Role of Periodontal Infection, Inflammation and Immunity in Atherosclerosis

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Abstract: ***Background:*** **Inflammation plays a major role in the development and progression of cardiovascular disease (CVD) morbidity and mortality. The well-established relationship between periodontal disease (PD) and CVD may be causal. Left untreated, PD can lead to high systemic inflammation, thus contributing to inflammatory CVD, such as atherosclerosis. Multiple mechanisms have been proposed to elucidate the causal relationship between PD and its contribution to CVD.** ***Objective:*** **This review article highlights the current evidence supporting the role of PD in the development and progression of atherosclerosis.** ***Methods:*** **After creating a list of relevant medical subject heading (MeSH) terms, a systematic search within PubMed in English for each MeSH term between 2000 and 2019 was used to generate evidence for this review article.** ***Conclusion:*** **There is overwhelming evidence in the current literature that supports an association**

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between PD and CVD that is independent of known CVD risk factors. However, the supporting evidence that PD directly causes CVD in humans continues to remain elusive. Multiple biologically plausible mechanisms have been proposed and investigated, yet most studies are limited to mouse models and in vitro cell cultures. Additional studies testing the various proposed mechanisms in longitudinal human studies are required to provide deeper insight into the mechanistic link between these 2 related diseases. (Curr Probl Cardiol 2021;46:100638.)

Introduction

Inflammation and Cardiovascular Disease Overview

Despite major advancements in prevention and treatment, such as lipid control and blood pressure control, cardiovascular disease (CVD) continues to remain the leading cause of death worldwide, including the United States. Emerging evidence has demonstrated that inflammation plays an important role in CVD.[1,2] Therefore, targeting residual inflammatory risk may be just as important as targeting residual cholesterol risk for reducing CVD development.[1,2] Coronary artery disease (CAD) is now recognized as a complex inflammatory disorder, in which the immune system interacts with metabolic derangements and vascular injury.[3,4]

Inflammation has been shown to play a crucial role in the pathogenesis of atherosclerosis and atherothrombosis.[5] During the initial stages of atherogenesis, oxidized low-density lipoprotein cholesterol (LDL-C), injury, and infection cause monocytes to bind to the site of injury in the endothelial wall.[6] Monocytes mature into macrophages and eventually develop into foam cells as they consume oxidized lipids and lipoproteins.[6] Macrophages and foam cells release proinflammatory cytokines, activating vascular endothelial cells and recruiting additional leukocytes.[7] The increased activity of inflammatory cells contributes to the development of the coronary plaque, consisting of a lipid core, foam cells, and fibrous cap composed of collagen.[3] The plaque can then rupture, as macrophages release proteolytic enzymes that break down the collagen in the fibrous cap.[3] Plaque rupture releases tissue factors and atherosclerotic debris into circulation, leading to thrombosis and consequent myocardial ischemia and infarction.[8]

Studies have demonstrated a robust relationship between inflammation and the risk of CAD, as well as major CVD events. An elevated plasma level of high-sensitivity C-reactive protein (hs-CRP), an inflammatory biomarker, has not only been shown to be an independent risk factor for CAD, but also predicts future CVD events, regardless of LDL-C levels.[9,10] Clinically, hs-CRP has been shown to be the gold standard for assessing low-grade systemic inflammation, as it captures the upstream activity of the interleukin (IL)-1 to IL-6 inflammatory cytokine axis.[11] In intermediate CVD risk patients, the risk of future CVD events was lowered by hs-CRP reduction in addition to standard CVD risk factor control.[12] The JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial demonstrated that patients without hyperlipidemia but with elevated hs-CRP levels exhibited a reduced incidence of major adverse cardiovascular events (MACE) when treated with a statin.[13] Nevertheless, it is important to note in this study that LDL-C was also reduced relative to baseline, and therefore, the inflammatory contribution to MACE could not be completely elucidated (Fig 1 and Table 1).

Thus, targeting inflammation has been an attractive pharmacological strategy to decrease CVD risk. Two major clinical trials have increased our understanding of the value of this approach. The CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcome Study) demonstrated that inhibition of IL-1β with the monoclonal antibody, canakinumab, significantly reduced MACE, independent of lipid lowering, in patients with history of MI and elevated hs-CRP.[14] In addition, patients treated with canakinumab had reduced plasma levels of downstream inflammatory cytokines, IL-6 and hs-CRP from baseline.[14] Lastly, those who experienced the greatest reduction in hs-CRP also had the most significant reduction in CVD- and total mortality. In contrast, CIRT (Cardiovascular Inflammation Reduction Trial) showed that patients receiving a non-specific anti-inflammatory therapy with low-dose methotrexate did not have reduction in MACE and did not have reduced IL1-β, IL-6, and hs-CRP levels.[15] The conclusions of these recent trials suggest that targeted inflammatory therapy, namely inhibition of the IL-1 to IL-6 axis, may provide atherosclerotic protection and thereby help reduce the risk of MACE.

Inflammation as a driver of atherogenesis is evident in patients with chronic inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, and psoriasis. Patients with chronic systemic inflammatory disease have earlier onset and increased rates of CVD events when compared to that of the healthy

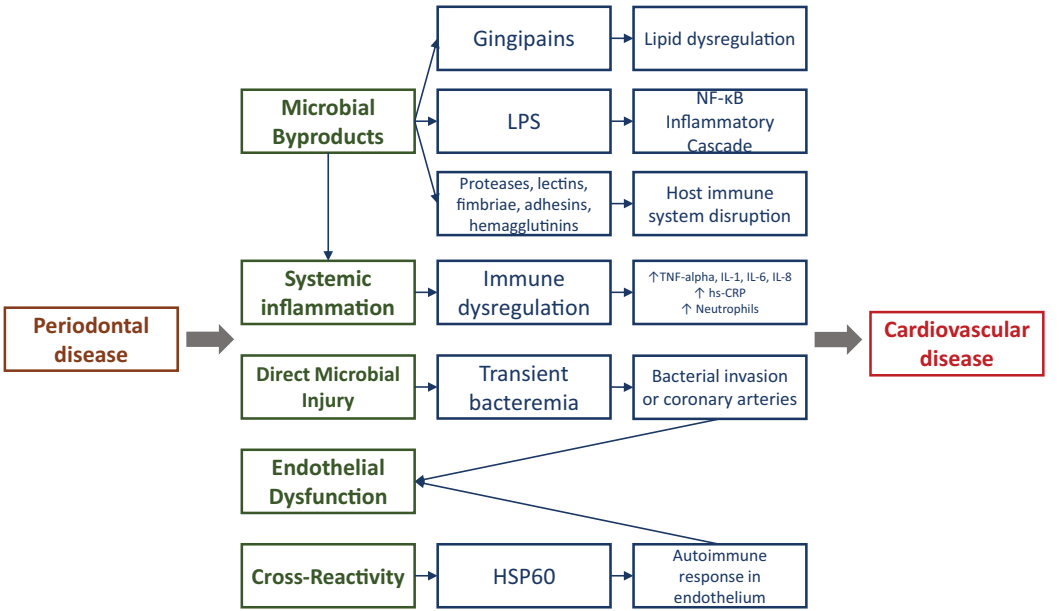

FIG 1. Schematic demonstrating proposed mechanisms linking periodontal disease to cardiovascular disease. Note: proposed mechanisms may overlap.

Table 1.

Topic	Author	Study design
Endothelial dysfunction		
Patients with severe PD and no known cardiovascular disease (CVD) or risk factors demonstrated significantly reduced brachial artery in flow-mediated dilation (FMD) when compared to healthy controls, not attributable to vascular smooth muscle cell dysfunction. No significant difference in FMD was found between patients with mild PD and healthy controls.	Amar et al., 2003[62]	Cross-sectional study
Patients with chronic PD and no known CVD have impaired endothelium-dependent dilatation (EDD) and endothelium-independent dilatation (EID) compared to healthy controls. The reduction in EDD and EID was improved following initial treatment for PD.	Mercanoglu et al., 2004[63]	Prospective observational study
Patients with chronic PD demonstrated reductions in FMD similar to those in patients with a history of a myocardial infarction.	Punj et al., 2017	Cross-sectional study
Periodontitis patients demonstrated impaired FMD as well as higher salivary levels of matrix metalloproteinase-2 complex compared to healthy controls, suggestive of endothelial dysfunction.	Moura et al., 2017[65]	Cross-sectional study
Patients with severe periodontitis demonstrated improvements in FMD, concurrent with treatment of their periodontal disease. The effects on CV risk reduction and events were not investigated.	Seinost et al., 2005[66]	Prospective observational study
Direct microbial injury from periodontal pathogens		
Pathogenic periodontal organisms, *P. gingivalis* and *P. intermedia*, invade and adhere to human coronary artery endothelial cells in vitro.	Dorn et al, 1999[71]	In vitro
P. gingivalis induce the expression of surface-associated molecules, including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and P- and E- selectins in human umbilical vein endothelial cells.	Khlgatian et al., 2002[72]	In vitro
Apolipoprotein E (ApoE) deficient mice subjected to an oral *P. gingivalis* infection developed periodontitis and demonstrated increased inflammatory cytokines, cholesterol, alveolar bone loss, and atherosclerotic lesions in the aorta, which improved after stimulation of nucleotide binding oligomerization domain-containing protein 2.	Li et al., 2002[74] Yuan et al., 2013[73]	In vivo
Treatment of *P. gingivalis* in infected mice resulted in fewer atheromatous lesions in the aorta and aortic tree compared to infected mice not receiving treatment. Additionally, mice infected with *P. gingivalis* with deficient fimbria demonstrated fewer atheromatous lesions compared to those with wild type *P. gingivalis*.	Amar et al., 2009[75]	In vivo

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Table 1. (*continued*)

Topic	Author	Study design
In ApoE deficient mice, polymicrobial oral infection with major periodontal organisms (*P. gingivalis, T. denticola, T. forsythia*) led to increased aortic plaque with increased macrophage presence as well as increased serum cholesterol and triglycerides.	Rivera et al., 2013[76]	In vivo
Evidence of periodontal pathogens (DNA, RNA, antigen) identified in atheromatous plaque samples and vascular walls in patients with PD.	Rath SK et al., 2014[77] Haraszthy VI et al., 2000[78] Ott SJ et al., 2006[79]	In vitro
Edentulousness and serum IgG antibodies to periodontal pathogens (*Actinobacillus actinomycetemcomitans* and *P. Gingivalis*) associate with coronary heart disease and stroke.	Pussinen PJ et al., 2003[80] Pussinen PJ et al., 2004[81]	Prospective observational study
Viable invasive *A. actinomycetemcomitans* and *P. gingivalis* identified in human atherosclerotic plaque, suggesting periodontitis involvement in CV disease.	Kozarov EV et al,. 2005[82]	In vitro
Injury and inflammation due to microbial byproducts		
Increased circulating levels of LPS associates with elevated cardiometabolic risk factors, including elevated C-reactive protein, diabetes, obesity, as well as CV events.	Kallio KAE et al., 2015[86]	Prospective observational study
High antibody response to periodontal pathogens (IgG angibodies to *A. actinomycetemcomitans* and *P. gingivalis*) independently predicts incidence of CV events over a 10-year follow-up period. LPS levels positively correlated with periodontal IgG antibody levels.	Pussinen PJ et al., 2007[87]	Prospective observational study
Subgingival microbial burden associates with and contributes to salivary and serum LPS levels. Additionally, LPS levels associate with stable coronary artery disease.	Liljestrand JM et al.,2017[85]	Cross-sectional study
Patients with history of myocardial infarction have a higher frequency of allele T in position -260 in the promoter region of the CD14 receptor gene of monocytes, which are activated by LPS from Gram-negative bacteria.	Hubacek JA et al., 1999[90]	In vitro
Single nucleotide polymorphism of allele T in position -159 in CD14 increases the transcriptional activity of the downstream NF-κB inflammatory pathway, which can synergize with CRP in activating the vascular endothelium.	LeVan TD et al., 2001[91]	In vitro

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Table 1. (*continued*)

Topic	Author	Study design
LPS shown to contribute to oxidation of low-density lipoprotein, foam cell maturation, and thrombogenesis.	Liao W. et al., 1996[92]	In vitro
Stimulation of *P. gingivalis* and LPS in human umbilical vein endothelial cells induced endothelial cell death and increased oxidized LDL, and TNF-α levels.	Bugueno IM, et al., 2016[93]	In vitro
P. gingivalis produces toxins and virulence factors, such as proteases, lectins, fimbriae, adhesins, and hemagglutinins that can interfere with signal pathway activation in host immune responses and potentially lead to CVD.	Zhou Q et al., 2006[94] Zhou Q et al., 2005[95] Guo Y et al., 2000[96]	In vitro
Gingipains shown to reduce LPS-induced IL-8 production in gingival fibroblasts and to cleave ICAM-1 on endothelial cells, allowing for immune invasion in periodontal tissues. Gingipain shown to cleave apoB-100, the main component of LDL that is responsible for allowing the binding of LDL to cell surface receptors.	Tada H et al., 2002[97] Tada H., 2003[98] Hashimoto M et al., 2006[99] Bengtsson T et al., 2008[100]	In vitro
Mutant gingipain, lacking either the Rgp- or Kgp gingipains, demonstrated unfavorable proteolytic effects on lipoproteins (LDL, VLDL, and HDL) by inducing reactive oxygen species and lipid peroxidation.	Lönn J et al., 2018[101]	In vitro
Cross-reactivity due to bacterial antigen		
T-cells observed in atherosclerotic plaque, suggesting involvement of T-cell immune response to *P. gingivalis* HSP60 in atherosclerosis.	Choi J-I et al., 2002[104]	In vitro
Cross-reactive T-cells recognizing HSP60 found in diseased periodontal tissue and peripheral blood.	Ford P et al., 2005[105]	In vitro
HSP60 antibody levels to both human and *P. gingivalis* were highest in patients with atherosclerosis. Clonal analysis demonstrated HSP60-reactive T cells that recognized both human and *P. gingivalis* HSP60 in these patients.	Yamazaki K et al., 2004[106]	Prospective observational study
Patients with mild periodontitis had increased levels of serum HSP60 and small, dense LDL when compared to controls matched for age and body mass index.	Rizzo et al., 2012[107]	Prospective observational study

Overview and summary of studies that elucidate potential mechanisms of periodontal disease and atherosclerosis.

population.[16-20] Moreover these patients specifically psoriasis on a biologic treatment (eg, anti-tumor necrosis factor (aka anti-TNF)-α, anti-IL-12, anti-IL-23, and anti-IL17) had favorable modulation of CAD with reduction of high risk plaque features such as noncalcified coronary plaque burden.[21,22] Observational cohorts of patients with rheumatoid arthritis on anti-TNF-α therapy experienced a reduced incidence of MACE.[23] These findings suggest that early treatment of systemic chronic inflammatory conditions may reduce the progression of subclinical CVD and potentially reduce the risk of future CVD events.

In addition to the systemic inflammatory conditions mentioned previously, periodontal disease (PD) is a chronic inflammatory disease with a microbiological etiology that increases the overall inflammatory burden. Given its inflammatory nature, PD may potentially contribute to atherosclerosis and CVD events. This review will explore the current mechanistic and clinical evidence supporting the role of PD in the development and contribution to CVD.

Periodontal Disease as a Systemic Inflammatory Condition

PD encapsulates a broad range of chronic inflammatory conditions that affect the gingiva, bone and periodontal ligaments supporting teeth.[24] According to the classification set by the American Academy of Periodontology and the European Federation of Periodontology, PD can be divided into 3 major categories with their respective subcategories:[25]

(1) Periodontal health and gingival diseases
    a Periodontal Health and Gingival Health
    b Gingivitis: Dental Biofilm-Induced
    c Gingival Diseases: Non-Dental Biofilm-Induced
(2) Periodontitis
    a Necrotizing Periodontal Diseases
    b Periodontitis
    c Periodontitis as a Manifestation of Systemic Disease
    d Periodontal Abscess and Endodontic-Periodontal Lesions
(3) Periodontal Manifestations of Systemic Diseases and Developmental and Acquired Conditions
    a Systemic Diseases or Conditions Affecting Periodontal Supporting Tissues
    b Mucogingival Deformities and Conditions
    c Traumatic Occlusal Forces
    d Tooth- and Prosthesis-Related Factors

PD is caused by the interaction between the oral flora and host immune system, which results in an inflammatory response and destruction of the supporting structures of the teeth (gingiva, bone and ligament).[24] Dental plaque is formed as the oral bacteria produce a microbial biofilm on the teeth.[26] Gingivitis ensues as bacteria in the dental plaque induce localized inflammation of the gingiva.[26] As gingivitis continues to progress, the loss of gingiva, bone and periodontal ligament leads to the development of deep periodontal pockets that are characteristic of chronic periodontitis, leading to systemic inflammation.[26]

Immune/Inflammatory Pathways Associated with Periodontal Disease

PD has features of immune dysregulation associated with chronic inflammation, including elevated plasma cytokines, such as IL-1, IL-6, and TNF-α in the gingiva and gingival fluid.[27] Inhibiting IL-1/TNF-α activity in animal models of PD resulted in reduced inflammatory cell recruitment and bone loss.[28] Clinically, patients with chronic periodontitis exhibit increased systemic inflammatory markers, including TNF-α, IL-1, IL-6, IL-8 and circulating neutrophils.[29,30] Treatment of periodontitis in these models led to reduced systemic inflammation, as measured by hs-CRP, over a 6-month period.[31] Mice infected with periodontal bacteria displayed alterations in their gut microbiota that were more conducive to systemic inflammation (eg, decreased tight junctions in the ileum).[32] However, it is important to note that oral pathogens were not detected in the gut microbiota of infected mice, and the exact mechanism by which gut microbiota would be altered due to PD was not fully understood. Studies have also suggested that locally produced CRP from PD may modulate systemic CRP.[33] However, the direct contribution of the locally increased CRP to increased systemic CRP levels has not been established.

Association of Periodontal Disease and Cardiovascular Disease

Multiple epidemiologic and observational studies have consistently demonstrated that PD is independently associated with subclinical, and clinical CVD across diverse populations.[34-36] Nevertheless, epidemiologic and observational studies are limited in that they do not support a causal relationship, and there is uncertainty regarding the magnitude of the influence of PD and future risk of atherosclerosis and MACE.[37,38]

Recently, a prospective observational study attempted to provide a more accurate assessment of the role of PD in CVD.[39] In a prospective cohort consisting mainly of middle-aged women, an increased incidence and prevalence of PD was significantly associated with an increased risk of developing future cardiovascular event.[39] Furthermore, patients who had acute coronary syndrome (confirmed via coronary angiography) demonstrated worse dental and periodontal health.[40] In a most recent case-control study, patients with moderate-to-severe periodontitis demonstrated significantly greater odds of developing myocardial infarction, suggesting a possible relationship between PD severity and CVD state.[41] Smoking is also risk factor for both periodontitis and cardiovascular disease.[42] Smoking contributes to a chronic inflammatory reaction, impairment in fibroblast function and tissue healing, all of which are involved with both PD and CVD. [42]

PD is linked to a multitude of cardiac and vascular pathologies. Periodontal bacterial burden was associated with carotid artery intima-media thickness (IMT), a subclinical marker of carotid atherosclerotic disease.[43] Treatment and improvement of periodontal status was associated with a reduced progression of carotid IMT at 3-year follow-up.[44] Periodontal disease has also been implicated in the progression of abdominal aortic aneurysm (AAA) with periodontopathic bacteria present in the samples of diseased AAA.[45,46] A recent systematic review of 5 studies demonstrated that presence of periodontal bacteria in the bloodstream or in situ in the vascular lesion was associated with AAA.[47] Periodontitis was also an independent predictor of arrhythmia-related CVD events in patients with atrial fibrillation.[48] A cross-sectional study demonstrated that patients with peripheral artery disease had worsened periodontal health and higher levels of systemic inflammation, including CRP and TNF-α.[49] Lastly, a murine study demonstrated that the injection of periodontal pathogens induced myocardial hypertrophy via activation of matrix metalloproteinases.[50]

Genetic Susceptibility to Periodontal Disease and Cardiovascular Disease

Genetic studies have suggested the existence of shared susceptibility genes that are involved in both the pathogenesis of CVD and PD.[51-53] One of the strongest and best replicated genetic CVD risk loci has been identified on human chromosome *9p21.3*; this locus has been confirmed by 4 independent genome-wide association studies and by a subsequent meta-analysis.[54-58] Studies have shown that 9p21.3 locus is also associated with PD, suggesting shared susceptibility effects in both CVD and

PD.[53,59] Nevertheless, the multifaceted and chronic nature of both diseases makes it difficult to establish a definitive causal relationship. Despite the increased evidence for an association between PD and CVD, the confirmation of a genetic link between the 2 pathologies continues to remain elusive.

Mechanisms of PD Involvement in CVD

Though no study has elucidated the exact underlying biological mechanisms by which PD contributes to atherosclerosis, multiple potential mechanisms link PD to CVD and MACE. These include: (1) endothelial dysfunction; (2) direct microbial injury from periodontal pathogens; (3) injury and inflammation due to microbial byproducts (namely, lipopolysaccharide and gingipains); and (4) immune cross-reactivity with bacterial antigens.

Periodontal Disease Leads to Endothelial Dysfunction

PD has been implicated in endothelial dysfunction, one of the initial steps of atherogenesis that provide valuable CVD risk stratification.[60] Flow-mediated dilation (FMD) is a robust method to assess for endothelial dysfunction in patients with subclinical CVD.[61] In one study, patients with severe PD without CVD or known CVD risk factors demonstrated significantly reduced brachial artery FMD when compared to age-matched healthy control subjects.[62] Furthermore, FMD in subjects with only mild PD was not significantly different from the control group.[62] These findings suggest that impairment of FMD and subsequent endothelial dysfunction may depend on PD severity. Other studies have confirmed the reduction in FMD in PD patients, with impairment of FMD similar to that seen in patients with a history of a MI.[63,64] Periodontitis patients with endothelial dysfunction also demonstrated elevated levels of salivary matrix metalloproteinase and tissue inhibitor of metalloproteinase complex.[65] Furthermore, patients with severe periodontitis demonstrated improvements in FMD when PD was treated, though the influence of treatment upon subsequent CVD risk reduction and MACE was not investigated.[66]

Microbes may Directly Induce Inflammation and Atherosclerosis

Bacteremia due to PD resulting in direct vascular injury has been shown to potentially contribute to atherosclerosis. Transient bacteremia

can occur due to tooth brushing, chewing, or after any dental procedure and is more frequent in patients with PD.[67,68] Chronic infections due to bacteremia may increase inflammatory burden required to accelerate atherogenesis.[69] In the setting of PD, gingival lesions and bleeding in periodontal pockets allow periodontal bacteria to enter the systemic circulation.[70]

Major pathogenic periodontal organisms, such as *Pophyromonas gingivalis* (*P. gingivalis*), have been shown to adhere and invade the coronary artery endothelial cells in vitro.[71] *P. gingivalis*, one of the most widely studied periodontal microorganisms, also induces the expression of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1, and P- and E- selectins in human umbilical vein endothelial cells.[72] Apolipoprotein E (ApoE) deficient mice with oral *P. gingivalis* infection moreover developed periodontitis and demonstrated increased plasma levels of inflammatory cytokines and cholesterol, along with alveolar bone loss and atherosclerotic lesions in the aorta.[73,74] In contrast, ApoE deficient mice with oral *P. gingivalis* infection that were treated with muramyl dipeptide to stimulate nucleotide binding oligomerization domain-containing protein 2 (NOD2) demonstrated a reduction in the same parameters. These results suggest that inhibition of NOD2 may play an important role in triggering a host immune response that is conducive to both periodontitis and atherosclerosis. In mice infected with *P. gingivalis*, treatment of the oral infection resulted in fewer atheromatous lesions in the aorta and aortic tree compared to those of infected mice not receiving antibiotic treatment.[75] Additionally, mice infected with *P. gingivalis* with deficient fimbria (bacterial component required for endothelial invasion) also had fewer atheromatous lesions compared to mice infected with wild type *P. gingivalis*.[75] These findings suggest that direct bacterial invasion of the vascular endothelium may be involved in accelerated atherosclerosis.

Polymicrobial oral infection of major periodontal organisms has demonstrated increased aortic plaque with increased macrophage presence, increased serum cholesterol, and increased triglycerides in ApoE deficient mice.[76] Furthermore, several studies have highlighted the presence of periodontal organisms (via DNA, RNA, antigens) in atheromatous plaque samples and vascular walls in patients, which may contribute to the progression of atherosclerosis.[77-79] In addition, edentulousness and serum IgG antibodies to periodontal pathogens (*Aggregatibacter actinomycetemcomitans* [*A. actinomycetemcomitans*] and *P. gingivalis*) were associated with CVD in humans.[80,81] While the majority of the studies assessed indirect markers of bacterial presence (eg, serology, presence of dead bacteria, DNA, RNA,

and antigen), one study demonstrated viable invasive *A. actinomycetemcomitans* and *P. gingivalis* in samples of human atherosclerotic plaque.[82] Despite mounting evidence suggesting a role for periodontal microbes in inducing atherosclerosis, no study to date has demonstrated that the bacteria themselves are the causative agents of atherosclerosis in humans. These studies suggest that the presence of periodontal bacteria in plaque may accelerate the process of atherogenesis. However, this tentative conclusion requires additional study and more definitive evidence.

Microbiota (including those of the oral cavity) of the human body play important roles in human disease. Probiotics have been suggested to have a positive impact on both the oral cavity and cardiovascular risk.[83] There is emerging evidence that probiotics may confer reduction in CVD progression as well as PD.[83] In a randomized, double-blind, placebo-controlled study, the use of probiotics demonstrate such has Lactobacillus salivarius WB21 reduced both plaque index and periodontal pocket depth in patients at risk of PD.[84] However, the effects of Lactobacillus salivarius WB21 on reducing CVD risk has yet to be established.

Microbial Byproducts may Play a Role in Inducing Systemic Inflammation

Microbial components and byproducts have also been implicated in inducing systemic inflammation and atherosclerosis. Lipopolysaccharide (LPS), also known as endotoxin, is the primary membrane wall component of gram-negative bacteria and is a major factor in sepsis. LPS has also been shown to be an important surface antigen and a potential proinflammatory mediator between PD and CVD.[85] High circulating levels of LPS (endotoxemia) is associated with elevated CRP and increased CVD events, when adjusted for traditional risk factors for CVD.[86] In the setting of periodontitis, a case-cohort study in the FINRISK 1992 cohort demonstrated that a high antibody response to periodontal pathogens (IgG antibodies to *A. actinomycetemcomitans* and *P. gingivalis*) independently predicted incidence of CVD events over a 10-year follow-up period.[87] Furthermore, LPS levels correlated positively with periodontal IgG antibody levels. [87] However, it is important to note that LPS levels can be derived from a variety other types of chronic infections such as *Helicobacter pylori* infection or bacterial translocation from the large intestine. A recent follow-up study has confirmed that subgingival microbial burden can associate with and contributes to salivary and serum LPS levels.[85] After adjusting for confounding factors, this study found that the association between PD and CVD may be due to elevated LPS levels,

suggesting that periodontitis leads to low-grade systemic inflammation that may contribute to a higher risk of CVD.[85]

LPS is released into the systemic circulation as gram-negative bacteria in the blood circulation are lysed. LPS is recognized by the host immune system by the LPS binding protein (LBP). Once LBP binds to LPS, the antigen is recognized by the CD14 co-receptor present on macrophages, neutrophils, and endothelial cells.[88] CD14 then complexes with toll-like receptor 4 (TLR4) and MD2 complex to activate the nuclear factor κB (NF-κB) inflammatory pathway.[89] The CD14 and TLR4/NF-κB inflammatory pathway has been associated with the pathogenesis of atherosclerosis and CVD events. Patients with history of a MI have a higher frequency of a single nucleotide polymorphism (C$\rightarrow$T) in position -260 in the promoter region of the CD14 receptor gene.[90] This polymorphism has been shown to increase the transcriptional activity of the downstream NF-κB inflammatory pathway, which can synergize with CRP in activating the vascular endothelium.[91] This finding suggests that certain immune reactions of inflammatory cells to infectious insults may play a role in atherosclerosis.[89] Additionally, LPS has been shown to contribute to LDL-C oxidation, foam cell maturation, and thrombogenesis, all of which are involved in the atherosclerotic process.[92] In vitro studies in human umbilical vein endothelial cells demonstrated that exposure to *P. gingivalis* and LPS induced endothelial cell death and increased oxidized LDL-C, and TNF-α levels.[93] Thus, the increased systemic levels of LPS due to chronic PD could potentially exert proatherogenic effects.

Periodontal bacteria also produce a wide variety of toxins and virulence factors, such as proteases, lectins, fimbriae, adhesins, and hemagglutinins that can disrupt the host immune response and consequently contribute to CVD. In human monocyte-derived macrophages exposed to *P. gingivalis*, cytokine antibody arrays demonstrated production of monocyte chemoattractant protein 1, macrophage inflammatory protein 1β, and MIP-3α.[94] Furthermore, purified *P. gingivalis* LPS and fimbrillin, a major fimbrial protein of the bacteria, produced patterns of cytokines suggesting that host immune cells may respond differently to live *P. gingivalis* compared to its components.[95]

Gingipains, cysteine proteases produced by *P. gingivalis*, demonstrate disruptive effects on inflammatory cells, coagulation pathway components, vascular permeability, and the complement system.[96] Gingipains reduced LPS-induced IL-8 production in gingival fibroblasts and cleaved ICAM-1 on endothelial cells, thereby allowing immune invasion in periodontal tissues.[97,98] Recent studies have also suggested that gingipains may play an important role in lipid dysregulation by cleaving apoB-100,

which is the main component of LDL-C that is responsible for binding LDL-C to cell surface receptors.[99,100] Disruption of genes involved in gingipain or inhibition of gingipain in *P. gingivalis* was found to attenuate atherosclerotic lesions in ApoE knockout mice.[99] The authors in the study suggest that gingipains, in particular the Arg-gingipain, play an important role in the degradation of apoB-100 in LDL-C particles, and thus are instrumental in development of atherogenesis. Gingipains also demonstrate unfavorable proteolytic effects on lipoproteins by inducing reactive oxygen species and lipid peroxidation.[101] Conclusions derived from these study suggest a potential mechanism linking virulence factors from gingival infections to deranged lipid profiles and a pro-inflammatory pathway leading to accelerated atherogenesis .

Molecular Mimicry/Cross Reactivity as Potential Mechanisms of Atherogenesis

Cross-reactive autoantibodies against bacterial antigens are also suggested as a possible mechanism by which periodontal infections promote atherosclerosis, particularly those against heat-shock protein (HSP)60. HSP60 is not only highly conserved with high homology between prokaryotes and eukaryotes, but also is highly immunogenic.[102] The proposed mechanism involves endothelial damage from an immune response to bacterial HSP. Cross-reactivity between *P. gingivalis* and human HSP60 was capable of promoting atherosclerotic changes due to the subsequent autoimmune response in the vascular endothelium.[103] This study demonstrated that T-cell immune responses to *P. gingivalis* HSP60 may be involved with atherosclerosis, since *P. gingivalis* HSP-specific T-cells were observed in atherosclerotic plaque in the laboratory.[104] T-cells recognizing HSP60 were also found in diseased periodontal tissue and peripheral blood from patients.[105] In another study, HSP60 antibody levels to were highest in patients with atherosclerosis.[106] Clonal analysis demonstrated HSP60-reactive T cells from these patients recognized both human and *P. gingivalis* HSP60.[106] In a cross-sectional study, patients with mild periodontitis had increased levels of anti-serum HSP60 and of small, dense LDL-C when compared to controls matched for age and body mass index.[107]

Conclusions

There is overwhelming evidence in the current literature that supports an association between PD and CVD. Multiple observational studies

have found that PD associates with CVD, independent of known CVD risk factors. However, the supporting evidence that PD directly causes CVD in humans continues to remain elusive. Multiple biologically plausible mechanisms have been proposed and investigated, yet most studies are limited to mouse models and in vitro cell cultures. Additional studies testing the various proposed mechanisms in longitudinal human studies are required to provide deeper insight into the mechanistic link between these 2 related diseases. Current studies have only assessed how periodontal interventions affect markers of systemic inflammation (eg, hs-CRP) and markers of subclinical CVD (eg, endothelial dysfunction). Whether treatment of PD reduces the incidence and/or severity of CVD and MACE remains to be established. A randomized clinical trial involving standardized definitions of PD and standardized periodontal interventions is required to demonstrate that PD treatment is beneficial to CVD risk reduction. In particular, future studies could benefit from expanding on how decreases in markers of systemic inflammation and endothelial function from periodontal therapy affect the incidence of CVD events. With increasing investigation of the role of PD in CVD, PD may be recognized as an important CVD risk factor.

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Conflict of Interest

All authors declare no potential conflicts of interest related to this article.

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