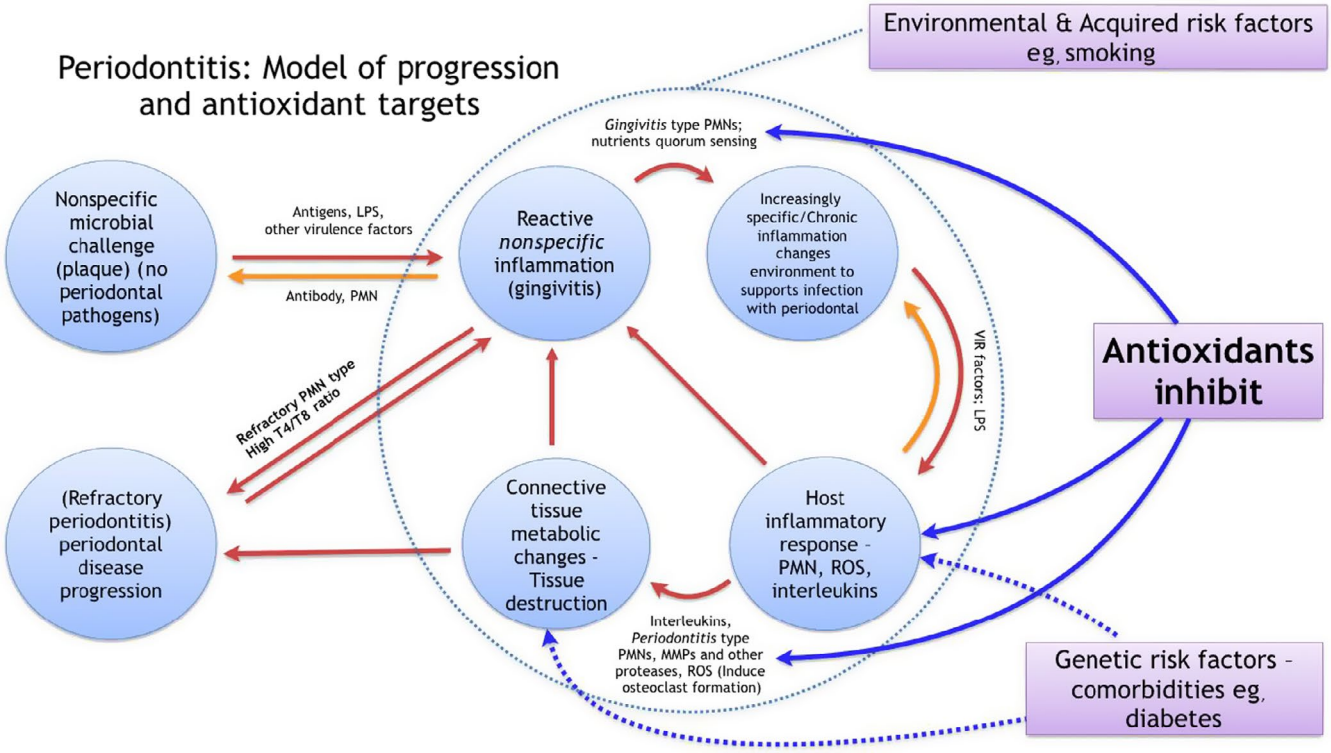


**FIGURE 1** Diagram/flow chart that outlines a suggested pathway explaining periodontal inflammation, including some of the mechanisms. In particular, we focus on where antioxidants (eg, resveratrol, curcumin) could block the pathway, thereby arresting initiation and progression of periodontal disease. In this regard, antioxidants would quench reactive oxygen species formation. Moreover, resveratrol, particularly in smokers, could also mediate effects on oxidative stress not only through its direct antioxidant activity, but also by upregulating nuclear factor erythroid 2-related factor 2 (Nrf2), probably through effects on the aryl hydrocarbon receptor and, more importantly, as an antagonist of aryl hydrocarbon-mediated downregulation of Nrf2 and increased oxidative stress. LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; PMN, polymorphonuclear neutrophil; ROS, reactive oxygen species [Colour figure can be viewed at wileyonlinelibrary.com]

and osteoclastogenesis.[228] Similar results were found in another study using a ligature-induced periodontitis rat model in which rats were administered natural curcumin. Even though micro-computed tomography analyses demonstrated that curcumin administration was not associated with prevention of alveolar bone loss, curcumin did suppress inflammation, increase collagen content, and stimulate proliferation of fibroblastic cells, and effectively inhibited expression of interleukin-6 and tumor necrosis factor-alpha genes in periodontal tissues.[229] Thus, the beneficial effects of natural curcumin seem to be limited to host response modulation, as the proinflammatory cytokine profile demonstrates a benefit from the administration of natural curcumin, whereas "clinical" results, such as alveolar bone levels, did not show improvement in a ligature-induced periodontitis model. These results are partially contradicted by others, who tested the "prophylactic/preventive" effects of curcumin. In that study, curcumin was administered by intragastric gavage to rats in a ligature-induced model, and alveolar bone loss was compared in periodontally healthy and periodontally compromised groups. The combination of curcumin and periodontitis presented significantly lower alveolar bone loss than observed in rats with untreated periodontitis (ie, no curcumin) ($P < .0125$). However, curcumin was not capable of decreasing alveolar bone loss to levels comparable with those in the healthy controls.[240] This difference might have resulted from variations in the measurement technique used between studies (standardized photographs vs micro-computed tomography scans).

Different studies present heterogeneous methods, such as discrepancies in drug presentations, drug concentrations, modes of administration, and data interpretation; thus, comparisons should be made with caution. A recent study on the effects of curcumin and piperine (a pepper derivative with putative positive effects on curcumin bioavailability), on experimental periodontitis in rats, was conducted.[234] Besides the beneficial effects(inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells, diminished cellular infiltrate, and increased collagen content) curcumin-treated sites showed significantly increased bone neoformation (as demonstrated using micro-computed tomography), irrespective of whether or not it was administered in conjunction with piperine. The authors concluded that curcumin augments alveolar bone repair.[234] Similarly, the combination of curcumin and piperine suppressed osteoclastogenesis in vitro in periodontal ligament cells.[233] Another possible synergistic effect (curcumin and resveratrol combined) was tested by other authors. In a ligature-induced periodontitis model in rats, morphometric measurements of alveolar bone loss showed no statistical difference between curcumin, resveratrol and both drugs combined, even

without "periodontal treatment", represented by ligature removal.[11] Given the protective role played by resveratrol in bone metabolism, as previously described, similar positive results presented by curcumin undoubtedly indicate this compound as a promising alternative for periodontitis management. Even though 3 different human studies have reported positive additive effects of a 0.2% loaded curcumin strip,[241] a 1% curcumin gel,[242] and the combination of curcumin with 1% ornidazole gel[243] on periodontal parameters after scaling and root planing, the sample sizes and study designs raise questions regarding the validity and reliability of the results. Thus, the current literature still lacks well-controlled human trials on this subject.

Finally, a recent study tested the viability and biological effect of local administration of curcumin in a nanoparticle formula (nanocurcumin). Three microliters of nanocurcumin was administered twice a week in rats given lipopolysaccharide (periodontitis group); as control, injections of phosphate-buffered saline, administered to rats using the same schedule as for nanocurcumin, were given. After treatment with nanocurcumin, a marked reduction in nuclear factor kappa-light-chain-enhancer of activated B cells activation levels was observed. In addition, the number of osteoclasts in sections from the hemimaxillae of the group treated with lipopolysaccharide/nanocurcumin did not differ from those of the phosphate-buffered saline-injected group (control). Furthermore, the bone volume/tissue volume ratio showed no statistical difference between the lipopolysaccharide/nanocurcumin group and the phosphate-buffered saline group (control). Thus, bone resorption was attenuated by curcumin administration. Finally, the experimental periodontitis model effectively increased polymorphonuclear neutrophil counts, which were markedly diminished after local curcumin administration twice a week. No statistical difference was found in polymorphonuclear neutrophil counts between the lipopolysaccharide/nanocurcumin group and the control group. The authors refer to topical administration of nanocurcumin as a noninvasive, effective adjunctive tool in the conventional treatment of periodontitis, with the virtual absence of side effects.[244] Recently, a curcumin-loaded biodegradable crosslinked gelatin film was developed for delivering curcumin into periodontal pockets. The film was able to release curcumin for up to 7 days.[244] Similarly, mucoadhesive films containing curcumin-loaded nanoparticles showed swelling of up to 80% when placed in the oral cavity.[245] Swelling is an important characteristic as absorption of water creates a network within the drug delivery system, entraps the drug of interest, and slowly releases it.[246] It is possible that the only reliable human trial available, in which curcumin gel was investigated, is the one conducted by Nasra and colleagues, with a sample size of 20. A gel containing 2% curcumin was inserted into the periodontal pockets of patients with periodontitis, and these patients were given nonsurgical periodontal therapy. The control group comprised patients with periodontitis who underwent scaling and root planning. Both groups showed significant reductions in probing depth, bleeding on probing, and plaque index ($P < .05$). The experimental group (2% curcumin plus nonsurgical periodontal therapy) showed higher, but nonsignificant, reductions in those parameters, which was attributed to the controlled release of curcumin for a prolonged duration.[247]

## 4 | CONCLUSIONS

A diagram is presented to illustrate a potential model for progression of periodontitis and the pathways targeted by the antioxidant compounds discussed (eg, resveratrol and curcumin) (Figure 1). Resveratrol and curcumin, as well as their derivatives, represent potentially important therapeutic agents that can lead to the development of new drugs (eg, chemically modified resveratrol)[248] which may be used in conjunction with conventional techniques for periodontal disease treatment. The studies discussed in this review suggest these 2 natural compounds as possible candidates in alternative periodontal therapies. Initially, both compounds prevented an exacerbated inflammatory setting that is characteristic of chronic periodontal disease. Moreover, they also yielded effects on the control of established periodontal infection. Furthermore, resveratrol was described as being capable of reversing the destructive effects of periodontitis. Thus, even though additional pharmacological tests are still to be conducted for both agents, evolution of the modes through which they are delivered (systemic and even local) should produce new paradigms in the management of periodontal diseases that transcend infection control alone (ie, by surgical or nonsurgical debridement) in the near future. Current evidence indicates that oxidation plays a significant role in many human diseases, including periodontitis. Antioxidants and upregulation of nuclear factor erythroid 2-related factor 2-associated antioxidant and detoxification enzymes enhance cytoprotective effects by decreasing inflammation downstream of oxidative tissue damage.[28,214] Accordingly, therapies that increase the level of antioxidants and/or antioxidant activity may be viable additions to current approaches related to both the prevention and treatment of periodontitis, as well as other diseases of oxidative stress.

### ACKNOWLEDGMENTS

The authors acknowledge the support of Dentalcorp for providing funding for research programs in the Centre for Advanced Dental Research and Care, Mount Sinai Hospital, and University of Toronto. This work was conducted during a scholarship supported by the International Cooperation Program at the University of Toronto financed by CAPES - Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil.

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**How to cite this article:** Sczepanik FSC, Grossi ML, Casati M, et al. Periodontitis is an inflammatory disease of oxidative stress: We should treat it that way. *Periodontol 2000*. 2020;84:45-68. https://doi.org/10.1111/prd.12342