⬤ **MICROBIOME**

The oral microbiota: dynamic communities and host interactions

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Abstract | The dynamic and polymicrobial oral microbiome is a direct precursor of diseases such as dental caries and periodontitis, two of the most prevalent microbially induced disorders worldwide. Distinct microenvironments at oral barriers harbour unique microbial communities, which are regulated through sophisticated signalling systems and by host and environmental factors. The collective function of microbial communities is a major driver of homeostasis or dysbiosis and ultimately health or disease. Despite different aetiologies, periodontitis and caries are each driven by a feedforward loop between the microbiota and host factors (inflammation and dietary sugars, respectively) that favours the emergence and persistence of dysbiosis. In this Review, we discuss current knowledge and emerging mechanisms governing oral polymicrobial synergy and dysbiosis that have both enhanced our understanding of pathogenic mechanisms and aided the design of innovative therapeutic approaches for oral diseases.

Subgingival
Relating to the area under the gum margins.

Gingival crevicular fluid
(GCF). A serum exudate that contains immune and inflammatory mediators, along with large numbers of neutrophils recruited along a chemokine gradient.

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https://doi.org/10.1038/s41579-018-0089-x

Diverse microorganisms inhabit the oral cavity[1,2] and in many cases are unique to this niche as they have evolved an exquisite specificity for oral colonization[3]. Within the oral cavity, there are distinct microenvironments such as the hard non-shedding surfaces of the teeth and the epithelial surfaces of the mucosal membranes (FIG. 1). These surfaces are exposed to a fluid phase of saliva, or if subgingival, to gingival crevicular fluid (GCF). The microbial communities that grow on these surfaces are also distinct, and any one site contains ~50 species — a subset of ~1,000 species that are capable of oral colonization[4,5]. Tissue-specific tropisms are often defined by specificity and avidity of adherence, which is a feature of many successful oral colonizers, providing resistance to the mechanical shearing forces of fluid flow and mastication. Primary colonizers of oral surfaces are predominantly facultative anaerobes such as streptococci and *Actinomyces* species. Within the confines of the subgingival area, reduced oxygen tensions favour population shifts with increasing abundance of strict anaerobes such as Bacteroidaceae spp. and spirochaetes. In addition to microbial composition, the spatial and structural organization (biogeography) of natural microbial communities is being increasingly recognized as essential for physical and metabolic interspecies interactions that can be antagonistic or cooperative[6,7]. Microorganisms on tooth surfaces tend to form multispecies biofilm communities that are often embedded in a matrix of extracellular polymeric substances (EPS). By contrast, the shedding, more transient epithelial surfaces necessitate a specialized colonization strategy, and although

organisms do form biofilms on these surfaces, there is less time for biofilm maturation than with abiotic or tooth surfaces. In addition, bacteria penetrate and grow within epithelial tissues and even intracellularly. Most of the time, a homeostatic balance exists between the host and microbial communities, and the resident microbiota is thought to compete with and exclude exogenous pathogens as a component of ecosystem stability, as well as contribute to normal tissue and immune system development[8,9]. Although gingivitis is an almost inevitable consequence of prolonged accumulation of biofilms (also known as plaque) on tooth surfaces, it is a controlled immune-inflammatory state (BOX 1) that does not permanently compromise the integrity of the tissues supporting the teeth. Host saliva also contributes to ecosystem stability by buffering the oral environment, providing nutrition to the community and delivering antimicrobial factors that are antagonistic to exogenous species. Nonetheless, under particular conditions, the host-community interaction becomes dysbiotic and site-specific diseases involving the teeth or gums (gingivae) can follow[10–12]. The accessibility of the oral ecosystems has facilitated the characterization of microbial communities that are associated with health or disease at distinct oral sites. In this Review, we discuss the mechanisms by which oral microbial communities develop and become functionally specialized. We examine the progression of polymicrobial communities towards pathogenicity with a particular emphasis on the induction of immune responses that are ineffective, uncontrolled and destructive, and on cariogenic biofilm development stimulated by host diet.
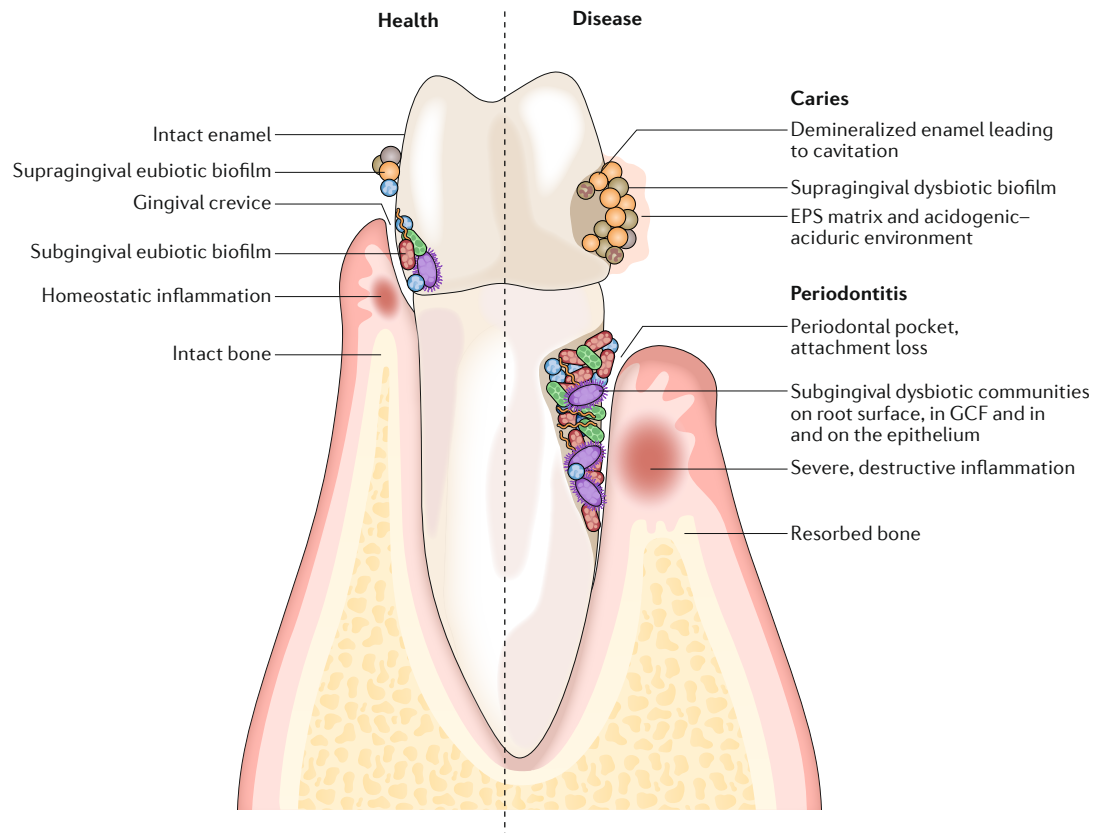
Fig. 1 | **Biogeography of oral microbiota colonization in the diverse habitats of the oral cavity.** Microbial colonization occurs on all available surfaces, and microorganisms can also penetrate epithelial tissues and cells. The microbiota assembles into biofilm communities on the abiotic and biotic surfaces. In health (left), eubiotic biofilms maintain a homeostatic balance with the host. In disease (right), caries and periodontitis ensue when biofilms become dysbiotic, resulting in increased levels and duration of low pH challenge and the induction of destructive inflammatory responses, respectively. EPS, extracellular polymeric substance; GCF, gingival crevicular fluid.

Finally, the opportunities for therapeutic intervention directed towards interfering with acidogenic biofilm development or the subversion of the immune response are explored.

The oral microbiota and disease

Several processes underlie the transition of a microbial community to a state of dysbiosis. Alterations in host immune competence or diet can affect the community composition and the metatranscriptional landscape, with increases in the production of virulence factors. As a community develops, microbial metabolism and by-products of the host immune response can cause changes to the local environment that facilitate the outgrowth or over-representation of microorganisms associated with a dysbiotic state. The microbiota associated with a healthy state is thus considered more generalist, whereas the disease-associated microbiota is influenced by 'specialist' microorganisms that possess metabolic functions and an elevated virulence potential that are largely absent in health[13]. Once a community has transitioned to a dysbiotic state, the structural stability of functionally specialized components[6,14,15] will allow the condition to persist for an extended period of time, and oral diseases such as periodontitis and dental caries (FIG. 1) are often chronic and slowly progressing (although acute

onset of both diseases can be triggered under particular host-compromising conditions).

Supragingival communities and dental caries. In dental caries, overexposure to dietary carbohydrates and host factors promotes the production of EPS and acidic metabolites, in addition to causing the accumulation of acidogenic and aciduric microorganisms. Excess fermentable carbohydrate thus drives the transition to a pathogenic biofilm community[7,16,17]. If sugar consumption is low and infrequent, the microbial communities on teeth remain stable and, despite being able to produce acids that demineralize enamel, the episodic pH decrease can be readily neutralized by saliva, which restores and maintains the mineralization of enamel[17]. However, with frequent exposure to fermentable carbohydrates, microorganisms become embedded in an EPS-rich biofilm matrix while constantly producing acids that are physically protected from rapid buffering by saliva. Localized regions of low pH within biofilms formed on tooth surfaces[18,19] continue to select for aciduric microorganisms[17]. If the biofilm is not removed and frequent sugar consumption continues, a prolonged and repeated state of acidification ensues (which can be exacerbated by dysfunction in salivary secretion or composition), disrupting the homeostatic mineral balance

towards enamel demineralization. Mutans streptococci (especially *Streptococcus mutans*) and lactobacilli have long been recognized as pathogens that are associated with caries; however, more recent molecular analyses have revealed the existence of a pathogenic community that includes non-streptococcal bacteria (for example, *Bifidobacterium* spp., *Scardovia* spp. and *Actinomyces* spp.) and fungi (for example, *Candida albicans*)[20–23]. The microbial composition can vary depending on the different sites of the tooth surface. These microorganisms interact with each other in a dynamic and concerted polymicrobial synergy to form a cariogenic biofilm (that is, a biofilm that can cause caries) within which the community changes as caries progress from early onset (initial demineralization) to deeper lesions with dentin exposure[7].

Subgingival communities and periodontal disease.
In periodontal diseases, polymicrobial communities induce a dysregulated and destructive host response through an overall mechanism referred to as polymicrobial synergy and dysbiosis[24] (BOX 1). Conventional culture-based approaches identified a pathogenic triad of *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*[25,26] (the red complex), and culture-independent molecular studies have extended the list of candidate pathogens to include the Gram-positive bacteria *Filifactor alocis* and *Peptoanaerobacter stomatis*; Gram-negative members of the Firmicutes phylum (*Dialister* spp., *Megasphaera* spp. and *Selenomonas* spp.); species in the genera *Prevotella*, *Desulfobulbus* and *Synergistes*; and many others[1,2,5,27,28]. Indeed, contrary to the situation in the gastrointestinal tract, periodontal diseases are associated with increases in diversity of the microbiome, thought to be the consequence of additional nutrients derived from host tissue damage

and increasing physical space as the gingival crevice deepens. However, it is important to note that in vivo studies show substantial variation in the microbiomes among individuals with periodontitis and even between sites in the same individual[29–32]. Although human studies do not provide insights into disease mechanisms, the integration of metagenomic and metatranscriptomic data indicates that, rather than there being a discrete pathogenic cohort of organisms, a particular set of gene functions is required to induce dysbiosis.

The oral microbiota in cancer and other systemic diseases.
Increasing evidence supports an association between the oral microbiome and oral cancers such as oral squamous cell carcinoma. A mechanistic understanding for such an association may arise from the ability of many oral microorganisms to alter the inflammatory microenvironment and to interfere with host signalling pathways that control cell viability, proliferation and differentiation[33–38]. Remarkably, the influence of the oral microbiome can extend beyond the oral cavity, and systemic conditions such as coronary artery disease, preterm delivery of low-birthweight neonates and rheumatoid arthritis are associated with the oral microbiome[39–41]. In the case of rheumatoid arthritis, the enzyme peptidylarginine deiminase PPAD — produced uniquely by *P. gingivalis* — can citrullinate host proteins, which may then induce autoantibody production[42].

Polymicrobial synergy
Microorganisms within communities often interact synergistically to enhance colonization, persistence or pathogenicity. The concept of polymicrobial synergy among members of the periodontal microbiome has been established in vivo; experiments in animal models consistently show elevated pathogenicity with combinations of organisms compared with monospecies infection[10,43,44]. Such polymicrobial synergy can arise from several classes of interspecies interactions including one organism providing a substratum for the attachment and colonization of another, nutritional cross feeding, and the co-ordinated metabolism of complex substrates[10,45–48]. Physical interactions among organisms and the diffusion of soluble factors can modulate virulence gene expression and nososymbiocity[45,49]. Interdependence among community members has led to functional specialization, with different species contributing discrete sets of community-essential genes. Similarly, in cariogenic communities, synergistic interactions occur between acidogenic and aciduric bacteria during the different stages of biofilm development and acidification[7]. Recently, inter-kingdom synergies with *Candida albicans* were found to enhance biofilm nososymbiocity, leading to the onset of severe caries in vivo. Furthermore, mechanistic studies have indicated complex signalling and cross-feeding interactions combined with elevated EPS production[7,50,51].

The notion that commensalism or pathogenicity represent a fixed duality has in recent years been replaced by the concept of a fluid continuum[45,52–55], and the oral microbiome provides many illustrative examples.

Box 1 | Models of microbiota-induced periodontitis

Gingivitis is highly prevalent in the human population. An immune-inflammatory state characterizes this condition, and neutrophils are continuously recruited into the gingival tissues. Mild periodontal inflammation can therefore be seen as a normal and controlled state that may prevent, or at least does not contribute to, tissue destruction. Disruption of this equilibrium is necessary for the onset of destructive inflammation and permanent tissue damage. The widespread occurrence and clinical variety of destructive periodontal conditions has complicated attempts to construct an overarching model of disease initiation and progression. Additionally, experimentally tractable animal and in vitro models by definition do not fully recapitulate the human situation, although different aspects of the disease can be productively addressed by in vitro and animal models. The recently formulated polymicrobial synergy and dysbiosis (PSD) hypothesis is consistent with human microbiome studies and mechanistic studies in animal models and with the 'ecological plaque' hypothesis[24,99]. According to the ecological plaque concept, environmental factors (for example, inflammation, pH, redox potential and nutrient availability) drive the selection and enrichment of specific pathogenic bacteria, also known as pathobionts[36,45]. The PSD model proposes that disease is not initiated by individual causative pathogens but rather by a synergistic polymicrobial community, within which specific constituents, or combinations of functional genes, fulfil distinct roles that converge to shape and stabilize a dysbiotic microbiota, which perturbs host homeostasis[11]. Disease is caused by reciprocally reinforced interactions between such physically and metabolically integrated polymicrobial communities and a dysregulated host inflammatory response. Thus, the PSD model bypasses the 'chicken-or-the-egg' question on whether dysbiosis initiates inflammation or vice versa but rather places an emphasis on the continuous cyclic process in which dysbiosis and inflammation are reciprocally reinforced and constitute the actual driver of periodontitis.
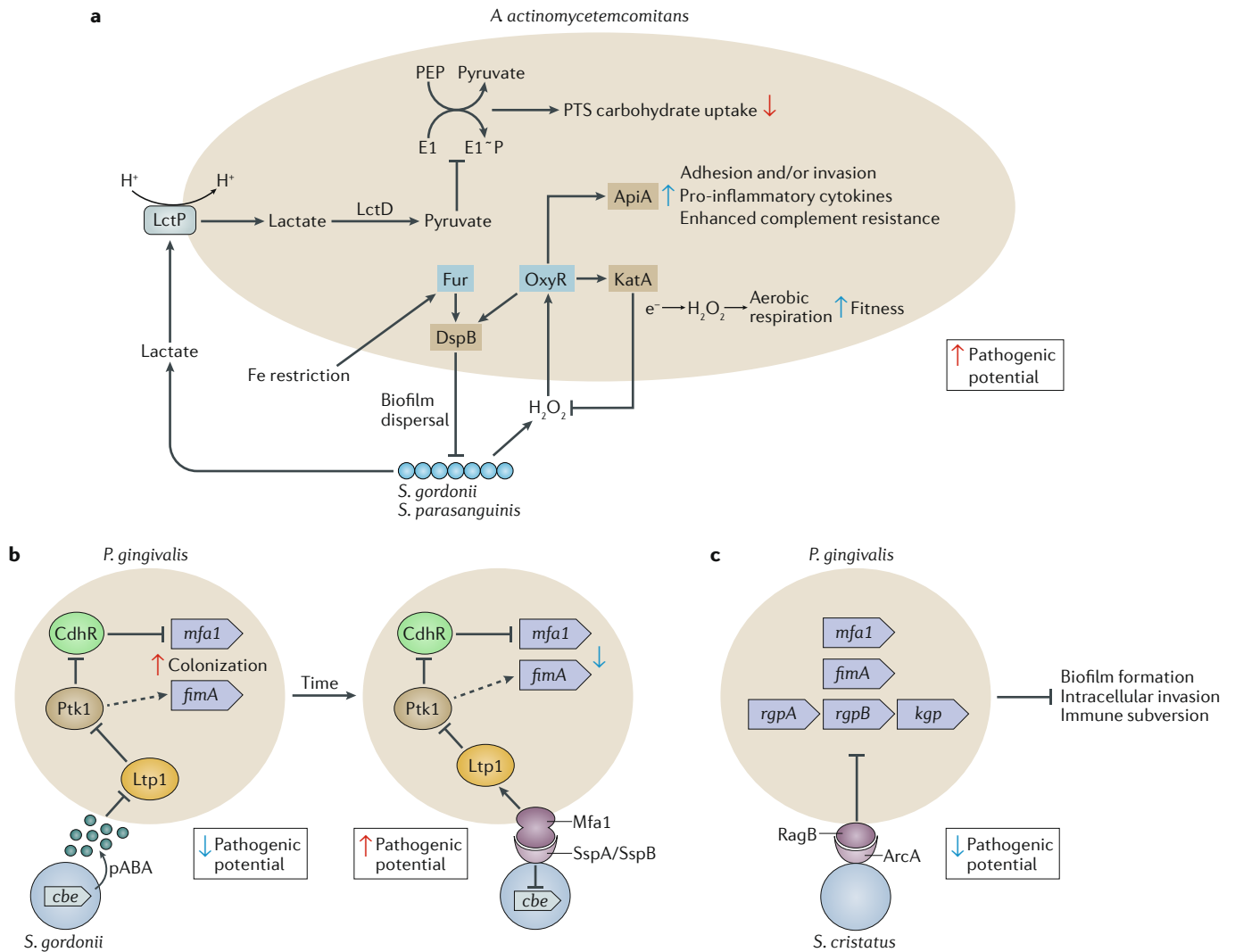

Fig. 2 | **Interactions among bacterial species that affect nososymbiocity.** Oral bacteria interact through multiple pathways that can be separated both spatially and temporally. **a** | *Streptococcus gordonii* and *Streptococcus parasanguinis* produce hydrogen peroxide, to which *Aggregatibacter actinomycetemcomitans* responds by activation of the OxyR transcriptional regulator; consequently, transcription of both *apiA* and *katA* is elevated. Higher levels of the ApiA surface protein increases complement resistance and will also potentially induce intracellular invasion and pro-inflammatory cytokine production. KatA (a catalase) degrades the hydrogen peroxide produced by both streptococci and neutrophils, thus protecting *A. actinomycetemcomitans* from oxidative damage[64,66,154–156]. OxyR also regulates production of dispersin B (DspB), an enzyme that degrades the biofilm matrix and facilitates dispersal of *A. actinomycetemcomitans*[65]. Hydrogen peroxide increases the bioavailability of oxygen, and, in response, *A. actinomycetemcomitans* shifts from a primarily fermentative to a respiratory metabolism, an interaction referred to as cross respiration[157]. Respiratory metabolism enhances the growth and fitness of *A. actinomycetemcomitans* in vivo. Transport of streptococcal lactate into *A. actinomycetemcomitans* through the proton-driven lactate permease (LctP) leads to conversion to pyruvate by lactate dehydrogenase (LctD). Pyruvate suppresses autophosphorylation of E1, which then decreases uptake of phosphotransferase system (PTS) carbohydrates such as glucose[155]. Preferential utilization of lactate through this carbon resource partitioning gives a competitive advantage to *A. actinomycetemcomitans* in the presence of organisms that can metabolize glucose more efficiently. Communities of *A. actinomycetemcomitans* and *S. gordonii* also become restricted for iron. The Fur transcriptional regulator of *A. actinomycetemcomitans* responds to iron limitation and induces upregulation of the gene encoding DspB, which will release

A. actinomycetemcomitans from biofilms[158]. *A. actinomycetemcomitans* responses to both oxidative stress and iron restriction thus involve DspB activity and re-localization, and in vivo *A. actinomycetemcomitans* maintains an optimal distance from streptococci in communities that are synergistically virulent[65]. **b** | Interactions between *Porphyromonas gingivalis* and *S. gordonii* resulting from metabolite (4-amino benzoate (pABA)) perception (left) and direct contact (right). pABA secreted by *S. gordonii* inactivates the *P. gingivalis* tyrosine phosphatase Ltp1. Dephosphorylation and inactivation of the tyrosine kinase Ptk1 is thus reduced. Ptk1 phosphorylates and inactivates the transcription factor CdhR, which is a repressor of the *mfa1* gene. Ptk1 activity also converges on expression of the *fimA* gene. Expression of both fimbrial adhesins is increased, and in this mode *P. gingivalis* is primed for attachment to *S. gordonii*. However, nososymbiocity is reduced, and pABA-treated *P. gingivalis* are less pathogenic in animal models. Engagement of Mfa1 with the streptococcal SspA or SspB surface protein increases Ltp1 and reverses information flow through the Ltp1–Ptk1 axis. In addition, Mfa1–SspA/SspB binding suppresses expression of chorismate binding enzyme (Cbe), which is responsible for pABA production. Prolonged physical interaction between *P. gingivalis* and *S. gordonii* leads to increased nososymbiocity, and dual infection of animal models causes more alveolar bone loss than *P. gingivalis* infection alone[60–63,159,160]. **c** | *Streptococcus cristatus* arginine deiminase (ArcA) interacts with the *P. gingivalis* surface protein RagB. Signal transduction results in downregulation of genes encoding the FimA and Mfa1 component fimbriae along with the arginine-specific (RgpA or RgpB) and lysine-specific (Kgp) gingipain proteinases. Adhesion, biofilm formation, epithelial cell invasion and degradation of cytokines are consequently reduced and nososymbiocity is suppressed[72–74]. Part **a** adapted with permission from REF.[56], Wiley-VCH.

Aciduric
Organisms capable of growth at acidic pH levels that are often toxic to other bacteria.

Polymicrobial synergy
Interactions among organisms that increase microbial fitness in the local environment.

Dentin
Calcified tissue, predominantly hydroxyapatite, forming the bulk of the tooth, which is beneath and is softer (less mineralized with more organic material) than enamel.

Periodontal diseases
A collection of conditions in which poorly controlled inflammatory responses induced by the microbiota cause destruction of the supporting structures of the tooth.

Red complex
The triad of *P. gingivalis*, *T. forsythia* and *T. denticola* — organisms that are often isolated together and are classically considered to be the predominant pathogens in chronic periodontitis.

Gingival crevice
The compartment between the tooth root and the gingival (gum) tissue. The gingival crevice deepens into a periodontal pocket as periodontal disease progresses and tissue is destroyed.

Citrullinate
Post-translational modification of a protein involving deamination of arginine by the enzyme peptidylarginine deiminase PPAD to produce citrulline.

Cross feeding
The utilization of a metabolic by-product of one organism as a nutrient source by another organism.

Oral streptococci of the Mitis group (including *Streptococcus gordonii*, *Streptococcus sanguinis*, *Streptococcus parasanguinis*, *Streptococcus oralis* and *Streptococcus mitis*) were once considered strict commensals in the oral cavity, a view that is now considered as too restrictive[56]. These microorganisms are abundant pioneer colonizers of tooth surfaces, in large part owing to the expression of numerous adhesins for receptors in the salivary pellicle that coats tooth surfaces[3,57]. Monoinfection of animal models with Mitis streptococci does not induce substantial pathology[56,58,59]. However, mixed infections of *S. gordonii* and *P. gingivalis* result in increased alveolar bone loss compared with infection with *P. gingivalis* alone[60]; *S. gordonii* is considered an accessory pathogen in this context[56]. However, the interaction between *S. gordonii* and *P. gingivalis* is nuanced and multidimensional. Sensing of the streptococcal metabolite 4-amino benzoate (pABA) by *P. gingivalis* increases the activity of the tyrosine kinase Ptk1, and the resulting protein tyrosine phosphorylation-dependent signalling converges on the FimA and Mfa1 fimbrial adhesins[61]. Although this primes *P. gingivalis* for attachment, the pathogenic potential of this organism is diminished[61]. Engagement of the Mfa1 adhesin with streptococcal SspA or SspB surface proteins initiates community development and activates the tyrosine phosphatase Ltp1. Dephosphorylation of Ptk1 by Ltp1 suppresses adhesin production and ultimately constrains the degree of community accretion[62,63] (FIG. 2). Interspecies communication based on physical contact increases the pathogenic potential of *P. gingivalis*. Moreover, communication is bidirectional, and as a keystone pathogen, *P. gingivalis* increases the nososymbiocity of communities in which it resides and drives pathogenicity even at low abundances (discussed below).

Both *S. gordonii* and *S. parasanguinis* are accessory pathogens to *Aggregatibacter actinomycetemcomitans* — a keystone pathogen in localized aggressive periodontitis[64-66] (FIG. 2). Mitis group streptococci have also been shown to enhance the virulence of *C. albicans* by promoting fungal tissue invasion and increasing the severity of mucosal infection[67,68]. However, consistent with the notion that pathogenic potential is context-dependent, *S. gordonii*, *S. sanguinis* and *S. oralis* are antagonistic towards *S. mutans*[69-71] and may help protect against caries (FIG. 3). A related streptococcal species, *Streptococcus cristatus*, is antagonistic towards *P. gingivalis* and suppresses virulence gene expression[72,73] (FIG. 2). *S. cristatus* can reduce the pathogenicity of *P. gingivalis* in animal models of disease[74], and the two organisms are negatively correlated in human oral biofilm samples[75]. Host epithelial cells are an additional factor in community nososymbiocity as they provide an interactive interface for colonizing microorganisms; interactions between colonizers and the host epithelia affect inflammatory responses[11,76]. Streptococci are also major components of microbial communities that are associated with oral epithelia[77] and can restrain pro-inflammatory responses and stimulate the expression of antimicrobial peptides (for example, β-defensins)[43,78,79]. Thus, on the epithelial interface, oral streptococci can act as homeostatic commensals and help maintain a host-community equilibrium. Interestingly, although homeostatic commensals can induce the expression of antimicrobial proteins that preferentially target periodontitis-associated bacteria[80], the reverse is also true. Indeed, *P. gingivalis* activates Notch 1 signalling in oral epithelial cells, leading to the production of PLA$_2$-IIA (also known as PLA2G2A), an antimicrobial protein that can promote dysbiosis[81]. Polymicrobial infections involving functionally specialized organisms within an interactive communication network are thus inherently more complex than infections caused by single species, which complicates the study of pathogenicity and the development of diagnostic and treatment options.

Host diet and biofilms in dental caries

Dental caries is a polymicrobial biofilm disease driven by diet–microbiota interactions that cause the destruction of the mineralized tooth tissue[7,16,17,82] (FIG. 1). Oral microorganisms are required for the formation of dental caries, but not sufficient, as the formation of pathogenic biofilms is dependent on frequent consumption of dietary sugars by the host[7,16,22]. Other host and behavioural factors (for example, poor oral hygiene, salivary flow and composition, and enamel defects) and inadequate fluoride exposure also contribute to caries development[7,16,17,82]. Early (primary) colonizers associated with dental health, such as Mitis streptococci, have substantial ecological advantages over cariogenic organisms when the diet of the host is not rich in dietary sugars. These organisms can bind more avidly to salivary-pellicle-coated teeth, show more rapid growth and antagonize pathogens through multiple mechanisms, including the production of alkali, bacteriocins and hydrogen peroxide[83-85], helping to maintain microbial homeostasis and stability (FIG. 3). However, when ecological perturbations exceed thresholds, interspecies competition is altered, thus triggering pathogenic processes. Specifically, the balance between commensals and pathogens can be disrupted by overexposure to fermentable carbohydrates. Sucrose is particularly cariogenic as the component hexoses (glucose and fructose) are used to synthesize EPS (glucans and fructans) and are efficiently fermented to produce organic acids (such as lactic acid), which greatly influence the structure and composition of dental biofilms[7,17,18]. EPS provide binding sites for adhesion to the tooth surface and co-adhesion between bacterial cells, and the microbial communities become embedded in a polymeric matrix that provides cohesion, protection and stability. Such structural organization, together with environmental acidification, promotes microbial shifts towards acidogenic and aciduric organisms and new interspecies interactions[7,16,17]. However, organisms within dental biofilms must manage a wide range of stresses and large nutrient fluctuations to persist and contribute to the onset of caries (FIG. 3). Thus, diet can modulate both the ecology of the oral microbiota and polymicrobial synergies by providing a highly structured and localized acidic microenvironment. In turn, this acidic microenvironment shapes the composition and metabolic activity of the community in a manner that promotes caries development.
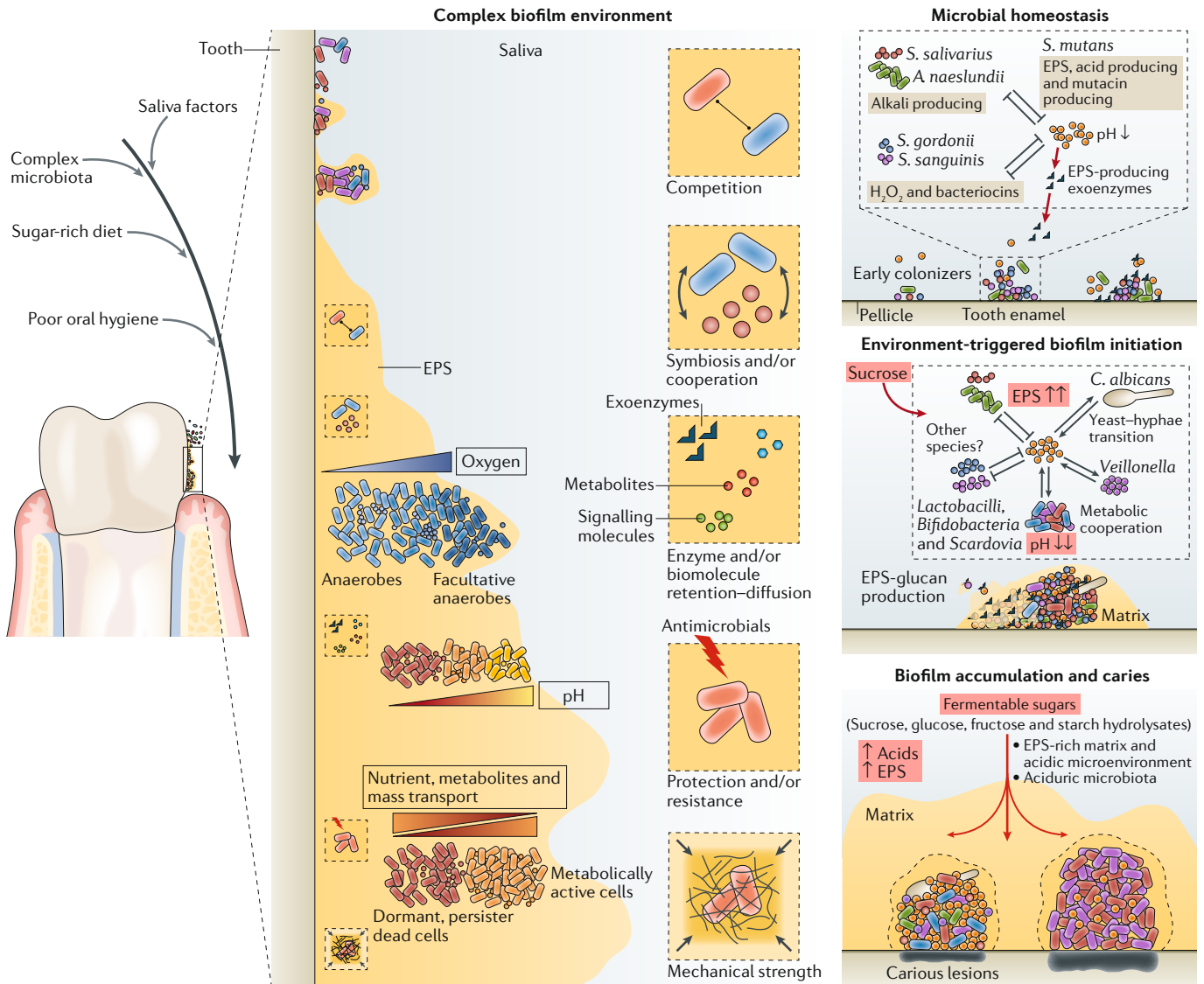
Fig. 3 | **Diet–microbiota interactions trigger the assembly of cariogenic biofilm microenvironment.** In the oral microbial community on tooth surfaces, social interactions begin with primary colonizers that can rapidly attach and then co-adhere with later colonizers. Microorganisms can interact physically and metabolically to determine the initial biofilm community. Both antagonistic and cooperative interactions can occur, and these dynamically change according to the host diet, and other factors such as salivary dysfunction, fluoride exposure and oral hygiene. In particular, dietary sucrose provides a substrate for extracellular polysaccharide production and synthesis of organic acids by acidogenic microorganisms. The extracellular matrix, which also contains other biomolecules (extracellular DNA (eDNA) and bacterial or host-derived proteins), provides a multi-functional scaffold for spatial organization, mechanical coherence and interbacterial interactions. The matrix can trap or sequester substances, which, in combination with diffusion-modifying properties, can generate a variety of chemical and protective microenvironments. Biofilms thus become persistently adhered to the surface and recalcitrant to antimicrobial action. *S. mutans* has a key pathogenic role as an EPS-matrix producer, acidogenic and aciduric organism. With frequent dietary sugar exposure, continued bacterial metabolism of carbohydrates and reduced accessibility to salivary buffering systems causes the microenvironment within the matrix to become increasingly and constantly acidic. As the biofilm accumulates, the microenvironment also becomes progressively anaerobic (hypoxic). In a feedforward loop, microbial diversity decreases as an aciduric microbiota predominates. If the biofilm is not removed, persistent low-pH conditions at the tooth–biofilm interface shift the demineralization–remineralization balance towards net mineral loss from the tooth enamel, leading to the development of a carious lesion. EPS, extracellular polymeric substance. Adapted with permission from REF.[7], Cell Press.

Polymicrobial interactions and acidogenesis. Caries development is a consequence of dietary sugar-driven biofilm accumulation and localized acidification that causes deleterious microbial community shifts and disrupts tooth-enamel mineral homeostasis.

This ecological plaque (biofilm) hypothesis has provided a logical and a tractable model for caries microbial pathogenesis[16] and is supported by microbiome-based studies that reveal microbial composition shifts during the transition from health to the various stages

Salivary pellicle
A layer of salivary proteins and glycoproteins adsorbed to the enamel surface and to which adhesins of initial colonizers of the oral surface can attach. Pellicle can also contain molecules of microbial origin and those derived from epithelial cells.

Accessory pathogen
Organisms that act synergistically with more pathogenic species (keystone pathogens or pathobionts) to elevate community nososymbiocity. Accessory pathogens can provide an attachment substratum for colonization and metabolic support, and can increase virulence gene expression in other organisms through physical interactions or small-molecule-dependent communication.

Keystone pathogen
Species that exert an influence on their communities that is disproportionate relative to their abundance and therefore form the 'keystone' of the community's structure.

Homeostatic commensals
Species that act to maintain a host–microbiota equilibrium by mitigating the action of more pathogenic species. Mechanisms include reducing the impact of pathogens on host cell signalling pathways or production of metabolites that favour a homeostatic inflammatory response.

Homeostasis
A state of equilibrium or stability in a system that is maintained by adjusting physiological processes to counteract external changes.

of caries[16,17,20–22,86–88]. Overall, the microbiota becomes dominated by increasingly acidogenic and aciduric organisms, including mutans and non-mutans streptococci, actinomyces, lactobacilli, bifidobacteria and *Scardovia* spp., which can synergize to enhance EPS production and promote further acidification of the biofilm milieu with frequent sugar exposure. This increased acidification is accompanied by loss of diversity and a reduction in the levels and metabolic activity of beneficial bacteria, which preferentially grow at neutral pH. However, the community composition in advanced caries lesions may reflect the exposure of dentin, an important microenvironment change that allows proteolytic bacteria to thrive[89]. Other genera commonly found in cariogenic communities, including *Propionibacterium*, *Corynebacterium*, *Granulicatella* and certain strains of *Leptotrichia*, exhibit high saccharolytic potential and produce acids[21,31,90]. Although acid-sensitive species survival is disrupted in acidic microenvironments, microorganisms that use lactate as a carbon source (for example, *Veillonella* spp.)[91,92] will benefit from decreases in pH, which may help inhibit the feedforward loop of increasing acidification. Some bacteria found in cariogenic biofilms are not acidogenic–aciduric, such as *Prevotella* spp., *Atopobium* spp. and Gram-negative bacteria[21,31], and their contribution to the biofilm community or to caries pathogenesis remains to be elucidated. Intriguingly, *C. albicans* can be detected in higher numbers, often together with *S. mutans*, in cariogenic plaque from toddlers with severe caries[20,93]. *C. albicans* can interact synergistically with *S. mutans* and colonize tooth surfaces in the presence of dietary sucrose through EPS-mediated interactions. Exoenzymes, termed glucosyltransferases, secreted by *S. mutans*, bind to the *Candida* surface and synthesize glucans in situ using sucrose as a substrate. The glucans formed on the surface enhance bacterial–fungal co-adhesion and embed the microorganisms in an EPS-rich matrix, promoting mixed-biofilm accumulation[50,94]. Within biofilms, the microorganisms cooperate by providing substrates or metabolites (cross feeding), providing growth-stimulating factors, enhancing EPS production and maintaining an aciduric environment[51,95]. In a rodent model, cross-kingdom interactions enhance biofilm virulence when fed a sugar-rich diet, leading to the onset of extensive carious lesions similar to those found clinically[50,94]. Whether other *Candida* species or additional fungi are associated with the cariogenic microbiome in childhood caries remains to be determined. Altogether, the available evidence indicates that synergistic polymicrobial interactions that are triggered by host dietary sugars drive the development of caries, which can be exacerbated by salivary dysfunction, inadequate fluoride exposure and poor oral hygiene.

The role of the extracellular matrix. Although early studies of caries focused on the composition of oral microbial communities, the importance of the extracellular matrix in collective microbial behaviour and virulence is being increasingly recognized as essential for the biofilm lifestyle[7,96]. The structural and biochemical properties of the matrix provide the emergent properties of biofilms, including surface adhesion, social interactions and antimicrobial tolerance[96]. The major components of the matrix are EPS, including exopolysaccharides such as glucans, extracellular DNA (eDNA), lipoteichoic acid, amyloid-like proteins, glycoproteins and host proteins[7]. The microorganisms that are embedded in the matrix of cariogenic biofilms are cohesive and adherent, making these biofilms difficult to remove from surfaces. Furthermore, the extracellular matrix provides protection against antimicrobials. The extracellular matrix also enables chemical or nutrient gradients to form, including pH and redox gradients, thereby affecting the behaviour and survival of microorganisms[7,96]. Moreover, the matrix can limit the diffusion of charged buffering ions, whereas uncharged sugars such as glucose and sucrose can readily diffuse into biofilms and these can be metabolized into acids[7]. Conversely, extracellular matrix glucans provide an endogenous source of sugars and can directly trap protons to help the accumulation of acids within biofilms[7,18,19,97]. These properties may help explain how localized acidification within biofilms occurs in the presence of buffering saliva, shear forces and the near-alkaline environment of the oral cavity. Thus, the extracellular matrix provides a multi-functional platform for the organization of cells into a cohesive multicellular ecosystem that promotes adherence and spatially localizes acid metabolites. Additionally, acids produced within biofilms are sheltered from saliva, which impedes rapid neutralization and helps create a cariogenic microenvironment. This sheltering effect potentiates the ability of acids to demineralize enamel and cause caries. The ability of *S. mutans* to reduce the pH to levels that are toxic to some microorganisms and create a cohesive EPS-rich environment that can facilitate colonization of other organisms and provide protection is compatible with a keystone or specialist role (FIG. 3). Establishing a localized pathogenic habitat through simultaneous matrix and acid production that promotes a dysbiotic and aciduric community is consistent with the variable levels of the bacterium in plaque during caries development (<1–30% of microbial composition)[16,21,22,31,86,87,90,98], although further mechanistic in vivo studies are needed to validate these concepts.

Altogether, dental caries can be conceptually defined as a host-diet-dependent pathological process that relies not only on ecological shifts and polymicrobial acidogenesis but also on the biofilm extracellular milieu within which organisms interact and acids accumulate. This evolving view of ecological flux and concerted polymicrobial synergies within a structured and protected environment, together with salivary and behavioural factors, has direct implications for the development of new and more effective antibiofilm therapeutics.

Inflammation and dysbiosis in periodontitis

A common theme that links caries and periodontal disease is a central axis of microbial community interactions with the host (diet in the case of caries and inflammation in the case of periodontal disease). Additionally, the structure of microbial communities (that is, species composition and abundance of
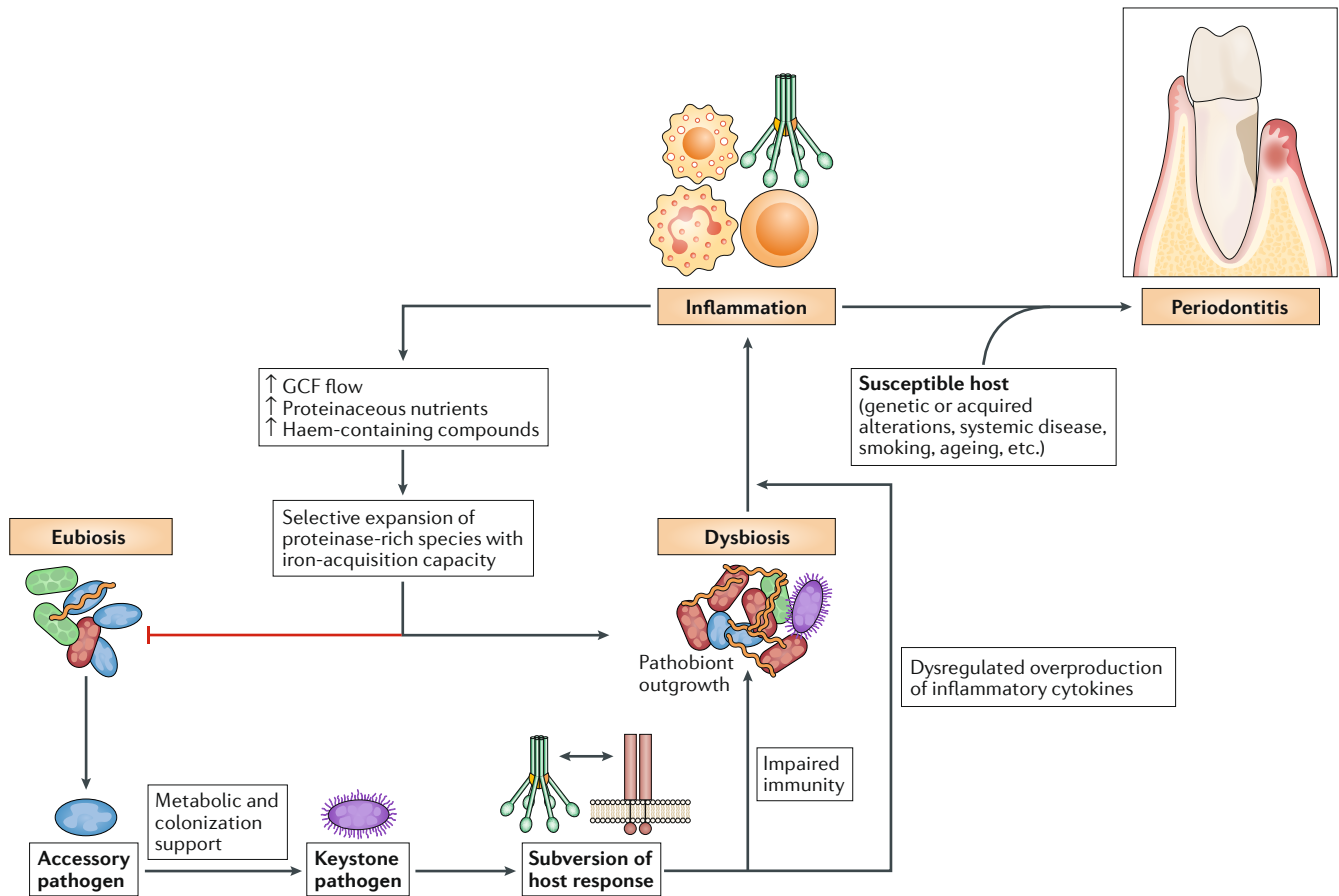
Fig. 4 | **Reciprocally reinforced interactions between dysbiosis and inflammation drive chronic periodontitis.** Colonization by keystone pathogens (for example, *Porphyromonas gingivalis*) aided by accessory pathogens (for example, *Streptococcus gordonii*) leads to impaired innate host defence and promotion of inflammation (for example, by subverting complement–Toll-like receptor (TLR) crosstalk in neutrophils and other myeloid cells)[60,102,117,119]. These alterations contribute to the emergence of dysbiosis (quantitative and compositional changes in the periodontal microbiota). Inflammation worsens dysbiosis by increasing the flow of gingival crevicular fluid (GCF), which, as a result of inflammatory tissue destruction, carries degraded collagen and haem-containing compounds into the gingival crevice, where dysbiotic communities develop. These molecules are selectively used by proteolytic and asaccharolytic bacteria with iron-acquisition capacity. By contrast, health-associated (eubiotic) species cannot capitalize on the new environmental conditions and are outcompeted. This imbalance drives dysbiosis, which further exacerbates inflammation, culminating in periodontitis in susceptible individuals. The ability of inflammation and dysbiosis to positively reinforce each other in a self-sustained feedforward loop may contribute to the chronicity of periodontitis.

Ecological plaque (biofilm) hypothesis
A model encompassing microbiological, biochemical and ecological properties of oral biofilms and their association with disease. The model also accommodates host-derived changes (for example, frequent dietary sugar exposure) that trigger changes in the nososymbiocity of biofilm communities.

individual species) changes from health to disease. In periodontal disease, the rise of newly dominant species, rather than the appearance of new species (such as exogenous pathogens that are absent in health), has been observed[1]. Thus, species or genera that dominate disease-associated polymicrobial communities are also found in health but at markedly reduced relative abundance, as predicted by the ecological plaque hypothesis, according to which changes in environmental conditions may favour the outgrowth of pathogens (now defined as pathobionts) beyond a threshold that can instigate periodontitis[99]. This concept gained experimental support through recent animal model studies showing that anti-inflammatory treatments not only inhibit periodontitis in mice, rats and rabbits but also diminish the periodontal bacterial burden and reverse dysbiosis[100–105]. Conversely, the bacterial load of subgingival biofilms

from individuals with periodontitis accumulates with increasing clinical inflammation[1].

Therefore, inflammation appears to be an important ecological change that can drive the outgrowth of periodontitis-associated microorganisms through tissue destruction that releases nutrients (for example, degraded collagen, haem-containing compounds, sources of amino acids and iron, respectively)[28,106]. These nutrients can be carried via the inflammatory exudate (that is, the GCF) into the gingival crevice to foster the growth of subgingival proteolytic and asaccharolytic bacteria with iron-acquisition capacity (FIG. 4). Accordingly, in situ community-wide transcriptomic analyses of periodontitis-associated subgingival biofilms revealed elevated expression of proteolysis-related genes and genes for peptide transport and acquisition of iron as well as genes for the synthesis of lipopolysaccharides that

could further increase the pro-inflammatory potential of the microbial community[107]. Thus, a subset of species, termed inflammophilic pathobionts[106], may selectively expand at the expense of those that fail to adapt to the new environmental conditions, thereby creating a dysbiotic imbalance in the community[28,106]. In support of this notion, the addition of serum, haemoglobin or hemin to generated oral multispecies biofilms in vitro induces the selective outgrowth of organisms that can act as pathobionts, which additionally upregulate virulence genes including those encoding proteases, haemolysins and proteins involved in hemin transport[108]. This remodelling of the original eubiotic biofilm into a dysbiotic one also enhances the ability of the biofilm to induce pro-inflammatory cytokines by host cells[108], thus mimicking the in vivo setting where dysbiosis leads to inflammation.

In addition to iron and amino acid transport, another enhanced metabolic change detected in developing dysbiosis of the human periodontal microbiome involves the transport of potassium ions[109], which become more concentrated in the GCF with increased periodontal disease[110]. Interestingly, elevating the concentration of potassium in an ex vivo dental plaque biofilm model causes compositional and phenotypic changes in the microbial community, resulting in enhanced production of pro-inflammatory cytokines and a decrease in the production of human β-defensin 3 in gingival epithelial cells[111]. Another by-product of inflammation is nitrate, which can be used as an electron acceptor for anaerobic respiration by Enterobacteriaceae to outcompete fermenting microorganisms during colitis-associated dysbiosis[112]. In a murine model of spontaneous periodontal inflammation (due to deficiency of growth arrest-specific gene 6 (GAS6)), microbial dysbiosis was associated with the selective expansion of nitrate reductase-expressing Proteobacteria, which can use the elevated nitrate in the periodontal environment of $Gas6^{-/-}$ mice[113]. Potassium and nitrate thus appear to be inflammation-related environmental cues that can contribute to the remodelling of the oral microbiome from a eubiotic community to a dysbiotic one.

Whether dysbiosis is a cause or a consequence of inflammatory disease has been the subject of debate[114,115]. In periodontitis, there appears to be a reciprocal cause-and-effect relationship between dysbiosis and inflammation. As inflammation fuels the selective growth of dysbiotic communities and dysbiosis exacerbates inflammation[28,105–108,111,113], it is likely that neither destructive inflammation nor dysbiosis could fully develop without interactions between these two processes. In conclusion, inflammation acts as a reciprocal ecological driver of dysbiosis, and this inflammation–dysbiosis interplay appears to generate a self-sustained feedforward loop that drives periodontitis (FIG. 4).

Subversion of the host response
Uncoupling bactericidal activity from inflammation.
The crucial importance of inflammation for the development of dysbiosis creates a biological conundrum — dysbiotic communities need inflammation to acquire nutrients but also must downregulate the host immune response for their protection. Although immunosuppression is a common microbial evasion strategy[116], this tactic would not be a viable option for inflammophilic bacteria as it would create a non-inflammatory environment that starves the bacteria of essential nutrients. Periodontitis-associated bacteria have resolved this paradox by manipulating the host response in a manner that uncouples inflammation from bactericidal activity, as exemplified by the action of the keystone pathogen *P. gingivalis*[115]. In this context, *P. gingivalis* can benefit the entire microbial community by impairing the bactericidal activity of innate leukocytes while promoting their inflammatory responses[60,102,117–120] (FIG. 4). The importance of this keystone function was demonstrated in mice, where this dual subversive action disrupts host–microorganism homeostasis and contributes to the emergence of a dysbiotic microbiota and the development of periodontitis[102,117].

Manipulation of complement, Toll-like receptor signalling and cytokines. The complement C5a receptor 1 (C5aR1) and Toll-like receptor 2 (TLR2) are at the core of the immune-subversive action of *P. gingivalis*[117,119]. In human or mouse neutrophils, *P. gingivalis* initiates C5aR1–TLR2 crosstalk signalling that separates a host-protective TLR2–MyD88 pathway from a TLR2–MyD88-adaptor-like (MAL; also known as TIRAP)–PI3K pathway, which blocks phagocytosis and promotes inflammation[117] (FIG. 5). *P. gingivalis* can also bypass MyD88 to induce pro-inflammatory and anti-phagocytic TLR2–PI3K signalling in macrophages; intriguingly, even within cells that do phagocytose *P. gingivalis*, PI3K signalling suppresses phagolysosomal maturation and thus promotes intracellular survival[118] (FIG. 5). Pharmacological inhibition of either C5aR1 or TLR2, or key downstream signalling intermediates, blocks *P. gingivalis*-induced dysbiosis and periodontitis in mice[102,117]. The capacity of *P. gingivalis* to induce TLR2-dependent inflammation while bypassing MyD88, which undergoes ubiquitylation and proteasomal degradation[117], is unusual given that prototypical TLR2 agonists (for example, the Pam3CSK4 lipopeptide) activate TLR2 in a strictly MyD88-dependent manner[117,118]. Consistent with the redundant role of MyD88 in *P. gingivalis*-induced inflammation, this bacterium induces inflammatory bone loss in mice regardless of the presence or absence of MyD88, whereas the presence of TLR2 is absolutely necessary[118].

The ability of *P. gingivalis* to subvert the host response in a manner that benefits the entire microbial community is also supported by the localized chemokine paralysis concept. The expression of *IL-8* (also known as *CXCL8*) by the junctional gingival epithelium, adjacent to the tooth-associated biofilm, is a homeostatic feature of the healthy periodontium as it generates a chemotactic gradient for neutrophil recruitment to the gingival crevice[121,122]. Consistent with its keystone role, *P. gingivalis* selectively suppresses expression of IL-8 and T helper 1 (T_H1) cell-biasing chemokines (CXCL9, CXCL10 and CXCL11), even in the presence of otherwise stimulatory pathobionts such as *Fusobacterium nucleatum*[123,124]. Within epithelial cells, *P. gingivalis* secretes the serine
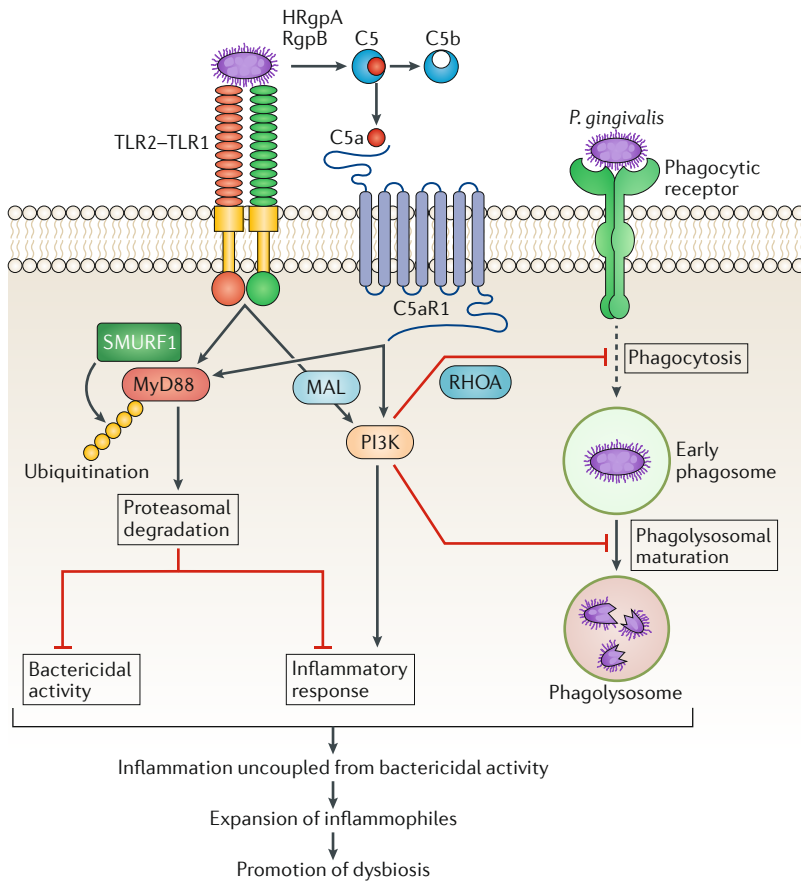
Fig. 5 | *P. gingivalis* induces dysbiosis by impairing innate host defences while promoting inflammatory responses in phagocytic cells. *Porphyromonas gingivalis* expresses cell-surface molecules that activate the Toll-like receptor 2 (TLR2)–TLR1 complex and secretes enzymes (HRgpA and RgpB gingipains) that act on the complement component C5 to generate high local concentrations of C5a, a ligand of complement C5a receptor 1 (C5aR1). The bacterium can thus co-activate C5aR1 and TLR2 in phagocytic cells such as neutrophils and macrophages. In both of these myeloid cell types, *P. gingivalis* can bypass MyD88 and thus prevent the associated bactericidal activity[117,161], which in neutrophils is possibly mediated by downstream activation of IRAK4-dependent granule exocytosis[162]. In neutrophils, the inactivation of MyD88 involves its ubiquitylation via the E3 ubiquitin ligase SMURF1 and subsequent proteasomal degradation. Although MyD88-dependent inflammation is blocked by *P. gingivalis*, this organism induces PI3K-dependent inflammatory cytokines in both neutrophils and macrophages[118,120]. Similarly, in both cell types, *P. gingivalis*-induced activation of PI3K leads to inhibition of phagocytosis[117,118]. In neutrophils, this activity is mediated by the ability of PI3K to suppress RhoA GTPase and actin polymerization[117]. Intriguingly, even within those macrophages that do manage to phagocytose *P. gingivalis* bacteria, PI3K signalling suppresses phago-lysosomal maturation, thereby preventing pathogen destruction[118]. These tactics compromise innate immunity and promote inflammation that leads to the selective expansion of inflammophilic pathobionts. Conversely, inhibition of C5aR1, TLR2 or PI3K reverses dysbiotic inflammation and periodontitis in mice[102,117]. MAL, MyD88-adaptor-like.

phosphatase SerB, which dephosphorylates the p65 subunit of the NF-κB transcription factor[125]. Translocation of NF-κB–p65 homodimers into the nucleus is consequently reduced and *IL8* transcription is diminished (FIG. 6). The suppression of T_H1-associated chemokine expression by *P. gingivalis* is mediated through inhibition of the STAT1–IRF1 pathway in epithelial cells, neutrophils and monocytes[124] (FIG. 6). Collectively, these chemokine-paralyzing phenomena, even if transient as suggested by in vivo observations[102], could have a

debilitating effect on immune surveillance during the development of a dysbiotic biofilm. Indeed, defective neutrophil recruitment into the gingival crevice could allow overgrowth of pathobionts, whereas disruption of T_H1-biasing chemokines may perturb the balance of protective and destructive immunity in the periodontium[126], thereby contributing to increased nososymbiocity.

***P. gingivalis* as a community activist.** Consistent with the notion that *P. gingivalis* has important roles in initiating dysbiosis, a longitudinal metatranscriptomic analysis of microbial communities from stable or disease-progressing sites showed that, among those bacteria commonly associated with the red complex, only *P. gingivalis* expressed virulence factors at healthy sites that progressed to disease[109]. By contrast, *T. denticola* and *T. forsythia* virulence gene expression was upregulated at later times when tissue breakdown was clinically observed[109]. These findings suggest that, at early stages, *P. gingivalis* acts as a keystone pathogen that contributes to the dysbiotic process, leading to disease progression, whereas *T. denticola* and *T. forsythia* may contribute to the nososymbiocity of the microbial community once homeostasis is disrupted, thereby acting as pathobionts that accelerate disease progression. Although not an essential mechanism for dysbiosis (for example, host genetic deficiencies can cause dysbiosis and periodontitis in the absence of *P. gingivalis*[127]), *P. gingivalis* is likely an important risk factor in periodontitis. By manipulating host immunity and separating bactericidal from inflammatory activities[117,118,128], *P. gingivalis* can enhance the adaptive fitness of the entire microbial community in a nutritionally favourable and disease-promoting inflammatory environment (FIG. 4).

Novel approaches to prevention and treatment
Oral biofilms harbour complex polymicrobial communities involving interspecies interactions with host, diet and immunity that control dysbiosis and explain nososymbiocity. The lack of a single obvious target for therapeutic intervention and the potential for transfer of antibiotic resistance genes and physical protection provided by EPS complicate treatment options, and conventional antimicrobial elimination has proved difficult. The presence of a fluid phase that can inactivate bioactive molecules and the difficulty of accessing different oral sites where the disease occurs, combined with poor retention of topically delivered agents, pose additional challenges. These unique conditions are hurdles but also opportunities for the development of innovative drug delivery and effective therapies that could target these complex biological traits and protected oral niches.

For caries prevention, multitargeted therapeutic strategies may be required to prevent pathogenic biofilm accumulation or disrupt established biofilms and decrease dissolution of the enamel mineral. Although supragingival biofilms can be mechanically dislodged by manual or powered toothbrushing, these approaches do not remove biofilms completely, particularly in areas that cannot be easily accessed such as sulcal and interproximal surfaces. Furthermore, subpopulations
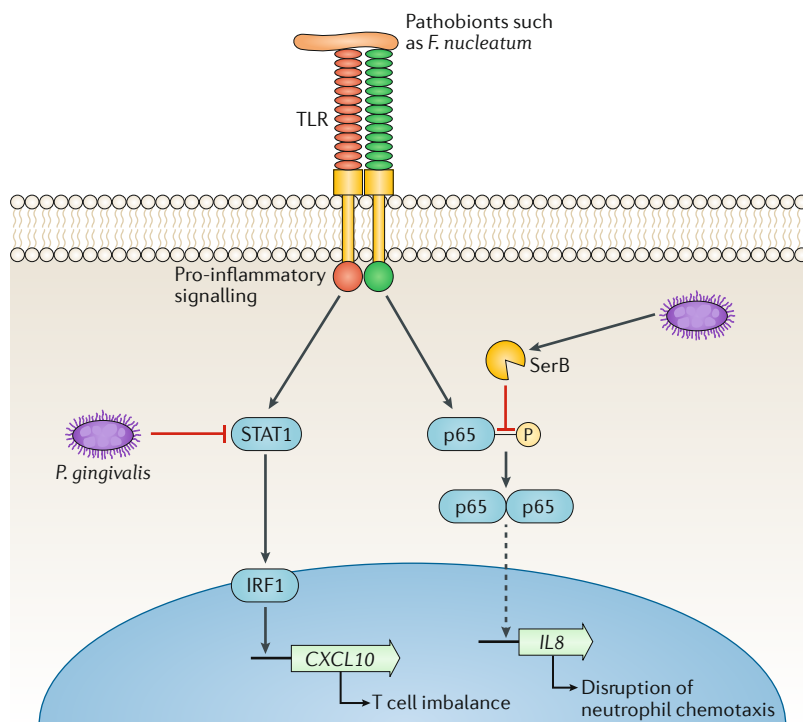
Fig. 6 | **Localized chemokine paralysis.** Oral pathobionts such as *Fusobacterium nucleatum* are recognized by Toll-like receptors (TLRs) on epithelial cell surfaces, which leads to the activation of pro-inflammatory signalling pathways. The keystone pathogen *Porphyromonas gingivalis* can manipulate these pathways and cause a targeted and precise reduction in the production of specific chemokines. Inactivation of STAT1 by *P. gingivalis* leads to reduced expression of *CXCL10*, which is controlled by the IRF1 transcription factor[124]. Intracellularly, *P. gingivalis* secretes SerB, a serine phosphatase that specifically dephosphorylates the serine 536 residue of the p65 NF-κB subunit, thus inhibiting formation and nuclear translocation of NF-κB–p65 homodimers. Transcription of the *IL8* gene is reduced and the IL-8 neutrophil gradient is disrupted[125]. These chemokine paralysis activities will be localized to tissue adjacent to, or containing, *P. gingivalis*, and in animal models supersede the effects of community pathobionts[163]. The continuous recalibration of host cell signalling pathways also limits the temporal extent of the phenomenon, which may contribute to the cyclical nature of periodontal tissue destruction. Adapted with permission from REF.[10], Cell Press.

A number of approaches to manipulate biofilm microbial communities have recently emerged, including pH modulation. The simplest strategy that has found clinical applicability involves the use of arginine as a prebiotic-like agent[83,132,133]. L-Arginine can be metabolized by arginolytic oral species (for example, *S. gordonii* and *Actinomyces* spp.) to produce alkali. This alkali can counter the biofilm acidification process and modulate pH homeostasis within oral biofilms, preventing overgrowth of acidogenic–aciduric bacteria and enhance the anti-caries activity of oral care products. Alternatively, bacteria naturally found in the oral cavity such as *Streptococcus dentisani* or *Streptococcus* A12 can display dual probiotic action by inhibiting the growth of cariogenic species and by pH modulation through their arginolytic activities[50]. Conversely, a new generation of antimicrobial peptides consisting of a broad-spectrum, novispirin-derived 'killing' region attached to a species-specific peptide pheromone can enhance the targeting specificity against caries pathogens such as *S. mutans* and increase the abundance of commensal streptococci[51]. Once a cariogenic biofilm is established, more aggressive measures are needed. These include synergistic combinations of antimicrobial action with biofilm matrix degradation or enhanced physical (mechanical) disruption techniques such as shear-generating high-velocity water sprays[129,134–136], particularly in difficult-to-reach and protected retentive sites. Other potential biofilm-specific targeting approaches, including surface modification, antibiofilm coatings and small molecules identified from chemical libraries or in silico screening, have been reviewed elsewhere[129].

New drug delivery nanotechnologies have emerged that can penetrate biofilms more effectively and expedite drug release in response to acidic pH, which could enhance the efficacy of current and prospective chemical modalities that target cariogenic biofilms[129,137–140]. Another approach employs pH-dependent catalytic nanoparticles that generate free radicals from hydrogen peroxide only at acidic pH values and simultaneously degrade the biofilm matrix and kill embedded bacteria with high efficacy under cariogenic conditions. Topical applications of catalytic nanoparticles with low concentrations of hydrogen peroxide prevented the onset of severe caries (including on interproximal and sulcal surfaces) without cytotoxic effects in vivo[137]. Thus, stimuli or environment-triggered technologies can enhance the selectivity of drug activation or delivery within biofilms, targeting the matrix and the embedded bacteria to eradicate the pathological niches with precision and efficacy without affecting the surrounding host tissues and the commensal microbiota.

The self-sustained feedforward loop between dysbiosis and inflammation discussed earlier (FIG. 4) appears to underlie the chronicity of periodontitis and suggests that host modulation is an effective adjunctive therapy to standard treatment (mechanical debridement to remove the disease-associated biofilm). Targeted inhibition of implicated inflammatory pathways may break the reciprocal cause-and-effect relationship between dysbiosis and inflammation, arrest further

at high risk of caries, such as young children, elderly adults and those with disabilities, lack the dexterity for optimal brushing techniques, necessitating new or adjunctive therapies. Current antimicrobials do not target important structural and functional traits of biofilms or drug tolerance mechanisms, resulting in limited clinical efficacy to prevent caries[7,129,130]. Conversely, fluoride can decrease the rate of enamel demineralization and enhance remineralization but has limited biofilm-killing effects (although fluoride can disrupt acid production and acid tolerance by cariogenic bacteria[131]). In this context, prospective therapeutic strategies should be developed to specifically target the biofilm matrix, the acidic pH microenvironment and the polymicrobial synergies associated with acidogenesis and to facilitate the action of remineralizing agents or mechanical removal. Likewise, enhanced strategies to deliver and retain bioactive agents at the sites where pathogenic biofilms develop (for prevention) or that can penetrate the complex structure of biofilms (for disruption) are needed.

Inflammophilic
A property of bacteria that thrive on inflammation and utilize inflammatory tissue breakdown products for nutrition.

Hemin
An iron-containing porphyrin compound released from red blood cells; exploited by bacteria to obtain iron for growth.

Localized chemokine paralysis
Precise and targeted suppression of specific chemokines by microbial community participants, superseding the otherwise stimulatory activity of other community inhabitants.

Black queen hypothesis
A theory of reductive evolution to account for co-dependency.

development of the disease and promote an environment conducive to inflammation resolution and periodontal tissue repair. Host modulation is indirectly an antimicrobial approach as inflammation control should limit the nutrient supply (inflammatory tissue breakdown products) that sustains dysbiosis, thereby restoring ecological conditions that favour microbiotas compatible with periodontal health. Preclinical interventions in animal models, ranging from mice to non-human primates, have targeted distinct but interconnected signalling pathways involving inflammation initiation (for example, inflammatory cell recruitment[103]), mechanisms that amplify and propagate inflammation (for example, complement[141] and specific pro-inflammatory cytokines[142]) and pathways that promote inflammation resolution[143]. In terms of anti-cytokine therapy, antibody-mediated blocking of IL-23, which drives aggressive periodontitis associated with leukocyte adhesion deficiency, led to resolution of inflammatory periodontal lesions in both mouse and human disease[104,144]. Furthermore, new nanotechnologies for drug delivery, regenerative tissue engineering and biomaterials are emerging for the treatment of periodontal diseases[145,146].

Future development should focus on achieving maximal in vivo efficacy and targeting specificity with minimal toxicity and long-term therapeutic effects (compared with current treatments). These, combined with safety and efficacy clinical trials, affordable manufacturing and the development of practical formulations, will determine whether any of the current preclinical strategies could be used for the treatment of human periodontitis and caries.

Conclusions and perspectives

In both dental caries and periodontitis, the nososymbiocity of the polymicrobial communities involved in disease is largely regulated by host factors, predominantly dietary sugars and inflammation. In periodontitis, inflammation and dysbiosis co-develop in a reciprocally reinforced manner, and their interplay develops to become the driver of periodontitis in susceptible individuals (FIG. 4). In caries, diet–microbiota interactions help assemble a persistent tooth-associated biofilm that provides protection to resident microorganisms. The biofilm is also where polymicrobial acidogenesis creates an aciduric environment that disrupts enamel tissue homeostasis, favouring demineralization, which drives caries onset and progression (exacerbated by salivary dysfunction, inadequate fluoride exposure and poor oral hygiene).

The polymicrobial communities in caries and periodontitis display sophisticated structural and functional integration that confers a quasi-organismal status to these entities. This interdependence among constituent members of oral polymicrobial communities lends support to the 'black queen hypothesis'[147], according to which functions that are energetically costly can be discarded as dispensable by 'cheater' organisms provided that they are not entirely lost from the community (that is, they are retained by a subset of community members ('helpers') that benefit the community).

This hypothesis offers a theoretical framework for the emergence of keystone or specialist pathogens that contribute an indispensable public service to the microbial community (for example, formation of a matrix or subversion of host immunity). Whereas in classic infectious diseases overt pathogens employ strategies to overcome colonization resistance as a prerequisite to cause disease, keystone or specialist pathogens exploit the inherent metabolic and/or colonization properties of their commensal neighbours (accessory pathogens) or help create a selective environment in which they increase their virulence and the nososymbiocity of the community.

In a state of compromised homeostasis, the expansion of pathobionts or changes in microenvironment marks a potential tipping point for full-blown development of nososymbiocity (in that restoration of host–microbiota homeostasis is unlikely to occur without treatment intervention). Multitargeted approaches to counter cariogenic biofilm establishment by disrupting both biofilm matrix and acidification, together with mechanical removal, could help re-establish the healthy microbiota and concomitantly enhance the efficacy of remineralizing agents to prevent and treat caries. These approaches are essential given that necessary dietary changes to control caries entail behavioural modifications of at-risk populations that have proved difficult to achieve. Strategies to control other environmental variables that drive and perpetuate dysbiosis (for example, inhibition of inflammation in periodontitis) or to restore immune function in immunodeficient individuals should arrest disease development and promote host–microbiota homeostasis[148–151]. Moreover, approaches to block the synergistic mechanisms that drive nososymbiocity (for example, targeting key interspecies interactions, acidogenesis or host signalling pathways that are exploited by keystone pathogens to subvert immunity) may also help control polymicrobial biofilm-associated diseases[60,72,117]. These strategies, combined with enhanced drug delivery and retention approaches, could substantially help control these prevalent and costly oral diseases.

Advances in omics technologies and improved database curation and bioinformatics have improved the identification of active microbial social networks and their products (genes, proteins and metabolites)[37,152]. However, to move beyond correlations and begin to address causation, further refinement of these technologies is needed. Integration of omics data combined with microorganism phenotype–pathogenicity association, together with complementary in vivo polymicrobial models[153], may be a powerful strategy to identify additional disease-related microorganisms, their virulence properties and synergistic interactions that modulate components of the microbiota and host immunity within the conceptual framework presented here. In turn, a more complete set of therapeutic targets can be revealed, providing better opportunities to develop highly precise and efficacious therapeutic approaches.

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Author contributions
R.J.L., H.K. and G.H. researched the data for the article, substantially contributed to discussion of content, wrote the article and reviewed and edited the manuscript before submission.

Competing interests
The authors declare no competing interests.

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Reviewer information
Nature Reviews Microbiology thanks G. Belibasakis, B. Keijser and other anonymous reviewers for their contributions to the peer review of this work.