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Review article

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The oral microbiome in the pathophysiology of cardiovascular disease

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Abstract

Despite advances in our understanding of the pathophysiology of many cardiovascular diseases (CVDs) and expansion of available therapies, the global burden of CVD-associated morbidity and mortality remains unacceptably high. Important gaps remain in our understanding of the mechanisms of CVD and determinants of disease progression. In the past decade, much research has been conducted on the human microbiome and its potential role in modulating CVD. With the advent of high-throughput technologies and multiomics analyses, the complex and dynamic relationship between the microbiota, their 'theatre of activity' and the host is gradually being elucidated. The relationship between the gut microbiome and CVD is well established. Much less is known about the role of disruption (dysbiosis) of the oral microbiome; however, interest in the field is growing, as is the body of literature from basic science and animal and human investigations. In this Review, we examine the link between the oral microbiome and CVD, specifically coronary artery disease, stroke, peripheral artery disease, heart failure, infective endocarditis and rheumatic heart disease. We discuss the various mechanisms by which oral dysbiosis contributes to CVD pathogenesis and potential strategies for prevention and treatment.

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Key points

- The incidence and prevalence of cardiovascular diseases (CVDs) are increasing despite advances in our understanding of their pathophysiology and an expanded arsenal of treatment options.
- An association between the oral microbiome (or oralome) and cardiovascular inflammation and CVD is supported by a growing body of epidemiological studies, systematic reviews and basic science investigations.
- Validated links exist between oral dysbiosis and CVDs, including atherosclerotic diseases, heart failure, infective endocarditis and rheumatic heart disease.
- The mechanisms by which oral dysbiosis contributes to CVD include immunomodulation; endothelial dysfunction; molecular mimicry and antibody cross-reactivity; protein citrullination; platelet activation, aggregation and thrombogenesis; arterial invasion; and systemic inflammation.
- Targeting oral dysbiosis in a clinical setting could be an important component of CVD management.

Introduction

The global prevalence of cardiovascular disease (CVD) almost doubled from 271 million in 1990 to 523 million in 2019 (ref. 1). Deaths from CVD increased from 12.1 million in 1990 to 18.6 million in 2019, and global trends for disability-adjusted life-years (DALYs) and years of life lost related to CVD also increased substantially during the same period[1]. Moreover, the prevalence of CVD is likely to increase substantially over the next few decades, particularly in Northern Africa, Western Asia and Latin America, where ageing of the population is expected to lead to a doubling in the number of people aged >65 years between 2019 and 2050 (ref. 1). Additionally, the burden of CVD-associated events has risen, particularly in low-income and middle-income countries where, since 2013, approximately 80% of all CVD-related deaths have occurred[2].

The past decade has witnessed substantial growth in the body of research on the human microbiome and its potential role in promoting CVD[3,4]. Changes in the composition, distribution, function or metabolic activities of the microbiome, known as dysbiosis[5], have been associated with various CVD-related pathologies, including atherosclerosis, hypertension, heart failure and type 2 diabetes mellitus[3,4]. The complex and dynamic community of microorganisms, consisting of bacteria, archaea, viruses and eukaryotic organisms, in the human microbiota vary in abundance and diversity[6]. Microorganisms have co-evolved with humans, leading to the development of symbiotic relationships in which they have a pivotal role in the appropriate functioning of human physiology. The genetic content of the human microbiome exceeds that of the host by 100-fold[7]. The number of microbes present equates to that of all the cells in the human body, and their total mass is approximately 200 g[8]. The diversity of the microbiota is dynamic, with proportions of different species influenced by an individual's genetic make-up, method of delivery at birth, age, diet, comorbidities and the medication they take[9]. Variation in the microbiota is pronounced between individuals at the species level, but at higher taxonomic unit levels (for example, phyla) the microbiota are similar[10]. The microbiome contributes to human metabolic function, protects against pathogens and has a vital role in the appropriate development of the immune system[6,11,12].

Over the past decade, disruption in the oral microbiome or oralome (Box 1) in particular has increasingly been reported to promote cardiovascular inflammation and CVD, and this finding is supported by a growing body of evidence from epidemiological studies, systematic reviews, and basic science and clinical investigations[13–16]. Therefore, an in-depth discussion of the current evidence linking the oralome with CVD and its increasing global prevalence is timely.

In this Review, we examine the association between oral dysbiosis and atherosclerotic CVD (ASCVD) — comprising coronary artery disease (CAD), stroke and peripheral arterial disease (PAD) — heart failure (HF), infective endocarditis (IE) and rheumatic heart disease (RHD). We describe the pathophysiological mechanisms by which oral dysbiosis causes CVD and explore strategies for prevention and management. Finally, we discuss current gaps in knowledge and directions for future research in the field.

Periodontitis and bacteraemia

Periodontal infections are an important cause of oral and systemic inflammation, driven by bacterial biofilm (dental plaque) on teeth and gums. In gingivitis, infection is limited to the gingiva and reversed by good oral hygiene. In periodontitis, the inflammation extends more deeply into the tissues, affecting the attachment apparatus of the teeth[17]. Periodontitis acts as a reservoir for numerous microorganisms that are seeded into the bloodstream from ulceration of an inflamed crevice and pocket epithelium into the adjacent gingival microcirculation[18]. Invasive dental procedures and even normal daily activities, such as chewing and tooth brushing, create the conditions for bacteria to seed into the bloodstream[19,20]. Microorganisms or their products present in the circulation can increase local vascular inflammation, which can result in a procoagulant state[21,22].

Bacteraemia originating from the oral cavity is caused by a wide variety of microorganisms, including Gram-negative anaerobes and the viridans group streptococci (VGS)[23]. Among patients with chronic periodontitis, the magnitude of bacteraemia is directly associated with the level of gingival inflammation[24]. Smoking increases the incidence and severity of periodontal infection and thus the susceptibility of an individual to bacterial growth. Smokers with chronic periodontitis have greater loss of tooth attachment apparatus and bone, more furcation involvement (bone loss at the branching point of the roots) and deeper pockets, meaning that smoking can exacerbate periodontal bacteraemia[25].

The oral microbiome in CVD

Atherosclerotic CVD

Atherosclerosis refers to the pathological formation of fibrofatty lesions in the arterial intima and through its sequalae — CAD, acute myocardial infarction, stroke and PAD — is a leading cause of morbidity and mortality worldwide[26]. In 2019, 9.14 million deaths from CAD occurred worldwide, with associated DALYs numbering 182 million. In the same year, 6.55 million deaths and 143 million DALYs associated with stroke were recorded[1].

Through a complex interaction between genetic predisposition and exposure to environmental factors such as tobacco use, physical inactivity, obesity and poor diet[27,28], chronic inflammation is propagated over time and cholesterol accumulates in the intima of the

arterial walls, together with infiltration by macrophages[29]. A persistent and detrimental immune cascade is established and continuously propagated, resulting in further proliferation of smooth muscle cells, accumulation of connective tissue components and oxidation of LDL, leading to plaque rupture, thrombus formation and subsequent occlusion of downstream blood vessels, culminating in tissue ischaemia and necrosis[29] (Fig. 1).

Epidemiological and mechanistic evidence links periodontitis with atherosclerosis and thromboembolic events[30]. In 1993, patients with periodontitis were reported to have a 25% increased risk of atherosclerotic plaque formation[31]. A consistent, positive link has been reported between various measures of periodontal disease and the development of incident ASCVD[32]. In an analysis of 10,362 healthy participants from the Atherosclerosis Risk in Communities (ARIC) study[33] who were followed up over a 15-year period, periodontal disease was significantly associated with the incidence of stroke (HR 2.6 for cardioembolic stroke; HR 2.2 for thrombotic stroke). Regular dental care was protective, being associated with a reduced risk of stroke (HR 0.77)[33]. In another analysis of the ARIC study population, tooth loss from gum disease was associated with a 30% increased risk of venous thromboembolic events[34]. A meta-analysis of prospective cohort studies showed that periodontal disease and tooth loss increase the risk of CAD by 24% and 34%, respectively[35]. Since the late 1980s, periodontal disease has been associated with an increased risk of acute myocardial infarction[36]. Periodontitis is also associated with PAD, with a fivefold increase in risk among those diagnosed with comorbid periodontitis[37]. The risk of PAD is highest among individuals with elevated serum levels of IL-6 and tumour necrosis factor (TNF)[38]. In a small study ($n = 30$) from Mexico, severe periodontal disease was associated with a sixfold increase in the risk of PAD, and tooth loss was significantly worse in patients with PAD than in control individuals[39]. Periodontitis has also been associated with increased circulating fibrinogen levels, suggesting an increased risk of thrombosis[40], and with impaired lipid metabolism resulting in hyperlipidaemia, another powerful risk factor for ASCVD[41,42].

Data from in vitro, animal and human studies suggest that specific oral pathogens contribute to atherosclerotic thromboembolism through modulation of platelet aggregation. *Streptococcus sanguinis* expresses platelet aggregation-associated protein (PAAP)[43], and serotype k of *Streptococcus mutans* expresses collagen-binding adhesins and can survive in the blood for long periods of time[44]. *Porphyromonas gingivalis* not only accelerates atheroma plaque formation, but also induces fatty streaks in the aorta of rabbits[45], whereas apolipoprotein E-deficient mice intraorally challenged with *S. sanguinis* have remarkably increased pro-inflammatory cytokine expression and atherosclerotic plaque formation[46]. Using data from 63 studies including 1,791 patients, researchers identified 23 unique oral commensal bacteria in the atherosclerotic plaques of patients undergoing interventional procedures[47]. Of these 23 bacteria, five (*Campylobacter rectus*, *P. gingivalis*, *Porphyromonas endodontalis*, *Prevotella intermedia* and *Prevotella nigrescens*) were unique to coronary artery plaques, whereas the other 18 were present in non-cardiac organs and associated with >30 non-cardiac disorders. From these microorganisms, the researchers identified 36 secretory proteins associated with bacterial pathogenesis, increased virulence and host immune regulation and modulation, which could drive ASCVD progression[47].

Several independent mechanisms have been proposed for the role of oral dysbiosis in the development of ASCVD (Fig. 2). One mechanism involves bacteraemia from the pathogenic oral microbiota seeding from foci of periodontitis, which invades the arterial wall and promotes plaque formation[48,49]. In another mechanism, oral inflammatory lesions release pro-inflammatory cytokines directly into the bloodstream, promoting plaque formation via the propagation of inflammatory

Box 1

The oral microbiome

The oral microbiome refers to the assembly of microbiota, including bacteria, fungi, viruses, protists and archaea, microbial structural elements, and their internal and external structural elements, collectively referred to as the 'theatre of activity'[245], found in the oral cavity. The oral microbiome is second only to the gut microbiota in abundance and diversity[246], with 772 known species found in the oral cavity. Of these microbes, 96% can be categorized into six phyla: Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria and Spirochaetes[247]. In the oral cavity, targets for colonization include the palate, subgingival and supragingival surfaces, teeth, lips, cheeks and tonsils[248]. Oral microbes form polymicrobial associations, where bacteria are stimulated to produce glycoproteins and polysaccharides that allow them to sequentially adhere to the tooth enamel, forming ecological niches known as biofilms[246]. Biofilms host a diverse range of microbes, including early colonizers from the genera *Streptococcus* and *Actinomyces*, and anaerobes from the genera *Porphyromonas* and *Veillonella*, among others[249,250].

Next-generation sequencing technology and high-throughput metabolomic, proteomic, transcriptomic and genomic analytical instruments coupled with powerful bioinformatic algorithms have provided insights into the complexities of microbial communities[251]. At the genomic level, 16S rRNA pyrosequencing allows simultaneous analysis of the microbiome, providing information on genetic composition, novel biocatalysts or enzymes, genomic linkages between function and phylogeny of uncultured organisms and community-level functional and structural evolutionary dynamics[252]. Whole-genome shotgun sequencing provides enhanced detection of bacterial species and their diversity and improved prediction of genes compared with 16S rRNA pyrosequencing[253]. At the transcriptomic level, DNA microarray and RNA sequencing can identify and characterize total mRNA, revealing the level of expression of all genes at a particular point in time and enabling the detection of broad coordinated trends in the functional dynamics of microbial communities[254]. At the proteomic level, mass spectrometry allows the identification and quantification of all the proteins in a sample[255]. Therefore, the application of the full array of multiomics technologies and integration of the generated information using bioinformatics enables the elucidation of the entire expressed activity of these oral microbial collections[256].

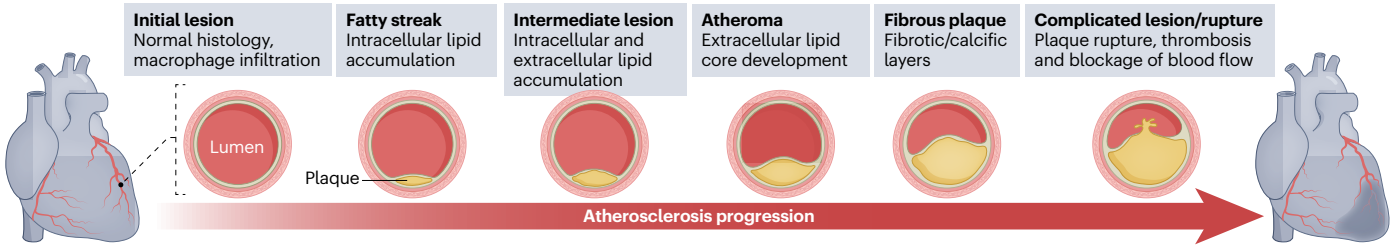

Fig. 1 | Development and progression of atherosclerotic cardiovascular disease. Development of atherosclerosis begins with a combination of individual susceptibility, driven by genetic factors, such as monogenic disorders (for example, familial hypercholesterolaemia) that elevate plasma LDL levels, and more complex polygenic phenomena[238], and exposure to cardiovascular risk factors (for example, elevated plasma cholesterol levels, hypertension, diabetes mellitus, smoking, male sex and inflammatory markers). These factors contribute to intact, but leaky and dysfunctional, endothelial cells, through which circulating lipoproteins extravasate into the subendothelial space, where they are modified and become cytotoxic and inflammatory[239]. Eventually, endothelial cells express adhesion molecules and recruit monocytes and T cells. Local immune mechanisms become overwhelmed by intracellular lipid accumulation, leading to cell death and subsequently extracellular lipid accumulation[240,241]. Propagation of immune responses by necrotic tissue results in the formation of a destabilized, necrotic, lipid-rich core and a fragile, rupture-prone fibrous cap, characterized as an atheromatous plaque[242]. Defects in the fibrous plaque expose the thrombogenic core to circulating blood, driving platelet activation and aggregation and coagulation cascades[243]. The thrombus can then occlude the artery itself or break away and cause downstream thrombosis, tissue ischaemia and, finally, widespread tissue necrosis[244].

cascades[50]. Additionally, autoimmunity, caused by the host humoral immune response targeting components of self-proteins with structural similarities to oral pathogenic antigens, promotes plaque formation[51,52]. Furthermore, oral pathogenic bacteria release specific bacterial toxins with pro-atherogenic effects[53]. Finally, patients with chronic or aggressive periodontitis have elevated serum levels of LDL and triglycerides and decreased serum levels of HDL[49], which are all associated with an increased risk of ASCVD[53,54].

Lipid abnormalities in the context of chronic low-level inflammation in periodontitis arise from interactions between bacterial lipopolysaccharides and serum lipoproteins via several mechanisms. Infection induces persistent increases in the levels of triglycerides, cholesterol and phospholipids contained in VLDL[55], owing to concomitant reductions in lipoprotein lipase and hepatic lipase activity[56]. The constant release of systemic pro-inflammatory cytokines, such as TNF, promotes the expression of adhesion molecules on endothelial cells as well as the recruitment and activation of inflammatory cells, and directly interferes with the metabolic pathways of triglycerides and cholesterol, thereby contributing to the development of ASCVD[57]. Additionally, low-dose lipopolysaccharide exposure, as seen in the subclinical endotoxaemia present with chronic, low-level periodontal infection[50], directly induces pro-atherogenic lipoprotein profiles by increasing the hepatic production of triglycerides, cholesterol and, ultimately, VLDL[58]. Lipopolysaccharide also affects a wide range of HDL-associated apolipoproteins, plasma transfer proteins and receptors, resulting in decreased plasma levels of HDL[59–61]. Finally, lipopolysaccharide also induces the uptake of oxidized LDL via scavenger receptors, contributing to the development of mature, lipid-laden macrophages, which exacerbate local inflammation and atherosclerotic plaque formation[62].

Heart failure

HF is a clinical syndrome of exercise intolerance associated with left ventricular (LV) dysfunction, resulting in fluid overload and compensatory activation of neurohormonal systems. In patients with HF, consistently elevated levels of pro-inflammatory cytokines, such as TNF, IL-6, IL-1 receptor-like 1 (ILRL1; also known as protein ST2), galectin 3 and C-reactive protein (CRP), indicate chronic immune activation and cardiac and systemic inflammation, and are markers of a poor prognosis[63–65]. Chronic, low-level inflammation in HF is linked to immune dysregulation (of both the innate and adaptive immune systems), leading to maladaptive changes, including cardiac fibroblast activation that promotes cardiac fibrosis and worsening HF[44,66].

Oral bacteria can travel via the saliva from the mouth to the gut. Some are destroyed by stomach acid, whereas others (such as *P. gingivalis*) are acid-resistant and subsequently induce local imbalances in gut microbial communities, resulting in gut dysbiosis[67]. In patients with HF, gut dysbiosis induced by bacteria from the oralome has been linked to increased gut permeability and translocation of gut bacteria, both triggers for chronic inflammation[68]. Consequently, endotoxins are released from gut bacteria into the circulation through leaky membranes, and directly from bacteria that have themselves translocated into the circulation[69]. This situation leads to metabolic endotoxaemia, which promotes CVD, LV dysfunction and HF[70]. Microbial dysbiosis can lead to inflammation-induced obesity, type 2 diabetes, atherosclerosis and worsening HF[70,71]. Bacterial translocation is an important driver of worsening HF owing to a vicious cycle of impaired LV function resulting in intestinal oedema and damage to the intestinal microcirculation, leading to epithelial ischaemia and barrier defects in the gut, which result in progressive cardiac dysfunction[72] (Fig. 3).

Lipopolysaccharide is a cell wall product of Gram-negative bacteria, and the serum levels of lipopolysaccharide are increased in patients with HF[73]. Release of lipopolysaccharide in HF is increased during periods of congestion and decreased following diuretic use[74]. In patients with HF, lipopolysaccharide activates dysregulated systemic inflammation via several inflammatory pathways, including Toll-like receptor 4 (TLR4)-dependent macrophage activation, upregulation of pro-inflammatory cytokines, elevated nuclear factor-κB (NF-κB)-dependent inflammation and stimulation of the ubiquitin–proteasome pathway[75–79] (Fig. 3). Patients with the most severe congestion and oedema in HF have the highest blood concentrations of lipopolysaccharide, TNF and soluble TNF receptor 1 (ref. 80).

Infective endocarditis

IE is a severe inflammatory disease that can affect one or more of the aortic, mitral or tricuspid valves, but seldom the pulmonary valve, with vegetations often the consequence of an infectious nidus. Pre-existing

conditions, such as RHD, mitral valve prolapse, degenerative aortic valve stenosis, bicuspid aortic valve, coarctation of the aorta and previous IE, as well as prosthetic valves and intravenous drug use, can predispose individuals to develop IE[81]. The prevalence of IE has risen steadily since 1990, reaching an estimated 1.09 million incident cases globally in 2019, with an associated 66,300 deaths and 1.72 million DALYs[1].

VGS and *Staphylococcus* spp., both from the oralome, are the most common bacterial causes of IE. VGS are reported in 50–80% of

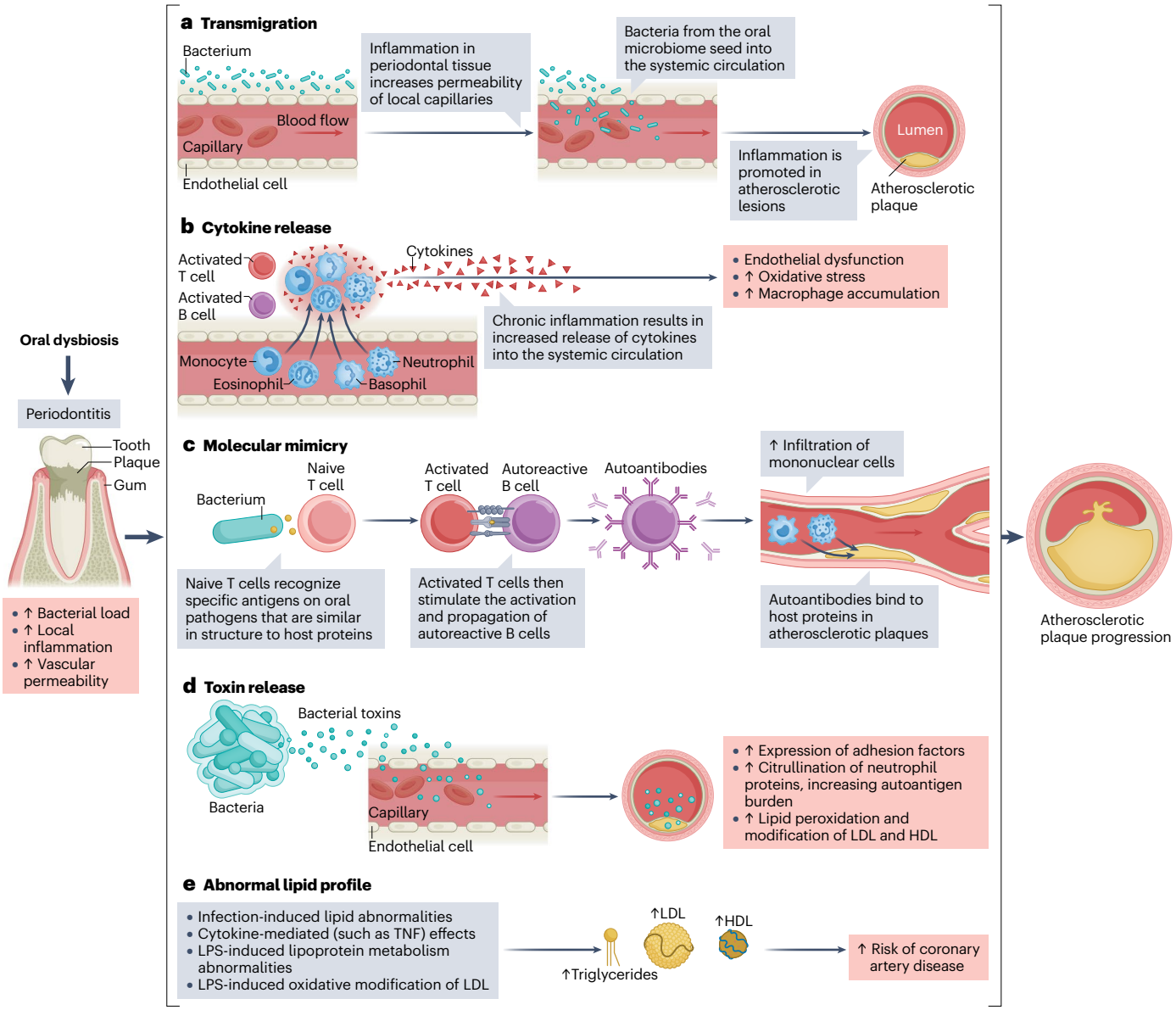

Fig. 2 | Oral dysbiosis in the pathogenesis of atherosclerotic cardiovascular disease. There are several mechanisms by which oral dysbiosis, in the context of periodontitis, contributes to atherosclerosis and, ultimately, atherosclerotic cardiovascular disease. **a**, Transmigration. Local inflammation results in increased permeability of the local periodontal vasculature, allowing oral bacteria to seed into the systemic circulation, where they inoculate atherosclerotic plaques and aggravate inflammatory processes. **b**, Cytokine release. Chronic local inflammation results in the sustained release of pro-inflammatory molecules into the systemic circulation, resulting in endothelial dysfunction, increased oxidative stress and macrophage accumulation in atherosclerotic lesions. **c**, Molecular mimicry. Oral bacterial antigens can have structural moieties identical to certain host proteins, which are recognized by naive T cells. The activated T cells then stimulate the activation and propagation of autoreactive B cells. Autoantibodies released by these B cells bind to host proteins in atherosclerotic plaques, leading to infiltration of mononuclear cells. This cross-reactive immune response targets tissues in the vasculature. **d**, Toxin release. Leaky periodontal vasculature allows the seeding of bacterial toxins into the systemic circulation, driving increased expression of adhesion factors, the citrullination of host proteins and increased lipid peroxidation, all of which are pro-atherogenic. **e**, Abnormal lipid profile. Chronic oral inflammation is associated with abnormal lipid profiles and an increased risk of atherosclerotic cardiovascular disease through several postulated mechanisms: infection-induced lipid abnormalities, cytokine-mediated effects, lipopolysaccharide (LPS)-induced lipoprotein metabolism abnormalities and LPS-induced oxidative modification of LDL.

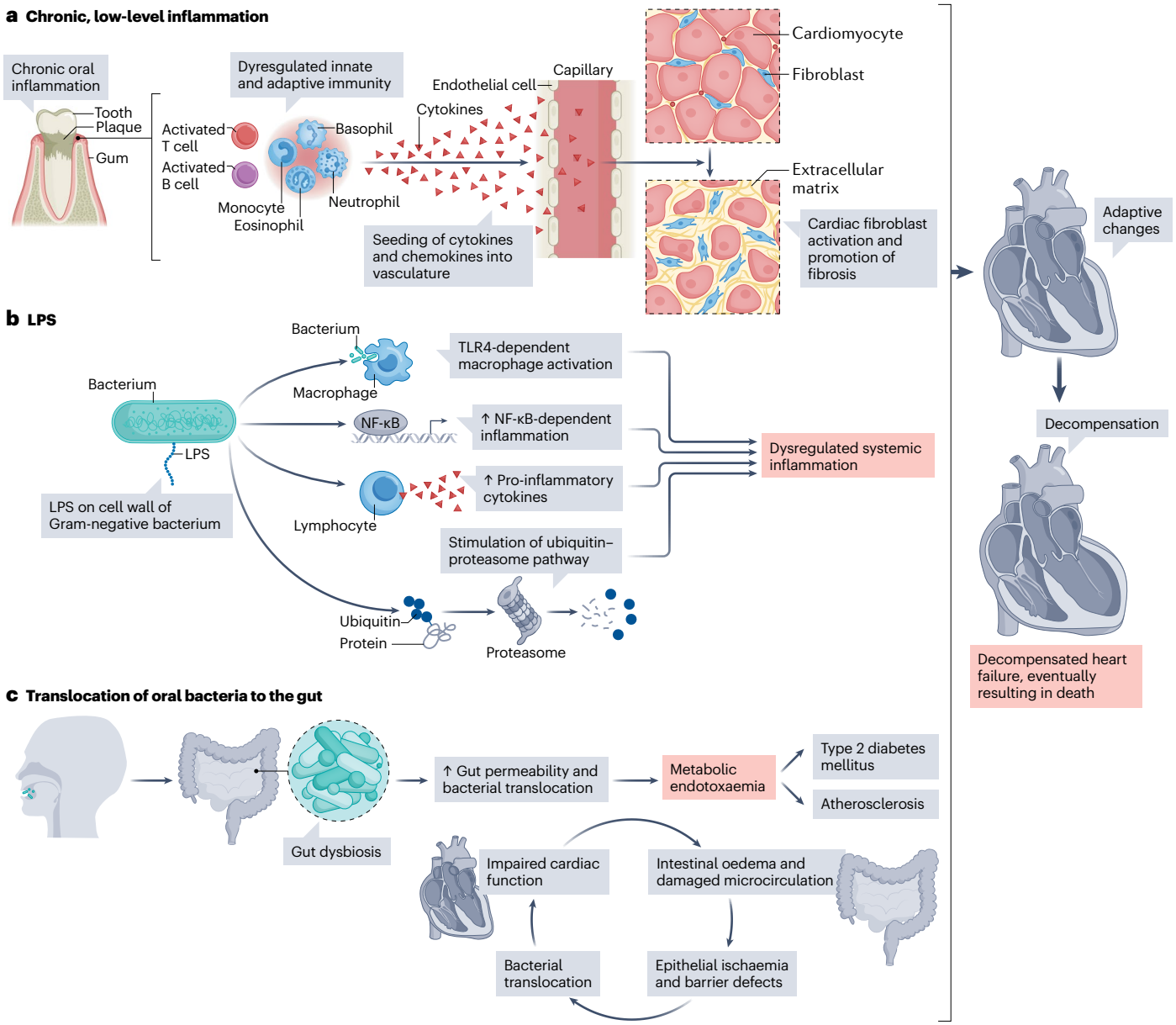

Fig. 3 | Oral dysbiosis in the pathogenesis of heart failure. a, Chronic, low-level inflammation. An increased cytokine and chemokine burden seeds into permeable local vasculature. When these circulating immunomodulatory molecules reach the heart, they can act on cardiac fibroblasts in the myometrium, stimulating unregulated cardiac fibrosis and contributing to heart failure progression. **b**, Lipopolysaccharide (LPS). An increased burden of LPS from the cell walls of Gram-negative bacteria induces dysregulated systemic inflammation via the Toll-like receptor 4 (TLR4)-dependent activation of macrophages, recruitment of nuclear factor-κB (NF-κB)-related transcriptional factors, increased pro-inflammatory cytokines and continuous stimulation of the ubiquitin–proteasome pathway. **c**, Translocation of oral bacteria to the gut. Together with gut dysbiosis and altered gut membrane permeability, translocation of oral bacteria induces metabolic endotoxaemia, with diabetogenic and atherogenic consequences. In the context of heart failure, a vicious cycle is initiated, with impaired cardiac output resulting in intestinal oedema and damage to the microcirculation, leading to gut ischaemia, further bacterial translocation and worsening cardiac function.

positive blood cultures in patients with IE, whereas *Staphylococcus aureus* is present in 20–36% of positive tests[82–85]. Dysbiotic bacteria associated with periodontal disease, such as VGS, gain access to the bloodstream by seeding through leaky endothelia caused by chronic, local inflammation[48]. Dental scaling and extraction and tooth brushing can lead to bacteraemia caused by a variety of microorganisms, including VGS. In a double-blind, placebo-controlled study, positive bacteraemia was found in 56% and 32% of patients after tooth extraction and tooth brushing, respectively[19]. Further studies have shown that VGS is one of the most commonly isolated

bacteria in patients with bacteraemia after undergoing periodontal procedures[86].

Repeated exposure of the cardiac endothelium to VGS promotes valve thickening, followed by platelet and fibrin deposition, producing sterile lesions known as thrombotic endocarditis[87], and increases susceptibility to adherence, colonization and infection of these non-bacterial vegetations through subsequent bacteraemia[88]. In addition to the production of monocyte cytokines and other factors, such as acute-phase proteins and anti-myolemmal and anti-sarcolemmal antibodies[89,90], three steps are important for infection of the sterile vegetation or endothelium that leads to IE — bacterial adherence, platelet activation and fibrin overlaying (Fig. 4). A variety of bacterial surface components and receptors, such as dextran, fibronectin-binding protein and teichoic acid, are important virulence factors in adhesion to the platelet–fibrin matrix on the valvular wall[18,89,90]. In 1975, VGS and *Enterococcus* spp. were found to have the highest rates of adherence to damaged aortic valve leaflets[91]. More than 80% of VGS express the surface protein fimbrillin (also known as FimA), which binds to fibronectin on damaged valves to ensure bacterial adherence and creates a fibrin overlay that evades the host immune response[92]. Bacterial cells interact with platelets through cell-surface adhesin B and an unidentified ligand of the platelet, leading to bacterial PAAP binding to the platelet α2β1 integrin, platelet activation and platelet degranulation. Activated platelets release dense granules and α-granules that, in combination with thromboxane production, promote the aggregation response. α-Granules contain platelet microbicidal proteins that kill bacteria. They also induce the production of fibrinogen and clotting factor V and factor VII, which activate thrombin and initiate the polymerization of fibrinogen to fibrin[43]. The consequent fibrin–platelet complex increases in mass as cells colonize and expand layer upon layer of the vegetation[93], creating a unique biofilm that can resist host immune surveillance and antimicrobial defence mechanisms[94].

Rheumatic heart disease

Acute rheumatic fever (ARF) is an inflammatory autoimmune condition triggered by childhood pharyngeal infection with group A β-haemolytic *Streptococcus* (GAS) spp. ARF eventually results in chronic immune activation and production of autoantibodies that induce irreversible valvular and myocardial disease (known as RHD) in susceptible individuals[95]. Affecting nearly 40.5 million individuals globally, and resulting in >300,000 deaths in 2019 (ref. 1), RHD has a prevalence that is unequally distributed according to socioeconomic status. The disease persistently and disproportionately affects low-income and middle-income countries, indigenous communities and migrants, and vulnerable populations affected by overcrowding and scarcity of health-care resources in high-income countries[96,97].

GAS pharyngitis can lead to the development of ARF and eventually RHD through molecular mimicry[98] (Fig. 4). In addition, binding of the amino terminus of the streptococcal M protein to the CB3 region of collagen type IV in the basement membrane of the stratified squamous epithelium of the pharynx leads to production of autoantibodies that target the subendothelial collagen matrix of the valvular endocardium[99,100]. Repeated immune responses result in neovascularization and fibrosis of the valves[101].

As early as 1949, individuals with ARF were reported to be significantly more likely than healthy controls to have tooth decay[102]. As with ARF and RHD, the prevalence of periodontal disease is disproportionately high in low-income and middle-income countries[103]. Moreover, ARF, RHD and periodontal disease have similar major risk factors, including poverty, overcrowding and shortage of health-care resources[97,104]. Coincidentally, the decline in ARF and acute post-streptococcal glomerulonephritis observed in high-income countries began in the mid-1940s after fluoridation of water supplies was introduced[105]. This public health intervention was also shown to reduce the burden of periodontal disease and periodontitis[106,107]. Fluoridation of GAS grown in culture results in a significant decrease in the cellular expression of GAS virulence factors, M protein and glyceraldehyde 3-phosphate dehydrogenase, a fibrinogen-binding protein[108].

In addition to GAS, evidence exists for a link between *P. gingivalis*, protein citrullination of host self-antigens and the development of RHD, owing to the presence of persistent, increased circulating antibodies specific to heart valve proteins, such as laminin[109], fibronectin[101] and collagen[99,110] (Fig. 5). *P. gingivalis* has been implicated in autoimmune disease via the breaking of immune tolerance generated through the citrullination of host proteins[111]. Protein citrullination involves the enzymatic deamination of arginine residues in proteins to citrulline by peptidylarginine deaminase (PAD), an important virulence factor secreted by *P. gingivalis*[112]. This alteration in protein structure is thought to contribute to loss of self-tolerance, leading to recognition by host antigen-presenting cells, driving immune activation and, ultimately, the production of autoantibodies against the affected host proteins[113]. Interestingly, arginine-specific gingipains (cysteine adhesin proteases essential to colonization, nutrient acquisition and immune subversion in periodontitis, secreted by *P. gingivalis*[114]) have been shown to bind to arginine residues on extracellular matrix proteins (laminin, fibronectin and collagen)[115]. These extracellular matrix–gingipain interactions have a crucial role in *P. gingivalis* colonization in the periodontal microenvironment[116]. Moreover, they could provide the groundwork for spatiotemporal interactions in which secreted PAD from *P. gingivalis* citrullinates arginine residues on extracellular matrix self-proteins, facilitated by the tight binding of specific gingipains, also secreted by *P. gingivalis*, to arginine residues. Ultimately, these interactions could promote the production of autoantibodies that distally drive the heart-valve-specific inflammatory changes seen in RHD.

Mechanisms of CVD from oral dysbiosis

Dental biofilms

Whole-metagenome data from the oral microbiome of healthy individuals and from patients with periodontitis and an abundance of dental biofilms have been compared[117]. These analyses demonstrate that the diseased microbiome is enriched in metabolic functions consistent with parasitism, characterized by the availability of nutrients derived from the degradation of host tissues. These functions include fatty acid metabolism and acetyl co-enzyme A degradation, aromatic amino acid degradation and the biosynthesis of toxic metabolites, such as the lipid A component of lipopolysaccharide[117]. The oralome is linked to CVD via this dental biofilm, which comprises a dynamic microbial community associated with chronic, low-grade immune activation and systemic inflammation, and often with acquired resistance to environmental challenges (such as antibiotics)[118]. Biofilm microbial communities arise from dysbiotic shifts in the oral microbiome, characterized by remarkably high overgrowth of pathogenic bacterial strains in the biofilm surface, which underpin the development of periodontitis[119].

Biofilms persist through various mechanisms by which the microbial community subverts the host immune response[120]. For example, the keystone periodontal pathogen, *P. gingivalis*, blunts polymorphonuclear leukocytes via the secretion of lipopolysaccharide, which binds to adhesion molecules, such as IL-8, intercellular adhesion molecule 1

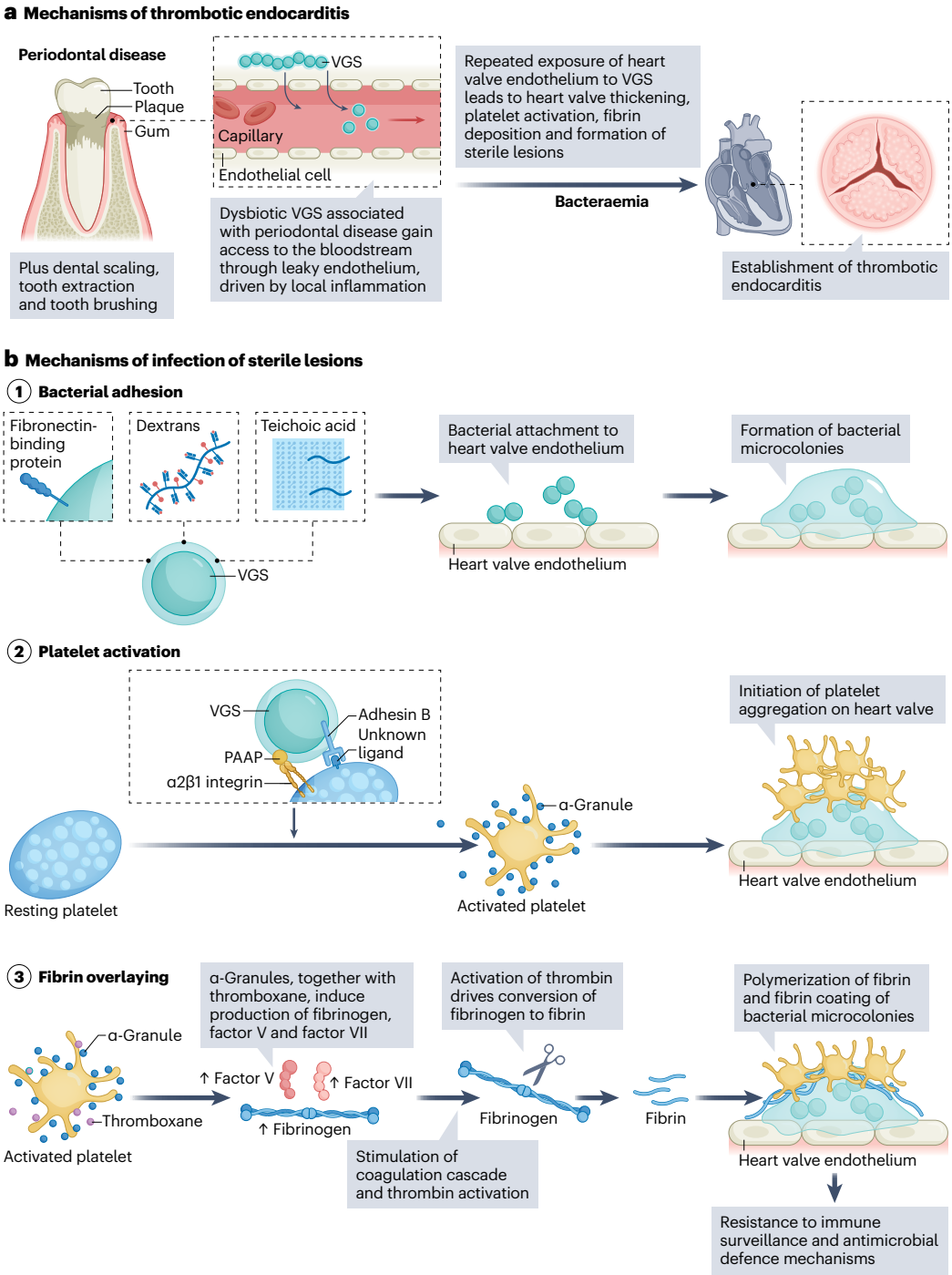

Fig. 4 | Oral dysbiosis in the pathogenesis of infective endocarditis. **a**, Periodontal disease, causing dysbiosis and increased burden of viridans group streptococci (VGS), results in bacterial seeding into the bloodstream through leaky endothelium, facilitated by periodontal procedures, such as dental scaling, tooth brushing and tooth extraction. Repeated exposure of the heart valve endothelium to VGS results in valve thickening, platelet activation, fibrin deposition and formation of sterile lesions known as thrombotic endocarditis. **b**, Sterile lesions become infected in three important steps. (1) VGS bacterial surface components and receptors (such as fibronectin-binding protein, dextrans and teichoic acid) facilitate bacterial adhesion to sterile lesions and formation of bacterial microcolonies on the heart valve. (2) VGS express adhesin B, which binds to an unknown ligand on circulating platelets, facilitating the interaction between bacterial platelet aggregation-associated protein (PAAP) and platelet α2β1 integrin, activating the platelets and driving platelet aggregation. (3) Finally, active platelets degranulate and release α-granules and thromboxane, which together increase the production of fibrinogen and clotting factor V and factor VII. Subsequent stimulation of the coagulation cascade drives activation of thrombin, which converts the fibrinogen to fibrin that forms deposits over the bacterial microcolonies, creating a unique barrier that allows the biofilm to evade host immune surveillance and antimicrobial defence mechanisms.

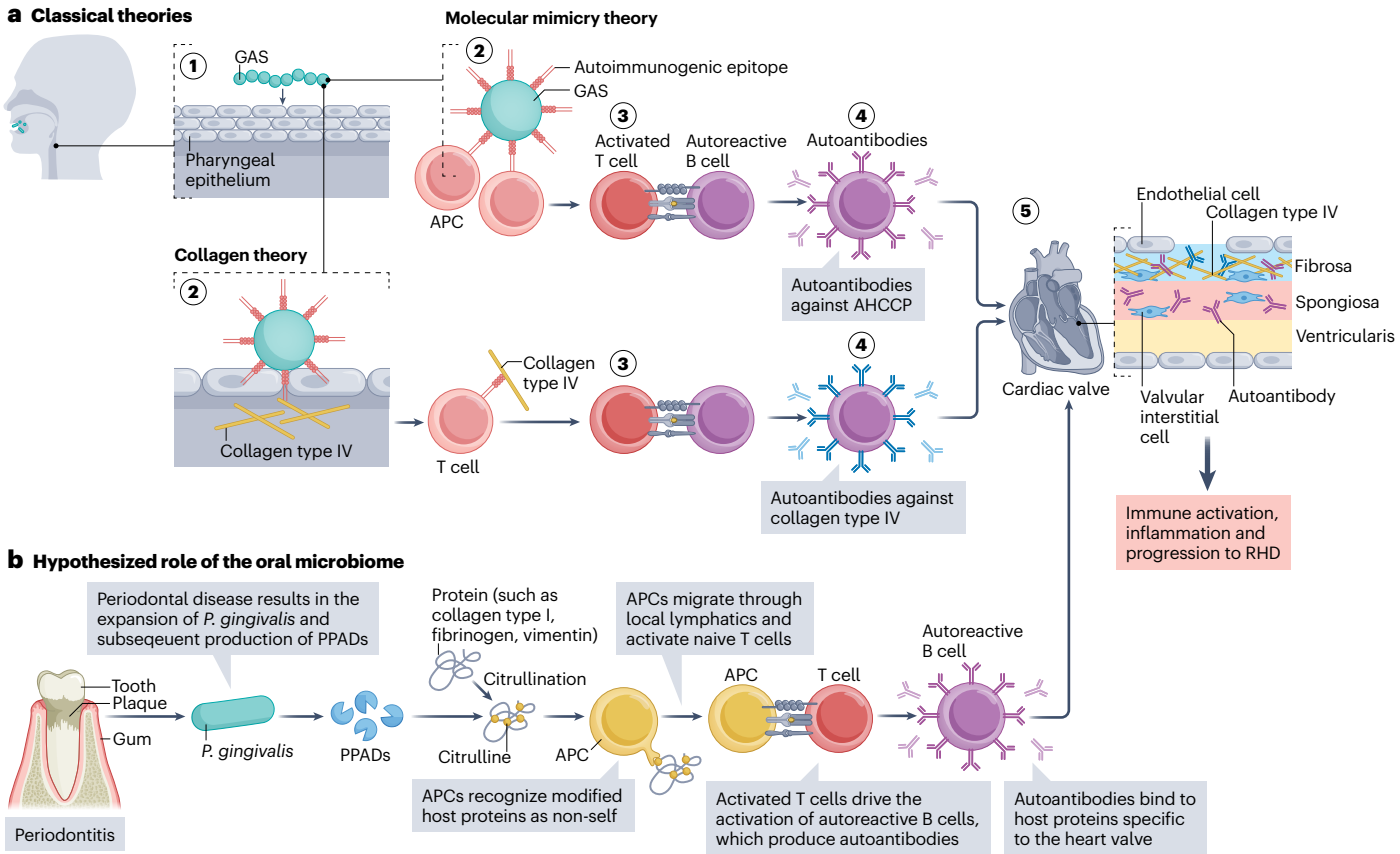

Fig. 5 | Oral dysbiosis in the pathogenesis of RHD. a, Classical theories. The proposed pathological mechanism linking streptococcal pharyngitis and the valvular inflammation characteristic of rheumatic heart disease (RHD). (1) Adhesion, invasion and infection of the pharyngeal epithelium by group A *Streptococcus* (GAS) spp. (2) Molecular mimicry theory and collagen theory: antigen-presenting cell (APC) recognition of the autoimmunogenic epitope. (3) Molecular mimicry theory and collagen theory: APC-mediated activation of autoreactive T cells. (4) Activation of autoreactive B cells producing autoantibodies against α-helical coiled-coil proteins (AHCCP) for the molecular mimicry theory or autoantibodies against the CB3 region of collagen type IV for the collagen theory. (5) Autoantibodies binding to AHCCP in valve interstitial cells in the fibrosa and spongiosa layers of the valve for the molecular mimicry theory and autoantibodies binding to the CB3 regions of collagen type IV in the fibrosa layer of the valve endocardium for the collagen theory. **b**, Hypothesized role of the oral microbiome. *Porphyromonas gingivalis* expresses peptidyl arginine deiminase (PPAD), which citrullinates valve-specific host proteins such as collagen type I, fibrinogen and vimentin. Citrullinated proteins are recognized as 'non-self' by APCs, which migrate through the local lymphatic system and activate naive T cells. These cells trigger the proliferation of autoreactive B cells that produce anticitrullinated antibodies. Ultimately, these anticitrullinated antibodies can bind to valvular proteins, increasing autoimmune inflammation.

and E-selectin, and impairs leukocyte recruitment[121,122]. In addition, activated complement receptor type 3 interacts with *P. gingivalis* fimbriae and induces downregulation of IL-12p70, a cytokine vital to intracellular bacterial clearance[123,124]. Biofilms are also immunomodulatory in chronic periodontal disease via the production of the signalling molecule autoinducer 2, which alters the transcription of immune mediators in epithelial cells[125]. Several oral pathogens associated with dental biofilms, including *P. gingivalis*, express autoinducer 2, inducing the secretion of IL-8 in oral epithelial cells[126].

Bacterial persistence together with a modulated host immune response establishes a pathogenic cycle in which dysbiosis and inflammation augment each other[119]. As a result, biofilms essentially act as resistant platforms from which pathogenic bacteria can induce systemic disease. For example, the protease gingipain R that is secreted by *P. gingivalis* induces CVD by activating factor X, prothrombin and protein C, promoting thrombotic tendency and intravascular clot formation[127]. Additionally, pro-inflammatory cytokines produced by activated macrophages or T cells in periodontal lesions can propagate inflammatory cascades, driving atherosclerotic changes at distal sites[15].

Endothelial dysfunction

The oral microbiota directly affects the vasculature via migration of and inoculation by microbes. Periodontitis results in a large bacterial load in the gingival sulcus, inducing persistent local inflammation and increasing the permeability of the surrounding vasculature. Invasion of endothelial cells by bacteria can lead to endothelial dysfunction, a key event in the development of atherosclerosis and vascular dysfunction. Endothelial dysfunction is associated with increased procoagulant properties, mononuclear cell adhesion, increased expression of cell adhesion molecules, and increased levels of pro-inflammatory cytokines and chemokines (such as IL-6, IL-8 and MCP1), all of which are induced by *P. gingivalis*[128,129].

As discussed above, the oralome also promotes CVD through the production of metabolites, including lipopolysaccharide from bacteria, which act as immunostimulators or immunomodulators that translocate into the circulation from the oral cavity, causing endotoxaemia and endothelial dysfunction[130]. Patients with periodontitis have persistent endotoxaemia, establishing chronic, low-grade inflammation that promotes oxidative stress, leading to accelerated atherogenesis, increased vascular inflammation and permeability, and an elevated risk of thrombosis[131–134]. Endothelial dysfunction also contributes to the development of hypertension (Box 2).

In a healthy oral microbiome, an enterosalivary nitrate–nitrite–nitric oxide pathway that involves nitrogen-reducing oral bacteria has been found to be crucial in maintaining nitric oxide homeostasis[135]. Additionally, clinical data have shown that an abundance of oral nitrate-producing bacteria is associated with reduced levels of cardiometabolic risk, characterized by lower levels of insulin resistance and plasma glucose and an association with mean systolic blood pressure in normotensive individuals[136]. Therefore, imbalances in these oral nitrogen-reducing bacteria in the context of oral dysbiosis facilitated by periodontitis have been associated with decreased nitric oxide levels[137], promoting endothelial dysfunction[138] and increased CVD risk[139]. Other clinical data have shown that intensive periodontal treatment is associated with improved endothelial function after 6 months, evidenced by a greater flow-mediated dilatation of the brachial artery, and a decrease in the levels of biomarkers of inflammation, coagulation and endothelial activation[140].

Molecular mimicry and antibody cross-reactivity

Bacteria in the oral cavity can promote a systemic inflammatory response through molecular mimicry and antigen shedding. Owing to the homologous nature of heat shock proteins (HSPs) across species, the immune system could initiate anti-self responses in susceptible individuals. For example, in individuals with chronic infection facilitated by periodontal disease, bacterial HSPs act as antigens against which self-reactive antibodies are generated[141,142] and target HSPs expressed by stressed endothelial cells, leading to cross-reactivity of the immune response, endothelial dysfunction and atherosclerosis[143,144]. CVD promotes expression of HSP60 and adhesion molecules by endothelial cells, resulting in progression from early fatty streak lesions to more severe and irreversible atherosclerotic alterations[145].

Porphyromonas gingivalis and protein citrullination

Citrullination of host proteins induces an autoimmune response with the production of autoantibodies that contribute to the development of CVD and atherosclerosis[146]. As discussed above, protein citrullination mediated by *P. gingivalis* is postulated to be an important mechanism in the development of RHD, which is associated with elevated levels of autoantibodies to fibronectin and type I collagen[101]. *P. gingivalis* contributes to the pathogenesis of rheumatoid arthritis among individuals with periodontal disease, specifically through the expression of enzymes that catalyse citrullination of host tissues[113]. These enzymes also have affinity for heart valve components, such as vimentin, fibrinogen and type I collagen, which are all powerful autoantigens in rheumatoid athritis[147]. Citrullination of the myocardium and its various subcomponents could also provide an explanation for myocardial involvement mediated via anti-citrullinated peptide antibody production in rheumatoid arthritis and associated CVD[148]. Dysbiosis of the oral microbiome also contributes to several other autoimmune conditions facilitated by similar pathophysiological mechanisms (Box 3), further underpinning the role of the oral microbiome in systemic function.

Platelet activation, aggregation and thrombosis

In addition to their primary role in haemostasis, platelets are important in the immune response to infection[149]. Oral bacteria activate platelets both directly (through binding and activation) and indirectly (through secretion of platelet-activating products). Platelets are also activated by the immune response to oral bacteria[150]. Platelet activation by bacteria can lead to localized thrombus formation, platelet consumption, and increased secretion of pro-inflammatory cytokines and mediators by the platelets themselves, which contributes to inflammation, atherogenesis and thrombogenesis[151]. VGS, specifically *S. sanguinis*, *Streptococcus gordonii*, *S. mutans* and *Streptococcus mitis*, induce platelet adhesion and aggregation in vitro[152], through a process involving multiple cell surface proteins, including PAAP, serine-rich glycoproteins, adhesins and glucosyltansferases[150,153–156].

Direct arterial infection

Transient bacteraemia has been associated with direct bacterial invasion of endothelial cells. Periodontal bacteria have been identified by PCR in atherosclerotic plaques[15]. Similarly, *S. mutans*, *S. sanguinis*,

Box 2

The oral microbiome and hypertension

Control of vascular function is influenced by dietary factors. A diet rich in nitrates and nitrites improves cardiovascular health[257,258], lowers blood pressure[259] in both healthy individuals[260] and individuals with hypertension[261], improves endothelial function[262] and systemic inflammation[263], and protects against ischaemia–reperfusion injury[264]. Nitrate supplementation improves the bioavailability of nitrite and nitric oxide, resulting in blood pressure reduction[265,266]. Antibacterial, chlorhexidine-based mouthwash was found to be more effective at reducing the burden of *Veillonella dispar* (nitrate-reducing bacteria) in the oral cavity than mouthwash containing essential oils or other antibacterials in healthy adults[267], and the use of chlorhexidine-based mouthwash has been associated with increased systolic blood pressure[268].

Short-chain fatty acids are produced by oral and gut microbiota through anaerobic fermentation of dietary fibre[269]. Short-chain fatty acids such as acetate, propionate and butyrate are associated with reduced blood pressure, improved myocardial remodelling and reduced systemic inflammation[270]. Increased fibre intake is associated with blood pressure control[271]. G protein-coupled short-chain fatty acid receptors are expressed in vascular smooth muscle cells and the juxtaglomerular apparatus, and mediate renin release and changes in vascular resistance, contributing to blood pressure modulation[272]. Microbiota suppression with antimicrobials[273] and faecal transplantation or transfer from humans with hypertension into mice are associated with the transmission of increased blood pressure[274].

Box 3

The oral microbiome and autoimmunity

The oral microbiome is a crucial modulator of immunity, underpinned by associations between oral dysbiosis and autoimmune disease, loss of immune tolerance against self-antigens and increased inflammatory events[275]. In most instances, the tissue with pathological autoimmune injury is not the same tissue that harbours the associated microbial community, meaning the pathological phenotype tends to be expressed distally from microbial colonies[276,277]. Periodontitis is an important contributor to autoimmunity via molecular mimicry and antigen seeding mechanisms[51]. Inflammation is driven by oral pathogens that form complex, polymicrobial dental plaque biofilms on the surface of teeth, resulting in a structured and functionally organized community[51] held together by a secreted exopolysaccharide matrix. These microbes live in symbiotic mutualism and are resistant to external threats, such as antibiotic treatment, underpinning the chronic and persistent nature of periodontal disease[278]. The oralome has also been linked to many other autoimmune conditions, such as rheumatoid arthritis, systemic lupus erythematosus, Sjögren syndrome and inflammatory bowel disease[279–283].

Aggregatibacter actinomycetemcomitans, *P. gingivalis* and *Treponema denticola* have been detected in both aortic aneurysms and in heart valves[157]. Oral bacteria have also been frequently detected at sites along the arterial tree where atherosclerotic lesions commonly occur[157]. For example, analyses of atherosclerotic lesion samples confirmed the presence of two oral bacteria heavily implicated in periodontitis: *P. gingivalis* and *A. actinomycetemcomitans*[158]. The presence of *A. actinomycetemcomitans* was shown to be associated with severe periodontal disease in a Thai population[159]. Other researchers found that *P. gingivalis*, *A. actinomycetemcomitans*, *P. intermedia* and *Tannerella forsythia* were commonly present in human coronary artery biopsy samples, but not in internal mammary artery samples[160]. In addition, *P. gingivalis*, *Fusobacterium nucleatum* and *T. forsythia* were identified in 100%, 84% and 48% of human carotid endarterectomy specimens, respectively[161].

One of the main arguments against the role of dysbiosis and bacteraemia in atherosclerosis progression, is that PCR does not prove the viability of the bacteria in atheromatous plaque and, therefore, a direct contributory role cannot be confirmed[162]. However, viable, previously uncultivable, oral bacterial species, such as *P. gingivalis* and *Propionibacterium acnes*[163], and invasive species implicated in chronic periodontitis, such as *A. actinomycetemcomitans*[158], have been identified in atheroma samples.

Data from animal studies also suggest a direct causal role for *P. gingivalis* invasion of arterial endothelial cells in atherosclerotic plaque formation. For example, administration of metronidazole (an antibiotic with a proven inhibitory effect on *P. gingivalis*) in mice inoculated with *P. gingivalis* resulted in complete prevention of atherosclerotic lesions in those fed normal chow diets, and significantly fewer atherosclerotic lesions in those fed high-fat diets, compared with those that received *P. gingivalis* alone[164].

Systemic inflammation

Strong evidence exists to support the role of systemic inflammation in the development and progression of CVD, HF and atherosclerosis. Epidemiological studies have shown that increased cardiovascular risk is associated with elevated levels of pro-inflammatory cytokines, such as IL-1, IL-6 and TNF[165,166], as well as acute-phase proteins, such as CRP and fibrinogen[167,168]. Chronic periodontitis is also associated with persistently increased systemic levels of pro-inflammatory cytokines[169] and acute-phase proteins[170,171]. Systemic inflammation stimulates expression of cell adhesion molecules on arterial endothelial cells, induces monocyte recruitment to the arterial wall and stimulates LDL uptake by macrophages, thereby promoting foam cell formation[168]. The role of lipopolysaccharide in vascular and myocardial dysfunction has been described above. Additionally, oxidative stress induced by increased circulating CRP[172] has been implicated in the development and progression of HF[173], stroke[174] and ASCVD[175]. The presence of reactive oxygen species (ROS) resulting from periodontitis induces cellular dysfunction via the oxidation of proteins and lipids and damage to DNA[176]. Additionally, ROS alter the contractile function of cardiomyocytes by modifying proteins crucial to excitation–contraction coupling, and by stimulating fibroblast proliferation that favours cardiac remodelling[177].

Animal studies have demonstrated the important role of the oral microbiota in the pathogenesis of inflammatory atherosclerosis via pro-inflammatory cytokines. For example, researchers showed that deletion of *Tlr2*, that encodes a principal sensor of the innate immune system, in mice inoculated with *P. gingivalis* is associated with fewer atherosclerotic lesions as well as differences in plaque composition compared with $Tlr2^{+/+}$ controls, suggesting a more stable phenotype[178]. Ablation of *Il1r1* in mice subjected to *P. gingivalis* challenge and fed a high-fat diet resulted in a reduction in atherosclerotic plaque formation[179]. However, inoculation with *P. gingivalis* has also been reported to increase the formation of pathogen-associated atherosclerotic plaques in $Il6^{-/-}$ mice[180]. This phenomenon could be attributed to the pleiotropic nature of IL-6, which has both pro-inflammatory and anti-inflammatory properties[181]. Together, this evidence directly links the oral microbiota with systemic inflammatory mediators and the progression of atherosclerotic disease.

Prevention and management

Despite the harmful effects of a dysbiotic oralome, its complete elimination is not ideal, as a eubiotic oral microbiota provides health benefits. In the following sections, we discuss strategies for establishing and maintaining a eubiotic state in the oral cavity and consider proven and putative approaches to reduce CVD from oral infection. Although faecal microbiota transplantation[182,183] and bariatric surgery[184,185] have been demonstrated to modulate gut dysbiosis, the evidence for their role in modulating oral dysbiosis is very limited and requires further research. Interestingly, data published in the past 2 years show that bariatric surgery can alter the oral and salivary microbiota[186,187]. Generally, however, there is a dearth of randomized clinical trials focusing on optimal management strategies for the oralome.

Oral hygiene

Bacterial load in dental plaque correlates with periodontal disease severity[188]. Oral hygiene is important for controlling the microbial load on the dentition and in the oral cavity, helping to prevent periodontitis

and subsequent bacteraemia[189]. Proven oral hygiene measures include tooth brushing with fluoride toothpaste to remove dental plaque and the use of chemical, antiplaque mouth rinses, which reduce both gingival inflammation and dental plaque scores[190,191]. Although oral hygiene might only temporarily disrupt the acidogenic biofilm[170], when practised regularly it can keep dental plaque in an immature state and in small amounts, significantly decreasing the risk of bacteraemia. The use of fluoride improves enamel remineralization[192,193]. Conversely, clinical data have indicated that the use of over-the-counter mouthwash, typically thought to reduce the abundance of nitrate-producing bacteria in the oral microbiome, is associated with an increased risk of hypertension independent of major risk factors and other confounders[194]. In a small study of 19 healthy volunteers, antiseptic mouthwash reduced oral nitrite production by up to 90% and was correlated with a 2.0–3.5 mmHg increase in systolic BP within 1 day of use[195]. Whether the risks associated with mouthwash use outweigh the benefits remains unclear, leading some researchers to suggest that limiting its use to prescription only might be a good compromise[196]. Further clinical investigation on the risks and benefits of mouthwash use in patients with periodontitis and CVD is necessary.

Intensive periodontal treatment has been shown to reduce glycated haemoglobin (HbA1c) levels in patients with type 2 diabetes[197]. HbA1c is a reliable risk factor for all-cause and cardiovascular mortality[198], suggesting that routine oral health assessment and treatment of periodontitis are important for the effective management of CVD. Generally, given the positive effects of periodontal treatment on systemic disease, a need exists for greater collaboration between dentists and cardiologists to elucidate these mechanisms and optimize treatments for those at risk[199].

Diet

Consumption of a Western diet, which is generally low in fibre and fermentable substrates and high in saturated and *trans* fatty acids, is associated with depletion of metabolic fuels, resulting in alterations in the oral and gut microbiota, ultimately contributing to increased cholesterol levels[200]. End products of bacterial fermentation, particularly short-chain fatty acids (propionate), are known to suppress cholesterol synthesis in the liver and intestine in rats[201]. This finding is supported by clinical data correlating specific short-chain fatty acid profiles with hypercholesterolaemia[202], and the fact that bacteria that ferment fibres are typically reduced in the mucosa and faeces of patients with gut-dysbiotic conditions, such as inflammatory bowel disease[203]. Therefore, a shift towards a plant-based diet could confer protective effects against ASCVD and systemic inflammation by increasing endothelial protective factors in the circulation, while reducing factors that are injurious to endothelial cells[204].

Additionally, bile acids (emulsifiers of lipids and lipid-soluble vitamins that aid intestinal absorption) and the human microbiome are inextricably linked[205]. Primary bile acids are biochemically modified by commensal microbiota, whereas the resulting composition of secondary bile acids directly affects microbiota abundance and diversity[206]. The bile acid receptor (also known as the farnesoid X receptor) has been implicated in atherosclerotic disease, specifically through ligand-mediated NF-κB suppression and subsequent downregulation of the inflammatory response[207,208], Therefore, administration of a bile acid receptor agonist, obeticholic acid, has been investigated for its potential to optimize metabolic parameters that might reduce cardiovascular complications. However, experimental data are inconclusive, with some studies showing additional metabolic derangements, such as increased blood lipid levels[209,210]. An investigation of the secondary bile acid, ursodeoxycholic acid, showed limited improvements in peripheral blood flow in patients with chronic HF[211]. There is a paucity of data on dietary interventions to improve CVD outcomes through modulation of the oral microbiota, highlighting the need for validation of these proposed mechanisms.

Antimicrobial peptides

Antimicrobial peptides modulate oral biofilm dysbiosis via broad-spectrum antimicrobial activity, although an undesired consequence is that they eliminate both pathogenic and commensal bacteria[212]. Nisin was approved as a food additive by the FDA in 1988 owing to its broad antimicrobial activity and has been demonstrated to disrupt oral biofilms without being cytotoxic to oral cells[213]. Endogenous antimicrobial peptides, such as LL-37, have antifungal, antimicrobial and antibiofilm properties and can act as a chemoattractant for human peripheral blood neutrophils, monocytes and T cells. In addition, LL-37 opsonizes *A. actinomycetemcomitans*, inhibits biofilm formation by this bacterium and prevents growth of *F. nucleatum*. However, various other cariogenic bacteria, including *S. mutans* and *Streptococcus sobrinus*, have been found to be resistant to LL-37 (refs. 214,215).

Although clinical trials of antibiotics have not reduced recurrent cardiovascular events, targeting of innate immune mechanisms using low-dose colchicine and anticytokine strategies in patients at risk of atherosclerotic events have shown some promise[216]. For example, in the randomized, controlled CANTOS trial[194,217,218], the anti-IL-1β monoclonal antibody canakinumab reduced inflammatory markers and recurrent cardiovascular events in patients with a history of myocardial infarction.

Prebiotics

Prebiotics are natural or synthetic food components or supplements that modulate the microbiome to benefit the host by exploitative competition, in which the growth of beneficial bacteria is promoted and bacteriocins exhibit selective interference to inhibit the growth of unwanted bacteria[219]. Prebiotics have long been applied to the gut microbiome, but only in the past 3 years to the oral microbiome[220,221]. Arginine prevents dental plaque by buffering the acids produced by a dysbiotic oralome[222]. In an in vitro study using human salivary samples, 16S rRNA pyrosequencing demonstrated that arginine pretreatment before sucrose ingestion resulted in a shift in the oral microbiome towards 'health', with an increased prevalence of commensal bacteria, such as *Prevotella* spp., *Veillonella dispar* and *Streptococcus anginosus*, and increased biofilm diversity compared with biofilms that were not pretreated with arginine[223]. Nitrate, methyl-β-D-galactoside and *N*-acetyl-D-mannosamine are other promising oral prebiotics that have been shown to induce a shift towards eubiosis in biofilm communities in vitro[224,225].

Probiotics

Probiotics are live microorganisms which, after ingestion, confer health benefits on the host. Administration of probiotics in the form of a mouth rinse improves the oralome and oral health by preventing the growth of harmful bacteria[226], modulating the local and systemic immune responses, and stimulating osteoblastic function to reduce gingivitis, plaque, alveolar bone loss and modulate pro-inflammatory effects[227]. In vitro data suggest that *Lactobacillus* spp. have antibacterial activity against *P. gingivalis*[228], whereas *S. mitis* isolated from mouth swabs taken from healthy volunteers have antibacterial activity against *P. intermedia*, *A. actinomycetemcomitans* and *F. nucleatum*[229].

Glossary

Dysbiosis
Any change to the composition or function, in terms of abundance and diversity, of resident commensal microbes relative to the composition or role in healthy individuals. Owing to the close, bidirectional orchestration of these communities and human physiology, perturbations in abundance and diversity can lead to inflammatory and metabolic abnormalities that contribute to a plethora of diseases.

Endotoxaemia
Translocation of lipopolysaccharide in the circulation, which can arise from infections or commensal bacteria. The oral cavity is an important source of endotoxaemia. The mouth harbours approximately 100–200 commensal bacterial species and has higher phylogenetic diversity than any other location in the body.

Eubiosis
A healthy or optimal microbiome, one with diversity and uniformity of respective microbiota, at abundances characteristically found in healthy individuals. A eubiotic microbiome confers a protective and beneficial physiological effect, helping to maintain the optimal homeostatic balance while ensuring appropriate training and maintenance of the immunological system.

Microbiome
A characteristic community of microbiota, including bacteria, archaea, fungi, viruses, protists and algae, occupying a specific habitat, including their 'theatre of activity', consisting of various microbial structural elements (proteins, lipids and polysaccharides and nucleic acids), internal and external structural elements (including microbial metabolites and mobile genetic elements) and the immediate environmental conditions. Together, these elements form unique ecological niches that are dynamic and adaptable through time, integrating with host physiology to influence function and health.

Multiomics
A comprehensive, or global, assessment of a set of molecules. The term is commonly applied to high-throughput technologies in the fields of genomics, proteomics, transcriptomics, metabolomics and lipidomics. When these fields are combined, specifically looking at data from a 'multiomics' perspective, the flow of information that underlies disease, from gene to phenotype, can be elucidated.

Oralome
The overarching term using to summarize the dynamic interactions between the ecological community of oral microorganisms, including bacteria, fungi, viruses, archaea and protozoa that live within the oral cavity of the host.

Periodontitis
A chronic inflammatory disease associated with destruction of connective tissue of gingiva, periodontal ligament and alveolar bone following untreated or improperly treated gingivitis. Bacterial biofilms (dental plaque), predominantly composed of the viridans group streptococci, are the primary aetiological factors for the inflammatory process of gingivitis, leading to subsequent destruction of periodontal tissues.

Inflammatory response modulators

The body's response to oral infection is periodontal destruction through inflammation. Therefore, inflammatory response modulators are a promising therapeutic avenue for the treatment of periodontitis. Administration of doxycycline hyclate (Periostat; Alliance Pharmaceuticals) downregulates the activity of matrix metalloproteinases, which are key destructive enzymes in periodontal disease[230]. In addition, chemically modified tetracyclines 1, 3, 4, 7 and 8 (all devoid of antimicrobial activity) reduce the collagenolytic activity of *P. gingivalis* in vitro and inhibit alveolar bone loss in vivo via the inhibition of matrix metalloproteinases[231].

Nanoparticle drug delivery systems

Nanoparticles are colloidal particles made from lipids, metals and polymers, with sizes between 1 nm and 1,000 nm. They offer high surface area and reactivity and low thermal resistivity. These particles facilitate the intracellular delivery of therapeutics and can induce the release of high levels of ions at low incorporated amounts[48]. Silver, copper, titanium oxide and zinc oxide nanoparticles have demonstrable antimicrobial ability in the oral cavity and disrupt *S. mutans* biofilms[232,233].

A mucoadhesive liquid crystalline delivery system loaded with the decapeptide KSL-W achieved 100% inhibition of multispecies bacterial biofilm growth, providing a potential vehicle for the buccal administration of antibiofilm peptides[233]. Given the myriad of epidemiological and pathophysiological associations between biofilm formation, periodontitis and CVD, antibiofilm nanoparticle drug delivery systems could be important modalities for the early prevention of CVD.

Biofilm disruption

Disruption of the oralome extracellular polymeric matrix prevents the formation of dysbiotic biofilms and can be achieved through inhibition of synthesis and secretion and targeting its composition and structure[48]. For example, L-arginine, a substrate for alkali production by arginolytic bacteria, can neutralize acids and control pH levels in biofilm microenvironments, preventing the proliferation of acidophilic bacteria that drive biofilm-mediated periodontal disease[234]. Treatment of *S. mutans* using L-arginine produced a substantial reduction in the quantity of insoluble biofilm and significantly altered 3D architecture[235]. Additionally, high-velocity micro sprays in combination with antimicrobial agents have also proved effective. High shear stresses generated at the biofilm surface result in the physical restructuring of the biofilm, facilitating improved penetration of antimicrobial agents and death of bacteria[236]. Another interesting method involves pH-responsive catalytic inorganic nanoparticles, which bind to biofilms under acidic conditions, generating free radicals from hydrogen peroxide, which not only degrade the biofilm, but simultaneously kill embedded bacteria[237]. Many other methods are currently in development, detailed discussion of which is beyond the scope of this article.

Knowledge gaps and future directions

High-throughput sequencing technologies and multiomics analyses have provided substantial advances in delineating the complex and dynamic relationship between the oral microbiota and CVD. However, many gaps in our knowledge remain and important questions for future research are outlined below.

- Although many of the studies discussed in this Review have established an association between oral infection, a dysbiotic oralome and CVD, the precise causal pathways are not yet clearly elucidated, and further interventional and pathomechanistic studies are required.
- The balance between eubiosis and dysbiosis, as well as the factors that trigger a shift from one to the other, need to be better defined.
- Despite the high prevalence of oral dysbiosis in patients with CVD, many individuals with unhealthy oral microbiota do not develop CVD. For example, the reasons why only 0.3–3.0% of patients with GAS-related pharyngitis and <6.0% of individuals in GAS-endemic regions develop ARF are unknown.

- Given that oral dysbiosis is linked to the development of many diseases, it is not clear which patient-related factors dictate the development of each condition and why some patients develop comorbid diseases and others isolated CVD within this dysbiotic spectrum.
- An urgent need exists for the development of novel, efficacious therapies for oral dysbiosis as well as robust randomized clinical trials of existing therapies.
- Further studies of interventions to eradicate oral bacteria, and that focus on clinical outcomes, are required.

Conclusions

The unabated rise in CVD morbidity and mortality over the past two decades, despite ongoing research, indicates that innovative approaches to understanding CVD pathophysiology are needed for effective therapies to be developed. Dysbiosis of the oral microbiome contributes to CVD through biofilm formation, endothelial dysfunction, molecular mimicry, platelet aggregation, direct arterial invasion and systemic inflammation. These mechanisms operate synergistically or independently in a highly complex manner; however, gradual elucidation of these links could lead to some compelling answers to fill the persistent gaps in our understanding of CVD in general. Improved understanding is needed not only from a mechanistic perspective, but also from a therapeutic perspective, where the potential exists to generate novel therapies that target these mechanisms. Therefore, further investigations are required to determine the biological validity of the postulated pathophysiological mechanisms discussed in this Review, to understand the effectiveness and feasibility of targeting oral dysbiosis in the prevention and management of CVD and to elucidate the depth of the relationship between human physiology and the oral microbiome.

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E.N.L. and N.A.B.N. conceived the microbiome project. A.T., E.N.L. and N.A.B.N. researched data for the article, and A.T. compiled the figures. All the authors contributed to discussion of content, writing of the manuscript and reviewing/editing the article before submission.

Competing interests
The authors declare no competing interests.

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