Review

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Oral infections and cardiovascular disease

Karim El Kholy[1,2], Robert J. Genco[3], and Thomas E. Van Dyke[1]

[1] Forsyth Institute, Cambridge, MA 02142, USA
[2] Harvard University, Cambridge, MA 02138, USA
[3] State University of New York at Buffalo, Buffalo, NY 14226, USA

Oral infections are the most common diseases of mankind. Numerous reports have implicated oral infections, particularly periodontitis, as a risk factor for atherosclerotic cardiovascular disease (CVD). In this review we examine the epidemiology and biologic plausibility of this association with an emphasis on oral bacteria and inflammation. Longitudinal studies of incident cardiovascular events clearly show excess risk for CVD in individuals with periodontitis. It is likely that systemic exposure to oral bacteria impacts upon the initiation and progression of CVD through triggering of inflammatory processes. Given the high prevalence of periodontitis, any risk attributable to future CVD is important to public health. Unraveling the role of the oral microbiome in CVD will lead to new preventive and treatment approaches.

How strong is the evidence linking oral infections to cardiovascular diseases?

The most common oral infections are periodontal diseases (gingivitis, periodontitis, see Glossary) and dental caries (tooth decay). Teeth are surrounded and anchored to the alveolar process of the jaws by an attachment apparatus comprising the most outer calcified layer of the tooth root (cementum), connected to bone by highly organized collagen fibers called periodontal ligaments. Systemic exposure to dental infections often results from the involvement of these supporting structures in infectious processes that create direct bacteremia. Dental caries affects the hard surfaces of teeth and, as such, does not lead to systemic exposure to bacteria. However, if left untreated, dental caries progresses to the dental pulp (nerves and blood vessels of the tooth), leading to a root canal infection that spreads to the supporting structures including the bone, and significantly increases the level of systemic exposure [1].

Periodontal diseases are multifactorial chronic inflammatory diseases, characterized by progressive destruction of the supporting structures of multiple teeth. The process begins as an inflammation localized to the soft tissues (gingivitis), caused by a resident biofilm (plaque) that forms on tooth surfaces at the gingival margin (gum line). Gingivitis is a reversible condition and good oral hygiene helps to remove the resident biofilm. In the presence of gingivitis, any oral manipulation, such as chewing, tooth cleaning and tooth brushing can lead to a transient bacteremia [1,2]. In some but not all cases, untreated gingivitis will progress to periodontitis. Periodontitis is characterized by a destruction of connective tissue, periodontal ligament, and bone, and this is generally irreversible and significantly increases the level of systemic exposure. An estimated 49% [3] of the population suffers from generalized (involving more than a third of the dentition) periodontitis, with 80–90% having a milder form of disease [4].

Glossary

Actinobacillus actinomycetemcomitans: also known as *Aggregatibacter actinomycetemcomitans*, a Gram-negative bacterium found in association with periodontitis that is considered to be one of the species that might be implicated in a destructive form of periodontitis.
Dental caries: also known as tooth decay, cavities, or caries, the breakdown of teeth due to bacteria.
Gingival sulcus: the space between a tooth and the surrounding gingival tissue, and is lined by sulcular epithelium.
Lipoxins: a class of eicosanoids that are produced by lipoxygenase-mediated metabolism of arachidonic acid and that have potent biological activities in the resolution of inflammation.
Periodontitis: an inflammatory disease of the tissues supporting the teeth (gingiva, alveolar bone, periodontal ligament, and cementum) that is induced by bacterial deposits on the teeth (plaque).
Phagocytosis: the process by which a cell (a phagocyte) engulfs a solid particle to form an internal vesicle known as a phagosome. Phagocytosis was first described by Élie Metchnikoff in 1882.
Phagocytes: the cells that protect the body by ingesting (phagocytizing) harmful foreign particles, bacteria, and dead or dying cells.
Porphyromonas gingivalis: a Gram-negative, anaerobic, pathogenic bacterium of the phylum Bacteroidetes. It is found in the oral cavity, where it is implicated in particular forms of periodontal disease. *P. gingivalis* has also been implicated in disease of the upper gastrointestinal tract, respiratory tract, and in the colon, and has been linked to rheumatoid arthritis.
Resolution of Inflammation: refers to the active termination of the acute inflammatory process to prevent chronic inflammation and damage to host tissues. There are several pathways of active resolution including down-regulation of proinflammatory molecules, apoptosis (programmed cell death) of inflammatory cells, and production of lipid mediators of resolution from polyunsaturated fatty acids. The lipid mediators of resolution of inflammation include lipoxins derived from endogenous arachidonic acid, and the resolvins, protectins, and maresins that are derived from ω-3 fatty acids.
Resolvins: lipid mediators that are induced in the resolution phase following acute inflammation. They are synthesized from the essential ω-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid.
Transcriptome: the set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNAs that are produced in one or a population of cells.
Transcriptomics: the study of the complete set of RNAs (transcriptome) encoded by the genome of a specific cell or organism. It is therefore a global way of looking at gene expression patterns in specific conditions.

Corresponding author: Van Dyke, T.E. (tvandyke@forsyth.org).

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CVDs include coronary heart disease, stroke, congestive heart failure, and peripheral artery disease. CVD is a chronic condition that can lead to life-threatening acute clinical events including myocardial infarction and stroke. Although the latest report by the American Heart Association on heart disease and stroke statistics showed a continued decline over the past 30 years in deaths attributable to CVD [5], the condition was still responsible for almost one third of deaths in the USA, with one person dying every 40 s of atherosclerotic vascular disease (ASVD) [5]. The above numbers highlight the fact that the burden of ASVD remains high, and, although major progress has been achieved by successful efforts in controlling known risk factors, including hypercholesterolemia, hypertension, smoking, and sodium intake, additional knowledge of the etiology and new risk and contributing factors is likely to have a public health benefit.

The high prevalence of periodontitis and CVD [3–5] makes it difficult to assess the relationship between these conditions when they occur in the same individual. Nonetheless, there is now significant epidemiological evidence for an association of oral infections, particularly periodontitis, and incident atherosclerotic CVD, including coronary heart disease, cerebrovascular disease, and peripheral vascular disease [6]. Statistically significant excess risk for atherosclerotic CVD in people with periodontitis was reported in several well-controlled studies that was independent of established risk factors. Increased risk was stratified based on factors such as type of CVD outcome, age and gender. For instance, periodontitis imparts greater risk for stroke than coronary heart disease; risk is greater in men and in younger people. The latter is a universal finding, and the strength of associations of all risk factors weakens in older populations and essentially disappears after age 65 ([6–10] for recent reviews).

Overall, there is consistent and relatively strong epidemiologic evidence linking periodontitis to CVD. The biologic possibility is supported by animal and clinical studies that will be further discussed below.

Mechanisms linking oral infections to CVD

Inflammation plays a major role in both oral infections such as periodontitis and in CVD [11–14]. Oral inflammation is induced by bacteria in biofilms comprising the microbiome that forms on the teeth [15]. Vascular inflammation is induced by hyperlipidemia, hypertension, smoking, and several other known and unknown factors. In the following sections we consider the recent evidence for the role of oral infections in CVD as well as the direct and indirect actions of oral bacteria through stimulation of local and systemic inflammation.

Direct actions of oral bacteria on the vasculature

The surface area of ulcerated epithelium lining periodontal pockets in generalized periodontitis patients has been estimated to be between 8–20 cm^2 [16]. The bacterial burden in the gingival sulcus approximating the ulceration provides a portal for bacterial entry into systemic circulation, resulting in bacteremia. As a result of this interaction, the interface is heavily populated by phagocytic cells that can also transport bacteria to remote sites [17].

Box 1. List of 'proofs' that must be fulfilled to demonstrate that periodontal bacteria are a contributing factor to atherosclerosis

(i) Periodontal bacteria can reach systemic vascular tissues.
(ii) Periodontal bacteria can be found in the affected tissues.
(iii) Evidence of live periodontal bacteria at the affected site.
(iv) *In vitro* evidence of invasion of affected cell types.
(v) Demonstration that periodontal bacteria can promote atherosclerosis in animal models of disease.
(vi) *In vitro* and *in vivo* evidence that non-invasive mutants cause significantly reduced pathology (animal model).
(vii) Fulfill modified Koch's postulate to demonstrate that a human atheroma isolate causes disease in animal models.

It has been suggested that fulfillment of proofs (i)–(vi) provides support that periodontal pathogens can contribute to atherosclerosis [8].

The contribution of periodontal bacteria to the atherosclerotic pathway was recently reviewed by Reyes and coworkers [8]. A series of studies beginning in 2000 [18] reported genomic DNA of several periodontal bacteria in human atheromas. These studies were replicated and extended by others who found multiple bacterial species in atheromas. Eventually, human atherosclerotic plaques were shown to harbor viable invasive *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* [19]. These studies showing localization of oral organisms in atheromatous plaques led to a large number of mechanistic and animal model studies directed to evaluating seven 'proofs' that have been proposed as necessary to demonstrate that periodontal bacteria are a contributing factor in atheromatous disease [8]. Studies supporting the first six are summarized in the 2013 review by Reyes *et al.* [8], and the seven proofs are listed in Box 1. The seventh, to demonstrate that a human atheroma microbial isolate causes atherosclerotic disease in animal models, has yet to be fulfilled successfully. Hence, although there is strong evidence supporting a role for periodontal bacteria in atherosclerotic disease, conclusive evidence is not available.

A recent study [20] of the microbiome of atherosclerotic plaques demonstrates that 84 different bacterial taxa were detected in the atheromas and vascular walls of patients with atherosclerotic vascular disease and periodontal disease, while 18 different taxa were identified in the vascular tissues from patients with vascular disease and little or no periodontal disease. Several bacterial taxa commonly found in the oral cavity were detected in diseased vascular tissues, including *Streptococcus, Prevotella*, *Capnocytophaga, Veillonella*, and *Porphyromas* species, suggesting that the oral cavity may be one source for dissemination of bacteria to vascular tissue. Other sources proposed to contribute are the skin and gut [20].

At least two key questions remain: (i) how do these organisms migrate to the vascular sites, and (ii) what is the role of these organisms, if any, in the pathogenesis of atherosclerosis?

Evidence is beginning to accumulate suggesting that in, addition to acute bacteremias associated with dental therapy and other procedures where the mucosal flora are introduced into the bloodstream, there is chronic seeding of bacteria into the bloodstream from other sources. A

recent study proposes that dendritic cells phagocytose periodontal pathogens from diseased pockets and carry them through the bloodstream until they are deposited in vascular sites [17]. The study followed healthy as well as periodontitis patients with or without ASVD, and demonstrated that blood myeloid dendritic cells carry known periodontal pathogens such as *P. gingivalis* and other oral species, and that the number of these carrier cells was highest in the group who suffered from both ASVD and periodontitis simultaneously. The study proposed that blood dendritic cells play a significant role in disseminating oral organisms to atherosclerotic plaques, and that these organisms provide key signals for dendritic cell differentiation and atherogenic conversion once they enter the atheroma. Furthermore, parallel *in vitro* studies of cultured *P. gingivalis* isolated from atherosclerotic plaques found them surviving for up to 24 h inside dendritic cells, while they are rapidly killed when outside dendritic cells by polymorphonuclear leukocytes (PMNs) [17].

In summary, there is evidence for two modes of invasion of cardiovascular tissues by periodontal bacterial species, in other words bacteremia and phagocyte-mediated modes of bacterial translocation from the periodontal lesion to the vascular tissues. Bacteremia-related bacteria invade the endothelial layer with production of proinflammatory cytokines such as monocyte chemoattractant protein 1 MCP-1 [21]. In the second mode, transmigrating phagocytes harboring oral bacteria are proposed to disseminate to atheromas, where they contribute to the growth of the atheroma. Further evaluation of these pathways and their specific contribution to atheroma formation is necessary to better understand the role of bacteria in atheromas.

Oral infections and systemic inflammation

A clear distinction should be made between active bacterial invasion and an infectious condition that triggers inflammatory disease. Once the inflammatory cascade has been instigated, the elimination of the initiating factor might not be sufficient to stop the series of events that follow. In this context the initial infection becomes an indirect contributing factor (Figure 1). The colocalization of bacteria (initially thought to be *Chlamydia* species, but recent evidence suggests many species [20]) in atheromas may be a sufficient stimulus to initiate an inflammatory cascade leading to atheroma formation. Several antibiotic intervention trials were initiated to support or refute the theory that bacteria directly cause CVD [22,23]. The impact of azithromycin treatment was prospectively evaluated in coronary heart disease patients that tested positive for *Chlamydia* [22,24]. In this study, 302 patients were randomized to azithromycin regimen or placebo for 3 months. Although more systemic markers of inflammation were reduced in the azithromycin group, no significant differences in the incidence of cardiovascular events was noted. While the trial is generally considered negative, because there was no impact on the progression of coronary artery disease, the possibility that oral bacteria play a role as an additional risk factor cannot be ruled out. It may be that, in this trial, the bacteria disseminated to the atherosclerotic plaques are protected from the antimicrobial effects of antibiotics by blood-borne phagocytic cells.

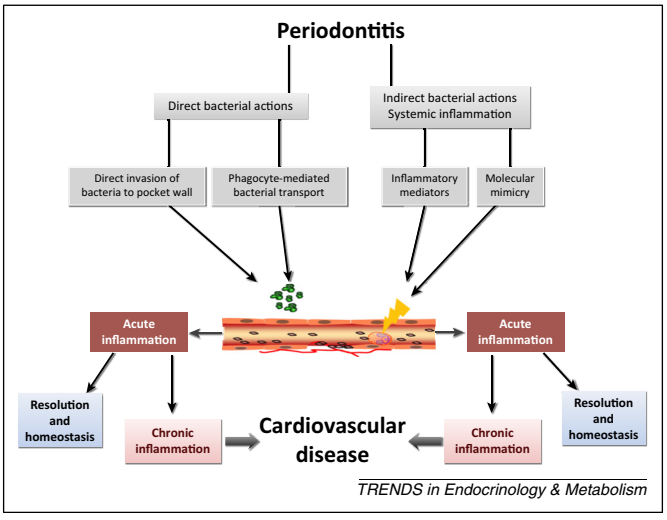

Figure 1. Proposed mechanisms linking periodontitis and cardiovascular disease (CVD). The illustration shows vascular injury or infection that induces inflammation of the large vessel wall initiating the atherogenic process. In the presence of functioning resolution of inflammation programs, natural mediators of resolution mediate rapid and complete healing and return to homeostasis. Failure of complete resolution leads to chronic inflammation, with the accumulation of macrophages in the tunica intima, lipid (low-density lipoprotein – LDL) accumulation, and the formation of foam cells that, with time and persistent inflammation, form the atherosclerotic lesion.

Reports of an epidemiologic association of periodontal disease and CVD are based on the same idea that periodontal bacteria could be an important cofactor in CVD [25–27]. Oral bacteria have been demonstrated in atheromas [21], but antibiotic therapy would be expected to have an impact on disease initiation associated with oral bacteria if indeed the bacteria were causal. Supporting the concept that bacteria are an etiologic cofactor, Richardson reported that an infectious agent at a distant site can lead to activation of inflammation and can accelerate atherosclerosis in hypercholesterolemic rabbits that had concomitant respiratory tract infections with *Pasteurella multocida* [28]. Infectious agents as an indirect etiologic factor for CVD cannot be dismissed. However, the pathogenesis of atherosclerosis is mainly inflammatory, and the role of bacteria as initiators of inflammation has been established.

In this context, the plausible link between periodontitis and atherosclerotic disease is inflammation. Recent findings suggest that a local inflammatory nidus can increase vascular inflammation systemically. Recent papers [27,29] also suggest that an elevated innate host response of any origin is a risk factor for CVD, as well as for periodontitis, suggesting the inflammatory response as a common susceptibility determinant. It was noted in cross-sectional studies that periodontal inflammation and carotid inflammation cosegregate in cardiovascular patients and that the degree of inflammation was predictive of cardiovascular outcome [30]. In a follow-up study, lowering of inflammation with statin therapy reduced both cardiovascular and periodontal inflammation in the same patients [14]. A relatively recent report suggested that initiation of isolated inflammation in the murine dorsal air pouch model resulted in upregulation of cyclooxygenase 2 (COX2) mRNA in the heart and lung

[31], supporting the hypothesis that local inflammatory foci have a wide-ranging systemic impact.

Human studies linking periodontitis and CVD to date are generally cross-sectional; however, several longitudinal studies have shown that periodontitis increases the risk for subsequent CVD. Intervention studies in humans are impractical because of time and expense. Hence, most reports investigating mechanism and biologic plausibility have been performed in rabbits and mice. In these studies, rabbits develop significant lipid deposits and exhibit atherogenic changes in the aorta over a 13 week period when fed a 0.5% cholesterol diet. Local periodontal inflammation induced by *P. gingivalis* dramatically increases lipid deposition in this model [32,33]. Similar results have been reported in apolipoprotein E (ApoE)-deficient mice [34–36]. The data support a role for periodontitis, as a local, chronic inflammatory insult in the progression of CVD, as well as the role of inflammation in the initiation and progression of both periodontitis and CVD. Intervention studies in humans and animals, to date, have been limited to assessing cardiovascular and periodontal outcomes with interventions that control inflammation. In these studies the method of control of inflammation is of paramount importance when considering the significant negative cardiovascular impact of COX inhibitors. New discoveries of pathways of programmed resolution of inflammation have opened the door for modification of inflammation using natural pathways [37]. The proinflammatory mediators include the classic eicosanoids, prostaglandins (PG) and leukotrienes (LT), whereas the more recently identified and characterized pro-resolution mediators include lipoxins (LX), molecules that are derived from arachidonic acid, and the novel resolvins (Rv) and protectins from the ω-3 essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively. Together, these local mediators constitute a new genus of endogenous anti-inflammatory and pro-resolving compounds that have proved to be very potent in treating several inflammation-associated models of human disease [38–42], including arthritis [40], colitis [43], peritonitis [44,45], asthma [41,46,47], dermatitis [48], infantile eczema [49], diabetic wounds [42,50], and retinopathies [51,52]. Of direct relevance, the resolvin RvE1 reduces oral inflammation in rabbit periodontitis [53], with regeneration of associated bone loss [54]. After induction of bone loss and inflammation using ligature and *P. gingivalis* applications around rabbit teeth, RvE1 was topically applied directly to study teeth three times weekly for 6 weeks. Control sites exhibited profound inflammatory infiltrate, loss of collagen, and loss of bone, while in the study group there were no inflammatory changes, osteoclast formation, or bone loss, with nearly complete regeneration of hard and soft tissue lost to disease. The implications of these findings are compelling because they suggest that inflammation plays a major role in the composition of the microbiota in the biofilm and that the composition can be manipulated with control of inflammation. More importantly, the regeneration of tissues, particularly bone, lost due to disease is an extremely difficult clinical problem. The results of these experiments imply that natural control of inflammation with endogenous agonists (resolvins) creates an environment permissive for spontaneous regeneration.

The resolvins and protectins were discovered in the Serhan laboratory [44,55] using an unbiased systems approach to study acute inflammation and self-resolving inflammatory exudates [56,57]. In resolving exudates, lipid mediator (LM) production is temporally dissociated. First, PG and LT appear with neutrophil entry, followed by LX and Rv biosynthesis, and then resolution with neutrophil apoptosis and clearance [46,55,58]. Although the concept of resolution of disease is well appreciated by clinicians [59,60], resolution of tissue inflammation was considered to be a passive process. The uncovering of pro-resolution mediators [44,54,61,62] offers many opportunities for targeting novel therapeutic strategies [63,64] and for new insights into disease pathogenesis (for a recent international consensus report, see [65]). LX and resolvins can control inflammation by stimulating resolution without immune suppression.

Resolution pathways probably account for the anti-inflammatory properties of statins [66] because statins boost the blood levels of lipoxins and resolvins. High-dose atorvastatin therapy in humans [14], and resolvins and lipoxins in animal models [33,53,54], prevent both periodontal and cardiovascular inflammation and also reverse existing disease. These studies are noteworthy because they demonstrate three important principles. The association of cardiovascular inflammation and periodontal inflammation in the patient population is clear. The actions of statins to resolve inflammation are also clear, and the degrees of reduction of carotid inflammation and periodontal inflammation correlate well. Importantly, these studies in humans, and others in animals, also support the suggestion that a local inflammatory insult can have major systemic impact, and vice versa.

Opportunities for better understanding the association of oral infections with atherosclerotic diseases

Intervention trials

Intervention trials perhaps offer the strongest evidence for a direct relationship between periodontitis and CVD because they attempt to address the impact of periodontal therapy on CVD indicators. There are no studies that evaluate the impact of treating periodontal disease on primary events (first myocardial infarction – MI), and only a feasibility study that examined secondary events (preventing second MI) has been published [67]. Several studies have shown that periodontal treatment can impact upon other known risk factors for CVD. For instance, treatment of periodontitis has been shown to lower C-reactive protein (CRP) levels and to improve both clinical and surrogate measures of endothelial function (reviewed in [10]). Intervention trials also offer avenues to understand more about the magnitude of proposed mechanisms that link periodontal and CVD, by intervening to control the suspected mechanism and observing the impact on the magnitude of link. The use of antibiotic intervention trials specifically targeting microorganisms isolated from atherosclerotic plaques has helped us to understand more about the lack of magnitude of the direct infectious etiology theory as a link between periodontal disease and ASVD. By

contrast, new discoveries of pathways of programmed resolution of inflammation have opened the door for modification of inflammation using natural pathways without a direct effect on the bacterial infection.

Animal studies

Animal studies offer an excellent, and easier to control and replicate, alternative to human intervention trials, which are often hard to organize because of logistical or financial constraints. Furthermore, as seen above, animal models offer a great opportunity to study in depth and confirm pathophysiological mechanisms and experimental treatments that are often impossible to perform on patients for ethical concerns. Studies in experimental animals have provided support for the hypothesis that molecular mimicry is a potential mechanism connecting periodontitis and CVD. Molecular mimicry is thought to occur when sequence similarities between foreign and self peptides produce cross-activation of auto-reactive T or B cells that can lead to tissue pathology or autoimmunity. Crossreactive autoantibodies to periodontal bacterial lipopolysaccharides and heat-shock proteins (HSPs) have been identified and suggested as a potential explanation for the putative relationship between periodontal disease and ASVD [68,69]. Different experimental models in dogs, mice, rabbits, and monkeys have demonstrated that oral infections can induce HSPs in periodontitis and CVD [70]. HSPs had demonstrated ability to trigger an immune response cascade leading to an accelerated rate of atherosclerosis [70].

A recent *in vitro* study demonstrated that dental infection with *P. gingivalis* increased endothelial injury in obese mice [71]. Aortal tissues studied 6 weeks post-infection were found to have an increased number of apoptotic (TUNEL-positive) cells compared to non-infected controls.

Immunohistochemical analysis detected *P. gingivalis* deep in the smooth muscle of the aorta, and the number of *P. gingivalis* cells in the aortal wall was higher in obese mice than in control mice. The results highlight the complexity and importance of studying the potential modification of the link between periodontal disease and CVD in the presence or absence of other known risk factors.

Future research recommendations

Further studies will help answer outstanding questions (Box 2) and to enhance our understanding of the link between periodontal disease and CVD. The generalizability of epidemiological evidence of an association is weakened by the heterogeneity of data collected. Future studies with standardized treatments, measurements, diagnostic criteria, and definitions of disease and health will lead to a more consistent pool of data and stronger evidence. In addition, lack of information regarding the temporal relationship of the exposure to periodontal disease and ASVD development is striking, likely due to the very long observation times required. The vast majority of trials are cross-sectional and association studies by design. Therefore, the impact of exposure to periodontal disease on ASVD markers over a period of time is still poorly understood. Prospective studies that monitor periodontitis and commonly used biomarkers for ASVD over time, and their relationship to common endpoints, are necessary to improve risk estimation. Furthermore, intervention trials comparing the effectiveness of different periodontal treatment approaches in modulating ASVD biomarkers are still needed. Understanding natural pro-resolution pathways and the use of pre-resolving molecules in the treatment of inflammation offer a unique opportunity to conduct interventional trials aimed at controlling the inflammation arm of the proposed periodontitis–CVD link without antimicrobial intervention. This can further clarify the contribution of each in the pathophysiology of atheromas and open doors to new therapeutic approaches in the treatment of both diseases.

Box 2. Outstanding questions

- Are there currently unidentified mechanisms by which bacteria are transported to vascular sites?
- Does the presence of inflammation influence the phenotype and pathogenicity of bacteria deposited in atherosclerotic plaques?
- Does the treatment of periodontitis influence the occurrence of primary myocardial infarctions?
- Does the use of recently identified and characterized anti-inflammatory pro-resolution mediators in the treatment of periodontitis impact on the onset or severity of CVD?
- Is there a reverse relationship between periodontitis and CVD? Can the onset of CVD influence the presence or progression of periodontal disease?
- How important is the pathogenic phenotype when the bacteria enter the circulation on the interaction with endothelium.

Concluding remarks

As we move forward, a clearer picture is emerging of how oral bacteria can have a significant impact on systemic inflammation and perhaps initiate local vascular lesions leading to CVD. Of particular interest is the dynamic relationship between inflammation and the oral microbiome, and the potential for modification of bacterial virulence both locally and systemically.

We now have the tools to make significant advances in our understanding of this relationship rapidly, and an opportunity to investigate the functions and events regulated by specific gene expression. In a candidate gene association study, Schaefer *et al.* identified a shared genetic susceptibility locus associated with both periodontitis and CVD [72]. They confirmed the known association of two neighboring linkage disequilibrium regions on human chromosome 9p21.3 with CHD, and showed additional strong association of these loci with the risk of aggressive periodontitis. The two associated linkage disequilibrium regions map to the sequence of the large antisense noncoding RNA in the *INK4* locus (ANRIL), which partly overlaps regulatory and coding sequences of the *CDKN2A/CDKN2B* locus, which is strongly associated with CHD. The elucidation of the interplay of ANRIL transcript variants, and their involvement in increased susceptibility to the interactive diseases CHD and periodontitis, promises new insight in the underlying shared pathogenic mechanisms of these complex common diseases. The development of cDNA microarrays represented a major step in the study of high-throughput gene expression, allowing the first transcriptomic studies to be performed [73]. The limitation of the method was that results were more qualitative than quantitative. Today, with the

development of next-generation high-throughput sequencing, we can now obtain accurate direct counts for a given transcript, a method that in theory has an infinite dynamic range. In addition, we can now analyze the full transcriptome, including transcripts from non-coding regions, thereby increasing the power of the method to a point where prokaryotic (bacteria) RNA expression and human RNA expression can be monitored simultaneously in the same sample [74]. For example, recent transcriptomic analyses of the oral microbiome in health and disease reveals marked upregulation of virulence factors in organisms from periodontitis subjects versus health [75]. Strikingly, virulence factors are upregulated in commensal organisms that are not usually considered pathogens. The implications of these findings are far-reaching. At least two interactions are at play. Local inflammation in periodontal disease is known to impact upon the composition and pathogenicity of the flora [54]. Can this be taken one step further to suggest that, in the presence of a permissive inflammatory milieu, bacteria influence the phenotype of each other, thereby greatly enhancing pathogenic potential? How important is the pathogenic phenotype when the bacteria enter the circulation in the interaction with endothelium? Unraveling these and other mechanisms of action of the microbiome on the vasculature may lead to new approaches to prevent and modify cardiovascular diseases.

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