DOI: 10.1111/prd.12296

REVIEW ARTICLE

Periodontology 2000 WILEY

The role of the microbiota in periodontal disease

Mike A. Curtis[1] | Patricia I. Diaz[2] | Thomas E. Van Dyke[3]

[1]Centre for Host Microbiome Interactions, Faculty of Dentistry, Oral& Craniofacial Sciences, King's College London, London, UK

[2]School of Dental Medicine, UConn Health, Farmington, Connecticut, USA

[3]Department of Applied Oral Sciences, The Forsyth Institute, Cambridge, Massachusetts, USA

Correspondence
Mike A. Curtis, Centre for Host Microbiome Interactions, Faculty of Dentistry, Oral & Craniofacial Sciences, King's College London, London, UK.
Email: Mike.Curtis@kcl.ac.uk

1 | INTRODUCTION

The interaction of the oral microbiome and the host response, and the development of periodontitis, is complex. The complexity arises, in part, from the wide range of bacterial taxa resident within the subgingival microbiota and the individual to individual variations in the repertoire of these taxa, and also from the intricacy of the immune and inflammatory responses of the host operative within the gingival tissues, and whether these are protective or destructive influences. Nonetheless, in the midst of these complexities, some broad defining principles concerning the role of the microbiota in disease have emerged and have gained consensus support.

First, bacteria are essential for the development of destructive disease evidenced, for example, by the largely effective treatment of disease using strategies that target the subgingival biofilm, and also by experimental studies in germ-free animals that demonstrate the requirement of a commensal microbiome for destructive periodontal bone loss.[1] Second, whole-scale changes to the overall microbial population structure of the subgingival biofilms and bacterial load are invariably associated with destructive disease. The shifts in microbial community profile appear largely independent of the acquisition of new members of the microbiota, rather they reflect changes in the abundance of individual organisms or consortia of organisms resident within the subgingival biofilm in health. This disease-associated community shift, or dysbiosis of the microbiota, may be driven by alterations to the local environmental conditions, and one likely driver of this altered ecology is the inflammatory response of the host.[2] Third, uncontrolled inflammatory and immune responses may be largely, if not entirely, responsible for tissue destruction.[3,4] Therapeutic strategies that target the inflammatory pathways that lead to tissue destruction or hasten the resolution of the inflammatory response may provide an alternative route to treatment.

While these principles provide a framework for understanding the role of the microbiota in disease, there remain several areas of controversy. We can be reasonably certain that bacteria cause gingivitis as their removal leads to reversal of this inflammatory condition. However, the factors that determine progression from gingivitis to periodontitis in a proportion of the population are less clearly established. Dysbiosis is clearly associated with periodontitis[5,6], but whether dysbiosis causes disease or results from disease is a matter of conjecture. Likewise, the molecular details of the microbial stimulus responsible for driving the destructive inflammatory response are open to question although there is a significant body of evidence, largely drawn from in vitro studies, which implicates a range of virulence determinants, but only in the very limited number of disease-associated bacteria that have been investigated in any detail to date.[7] While one should not underestimate the challenge in addressing these issues, there may be significant gains in making progress: an improved understanding may have significant implications for determining susceptibility to destructive disease, and for the development and application of novel diagnostic approaches and treatment modalities. In this review, we aim to highlight some recent advances in this field.

2 | THE SUBGINGIVAL MICROBIOME IN HEALTH

Diverse microbial communities develop attached to the root surfaces of teeth. These communities are better protected from shear forces and environmental oxygen than their supragingival counterparts. Nutritionally, subgingival communities depend on gingival crevicular fluid, a serum-like exudate positively flowing into the sulcus from the adjacent gingival tissues, and to a lesser extent on salivary or dietary nutrients. Subgingival microbial communities are composed of bacteria, archaea, fungi, and viruses.[8,9] Bacteria are the most abundant component with about 500 species estimated to exist in subgingival

plaque.[9,10] Individual subgingival sites, however, harbor only a few numerically dominant bacterial species, with a large number of taxa present in low abundance. Such long tail distribution is typical of microbial communities regardless of their host and environment.

Early microscopic studies of subgingival communities in health show that gram-positive cocci and rods are numerically dominant.[11] A classic cultivation study by Moore and Moore identified *Actinomyces naeslundii* as the most frequently recovered subgingival species in health.[9] Other *Actinomyces* spp. such as *A. meyeri* and *A. odontolyticus* also appeared in large numbers in healthy communities.[9] Oral *Actinomyces* are gram-positive rods and are among the first microorganisms to colonize pristine tooth surfaces. They have the ability to co-aggregate with other early colonizing bacteria such as *Streptococcus*, which together form the backbone of early dental plaque.[12,13] Other species detected via cultivation in abundance in subgingival plaque of healthy sites include the gram-positive *Streptococcus sanguinis*, *S. oralis*, *S. intermedius*, *S. gordonii*, *Peptostreptococcus micros*, *Gemella morbillorum*, and the gram-negative *Veillonella parvula*, *V. atypica*, *Capnocytophaga ochracea*, and *C. gingivalis*.[9] In addition, the same study reported *Fusobacterium nucleatum*, a gram-negative filamentous spindle-shaped rod, as the second most frequently recovered species in healthy plaque. These data confirm early microscopic observations, showing that gram-positives are abundant in healthy subgingival plaque, but also that some gram-negatives such as *F. nucleatum*, *Veillonella* spp., and *Capnocytophaga* spp. are important components.

The advent of high throughput DNA sequencing has facilitated characterization of the composition of subgingival communities in unprecedented detail.[14-16] Studies using 16S rRNA gene sequencing largely agree with the earlier cultivation-based reports, but at a far greater taxonomic resolution of healthy biofilm members. Figure 1 shows the most abundant bacteria found in the healthy subgingival crevice in 2 studies.[14,16] In agreement with cultivation results,[9] *Actinomyces* spp. and Mitis-group streptococci appear as abundant components, together with *F. nucleatum*, *V. parvula*, and *Capnocytophaga* spp. Sequencing, however, revealed that gram-positives such as *Rothia aeria*, *R. dentocariosa*, *Corynebacterium matruchotti*, and *C. durum* are also important subgingival components in health (Figure 1). It has been recently shown that *Rothia* spp. could serve as initiators of cell-cell co-aggregation interactions in early biofilms.[17] Corynebacteria have also been recently demonstrated as the key filaments around which a spatially organized consortium of 9 taxa arranges itself during early biofilm formation, as revealed by

Health

Abusleme et al (2013)[14]	Hong et al (2015)[16]
1. *Rothia dentocariosa*	1. *Actinomyces* sp.
2. *Fusobacterium nucleatum* ss. *vincentii*	2. *Streptococcus* sp.
3. *Lautropia mirabilis*	3. *Rothia aeria*
4. *Actinomyces* sp. HOT 169	4. *Corynebacterium matruchotii*
5. *Actinomyces* sp. HOT 170	5. *Streptococcus* sp. HOT 071
6. *Haemophilus parainfluenziae*	6. *Neisseria* sp.
7. *Streptococcus mitis*	7. *Fusobacterium nucleatum* ss. *vincentii*
8. *Prevotella oris*	8. *Lautropia mirabilis*
9. *Corynebacterium matruchotii*	9. *Veillonella parvula*
10. *Fusobacterium nucleatum* ss. *animalis*	10. *Capnocytophaga* sp.
11. *Fusobacterium nucleatum* ss. *nucleatum*	11. *Streptococcus* sp. HOT 058
12. *Actinomyces naeslundii*	12. *Prevotella* sp.
13. *Propionibacterium propionicum*	13. *Corynebacterium durum*
14. *Corynebacterium durum*	14. *Capnocytophaga sputigena*
	15. *Neisseria elongata*
	16. *Fusobacterium* sp.
	17. *Leptotrichia* sp.
	18. *Actinomyces* sp. HOT 171
	19. *Kingella oralis*
	20. *Bergeyella* sp. HOT 322
	21. *Fusobacterium nucleatum* ss. *animalis*

Periodontitis

Abusleme et al (2013)[14]	Hong et al (2015)[16]
1. *Treponema denticola*	1. *Prevotella intermedia*
2. *Pseudomonas fluorescens*	2. *Porphyromonas gingivalis*
3. *Neisseria flava*	3. *Rothia dentocariosa*
4. *Fusobacterium nucleatum* ss. *nucleatum*	4. *Fusobacterium nucleatum* ss. *vincentii*
5. *Neisseria subflava*	5. *Fusobacterium nucleatum* ss. *animalis*
6. *Treponema* sp. HOT 237	6. *Prevotella tannerae*
7. *Fusobacterium nucleatum* ss. *vincentii*	7. *Leptotrichia* sp.
8. *Treponema medium*	8. *Tannerella forsythia*
9. *Fusobacterium nucleatum* ss. *animalis*	9. *Streptococcus* sp.
10. *Saccharibacteria* (TM7) [G-1] sp. HOT 346	10. *Prevotella denticola*
11. *Saccharibacteria* (TM7) [G-1] sp. HOT 349	11. *Fusobacterium* sp.
12. *Neisseria elongata*	12. *Veillonella parvula*
13. *Rothia dentocariosa*	13. *Actinomyces* sp.
14. *Desulfobulbus* sp. HOT 041	14. *Prevotella melaninogenica*
15. *Filifactor alocis*	15. *Prevotella nigrescens*
16. *Peptostreptococcaceae* [XI][G-9] [Eubacterium] *brachy*	16. *Treponema* sp.
17. *Prevotella oris*	17. *Corynebacterium matruchotii*
18. *Peptostreptococcaceae* [XI][G-5] [Eubacterium] *saphenum*	18. *Pyramidobacter piscolens*
19. *Fretibacterium* sp. HOT 360	19. *Prevotella oris*
20. *Enterobacter sakazakii*	20. *Fusobacterium nucleatum* ss. *polymorphum*
21. *Mogibacterium timidum*	21. *Treponema denticola*
22. *Lautropia mirabilis*	22. *Treponema* sp. HOT 237
23. *Porphyromonas endodontalis*	
24. *Sneathia sanguinegens*	
25. *Staphylococcus aureus*	

FIGURE 1 Most abundant members of subgingival microbial communities in health and periodontitis. Dominant bacterial species in health- and periodontitis-associated subgingival microbial communities, as revealed by 2 16S rRNA gene sequencing studies.[14,16] Bacterial species shown are those with a mean relative abundance of at least 1% within each group of studied subjects. Names are presented according to ranked mean relative abundance with the most abundant species at the top of each list

spectral imaging fluorescence in situ hybridization.[18] This 9-taxon structure organized around corynebacteria is spatially differentiated, with individual taxa localized in ways suggestive of a functional metabolic organization. Other taxa seen as abundant components of subgingival plaque in these 16S rRNA sequencing studies, such as *Lautropia mirabilis*, appear as the core central taxon in other functionally arranged consortia detected via microscopy in early plaque.[18] Although the aforementioned spatially arranged consortia were observed supragingivally, the species which they consist of are similar to those seen in the healthy subgingival crevice, and it is therefore likely that these organized structures also occur subgingivally.

In summary, subgingival biofilms in health comprise gram-positives and a few but numerically abundant gram-negative species. These taxa have the potential to organize into spatially arranged consortia in which specific species physically and metabolically interact.

3 | DYSBIOTIC COMMUNITIES IN GINGIVITIS

Microbiological studies performed via cultivation have shown that, upon oral hygiene abstention, a shift in the dominant species present in subgingival communities occurs. Gram-negative morphotypes including rods, filaments, and spirochaetes increase in abundance after 2-3 weeks of undisturbed plaque accumulation, with these shifts correlating with the appearance of clinical inflammation of the gingiva.[19] These early investigations have now been complemented by more recent studies using 16S rRNA gene sequencing and the same experimental plaque accumulation model to define the gingivitis-associated shifts in subgingival community composition.[20-22] Figure 2 shows the most abundant species found in 15 subjects at baseline (day 0) during a state of gingival health, and after 3 weeks (day 21) of plaque accumulation, when subjects presented with clinical signs of gingivitis.[22] One of the most important changes in the evolution of communities from health to gingivitis is a decrease in the relative abundance of *R. dentocariosa*, with a mean relative abundance of 25 (± 31)% at day 0 dropping to a mean proportion of 2 (± 3)% in the gingivitis-associated communities (Figure 3). Similar findings have been reported elsewhere.[23] The development of gingivitis was accompanied by depletion of *Propionibacterium* and low abundance of *Stenotrophomonas maltophila* (Figure 3). By contrast, *Prevotella* was seen to increase proportionately after experimental plaque accumulation (Figures 2 and 3). *Selenomonas*, although present in low abundance, was another genus showing enrichment (Figure 3). Indeed, *Prevotella* and *Selenomonas* were the genera most strongly associated with clinical signs of gingivitis, and also with an increase in the levels of the inflammatory mediators interleukin 1alpha, interleukin 1beta, interleukin 1ra, and lactoferrin in gingival crevicular fluid.[22] Interestingly, although the genus *Fusobacterium* as a whole was not associated with gingivitis, *F. nucleatum* ss. *polymorphum* was enriched at day 21 and associated with gingival inflammation. Other taxa seen by other 16S rRNA sequencing studies as associated with gingivitis include *Leptotrichia* spp., *Porphyromonas catoniae*, *Tannerella* spp. HOT 286 and HOT 808, *Saccharibacteria* (TM7) [G-1] spp., and *Treponema socranskii*.[20,23] It should also be noted that these changes in species proportions from health to gingivitis occur concomitantly with an approximate 3-log increase in bacterial biomass and therefore the influence of enriched species is much larger than that expected based simply on their change in proportion (Figure 4). In summary, the development of gingivitis occurs concomitantly with an increase in bacterial biomass and a large shift in the composition of subgingival communities with depletion of gram-positive species such as *R. dentocariosa* and enrichment of gram-negatives such as *Prevotella* spp., *Selenomonas* spp., and *F. nucleatum* ss. *polymorphum*, among others.

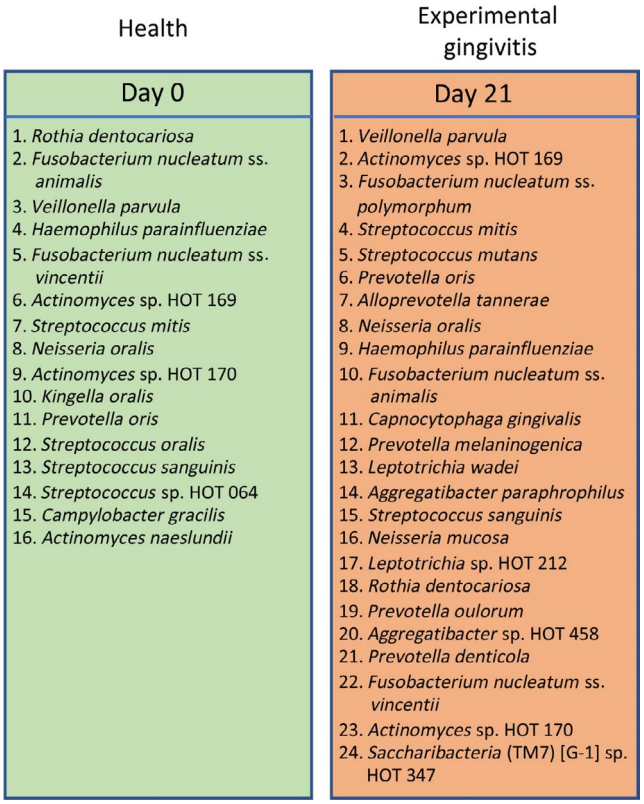

FIGURE 2 Most abundant members of subgingival microbial communities in health and gingivitis. Dominant bacterial species in health (day 0) and after 3 wk of undisturbed plaque accumulation accompanied by the development of gingivitis (day 21), as reported in a 16S rRNA sequencing study.[22] Bacterial species shown are those with a mean relative abundance of at least 1% within each group. Names are presented according to ranked mean relative abundance with the most abundant species at the top of each list

4 | DYSBIOTIC COMMUNITIES ASSOCIATED WITH PERIODONTITIS

The development of periodontitis is accompanied by profound shifts in the composition of subgingival communities, with the emergence of, for the most part, different gram-negative species to those enriched during gingivitis, which outgrow health-associated taxa. Among the enriched species are the classically

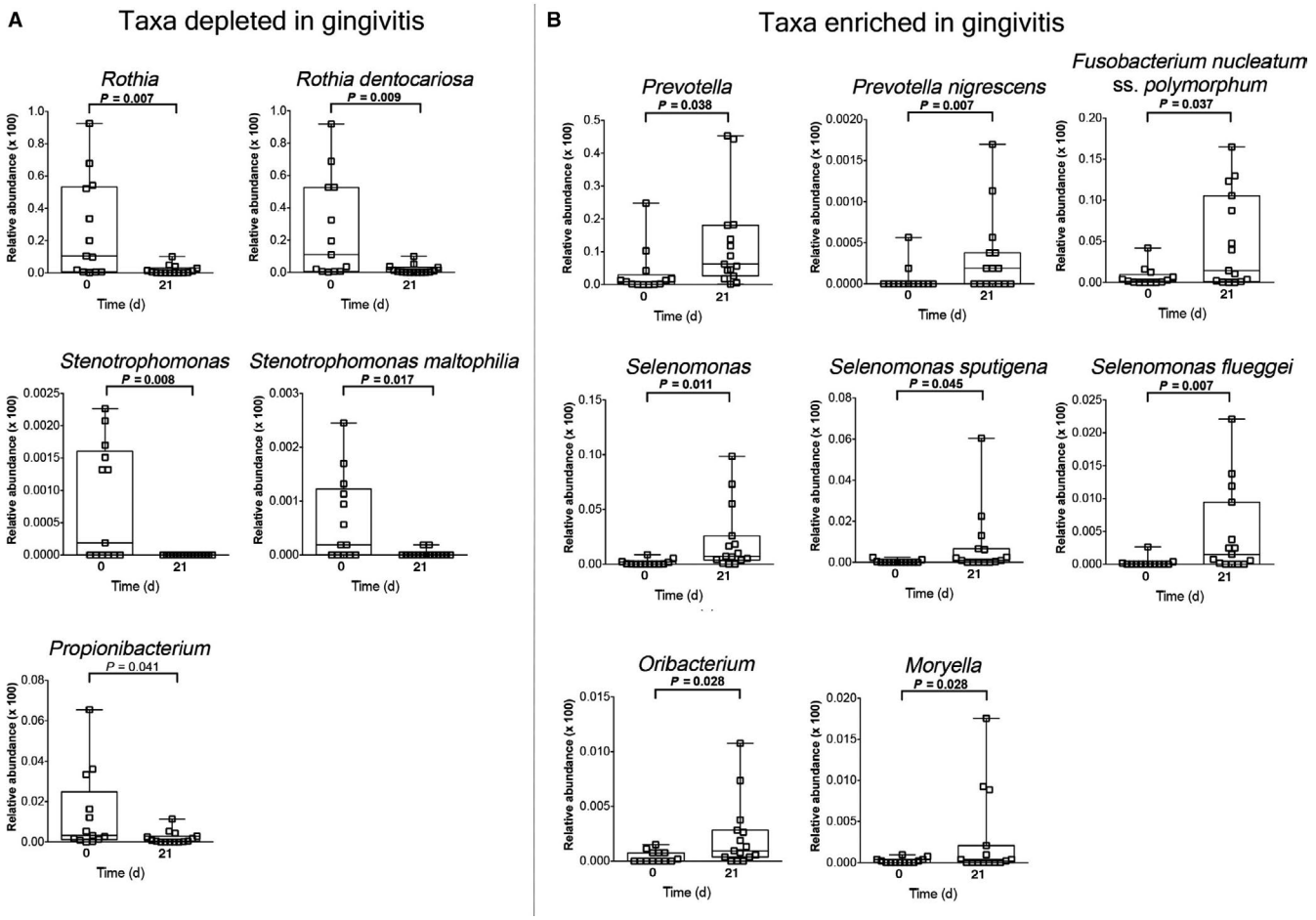

FIGURE 3 Bacteria associated with health and gingivitis. Graphs show species that significantly changed in relative abundance from day 0 to day 21 of undisturbed plaque accumulation, as reported in a 16S rRNA sequencing study[22]

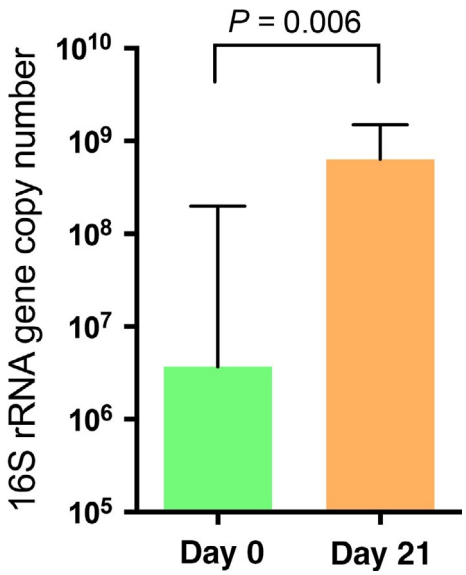

FIGURE 4 Changes in total bacterial load from health to gingivitis. Bars show the change in 16S rRNA gene copy number between the healthy state (day 0) and after 3 wk of undisturbed plaque accumulation (day 21), as reported in a 16S rRNA sequencing study[22]

described red-complex triad consisting of *T. denticola*, *P. gingivalis*, and *Tannerella forsythia*[24] (Figure 1). Several other *Treponema* spp. also appear as abundant components of periodontitis communities in agreement with classic microscopy studies, which indicated that the abundance of spirochaetes was associated with the severity of periodontal destruction.[25] *P. intermedia*, *Filifactor alocis*, *Desulfobulbus* sp. HOT 041, and *Fretibacterium* sp. HOT 360, among others, are also abundant components of periodontitis communities (Figure 1). Species that appear associated with periodontal health and periodontitis in recent 16S rRNA sequencing studies are reviewed elsewhere.[26-28] Figure 5 shows the species most strongly associated with health or periodontitis, as judged by their repeated appearance in molecular surveys conducted by different groups in distinct patient cohorts.[14-16,29] Two *Actinomyces* spp., 2 *Rothia* spp., and *S. sanguinis* appear as the main health-associated taxa depleted in periodontitis, while a diverse group of mostly gram-negatives are enriched in periodontitis. The greater number of species associated with periodontitis than species associated with health is consistent with the higher diversity of periodontitis communities, in which species are more evenly distributed, and therefore more species can be detected with a similar sequencing effort than in the less diverse healthy communities, which tend to be dominated by a few taxa.[14]

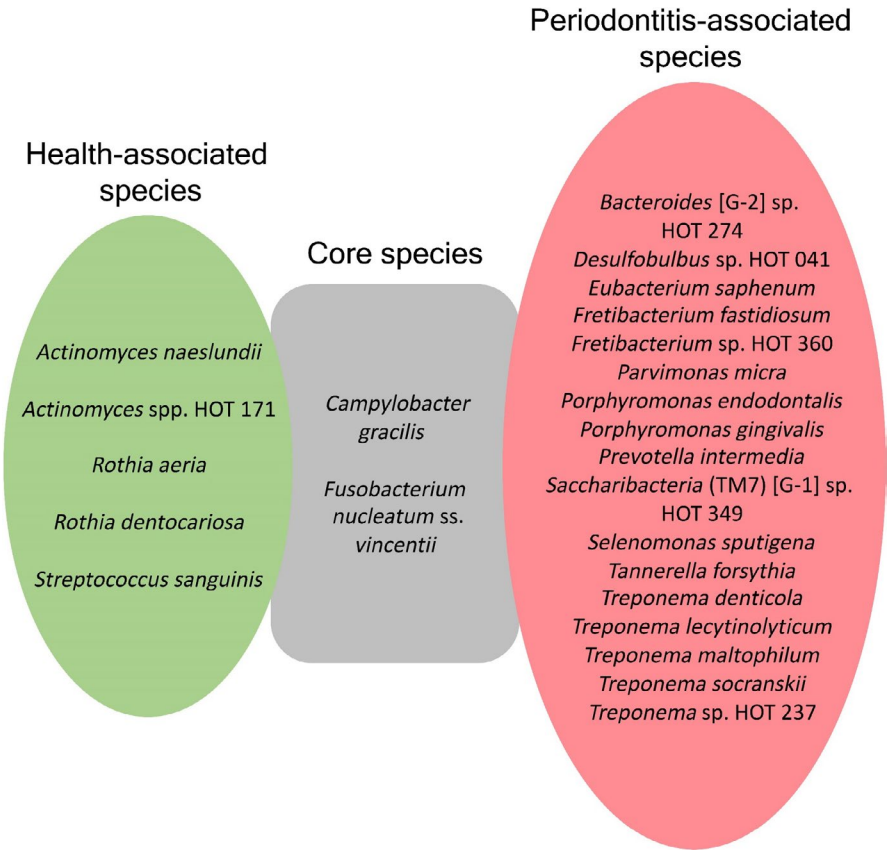

FIGURE 5 Health-associated, periodontitis-associated, and core species of the subgingival microbiome. Green and red ovals show species with significantly increased proportions in either health or periodontitis, appearing in at least 3 different 16S rRNA gene sequencing studies, as reviewed elsewhere,[26] and therefore strongly associated with health or disease.[14-17] The gray oblong indicates core species, which are those with unchanged proportions in health and periodontitis, and classified as core in both of our 16S rRNA gene sequencing studies[14,16]

This increased diversity is a distinctive feature of the microbiome alterations that accompany periodontitis and is in contrast to other oral diseases such as caries, or to gastrointestinal inflammatory conditions such as inflammatory bowel disease, in which disease development is associated with decreased diversity.[30,31] There may be a number of explanations for this difference. Diverse communities possibly indicate niches with a more diverse pool of available nutritional resources delivered through an increased inflammatory response, or alternatively, niches in which cooperation is necessary for the efficient utilization of nutrients, and therefore a single species cannot dominate. In vitro oral community models have indeed revealed that increased species diversity represents a benefit when communities grow using complex nutritional substrata, with multispecies consortia being more efficient in the utilization of complex glycoproteins, such as those in gingival crevicular fluid, than single microorganisms.[32,33] Hence, periodontitis communities may depend on a larger web of metabolic interactions than their healthy counterparts. Alternatively, it is possible that the subgingival environment in periodontitis may represent a site of immune dysfunction that permits the proliferation of species normally controlled by the host defense. Lowered efficiency of the innate and adaptive response in periodontal disease has been suggested through, for example, inhibition of interleukin-8 activity by *P. gingivalis*, so-called chemokine paralysis.[34] Interestingly, the mucosal microbiota associated with colorectal cancer tumors and polyps—sites of immune suppression in the colon—also demonstrate elevations in population diversity compared with healthy mucosal surfaces.[35,36]

It should also be noted that health-associated species are still present in periodontitis and vice versa, which reinforces the principle that dysbiosis results from changes in dominant species rather than from de novo colonization of periodontitis-associated taxa.[14,15] Furthermore, numerical analyses demonstrate a significant increase in the total load of health-associated species in periodontal disease compared with periodontal health, albeit that this increase is dwarfed by the concomitant rise in numbers of the disease-associated species (Figure 6). The potential role played by this increase in the microbial load of the health-associated species in periodontal disease is not known. However, as we describe later, the properties of the members of this group of bacteria do appear to switch to potentially more pathogenic behavior in disease compared with health, suggesting that they may contribute to the overall microbial challenge to the host tissues.

An important finding from subgingival microbiome characterizations via 16S rRNA gene sequencing is the identification of species whose proportions do not change from health to disease. These species are referred to as core species, as they are present in similar proportions regardless of health status. Core species represent bacteria capable of interacting with health-associated and periodontitis-associated community members. Two of the most consistently detected species in this group are *Campylobacter gracilis* and *F. nucleatum* ss. *vincentii* (Figure 5). The latter is also an abundant component of both healthy and periodontitis-associated plaque, and has been suggested as an important component of plaque structure because of its ability to co-aggregate with many other species.[37-39]

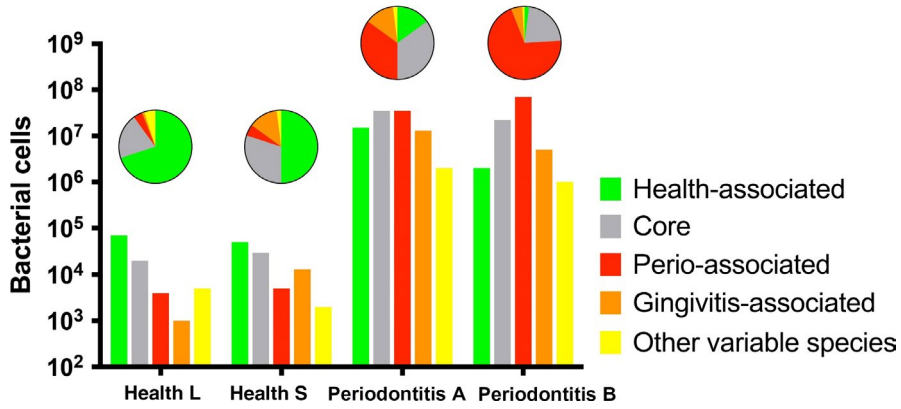

FIGURE 6 Types of health-associated and periodontitis-associated subgingival communities. We have found that each state is represented by 2 types of communities.[16] Healthy subjects could be clustered into 2 community types: type L which is enriched for health-associated taxa, and type S which are communities enriched for core and gingivitis-associated species. Two types of communities are also associated with periodontitis, with type A communities enriched for core and gingivitis-associated species, and type B communities enriched for taxa strongly associated with periodontitis. Individuals with the greatest periodontitis severity had type B communities indicating a more advanced dysbiotic state. Pie charts show the proportions of each group of species in the different community types. Bars show proportions normalized to bacterial load, depicting the true total abundance of each species group in the different community types. A 3-log difference in biomass between health and periodontitis was assumed to calculate total load

Similar to the shifts associated with gingivitis, the shifts that occur in the composition of communities in periodontitis need to be placed within the context of changes in the total biomass. Estimation of the total bacterial load in health and periodontitis reveals that after a single pass of a curette, 1,000 times more bacterial cells can be recovered from periodontitis sites in comparison with sites in healthy subjects.[14] These measurements agree with previously reported data by Moore and Moore using a cultivation approach.[9] Within this context, it is thus evident that although core species do not change in proportion as disease develops, comprising about 25% of the biomass in either health or disease, the host interacts with a 3-log higher load of core species such as *F. nucleatum* during periodontitis. The greatest change, however, is observed in the load of periodontitis-associated species, which occupies about 5% of the total biomass in health but increases to comprise close to 50% of the biomass in disease, thereby showing a ~4-log increase in their load (Figure 6).[26]

The influence of microbial load vs microbial composition of the subgingival microbiota on the development of periodontitis has recently been examined in experimental periodontitis.[40] In these experiments, the expansion of resident memory T helper 17 cells and associated neutrophil accumulation were necessary for inflammatory tissue destruction in a murine model of ligature-induced periodontitis. Treatment of ligature-induced periodontitis with a variety of different antibiotic regimens that were able to either reduce the total load or target only specific components of the microbiota without reduction to the total microbial burden demonstrated that while an increased microbial load is essential for activation of the cellular responses and development of destructive disease, this was insufficient unless accompanied by specific alterations to the overall microbial community structure associated with the ligature placement. Hence, in this system, a nonspecific challenge to the host tissues based on solely increased microbial burden is insufficient to drive disease.

5 | SUBJECT-TO-SUBJECT VARIABILITY IN SUBGINGIVAL MICROBIAL COMMUNITIES

Although the previous section described common patterns discerned from groups of subjects with health or disease, it should be noted that the composition of subgingival communities varies from individual to individual. This is consistent with the known inter-subject variability in the overall composition of the oral microbiome, which itself is liable to be governed at the individual level by both the exposure and acquisition of oral microbial communities from the environment from birth to adulthood, and by the genetic landscape of the host.[36] In a group of 79 healthy subjects, the emergence of 2 slightly different community types was observed, which were referred to as community L and community S (Figure 6).[16,26] The letter designations refer to the size of the clusters, with most subjects falling under the large cluster L.[16] The difference between these 2 healthy community types was a higher abundance of health-associated taxa such as *R. aeria*, *Actinomyces* spp., *Streptococcus* spp., *L. mirabilis*, and *G. adiacens* in the L community, whereas the small cluster S (with less subjects than cluster L) had a higher abundance of core species such as *C. gracilis* and *F. nucleatum* ss. *vincentii*, and also a higher abundance of gingivitis-associated species such as *F. nucleatum* ss. *polymorphum*, *L. wadeii*, and *P. catoniae*, despite a healthy clinical state.[16,26]

Similarly, 2 distinct communities were observed in subjects with periodontitis.[16] These communities are designated by the letters A and B (Figure 6). The A communities differed from the B communities by having increased proportions of core species and gingivitis-associated taxa, whereas the B communities were

enriched in taxa strongly associated with periodontitis such as the red complex, *F. alocis*, *T. maltophilum*, *T. medium*, and *Fretibacterium* sp. HOT 360.[16,26] Interestingly, subjects with the highest extent of disease had type B communities. These findings suggest different degrees of dysbiosis and point to a longitudinal progression in which taxa strongly associated with periodontitis dominate communities of subjects with advanced disease. It should also be noted that periodontitis is a site-specific disease. However, healthy shallow sites in subjects with periodontitis have communities that are more aligned with diseased sites than with shallow sites in healthy subjects.[8] Therefore, dysbiotic changes affect the whole mouth, rather than specific sites.

6 | CHANGES IN THE FUNCTIONAL PROPERTIES OF SUBGINGIVAL COMMUNITIES FROM HEALTH TO DISEASE

While 16S rRNA gene sequencing is able to discern the taxonomic profiles of bacterial subgingival communities, direct sequencing of DNA obtained from plaque via a shot-gun approach can reveal the presence of nonbacterial microbial components. A recent shot-gun DNA sequencing study of subgingival communities in health and periodontitis was able to detect archaea, fungi, and viruses, in addition to bacteria as community members.[8] This study confirmed that bacteria are the most abundant component of communities, comprising at least 95% of the reads obtained. Although present in low abundance, archaea showed differential patterns of colonization in health and disease, with *Methanobrevibacter oralis*, *M. smittii*, *Methanomassiliicoccus luminyensis*, and *Methanosphaera stadtmaniae* appearing in significantly higher proportions in periodontitis. Although present in low abundance, Archaea may have an important role in allowing the increase in biomass seen in periodontitis, as they occupy a terminal position in the community metabolic chain, removing hydrogen from the environment and thus creating more thermodynamically favorable conditions for the growth of anaerobic bacteria.[41] Contrary to archaea, the fungus *Candida albicans* was found associated with health, decreasing in abundance in periodontitis.[8]

Taxonomic differences in species dominating communities in health and periodontitis also correlate with differences in the metabolic activities of these communities. In an analysis of the metabolic potential of communities in health and disease, Dabdoub et al[8] showed that periodontitis communities have an expanded functionality, with an overrepresentation of genes encoding for lipid-A biosynthesis, antibiotic resistance, and iron acquisition, among others. Also, while virulence factors account for 8.9% of the genome content in health, 33.1% of the genome content in disease can be mapped to virulence-related attributes. Periodontitis communities may thus have an increased pathogenic potential compared with communities in health.

These largely descriptive studies of the DNA content of a given sample reveal little about the functional activities of the organisms present within these communities. A proper understanding of the metabolic activity of bacteria in periodontitis, and hence the nature of the microbial challenge to the host's tissues, can only be determined using experimental approaches that measure functions rather than microbial presence or absence. Consequently, applications of community-wide analyses of the transcriptome (mRNA), the proteome (proteins), and the metabolome (metabolites), are required. Largely as a result of the technical challenges, these studies are still in their infancy, but the limited number of investigations to date are beginning to reveal a hitherto hidden level of complexity.

Metatranscriptomic RNA sequencing permits evaluation of differences in the true metabolic activities of specific species within communities, or metabolic changes in the community as a whole. An evaluation of the metatranscriptome of communities in health and periodontitis showed enrichment in periodontitis of transcripts from genes related to flagellar motility, peptide transport, iron acquisition, beta-lactam degradation, lipid A biosynthesis, and cellular stress responses, in agreement with the hypothesis that periodontitis communities may have increased pathogenic potential.[42] Upregulation of iron acquisition mechanisms in periodontitis suggests that a requirement for any species to thrive in disease is to be able to compete efficiently for this nutrient. Evaluation of longitudinal changes in individual tooth sites showing disease progression (loss of attachment) agrees with these findings, showing enrichment of the gene ontology in terms of pathogenesis, response to oxidative stress, ferrous iron transport, protein secretion and growth, among others, in progressing sites.[43]

Altogether, these studies show that subgingival communities associated with health and disease do not only differ in their taxonomic composition, but that the metabolic activities of communities are distinct under the 2 states. Subgingival communities associated with periodontal sites that exhibit loss of attachment over time have been shown to be different metabolically from communities of stable sites, with these differences detected prior to disease progression.[43] Those sites that exhibited disease progression had a greater number of metabolically active species and an increased representation of transcripts related to lipid A biosynthesis, oxidative stress responses, flagellar motility, and transport of amino acids, iron, and potassium. These results suggest that prior to detectable destruction of tissues, the environmental conditions of the site and the characteristics of the community are different to those in sites that remain stable, possibly implying increased pathogenic potential of communities from progressing sites.

Perhaps the most revealing findings of these metatranscriptomic studies of the microbiota in periodontitis have come from scrutiny of the functional properties of individual organisms. For instance, the core species *F. nucleatum* does not change proportions in either healthy or diseased sites, but it upregulates the pathway of lysine fermentation to butyrate in disease.[44] This switch possibly correlates with the greater anaerobiosis of periodontal pockets, as in vitro studies show a negative correlation between environmental oxygen levels and butyrate production by *F. nucleatum*.[45] Moreover, analysis of red-complex species of periodontal disease in these

functional studies showed that all 3 species undergo an activation of the metalloproteases, peptidases, and proteins involved in iron metabolism, suggesting that these may represent important virulence factors in these well-established oral pathogens.

However, the most surprising outcome of these preliminary forays into functional analysis of bacteria in periodontitis has been the demonstration that the transcriptional profile of normally health-associated species may also be altered to a more potentially pathogenic status in sites that subsequently develop disease, and also in sites of progression. For example, in a comparison of baseline vs progressing sites, those organisms with the largest number of upregulated putative virulence determinants were health-associated streptococcal species. Similar results were obtained when comparing baseline nonprogressing to baseline progressing sites.[43] These findings further emphasize that focusing solely on those organisms that become dominant in disease as the drivers of periodontitis may be an oversimplification: while the contribution of the disease-associated species, many of which have been shown to have properties consistent with deregulation of the immune and inflammatory response,[7] cannot be ignored, the overall virulence challenge in periodontitis may actually be a product of the entire microbial community.[46]

7 | DRIVERS OF DYSBIOSIS

The undisturbed microbial accumulation at the gingival crevice and its triggering of host responses results in increased efflux of gingival crevicular fluid from adjacent tissues into the sulcus. Indeed, the flow rate of gingival crevicular fluid has been shown to gradually increase from health to gingivitis to periodontitis.[47,48] In vitro evaluation of the role of serum as a modulator of oral communities suggests a serum-like exudate like gingival crevicular fluid promotes the enrichment of proteinase-rich taxa, which are usually associated with periodontitis.[49-51] Another environmental parameter bound to affect the composition of communities is oxygen. Taxa associated with health and gingivitis have a higher tolerance to oxygen than some of the periodontitis-associated taxa.[52,53] Disruption of tissues and the presence of blood cells could also modify subgingival communities, as certain species such as black pigmented anaerobic bacteria associated with periodontitis prefer to utilize hemoglobin-derived hemin as a source of iron.

Finally, while the preceding section emphasized the importance of the entire microbial community in the development of the challenge to host tissues in periodontitis, this should not diminish the potentially crucial role played by individual species in orchestrating the development of dysbiosis. For example, one of the presumed important pathobionts, *P. gingivalis*, has been shown as able to elicit a profound effect on the quantitative and qualitative composition of the oral commensal microbiome in the mouse model of periodontal disease. In so doing, *P. gingivalis* drives dysbiosis of the normally benign oral microbiota into a community structure responsible for the tissue and bone destruction in this animal model.[6] That these changes occurred when only low numbers of *P. gingivalis* were present led to the proposal that this bacterium may be considered a keystone pathogen with elevated disease potential above that anticipated for a low-abundance species.[5] The mechanism through which *P. gingivalis* appears able to act as an orchestrator of dysbiosis in model systems is most likely through disabling and deregulatory effects on the host immune and inflammatory systems, in particular complement.[1] These disruptive properties may thereby create the environment for outgrowth of other members of the microbiota, a further example of how alterations to the local ecology can play a fundamental role in shaping the dysbiotic potential of the oral commensal microbiota.

8 | INFLAMMATION IN PERIODONTITIS

Cytokines, chemokines, and metalloproteinases are known to increase dramatically in periodontal tissues and gingival crevicular fluid in periodontitis.[54-56] The early response lipid mediators, leukotriene B4 and prostaglandin E2, are markedly increased.[57-60] Prostaglandin E2, which is significantly elevated in gingival crevicular fluid of periodontitis patients and correlates well with disease severity and ongoing activity, can be used to predict future disease episodes.[57,61] Cyclooxygenase activity, in particular cyclooxygenase-2, is increased in periodontitis, and in response to periodontal pathogens in vitro.[62-66]

In the natural resolution of acute inflammation, a distinct class of lipid mediators called specialized proresolving mediators emerge to regulate a coordinated return to tissue homeostasis.[67,68] Neutrophils from periodontal patients produce increased lipoxin A4,[66] especially in patients with localized aggressive periodontitis. Leukotriene B4 and prostaglandin E2 are also detected.[57,66] The chronic periodontal lesion is characterized by hyperactivated neutrophils with activated lipoxin pathways; however, the lipoxins are insufficient in quantity as resolution of inflammation is not achieved.

The insufficient resolution response was demonstrated experimentally in a rabbit model of ligature-induced periodontitis comparing wild-type rabbits with transgenic rabbits with elevated circulating levels of lipoxin A4.[69] The transgenic rabbits were protected from periodontal tissue damage and bone loss. Thus sufficient quantities of lipoxin prevent tissue destruction in bacterially induced periodontal disease. In humans, dysregulation of resolution was demonstrated in localized aggressive periodontitis.[70] Taken together, the data suggest a failure to resolve local inflammation induced by bacteria in the periodontium leads to periodontal disease progression.

Abnormalities in localized aggressive periodontitis were reversed with exogenous resolvin E1 derived from the omega-3 fatty acid, eicosapentaenoic acid (26). Hasturk and co-workers[71] showed that localized aggressive periodontitis neutrophils respond to resolvins, but not to lipoxins. Perhaps selective abnormalities of the response, together with variability in the composition of the microbiota described previously, explain aspects of individual variability in susceptibility to disease.

9 | INFLAMMATION AND THE MICROBIOME

As described earlier, the etiologic stimulus for periodontitis in susceptible individuals is the bacterial challenge, and there are strong associations with the outgrowth of disease-associated organisms, including *P. gingivalis*, *T. forsythia*, and *T. denticola*[72], and numerous other species shown in Figure 5. Active control of inflammation with specialized proresolving mediators prevents periodontitis in animal models and demonstrates that control of inflammation in an infectious disease can lead to clearance of the infection, not to disseminating infection.[71,73] Direct monitoring of the lesion-associated biofilm revealed that control of inflammation reversed the biofilm changes with resolution of microbial dysbiosis.[73]

The new molecular methodologies that sequence-cloned DNA from communities of microorganisms have completely changed our understanding of the relationship between the host response and the microbes that colonize the body.[74] The concept that periodontal disease is a consequence of the outgrowth of a relatively small number of "pathogenic species", which become dominant in disease, is now replaced by a view that the microbiome as a whole is in a bidirectional relationship with the host inflammatory response.[75-80] For example, intracellular pathogens that persist can cause significant dysregulation of inflammation[81-83] and upregulation of systemic inflammation, as in obesity and type 2 diabetes, resulting in dysbiosis of the gut microbiome.[79]

Thus it has been suggested that a reappraisal of the temporal sequence of the periodontal microbiome and inflammation in periodontal disease is necessary. Oral microbiome dysbiosis is clearly associated with periodontitis in cross-sectional studies,[5] but the temporal interplay between bacteria and inflammation is only beginning to be described.[84,85] Susceptibility to periodontitis and resultant tissue destruction is mediated by the host response to bacteria,[86-88] and excess, uncontrolled inflammation,[89-91] but there are few longitudinal studies that demonstrate that inflammation predicts disease progression,[92,93] and even fewer that dysbiosis occurs after onset of disease.[92]

In studies of periodontitis progression and treatment with resolvin E1 in rabbits, specialized proresolving mediators prevented and reversed disease and promoted bone remodeling.[71,73,94,95] Active control of inflammation resulted in spontaneous disappearance of periodontal pathogens without mechanical or antimicrobial therapy. The temporal dynamics of inflammation-induced dysbiosis were examined in rat periodontitis.[84] Active control of inflammation prevented and successfully treated ligature-induced periodontitis in the rat with significant regeneration of lost tissues including bone. Dysbiotic shifts in the local microbiota induced by inflammation were reversed to a significant degree.

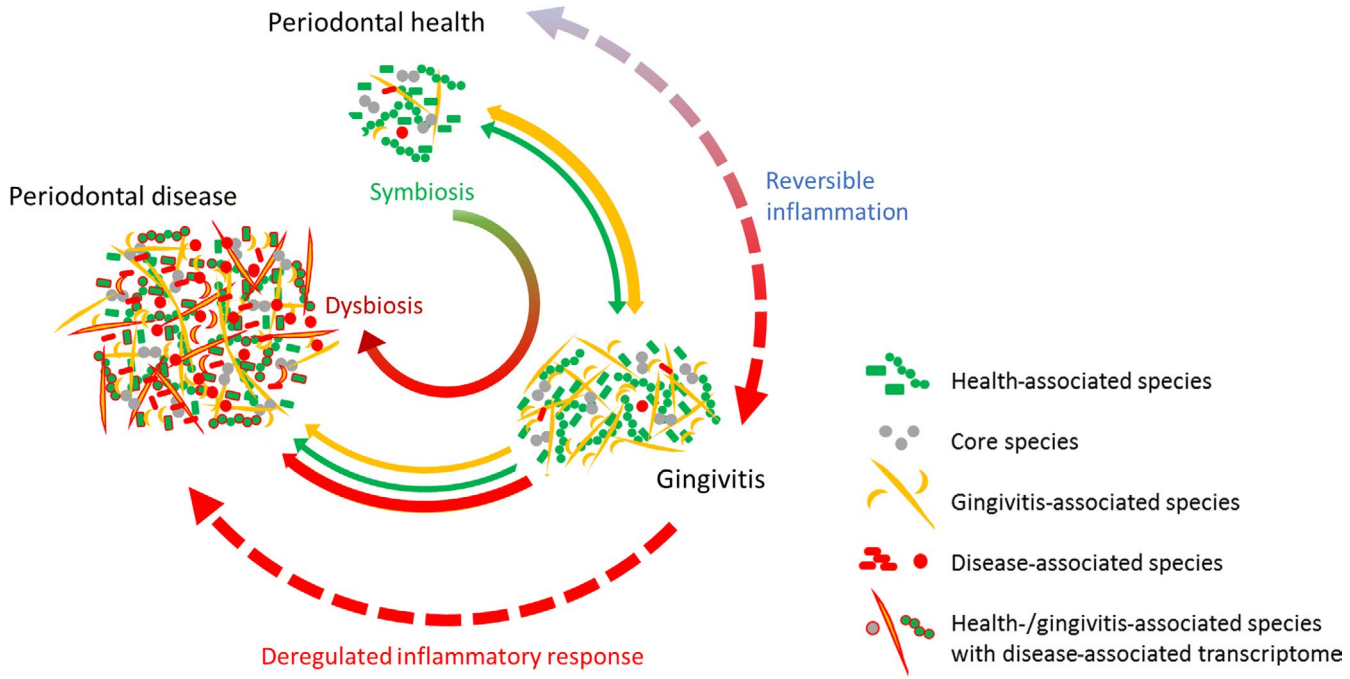

FIGURE 7 The bidirectional relationship between the subgingival microbiome and the inflammatory and immune response. The symbiotic microbiota in health is dominated by health-associated species (green) and low abundances of species associated with gingivitis (orange) and periodontitis (red). Gingivitis is characterized by an increased biomass (green and orange arrows) comprising both green and particularly orange species and an associated increase in inflammation. In periodontitis, biomass increases further (green, orange, and red arrows) and the red species become increasingly dominant in the dysbiotic microbiota. Furthermore, the gene expression profiles of the green and orange species are modified with increased expression of virulence determinants. This is accompanied by the development of a deregulated inflammatory response and tissue destruction. Interventions which are able to resolve the inflammatory response may also be important in the reversal of the dysbiotic microbiota

These findings reveal important principles relating to the role of inflammation in host/microbiome interactions in health and disease. Observations of the microbiome in periodontitis are quite different to other infectious diseases, probably because it is characterized by a dysbiosis of the commensal flora. Species that are associated with disease overgrow because inflammation induces changes in the local ecology. The disease-associated microbiome is more diverse (alpha diversity), while the microbiome of diseased sites within an individual become more similar (beta diversity).[14,15,96-98] The shifts in the microbiome induced by inflammation favor overgrowth of certain commensals and this may also change the expression of virulence factors. However, as we described previously, the shifts induced by inflammation may ultimately depend upon the repertoire of organisms that are present in a given niche. Variations in this "baseline" microbiota may therefore influence susceptibility. Global differential gene expression analyses reveal that virulence factors are upregulated in all bacteria, in presumed pathogens as well as in health-associated commensals.[42,43] Thus there appears to be a direct link between local environmental conditions and the amount, the composition, and the activity of the microbiota, and increasing evidence that this can be reversed with active control of inflammation. These changes are not observed with inhibition of inflammation with drugs, including nonsteroidal anti-inflammatory drugs.[99]

Taken together, the data suggest that the inflammatory response and the microbiome are in bidirectional balance in oral health (homeostasis) and bidirectional imbalance in periodontitis. The inflammatory response can contribute to microbiome changes and expression of bacterial virulence factors. Active control of excess inflammation can positively impact management of dysbiosis and periodontitis (Figure 7). Beyond oral diseases such as periodontitis, similar observations are made in sepsis, inflammatory bowel disease, and other diseases as well.[100,101]

ACKNOWLEDGMENTS

The authors express their thanks to Juliet Ellwood for her assistance in the preparation of this manuscript. This research was supported in part by USPHS grants DE025020 and 025383 (TVD) and 023967 (PD) from the National Institutes of Dental and Craniofacial Research and MR/P012175/1 (MC) from the Medical Research Council.

REFERENCES

1. Hajishengallis G, Liang S, Payne MA, et al. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe*. 2011;10:497-506.
2. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. *Adv Dent Res*. 1994;8(2):263-271.
3. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. *Periodontol 2000*. 1997;14:216-248.
4. Vandenberg JI, Conigrave A, King GF, Kirk K. From kinetics to imaging: an NMR odyssey–a festschrift symposium in honour of Philip William Kuchel. *Eur Biophys J*. 2013;42(1):1-2.
5. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. *Nat Rev Microbiol*. 2012;10(10):717-725.
6. Darveau RP, Hajishengallis G, Curtis MA. *Porphyromonas gingivalis* as a potential community activist for disease. *J Dent Res*. 2012;91(9):816-820.
7. Curtis MA, Slaney JM, Aduse-Opoku J. Critical pathways in microbial virulence. *J Clin Periodontol*. 2005;32:28-38.
8. Dabdoub SM, Ganesan SM, Kumar PS. Comparative metagenomics reveals taxonomically idiosyncratic yet functionally congruent communities in periodontitis. *Sci Rep*. 2016;6:38993.
9. Moore WE, Moore LV. The bacteria of periodontal diseases. *Periodontol 2000*. 1994;5:66-77.
10. Paster BJ, Boches SK, Galvin JL, et al. Bacterial diversity in human subgingival plaque. *J Bacteriol*. 2001;183(12):3770-3783.
11. Listgarten MA. Structure of the microbial flora associated with periodontal health and disease in man. A light and electron microscopic study. *J Periodontol* 1976;47(1):1-18.
12. Diaz PI, Chalmers NI, Rickard AH, et al. Molecular characterization of subject-specific oral microflora during initial colonization of enamel. *Appl Environ Microbiol*. 2006;72(4):2837-2848.
13. Kolenbrander PE, Palmer RJ Jr, Rickard AH, Jakubovics NS, Chalmers NI, Diaz PI. Bacterial interactions and successions during plaque development. *Periodontol 2000*. 2006;42:47-79.
14. Abusleme L, Dupuy AK, Dutzan N, et al. The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. *ISME J*. 2013;7(5):1016-1025.
15. Griffen AL, Beall CJ, Campbell JH, et al. Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. *ISME J*. 2012;6(6):1176-1185.
16. Hong BY, Furtado Araujo MV, Strausbaugh LD, et al. Microbiome profiles in periodontitis in relation to host and disease characteristics. *PLoS ONE*. 2015;10(5):e0127077.
17. Palmer RJ Jr, Shah N, Valm A, et al. Interbacterial adhesion networks within early oral biofilms of single human hosts. *Appl Environ Microbiol*. 2017;83(11):e00407-e00417.
18. Mark Welch JL, Rossetti BJ, Rieken CW, Dewhirst FE, Borisy GG. Biogeography of a human oral microbiome at the micron scale. *Proc Natl Acad Sci USA*. 2016;113(6):e791-e800.
19. Theilade E, Wright WH, Jensen SB, Löe H. Experimental gingivitis in man. II. A longitudinal clinical and bacteriological investigation. *J Periodontal Res* 1966;1:1-13.
20. Huang S, Li R, Zeng X, et al. Predictive modeling of gingivitis severity and susceptibility via oral microbiota. *ISME J*. 2014;8(9):1768-1780.
21. Kistler JO, Arirachakaran P, Poovorawan Y, Dahlén G, Wade WG. The oral microbiome in human immunodeficiency virus (HIV)-positive individuals. *J Med Microbiol*. 2015;64(9):1094-1101.
22. Schincaglia GP, Hong BY, Rosania A, et al. Clinical, immune, and microbiome traits of gingivitis and peri-implant mucositis. *J Dent Res*. 2017;96(1):47-55.
23. Kistler JO, Booth V, Bradshaw DJ, Wade WG. Bacterial community development in experimental gingivitis. *PLoS ONE*. 2013;8(8):e71227.
24. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. *J Clin Periodontol*. 1998;25(2):134-144.
25. Armitage GC, Dickinson WR, Jenderseck RS, Levine SM, Chambers DW. Relationship between the percentage of subgingival spirochetes and the severity of periodontal disease. *J Periodontol*. 1982;53(9):550-556.

26. Diaz PI, Hoare A, Hong BY. Subgingival microbiome shifts and community dynamics in periodontal diseases. *J Calif Dent Assoc*. 2016;44(7):421-435.
27. Pérez-Chaparro PJ, Gonçalves C, Figueiredo LC, et al. Newly identified pathogens associated with periodontitis: a systematic review. *J Dent Res*. 2014;93:846-858.
28. Curtis MA. Periodontal microbiology–the lid's off the box again. *J Dent Res*. 2014;93:840-842.
29. Kirst ME, Li EC, Alfant B, et al. Dysbiosis and alterations in predicted functions of the subgingival microbiome in chronic periodontitis. *Appl Environ Microbiol*. 2015;81(2):83-93.
30. Gross EL, Beall CJ, Kutsch SR, Firestone ND, Leys EJ, Griffen AL. Beyond *Streptococcus mutans*: dental caries onset linked to multiple species by 16S rRNA community analysis. *PLoS ONE*. 2012;7(10):e47722.
31. Loh G, Blaut M. Role of commensal gut bacteria in inflammatory bowel diseases. *Gut Microbes*. 2012;3(6):544-555.
32. Beighton D, Smith K, Glenister DA, Salamon K, Keevil CW. Increased degradative enzyme production by dental plaque bacteria in mucin-limited continuous culture. *Microb Ecol Health Dis*. 1998;1:85-94.
33. Bradshaw DJ, Homer KA, Marsh PD, Beighton D. Metabolic cooperation in oral microbial communities during growth on mucin. *Microbiology*. 1994;140(Pt 12):3407-3412.
34. Darveau RP, Belton CM, Reife RA, Lamont RJ. Local chemokine paralysis, a novel pathogenic mechanism for *Porphyromonas gingivalis*. *Infect Immun*. 1998;66(4):1660-1665.
35. Mira-Pascual L, Cabrera-Rubio R, Ocon S, et al. Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaeal biomarkers. *J Gastroenterol*. 2015;50(2):167-179.
36. Mira A, Simon-Soro A, Curtis MA. Role of microbial communities in the pathogenesis of periodontal diseases and caries. *J Clin Periodontol*. 2017;18:S23-S38.
37. Kolenbrander PE, Andersen RN, Moore LV. Coaggregation of *Fusobacterium nucleatum*, *Selenomonas flueggei*, *Selenomonas infelix*, *Selenomonas noxia*, and *Selenomonas sputigena* with strains from 11 genera of oral bacteria. *Infect Immun*. 1989;57(10):3194-3203.
38. Kolenbrander PE, London J. Adhere today, here tomorrow: oral bacterial adherence. *J Bacteriol*. 1993;175(11):3247-3252.
39. Kolenbrander PE, Palmer RJ Jr, Periasamy S, Jakubovics NS. Oral multispecies biofilm development and the key role of cell-cell distance. *Nat Rev Microbiol*. 2010;8(7):471-480.
40. Dutzan N, Kajikawa T, Abusleme L, et al. A dysbiotic microbiome triggers TH17 cells to mediate oral mucosal immunopathology in mice and humans. *Sci Transl Med* 2018;10(463):eaat0797.
41. Gibson GR, Cummings JH, Macfarlane GT. Competition for hydrogen between sulphate-reducing bacteria and methanogenic bacteria from the human large intestine. *J Appl Bacteriol*. 1988;65(3):241-247.
42. Duran-Pinedo AE, Chen T, Teles R, et al. Community-wide transcriptome of the oral microbiome in subjects with and without periodontitis. *ISME J*. 2014;8(8):1659-1672.
43. Yost S, Duran-Pinedo AE, Teles R, Krishnan K, Frias-Lopez J. Functional signatures of oral dysbiosis during periodontitis progression revealed by microbial metatranscriptome analysis. *Genome Med*. 2015;7(1):27.
44. Jorth P, Turner KH, Gumus P, Nizam N, Buduneli N, Whiteley M. Metatranscriptomics of the human oral microbiome during health and disease. *MBio*. 2014;5(2):e01012-e01014.
45. Diaz PI, Zilm PS, Rogers AH. The response to oxidative stress of *Fusobacterium nucleatum* grown in continuous culture. *FEMS Microbiol Lett*. 2000;187(1):31-34.
46. Berezow AB, Darveau RP. Microbial shift and periodontitis. *Periodontol 2000* 2011;55(1):36-47.
47. Hatipoğlu H, Yamalik N, Berberoğlu A, Eratalay K. Impact of the distinct sampling area on volumetric features of gingival crevicular fluid. *J Periodontol*. 2007;78(4):705-715.
48. Ozkavaf A, Aras H, Huri CB, et al. Relationship between the quantity of gingival crevicular fluid and clinical periodontal status. *J Oral Sci*. 2000;42(4):231-238.
49. Grenier D, Mayrand D, McBride BC. Further studies on the degradation of immunoglobulins by black-pigmented *Bacteroides*. *Oral Microbiol Immunol*. 1989;4(1):12-18.
50. ter Steeg PF, Van der Hoeven JS, de Jong MH, van Munster PJ, Jansen MJ. Enrichment of subgingival microflora on human serum leading to accumulation of *Bacteroides* species, *Peptostreptococci* and *Fusobacteria*. *Antonie Van Leeuwenhoek*. 1987;53(4):261-272.
51. ter Steeg PF, Van Der Hoeven JS, de Jong MH, van Munster PJJ, Jansen MJH. Modelling the gingival pocket by enrichment of subgingival microflora in human serum in chemostats. *Microb Ecol Health Dis*. 1988;1:73-84.
52. Diaz PI, Zilm PS, Rogers AH. *Fusobacterium nucleatum* supports the growth of *Porphyromonas gingivalis* in oxygenated and carbon-dioxide-depleted environments. *Microbiology*. 2002;148(Pt 2):467-472.
53. Loesche WJ. Oxygen sensitivity of various anaerobic bacteria. *Appl Microbiol*. 1969;18(5):723-727.
54. Romanelli R, Mancini S, Laschinger C, Overall CM, Sodek J, McCulloch CA. Activation of neutrophil collagenase in periodontitis. *Infect Immun*. 1999;67(5):2319-2326.
55. Gainet J, Chollet-Martin S, Brion M, Hakim J, Gougerot-Pocidalo MA, Elbim C. Interleukin-8 production by polymorphonuclear neutrophils in patients with rapidly progressive periodontitis: an amplifying loop of polymorphonuclear neutrophil activation. *Lab Invest*. 1998;78(6):755-762.
56. Assuma R, Oates T, Cochran D, Amar S, Graves DT. IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. *J Immunol*. 1998;160(1):403-409.
57. Offenbacher S, Odle BM, Van Dyke TE. The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. *J Periodontal Res*. 1986;21(2):101-112.
58. Solomon LM, Juhlin L, Kirschenbaum MB. Prostaglandin on cutaneous vasculature. *J Invest Dermatol*. 1968;51(4):280-282.
59. Raisz LG, Koolemans-Beynen AR. Inhibition of bone collagen synthesis by prostaglandin E2 in organ culture. *Prostaglandins*. 1974;8(5):377-385.
60. Klein DC, Raisz LG. Prostaglandins: stimulation of bone resorption in tissue culture. *Endocrinology*. 1970;86(6):1436-1440.
61. Offenbacher S, Odle BM, Gray RC, Van Dyke TE. Crevicular fluid prostaglandin E levels as a measure of the periodontal disease status of adult and juvenile periodontitis patients. *J Periodontal Res*. 1984;19(1):1-13.
62. ElAttar TM. Prostaglandin E2 in human gingiva in health and disease and its stimulation by female sex steroids. *Prostaglandins*. 1976;11(2):331-341.
63. Albers HK, Loning T, Lisboa BP. Biochemical and morphologic studies on prostaglandins E and F in the normal and inflamed gingiva. *Dtsch Zahnarztl Z*. 1979;34(5):440-443.
64. ElAttar TM, Lin HS, Tira DE. The relationship between the concentration of female sex steroids and prostaglandins production by human gingiva in vitro. *Prostaglandins Leukot Med*. 1982;8(5):447-458.
65. ElAttar TM, Lin HS, Tira DE. Arachidonic acid metabolism in inflamed gingiva and its inhibition by anti-inflammatory drugs. *J Periodontol*. 1984;55(9):536-539.
66. Pouliot M, Clish CB, Petasis NA, Van Dyke TE, Serhan CN. Lipoxin A(4) analogues inhibit leukocyte recruitment to *Porphyromonas gingivalis*: a role for cyclooxygenase-2 and lipoxins in periodontal disease. *Biochemistry*. 2000;39(16):4761-4768.

67. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. *Nature*. 2014;510(7503):92-101.
68. Serhan CN, Yacoubian S, Yang R. Anti-inflammatory and proresolving lipid mediators. *Annu Rev Pathol*. 2008;3:279-312.
69. Serhan CN, Jain A, Marleau S, et al. Reduced inflammation and tissue damage in transgenic rabbits overexpressing 15-lipoxygenase and endogenous anti-inflammatory lipid mediators. *J Immunol*. 2003;171(12):6856-6865.
70. Fredman G, Oh SF, Ayilavarapu S, et al. Impaired phagocytosis in localized aggressive periodontitis: rescue by Resolvin E1. *PLoS ONE*. 2011;6(9):e24422.
71. Hasturk H, Kantarci A, Ohira T, et al. RvE1 protects from local inflammation and osteoclast- mediated bone destruction in periodontitis. *FASEB J*. 2006;20(2):401-403.
72. Van Dyke TE, Serhan CN. Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. *J Dent Res*. 2003;82(2):82-90.
73. Hasturk H, Kantarci A, Goguet-Surmenian E, et al. Resolvin E1 regulates inflammation at the cellular and tissue level and restores tissue homeostasis in vivo. *J Immunol*. 2007;179(10):7021-7029.
74. Pagani I, Liolios K, Jansson J, et al. The Genomes OnLine Database (GOLD) v.4: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res* 2012;40:D571-D579.
75. DiGiulio DB, Romero R, Amogan HP, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. *PLoS ONE*. 2008;3(8):e3056.
76. Morgan XC, Tickle TL, Sokol H, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol*. 2012;13(9):R79.
77. Franceschi F, Niccoli G, Ferrante G, et al. CagA antigen of *Helicobacter pylori* and coronary instability: insight from a clinico-pathological study and a meta-analysis of 4241 cases. *Atherosclerosis*. 2009;202(2):535-542.
78. Giongo A, Gano KA, Crabb DB, et al. Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J*. 2011;5(1):82-91.
79. Larsen N, Vogensen FK, van den Berg FW, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS ONE*. 2010;5(2):e9085.
80. Rajendhran J, Shankar M, Dinakaran V, Rathinavel A, Gunasekaran P. Contrasting circulating microbiome in cardiovascular disease patients and healthy individuals. *Int J Cardiol*. 2013;168(5):5118-5120.
81. Helaine S, Cheverton AM, Watson KG, et al. Internalization of Salmonella by macrophages induces formation of nonreplicating persisters. *Science*. 2014;343(6167):204-208.
82. Miskinyte M, Sousa A, Ramiro RS, et al. The genetic basis of *Escherichia coli* pathoadaptation to macrophages. *PLoS Pathog*. 2013;9(12):e1003802.
83. Xu Y, Xie J, Li Y, et al. Using a cDNA microarray to study cellular gene expression altered by Mycobacterium tuberculosis. *Chin Med J (Engl)*. 2003;116(7):1070-1073.
84. Lee CT, Teles R, Kantarci A, et al. Resolvin E1 reverses experimental periodontitis and dysbiosis. *J Immunol*. 2016;197(7):2796-2806.
85. Sima C, Cheng Q, Rautava J, et al. Identification of quantitative trait loci influencing inflammation-mediated alveolar bone loss: insights into polygenic inheritance of host-biofilm disequilibria in periodontitis. *J Periodontal Res*. 2016;51(2):237-249.
86. Van Dyke TE. Proresolving lipid mediators: potential for prevention and treatment of periodontitis. *J Clin Periodontol*. 2011;38(Suppl 11):119-125.
87. Moutsopoulos NM, Konkel J, Sarmadi M, et al. Defective neutrophil recruitment in leukocyte adhesion deficiency type I disease causes local IL-17-driven inflammatory bone loss. *Sci Transl Med* 2014;6(229):229ra40.
88. Moutsopoulos NM, Chalmers NI, Barb JJ, et al. Subgingival microbial communities in Leukocyte Adhesion Deficiency and their relationship with local immunopathology. *PLoS Pathog*. 2015;11(3):e1004698.
89. Kantarci A, Oyaizu K, Van Dyke TE. Neutrophil-mediated tissue injury in periodontal disease pathogenesis: findings from localized aggressive periodontitis. *J Periodontol*. 2003;74(1):66-75.
90. Chapple IL, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontol 2000*. 2007;43:160-232.
91. Graves D. Cytokines that promote periodontal tissue destruction. *J Periodontol*. 2008;79(8 Suppl):1585-1591.
92. Tanner AC, Kent R Jr, Kanasi E, et al. Clinical characteristics and microbiota of progressing slight chronic periodontitis in adults. *J Clin Periodontol*. 2007;34(11):917-930.
93. Kinney JS, Morelli T, Oh M, et al. Crevicular fluid biomarkers and periodontal disease progression. *J Clin Periodontol*. 2014;41(2):113-120.
94. Gao L, Faibish D, Fredman G, et al. Resolvin E1 and chemokine-like receptor 1 mediate bone preservation. *J Immunol*. 2013;190(2):689-694.
95. Zhu M, Van Dyke TE, Gyurko R. Resolvin E1 regulates osteoclast fusion via DC-STAMP and NFATc1. *FASEB J*. 2013;27(8):3344-3353.
96. Liu B, Faller LL, Klitgord N, et al. Deep sequencing of the oral microbiome reveals signatures of periodontal disease. *PLoS ONE*. 2012;7(6):e37919.
97. Li Y, He J, He Z, et al. Phylogenetic and functional gene structure shifts of the oral microbiomes in periodontitis patients. *ISME J*. 2014;8(9):1879-1891.
98. Shi B, Chang M, Martin J, et al. Dynamic changes in the subgingival microbiome and their potential for diagnosis and prognosis of periodontitis. *MBio*. 2015;6(1):e01926-14.
99. Kornman KS, Blodgett RF, Brunsvold M, Holt SC. Effects of topical applications of meclofenamic acid and ibuprofen on bone loss, subgingival microbiota and gingival PMN response in the primate *Macaca fascicularis*. *J Periodontal Res*. 1990;25(5):300-307.
100. Spite M, Norling LV, Summers L, et al. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. *Nature*. 2009;461(7268):1287-1291.
101. Arita M, Yoshida M, Hong S, et al. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. *Proc Natl Acad Sci USA*. 2005;102(21):7671-7676.

How to cite this article: Curtis MA, Diaz PI, Van Dyke TE. The role of the microbiota in periodontal disease. *Periodontol 2000*. 2020;83:14–25. https://doi.org/10.1111/prd.12296