Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Nat Rev Microbiol. Author manuscript; available in PMC 2024 May 10.

Published in final edited form as:

Nat Rev Microbiol. 2024 February ; 22(2): 89–104. doi:10.1038/s41579-023-00963-6.

The oral microbiome: diversity, biogeography and human health

Jonathon L. Baker[1,2,3], **Jessica L. Mark Welch**[4,5], **Kathryn M. Kauffman**[6], **Jeffrey S. McLean**[7], **Xuesong He**[4,8,✉]

[1]Oregon Health & Science University, Portland, OR, USA.

[2]J. Craig Venter Institute, La Jolla, CA, USA.

[3]UC San Diego School of Medicine, La Jolla, CA, USA.

[4]The Forsyth Institute, Cambridge, MA, USA.

[5]Marine Biological Laboratory, Woods Hole, MA, USA.

[6]University at Buffalo, Buffalo, NY, USA.

[7]University of Washington, Seattle, WA, USA.

[8]Harvard School of Dental Medicine, Boston, MA, USA.

Abstract

The human oral microbiota is highly diverse and has a complex ecology, comprising bacteria, microeukaryotes, archaea and viruses. These communities have elaborate and highly structured biogeography that shapes metabolic exchange on a local scale and results from the diverse microenvironments present in the oral cavity. The oral microbiota also interfaces with the immune system of the human host and has an important role in not only the health of the oral cavity but also systemic health. In this Review, we highlight recent advances including novel insights into the biogeography of several oral niches at the species level, as well as the ecological role of candidate phyla radiation bacteria and non-bacterial members of the oral microbiome. In addition, we summarize the relationship between the oral microbiota and the pathology of oral diseases and systemic diseases. Together, these advances move the field towards a more holistic understanding of the oral microbiota and its role in health, which in turn opens the door to the study of novel preventive and therapeutic strategies.

✉ xhe@forsyth.org .

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41579-023-00963-6.

Related links

Cenote Human Virome Database: https://zenodo.org/record/4498884
Human Oral Microbiome Database: https://homd.org/
Human Oral Virome Database: https://github.com/RChGO/OVD
IMG/VR Viral Resources: https://img.jgi.doe.gov/cgi-bin/vr/main.cgi

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Introduction

> "I didn't clean my teeth for three days and then took the material that had lodged in small amounts on the gums above my front teeth… I found a few living animalcules" from Antonie van Leeuwenhoek's letter to the Royal Society on observations made from his own dental plaque, translated by Clifford Dobell)[1].

The direct visual observation of bacteria in the oral cavity by Antonie van Leeuwenhoek in 1670 using his self-designed microscope marked the discovery of the oral microbiota. The diverse morphologies of the microorganisms he observed, and later depicted in his notebook, were an early indication of the complexity of the oral microbial community. Subsequent study of the human oral microbiome has revealed that the microorganisms residing in the oral cavity are a major contributor to overall host health and that dysbiosis in the oral microbiome is frequently involved in the pathogenesis of both oral and systemic diseases. The oral microbiome is acquired through both maternal transmission and the environment, in an organized pattern, with the eruption of teeth providing new ecological niches and increasing diversity[2,3]. The acquisition and establishment of the oral microbiome have been recently reviewed in depth[4].

The accessibility of the oral microbiota enables the process of biofilm and community assembly to be directly captured at the sites of interest[5], and hence the oral microbiota offers a powerful model system for exploring and understanding complex microbiomes. Distinct habitats within the mouth are colonized by microbiotas that are widely different in both composition and spatial organization (Fig. 1a–e). Within the oral cavity, bacteria, archaea, eukaryotes and viruses coexist and interact with each other and with the human host (Fig. 1f). By contrast, at less accessible sites such as the human gut, microbiome structure and assembly must typically be inferred, for example, through faecal samples. Therefore, there is enormous potential in using omics approaches to examine the diverse communities of the oral microbiota both in situ and using in vitro model systems to elucidate principles of microbial community assembly and ecology. Recently developed technologies including culture-independent metagenomic sequencing, single-cell sequencing, fluorescence in situ hybridization-based microscopy (FISH-based microscopy), metatranscriptomics, metaproteomics and metabolomics have revolutionized the scale and level of resolution of oral microbiome research. These technologies have synergized with cultivation-based research, which has continued to provide the foundational model systems, whereby hypotheses generated by the new technologies can ultimately be tested and explored (Box 1). The resulting research has substantially increased our understanding of the community composition, genomic diversity, biogeography and metabolic underpinnings of the oral microbiota[6–9].

In this Review, we highlight recent findings that have illustrated that the oral microbiota is diverse, structured and can influence host pathophysiology on a systemic scale. Metagenomic sequencing and other technologies have continued to confirm that in addition to canonical bacteria, the oral microbiota is home to a highly diverse community of ultrasmall candidate phyla radiation bacteria (CPR bacteria), as well as fungi, amoebae, flagellates, archaea and viruses (Fig. 1f). The oral microbiota is also highly structured, with

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

combinatorial labelling and spectral imaging fluorescence in situ hybridization (CLASI-FISH) microscopy now able to illustrate microbial biogeography at the micrometre scale and species level. Finally, in addition to oral diseases such as dental caries, periodontal disease and oral cancer (which are also briefly reviewed), the oral microbiome is increasingly recognized to have a major role in the development of many systemic diseases. It is not possible to include all the important recent advances in our understanding of the oral microbiota and its role in health within the scope of this Review. Therefore, many topics are covered in broad terms, and readers are directed to comprehensive reviews that address specific topics in more depth. The topics that are covered in more detail represent several of the most recent and impactful breakthroughs.

Diversity of the oral microbiome

Most oral microbiome research, particularly early studies, used 16S rRNA gene amplicon sequencing and therefore focused on bacteria exclusively. Oral microbiome studies have shown that there is a diverse set of more than 700 bacterial species[10] (Human Oral Microbiome Database) drawn largely from a few dozen genera across seven phyla: Actinomycetota (formerly, Actinobacteria[11]), Bacteroidota (Bacteroidetes), Bacillota (Firmicutes), Fusobacteriota (Fusobacteria), Pseudomonadota (Proteobacteria), Saccharibacteria (TM7) and Spirochaetota (Spirochaetes). The abundant bacterial species that make up the bulk of the oral microbiome are generally conserved across individuals. However, differences in the relative abundances of the taxa, as well as strain-level differences and the presence of rare strains and species, account for a large fraction of the gene-level diversity observed across individuals and can also be used to distinguish individuals[12]. The declining cost of sequencing and increased computing power, combined with the development of new bioinformatics tools, has led to increased use of metagenomic and metatranscriptomic sequencing to study the oral microbiome. Crucially, metagenomics enables the detection of organisms that lack 16S rRNA genes, thus substantially expanding the known oral microbiome beyond bacteria. The oral microbiome is now known to harbour an abundance of viruses, as well as less common, but impactful, taxa such as fungi, protozoa and archaea (Fig. 1f). The following sections provide an overview of several of these groups of organisms that have historically been less well studied, but are receiving increased attention as their prevalence becomes more clear.

Candidate phyla radiation bacteria

CPR bacteria are a large monophyletic group[13] that is now thought to account for more than 25% of global bacterial diversity[14–16]. Of the more than 70 phyla identified within CPR, Saccharibacteria (formerly TM7), '*Candidatus* Absconditabacteria' (SR1) and '*Candidatus* Gracilibacteria' (GN02) are routinely detected in the human oral microbiome[17,18]. Saccharibacteria are of particular interest owing to their higher prevalence and abundance in the mouth, as well as their widely documented association with mucosal diseases[19–21]. The first isolation and cultivation of a CPR bacterium, the oral Saccharibacteria species *Nanosynbacter lyticus* strain TM7x, demonstrated that it had an ultrasmall cell size (200–300 nm), a reduced genome with limited de novo biosynthetic capabilities[22] and an epiparasitic lifestyle dependent on a physically associated bacterial host (in this case

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Schaalia odontolytica). These features are thought to be representative of all CPR bacteria and explain the difficulty in isolating CPR taxa in pure culture[21–26].

There are at least six major clades of Saccharibacteria in the oral cavity, known as clades G1–G6 (candidate names proposed in ref. 21). All six Saccharibacteria species-level taxa that have thus far been cultivated are from the G1 clade[21–26]. The current hypothesis is that mammal-associated G1 Saccharibacteria were ancestrally environmental, perhaps most recently acquired by mammal microbiotas from groundwater sources[17,21], an event that was likely to be accompanied by the acquisition of new functions that facilitate their adaptation from environmental to mammalian niches. Recent advances in long-read sequencing technologies have enabled reconstruction of complete genomes from metagenomic saliva samples (that is, without isolation or cultivation) and have produced the first complete genomes from Saccharibacteria clade G6 (aka '*Candidatus* Nanogingivalaceae' and HMT-870)[27]. Analysis of these genomes illustrated that the minimal pathways encoded by these epibionts are substantially different from those encoded by the clade G1 Saccharibacteria, which suggests that the non-G1 clades may have diverse hosts and host dependencies as well as diverse ecological and/or pathogenic roles[27].

Although multiple studies have associated Saccharibacteria abundance with disease, their basic physiology and mechanisms of pathogenesis remain largely unknown. Two recent studies[25,26], which have begun to shed light on these topics, are described in detail in Box 2. Association of TM7x with its *S. odontolytica* bacterial host increased survival of the host during acid stress, presumably through the arginine deiminase system encoded by TM7x[26]. This finding illustrated that although parasites routinely negatively affect the growth of their hosts, co-evolution selects for host–epibiont pairs that offer advantages to both taxa[28]. Another study demonstrated that the presence of Saccharibacteria actually decreased bone loss caused by Actinobacteria in a mouse model of periodontal disease, in contrast to earlier hypotheses suggesting that Saccharibacteria were overt periodontal pathogens[25]. Together, these studies demonstrated that the distinct, dynamic interactions between CPR and their bacterial hosts have considerable impact on the physiological, ecological and pathogenic roles of their hosts in the oral microbiota and human disease and that these processes remain poorly understood[25].

Microeukaryotes and archaea

In addition to bacteria, the oral microbiota also encompasses microeukaryotes (fungi, amoebas and flagellates), archaea and viruses (discussed subsequently). Although amplicon-based microbiome studies have identified more than 100 genera of fungi in the mouth[29–31], far fewer are detected on a routine basis[31]. The oral mycobiomes of individuals are generally dominated by either *Candida*[32–35] or *Malassezia*[31,35,36] species. As *Candida* typically consume sugars and *Malassezia* typically consume lipids, the genera are likely to have quite different ecological roles[35]. There is a rich history of the study of bacterial–fungal interactions in the oral microbiome (reviewed elsewhere[5,37]). More recent work is adding further depth to the understanding of the molecular mechanisms behind these relationships and their impacts on the human host. For example, *Streptococcus gordonii* facilitates the survival and escape of *Candida albicans* from macrophage phagosomes[38], whereas

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Pseudomonas aeruginosa inhibits the same processes. Furthermore, streptococci and *Candida* species have synergistic carbohydrate metabolisms and exhibit cross-feeding[39,40]. *C. albicans* has been associated with caries in microbiome studies[41] and is known to physically and metabolically interact with *Streptococcus mutans*, reinforcing the biofilm and acid-based virulence of these multispecies biofilms[42,43]. A recent study demonstrated that aggregates of *C. albicans* and *S. mutans*, isolated from toddlers with severe tooth decay, had emergent properties that were not characteristic of either taxon alone, such as an enhanced ability to colonize surfaces[44].

Less well studied than the oral fungi are archaea, amoeba (*Entamoeba gingivalis*) and amitochondriate flagellates (*Trichomonas tenax*[45]). All three of these groups live primarily in periodontal pockets and are associated with periodontal disease[46–50]. *E. gingivalis* feeds on live human cells, which suggests a distinct pathological role for this organism[49,50]. Both oral amoebas and flagellates seem to have strain-level differences that account for differences in pathological potential[51,52]. The role of Archaea in the oral microbiota, and possibly human disease, has been recently reviewed[47,53]. *Methanobrevibacter oralis* seems to be the most common and abundant archaeal taxon found in the oral microbiota[54,55]. Methanogenic archaea can facilitate the growth of fermentative bacteria by consuming hydrogen[56], and predicted potential syntrophic partners of oral archaea include the genera *Synergistes*, *Prevotella* and *Veillonella*[47,56]. Association studies show increases in archaeal abundance with obesity and smoking[57]. Although archaea and microeukaryotes are relatively low in abundance in the oral microbiome compared with bacteria, they are likely to have a disproportionately important ecological and pathogenic role owing to their larger size and distinct metabolic capabilities. It will be necessary to better integrate the study of these taxa into oral microbiome research to obtain a more complete picture of their ecology and the relationship to human disease.

Viruses

Most viruses in the oral microbiome are phages that infect bacteria. Although an early, influential study concluded that phages may not be as important in the oral microbiome as they are in other systems[58], this finding is being reevaluated as recent work illustrating the diversity and abundance of phages in the oral microbiome strongly suggests that phages are exerting substantial selective pressure in the mouth. A major driving force behind this paradigm shift is greatly improved sequence databases, sequencing capacity and phage-detecting bioinformatics tools, which have thus far led to the identification of more than 60,000 species-level groups of phages in the oral microbiome[59]. In addition to the identification of phage genomes, their influence can also be observed in the microbiome by the presence of phage-targeting CRISPR spacers in the genomes of oral bacteria[60,61]. Although the percent of oral CRISPR spacers identifiable as targeting phages has thus far been low[62], the increasing number of representative oral phage genomes within databases (for example, IMG/VRv4 (ref. 59), the Cenote Human Virome Database[63], the Oral Virome Database[64] and the anticipated expansion of the Human Oral Microbiome Database to include phages) is expected to likewise promote the identification of increasing numbers of spacer sequences as phage-derived. A recent study of *Porphyromonas gingivalis*[65], for example, has shown that strains of this species commonly harbour phages that

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

have integrated into their genomes (as prophages) and that some strains of *P. gingivalis* encode CRISPR spacer sequences that would be expected to protect against infection by these phages, suggesting that phages have a role in intraspecies antagonism in the oral microbiome. The few studies of oral phages that have been done also indicate that they have potential to impact overall community assembly[66] and interactions with the human host[67]. There are two noteworthy obstacles that have continued to limit cultivated model phage–bacteria systems to a handful of oral bacterial species[68]. First, phage interactions are sensitive to culture conditions and many have a narrow host tropism[69] (often as a result of the action of defence systems in bacteria[70,71]), requiring studies to include large panels of culture conditions and strains from each species of interest or derive the bacterial strains used as baits from the same samples to be tested for phage[72]. Second, some phages have life cycles that limit their detection with traditional plaque and turbidity (that is, bacteria-killing) assays, necessitating other detection methods[73]. Overcoming these hurdles, to establish and investigate more broadly representative oral phage-bacteria model systems, will be key to accelerating development of phage-based bioengineering tools, clinical practices and therapeutics aiming to shape the oral microbiota to prevent or reverse dysbiosis and resulting diseases.

Beyond phages, recent discoveries in vaginal, gut and skin microbiomes of viruses infecting archaea[74], *Entamoeba*[75], *Trichomonas*[76,77] and *Malassezia*[78] suggest that similar viruses will be identified for oral species of these groups, and indeed the recently described *Redondoviridae*[79] have now been identified as infecting *Entamoeba gingivalis* in the mouth[80]. Viruses infecting humans are also present in the saliva (recently reviewed elsewhere[81,82]). Greater than 90% of the human population is chronically infected with viruses in the *Anelloviridae* and the *Herpesviridae*[83] families and both groups are commonly detected in the saliva[82,84], with *Herpesviridae*[85] potentially contributing to periodontal disease[81]. Chronic viral infections are likely to affect the global state of the human immune system[83], highlighting the value of holistic approaches to understanding the role of the oral microbiome in health, inflammation and disease. Altogether, continued investment in ecologically representative model systems, reference sequence databases and cross-sectional and longitudinal studies (such as the foundational oral virome studies reviewed elsewhere[86]), which are inclusive of the CPR bacteria and non-bacterial members of the oral microbiota, will be important to achieving a comprehensive understanding of these microbial communities, and how they might be leveraged as tools and therapeutics to maintain health and prevent disease.

Biogeography of the oral microbiome

The oral cavity contains many distinct microenvironments that support different microbial communities (Fig. 1a). These include the hard surface of the tooth enamel (both above and below the gumline), the keratinized surfaces of the palate, gingiva and tongue papillae and the soft surfaces such as the buccal mucosa. Site specialization by oral bacteria has been observed for decades, but has been analysed more comprehensively and precisely in recent years using cultivation-independent methods and genome-scale information[87]. Interestingly, genera such as *Fusobacterium* and *Veillonella*, and families such as the *Prevotellaceae*, contain separate, distinct species that are specialized for the tongue, dental plaque or gums,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

which suggests that the oral microbial community has evolved to occupy these distinct oral habitats[88]. Factors influencing the microbial community composition at distinct sites include the surface characteristics of the substrate, gradients of oxygen and nutrients and proximity to salivary glands[89,90]. Because microorganisms from all oral sites are shed into saliva, and saliva is distributed throughout the mouth, most oral microorganisms are detectable at any oral site, but are detected at up to several orders of magnitude higher relative abundance at the site or sites thought to be their true niche[87,88]. The microbiotas of dental plaque, the tongue dorsum and the keratinized gingiva are the most distinctive from one another[8,88].

Some of the most compelling recent advances in oral microbiome research have revealed the spatial organization (biogeography) of oral biofilms. Many of the interactions between bacterial cells, including adhesion, syntrophy and secretion of molecules that manipulate or destroy adjacent cells, occur when cells are touching or are only a few micrometres apart[91]. Therefore, the spatial organization of oral biofilms determines which bacteria are located in close enough proximity to influence each other's biology and determines emergent biofilm properties[92]. Recent developments in microscopy and imaging enabled the visualization and analysis of the spatial organization of microorganisms in complex oral biofilms[93–96]. The development of CLASI-FISH imaging enabled the simultaneous identification of many different taxa within biofilm samples and demonstrated that bacteria in the mouth build complex, spatially organized communities in which taxonomically and metabolically disparate bacteria are directly adjacent to one another (Fig. 1b–d). The positions of the various members relative to one another or within the community overall provided clues as to the dynamics and interaction of the community members[96–98]. Studies using CLASI-FISH to examine the plaque biofilm suggested that *Corynebacterium matruchotii* forms a physical bridge between the base of the biofilm and its outer layers[93]. Some species have even more precise localization patterns, such as *Streptococcus* spp., which localize to the 'corncob' assemblage at the tips of *Corynebacterium* filaments[98] (Fig. 1b). This finding challenged a classic model of oral biofilm development, in which *Fusobacterium nucleatum* had a key structural role in bridging between early and late colonizers[99]. However, recent work has suggested that the cross-feeding and trophic interactions between *F. nucleatum* and early-colonizing commensals can influence biofilm development and dispersion of later colonizing pathogens, such as *P. gingivalis*[100].

More recently, probes for CLASI-FISH have been developed to identify oral bacteria at the species level, which is important given the taxonomic and pathogenic diversity within highly abundant oral genera such as *Streptococcus* and *Prevotella*. For example, in the context of dental caries, some streptococci, such as *S. mutans*, are regarded as pathogens, whereas others, such as *S. gordonii*, are regarded as health-promoting commensals[101]. A recent study analysed the distribution of species of the abundant oral genus *Streptococcus* using short-read metagenomic sequence data from the human mouth and found that the closely related *Streptococcus mitis*, *Streptococcus oralis* and *Streptococcus infantis* primarily occupy different regions of the mouth: *S. mitis* is found in high abundance on the buccal mucosa, *S. oralis* in dental plaque and *S. infantis* on the tongue dorsum[101] (Fig. 1e).

In recent work using fluorescence microscopy to examine multispecies biofilms, *S. mutans* was found either dispersed throughout the biofilms or densely packed within an extracellular

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

scaffold in what was termed a ‘rotund’ architecture. When in the rotund architecture, *S. mutans* generated a lower-pH environment and caused greatly increased demineralization of the underlying enamel surface, thus linking spatial organization with localized onset of caries[96]. Other recent research examining biogeography has shown that in addition to single cells, bacterial aggregates in saliva bind to teeth as large units that already include late colonizers and that these larger aggregates are at a growth advantage compared with single cells[102]. These results suggest an alternative biofilm development process, which helps explain in vivo findings in which late colonizers were detected as soon as 30 min after tooth brushing[102]. Therapeutic modulation of oral biofilms will depend on an accurate understanding of how bacterial cells are recruited to the biofilm (singly or in clusters) and which taxon–taxon interactions are required for the survival of key taxa. Using biogeographical data to inform and refine studies examining the metabolic underpinnings of oral microbial ecology will be crucial in obtaining a deep understanding of how the oral microbiota affects human health.

The oral microbiota and oral disease

The oral microbiota has a major role in oral health, as three of the most prevalent oral diseases, dental caries, periodontal disease and oral cancer, all have mainly microbial aetiologies.

Dental caries

Caries is associated with a dysbiosis of the dental plaque microbiota; specifically, there is an abundance of biofilm-forming, acid-producing and acid-tolerant species. Because *S. mutans* embodies all three of these traits, was frequently isolated from lesions and able to cause robust disease in animal models, it was historically considered a primary aetiologic agent of dental caries[103]. The development of the cultivation-independent microbiome analysis established that in an appreciable number of cases, caries occurs without substantial, or occasionally even detectable, levels of *S. mutans*. Therefore, the specific importance of *S. mutans* has been called into question[104], and caries is understood to be the result of more complex changes in ecology, rather than just infection by a single species. Although *S. mutans* is not required for caries pathogenesis, the unusual ability of *S. mutans* to generate extracellular glucans from sucrose means that when *S. mutans* is present, it is typically an important driver of biofilm formation and dysbiosis. This hypothesis was supported by a recent study of the caries-associated microbiome using deep metagenomics, showing that *S. mutans* was only present in a minority of subjects, but when it was present, it was very strongly correlated with caries[105]. Other acidophilic organisms such as lactobacilli and *Veillonella* species have been known to be associated with caries for decades[106]; however, it has been debated whether these represent true drivers of pathogenesis or simply bystanders in the climax community resulting from an increasingly acidic biofilm. As mentioned earlier, *C. albicans* can also have a role in caries pathogenesis. In addition to these more canonical caries-associated microorganisms, recent metagenomics studies have also associated other microorganisms, such as Epstein–Barr virus (formerly called human gammaherpesvirus 4) and *Prevotella* spp., with caries[105,107]. However, further research is needed to solidify these links and underlying mechanisms. An important recent discovery

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

was the association of nitrate-reducing bacteria, namely, taxa within the genera *Rothia, Neisseria* and *Haemophilus*, with good dental health[105,108,109]. This finding has led to the recent investigation of nitrate as an anticaries prebiotic and nitrate-reducing bacteria as anti-caries probiotics[109–111], similar to how arginine and arginine deiminase-encoding bacteria have been explored as a prebiotic and probiotics, respectively[112]. As dental caries remains the most common chronic infectious disease globally, continued advances in the understanding of its ecological pathogenesis may lead to new preventive modalities that synergize well with oral hygiene and fluoride treatments, which are currently the primary clinical defence[103,113].

Periodontal disease

Periodontal disease is an inflammatory disruption in the host–microbial homeostasis of the periodontal pocket. The tissues supporting the tooth are highly vascularized with a constant positive flow of gingival crevicular fluid recruiting neutrophils and other immune cell types to help maintain this balance between the constantly growing subgingival microorganisms and the innate and adaptive responses of the host. In most humans, this non-passive relationship resulting in healthy homeostasis is defined as an active inflammatory surveillance state[114]. Disruptions of this homeostasis triggered by changes in the microbiome or host lead to inflammation and ultimately gingivitis and periodontitis. Although gingivitis is an antecedent reversible disease state, which may lead to periodontitis if unchecked, periodontitis is defined by irreversible bone resorption and is classified according to stages and grades of severity[115]. There are many recent comprehensive reviews covering the disease aetiology, microbial and host biomarkers of active and progressing sites as well as what has been found across mechanistic studies in animal models of periodontitis[116,117]. The widespread use of single-cell RNAseq approaches has also now enabled the characterization of the differences between healthy subjects and those with periodontitis, which has led to the identification of specific host cell populations with inflammatory profiles that enhanced neutrophil and leukocyte recruitment and promoted antimicrobial defences[118]. Overall, since the first early descriptions by culture-based methods, microbial communities found in periodontitis sites have continued to be characterized with higher resolution, predominantly from cross-sectional studies comparing health and disease, studies comparing diseased sites pre-surgical and post-surgical and non-surgical treatments[119] as well as studies on progressing and non-progressing periodontitis sites. This has revealed the prevalence, abundances and, in some cases, the active processes in the microorganisms[120] that may influence ongoing inflammatory responses in chronic periodontal disease. More recent analyses have discovered that in addition to canonical bacterial taxa associated with the disease (that is, *Porphyromonas*, *Treponema* and *Tannerella* species), *Filifactor alocis, Peptoanaerobacter stomatis* and Saccharibacteria are potential periodontal pathogens[121]. Recent microbiome studies of periodontal disease have also identified novel species–species correlations[122] and functional interactions[123], as well as new insights into the periodontitis-associated virome[123,124], which leads to further questions regarding their roles in preventing resolution of inflammation or perpetuating inflammatory conditions.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The accessibility of the oral microbiota makes it a powerful model and experimental system. Experimental gingivitis is a clinical model used to study the dynamics of microbially induced inflammation leading to gingivitis directly in humans as plaque can grow unabated (Supplementary Box 1). A major strength of this model is that there are currently no other human models for other mucosal surfaces that enable the induction of acute inflammation with normal bacterial overgrowth of endogenous species, and then reversal of this state, especially in a manner that is readily accessible and clinically relevant. Studies using this model typically examine growth and maturation of the subgingival community during the transition from a healthy state to an inflammophilic subgingival community. A recent landmark study used the experimental gingivitis model to examine changes in the oral microbiome as well as variation in the inflammatory response of the subjects. Importantly, although all healthy subjects responded to oral plaque accumulation, the rate and severity of the inflammatory responses varied, resulting in three inflammatory responder types[125], high, low and slow, with distinct microbial and host signatures (Fig. 2). Subjects differed in their degree of inflammation, with high and low inflammatory responses observed across multiple independent experimental gingivitis studies[125–127]. Uniquely, the newly recognized slow phenotype[125] had a delayed plaque growth and maturation rate but shared similar increased mediator profiles as the high responder group during the induction phase. The slow responders displayed a signature of high abundance of *Streptococcus* species (*Streptococcus sanguinis* and *S. oralis*) that is maintained at the time of inclusion in the study (day −14) and after recovery (day 35)[125]. The existence of these three responder types suggests an important role for both microbial and human host immune phenotypes in the outcome of episodes of gingivitis. Identifying potential targets during disease initiation and development within the different human response types could translate to personalized treatment and intervention strategies.

Oral cancers

A large and growing percentage of oral cancers are associated with viral infections: ~90% of oral squamous cell carcinomas (OSCCs) in the USA are driven by infection with human papilloma virus and more than 90% of nasopharyngeal carcinomas are associated with Epstein–Barr virus[128,129]. Recent studies have also examined the microbiome associated with oral cancers, with one finding that a dysbiotic mycobiome rich in *C. albicans* was prevalent in patients with OSCC[130]. Conversely, higher abundances of *Malassezia* species were correlated with better overall survival in patients with OSCC[130,131], which suggests that the fungal genus may serve as a useful prognostic biomarker and that any mechanistic underpinnings of this association should be further explored. The interaction between the microbiome and tumours has been recently reviewed in more detail[132], and recent exploratory work has begun to examine the potential for health-associated oral microorganisms to be utilized as anticancer probiotics[133]. In addition to oral cancers, the oral microbiome has recently been associated with a number of other types of cancer as well, as discussed in the following section.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Roles in systemic health and disease

Evidence linking the oral microbiota to systemic diseases and overall health continues to accumulate (summarized in Fig. 3a–f). In particular, periodontal disease and associated pathogens, such as *P. gingivalis, Aggregatibacter actinomycetemcomitans* and *F. nucleatum*, have now been linked to a myriad of extra-oral diseases, including Alzheimer disease, diabetes, cardiovascular disease, colorectal cancers, inflammatory bowel disease, rheumatoid arthritis, nonalcoholic fatty liver disease and obesity (excellently reviewed in more depth in ref. 134). The periodontal microbiota influences the pathology of distal diseases through two main mechanisms, which can also be synergistic: direct disease-promoting effects from the translocation of oral bacteria to distal sites and a range of indirect effects caused by the presence of dysbiotic oral microbial communities in the mouth. Remarkably, the establishment of oral microorganisms in the gut may also serve as a broad sign of human disease; a recent meta-analysis of thousands of gut metagenomes representing more than 50 diseases, as well as healthy donors, revealed that many common oral taxa are biomarkers of disease in the gut[135]. Although the existence of a placental microbiota remains highly controversial[136], there is some evidence in animal models that oral infection with particular periodontal pathogens, such as *P. gingivalis* and *F. nucleatum*, is associated with adverse pregnancy outcomes[137].

The translocation of oral bacteria, particularly periodontal pathogens, to distal sites can directly exert disease-promoting effects. Colonization of the lung by oral bacteria can lead to aspiration pneumonia[138] (Fig. 3e). Oral bacterial colonization of the gastrointestinal tract can be exacerbated by abnormal secretion of gastric and bile acids owing to systemic diseases such as cirrhosis[139,140] and has been linked to inflammatory bowel diseases[140–142] and colorectal cancer[143]. The interaction of host and bacterial factors can be important in pathogenesis. For example, at the site of a colorectal tumour, *F. nucleatum* can be enriched by the gene expression profile of the cancerous cells, as these cells express Gal-Gal-NAc that is bound by the *F. nucleatum* surface protein, Fap2 (ref. 144). In turn, the presence of *F. nucleatum* drives tumour progression through multiple mechanisms. The *F. nucleatum* adhesin, FadA, upregulates expression of host annexin A1, which induces Wnt–β-catenin signalling, driving tumour proliferation[145]. The FadA adhesin forms amyloid-like protein aggregates that may have a critical role in pathogenesis[146]. *F. nucleatum* also binds to the human inhibitory receptors, TIGIT and CEACAM1, which recruits immune suppressor cells and disrupts the antitumour activity of T cells and natural killer cells[147,148] (Fig. 3c).

In addition to oral bacteria translocating further down the gastrointestinal and respiratory tracts, periodontal disease induces inflammation and the disruption of periodontal epithelial barriers, which leads to bacteraemia and systemic dissemination of oral bacteria[149,150]. Although this type of bacteraemia is typically transient, the consequences of the pro-inflammatory and immunomodulatory effects at distal sites, such as bone marrow, cardiovascular tissues, the brain and the liver, can be substantial[149,151,152]. Translocation of *P. gingivalis* to the bloodstream raised IL-6 levels and skewed mononuclear immune cell development to favour production of highly active osteoclasts, which drive bone loss disorders, such as rheumatoid arthritis[153,154]. In animal models and in vitro human endothelial cells, *P. gingivalis* gingipains degrade platelet endothelial cell adhesion molecule

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

1 and vascular endothelial cadherin, degrading barrier function and further promoting inflammation[151]. The presence of *P. gingivalis* proteins in the brain is correlated with Alzheimer disease, and *P. gingivalis* gingipains degrade tau proteins, which leads to tangles of fragmented tau proteins that promote disease, as well as increased neuronal inflammation[152,155] (Fig. 3d). In addition to passive translocation in the bloodstream, *P. gingivalis* is known to modulate the trafficking and behaviour of dendritic cells that have phagocytosed the pathogen, which leads to enhanced pro-inflammatory activity and accumulation at atheromatous plaques[156,157] (Fig. 3a). In mice, excess nitrate present during inflammation enabled oral *Veillonella parvula* to ectopically colonize the gut using nitrate respiration[158].

Beyond translocating to extra-oral sites to cause disease, periodontal pathogens can also affect distant sites through various indirect mechanisms. For example, through different mechanisms, *P. gingivalis* and *A. actinomycetemcomitans* promote citrullination of host proteins, which function as autoantigens and lead to the generation of anti-citrullinated protein antibodies, which is hypothesized to promote rheumatoid arthritis in genetically susceptible individuals[159,160] (Fig. 3f). In mice, periodontal pathobiont-specific T cells migrate to the gut, where they exacerbate colitis and lead to the development of an immunological profile reminiscent of what is observed clinically in humans with inflammatory bowel disease[142,161], which suggests that immune priming at the oral mucosa may lead to an immune response in the gut (Fig. 3b). In mice, experimental periodontitis induced bone-marrow-mediated trained innate immunity, a systemic, maladaptive pro-inflammatory state of immune responsiveness[162]. The associations of periodontal disease and oral dysbiosis with extra-oral and systemic diseases seem to be bidirectional. Systemic inflammatory diseases may also disrupt the immune barrier function of the oral mucosa, which leads to increased susceptibility to periodontal disease[150,163,164]. Thus, many of these comorbidities are synergistic and lead to positive feedback, further increasing morbidity and mortality[134]. Despite this growing body of evidence linking the oral microbiota to systemic disease, many of the links have been studied only in animal models, and intra-individual variability in humans presents a major hurdle in future research[125].

Perspectives

Over the past decade, advances in omics analyses (particularly sequencing), culture systems and microscopy have accelerated the pace of discovery in oral health and oral microbiome research. However, to realize the promise of improving human health through a better understanding of the impact of the human microbiota, the research community at large must incorporate the oral microbiome fully into its human microbiome research programmes. Large-scale longitudinal studies of the human microbiome must include oral as well as gut samples. Sampling protocols for the oral microbiota should recognize that although a saliva sample provides a snapshot of primarily the tongue microbiota, it is unlikely to be adequate for assessing habitats that are sites where oral bacteria and products may enter the bloodstream such as subgingival dental plaque. Furthermore, in vitro model systems must be developed that more accurately resemble the oral in vivo communities in both taxonomic composition and behaviour, and incorporate interaction of the microbiota with host tissue. More time-course studies are needed to address several key outstanding

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

questions, including (1) the growth and turnover rates of microbial cells in situ; (2) the fraction of microorganisms lost to phage or other predation; and (3) the rate of dispersion of microbial cells to the epithelium, to other sites in the mouth and to extra-oral habitats. It will be essential to conduct this future research in a manner that is inclusive of the non-canonical members of the oral microbiota and elucidates their complex ecological, structural, physiological and metabolic interspecies roles. Although CPR bacteria, archaea, microeukaryotes and viruses are less dominant in terms of biomass, they are proving to have ecologically and functionally important roles in human health. Altogether, these approaches will enable the development of novel therapeutic and preventive strategies, such as prebiotics and probiotics, that combat both oral diseases such as dental caries and periodontitis and also systemic diseases such as inflammatory bowel disease, cancer, Alzheimer disease and cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors were supported by NIH NIDCR K99-DE029228 (J.L.B.); NIH NIDCR R01DE022586 (J.L.M.W.); NIH NIDCR R03DE030987 and R01DE016937 (K.M.K.); NIH NIDCR R01DE031470 (J.S.M.); NIH NIDCR R01DE023810 (J.S.M. and X.H.) and NIH NIDCR R01DE029479 and R01DE030943 (X.H.). The authors acknowledge the many authors whose work in this domain was not included owing to text and citation constraints and thank F. Scannapieco, F. Dewhirst, C. Matrishin, E. Haase, W. Mei, H. Koo, B. Bor, G. Lewin, M. Freire, M. Kintzing and J. Tian for feedback and ideas for Fig. 1 and the illustration in Box 2.

Glossary

16S rRNA gene amplicon sequencing
A microbiome sequencing technique whereby the variable region or regions of 16S rRNA genes are amplified by PCR using primers specific to the flanking conserved regions; the amplicons are then sequenced, providing information about the presence of and qualitative information about the relative abundances of the various taxa within the sample.

Candidate phyla radiation bacteria
(CPR bacteria). A large, monophyletic group of bacteria that have reduced genomes and ultrasmall cell size and are thought to have an epiparasitic lifestyle dependent on bacterial host organism or organisms.

Combinatorial labelling and spectral imaging fluorescence in situ hybridization
(CLASI-FISH). A microscopy technique whereby each taxon of interest is labelled with multiple fluorophores to greatly expand the number of distinguishable targets. Microscopes capable of spectral imaging allow the use of fluorophores with overlapping emission spectra.

Dysbiosis
A disruption (that is, a change in taxonomic abundance, metabolism and or ecology) in the normal, health-associated microbiota that results in an ecological imbalance, frequently contributing to or resulting in a pathological state.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Fluorescence in situ hybridization-based microscopy
(FISH-based microscopy). Microscopy that uses fluorescently labelled DNA oligonucleotides complementary to specific DNA or RNA sequences as probes in FISH. Hybridization of probe to target enables cells or structures containing the sequence of interest to be observed directly using a fluorescence microscope.

Gingipains
A family of proteases secreted by the pathogen, *Porphyromonas gingivalis*, which can degrade cytokines and alter the host inflammatory response.

Gingival crevicular fluid
(GCF). A serum-like fluid that flows into the gingival sulcus (the gap between gums and teeth) from the blood vessels within the gingival connective tissue.

Metagenomic sequencing
A microbiome sequencing technique whereby an arbitrary subset of the DNA extracted from the sample is sequenced (as opposed to the sequencing of a targeted region by PCR, as in 16S rRNA gene amplicon sequencing), providing genomic information and taxonomic resolution that is not possible with amplicon sequencing.

Pathobiont
Opportunistic microorganism that emerges as a result of perturbations in the healthy microbiome.

Prebiotic
Compounds that foster growth or activity of microorganisms that are generally beneficial to the human host.

Probiotics
Live microorganisms that are intended to have health benefits when administered or consumed.

Syntrophy
A phenomenon (also known as cross-feeding) whereby one species is living off the metabolic products of another species.

References

1. Dobell C Antony van Leeuwenhoek and His ‘Little Animals’: Being Some Account of the Father of Protozoology and Bacteriology and His Multifarious Discoveries in these Disciplines (Harcourt, Brace and Company, 1932).
2. Mukherjee C et al. Acquisition of oral microbiota is driven by environment, not host genetics. Microbiome 9, 54 (2021). [PubMed: 33622378]
3. Sulyanto RM, Thompson ZA, Beall CJ, Leys EJ & Griffen AL The predominant oral microbiota is acquired early in an organized pattern. Sci. Rep 9, 10550 (2019). [PubMed: 31332213]
4. Kaan AMM, Kahharova D & Zaura E Acquisition and establishment of the oral microbiota. Periodontol 2000 86, 123–141 (2021).
5. Baker JL, Bor B, Agnello M, Shi W & He X Ecology of the oral microbiome: beyond bacteria. Trends Microbiol. 25, 362–374 (2017). [PubMed: 28089325]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

6. Zaura E, Keijser BJF, Huse SM & Crielaard W Defining the healthy ‘core microbiome’ of oral microbial communities. BMC Microbiol. 9, 259 (2009). [PubMed: 20003481]
7. Griffen AL et al. Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. ISME J. 6, 1176–1185 (2012). [PubMed: 22170420]
8. Segata N et al. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. Genome Biol. 13, R42 (2012). [PubMed: 22698087]
9. Lloyd-Price J et al. Strains, functions and dynamics in the expanded Human Microbiome Project. Nature 550, 61–66 (2017). [PubMed: 28953883]
10. Escapa IF et al. New insights into human nostril microbiome from the expanded human oral microbiome database (eHOMD): a resource for the microbiome of the human aerodigestive tract. mSystems 10.1128/msystems.00187-18 (2018).
11. Oren A & Garrity GM Valid publication of the names of forty-two phyla of prokaryotes. Int. J. Syst. Evol. Microbiol 71, 005056 (2021).
12. Tierney BT et al. The landscape of genetic content in the gut and oral human microbiome. Cell Host Microbe 26, 283–295.e8 (2019). [PubMed: 31415755]
13. Castelle CJ & Banfield JF Major new microbial groups expand diversity and alter our understanding of the tree of life. Cell 172, 1181–1197 (2018). [PubMed: 29522741]
14. Brown CT et al. Unusual biology across a group comprising more than 15% of domain bacteria. Nature 523, 208–211 (2015). [PubMed: 26083755]
15. Hug LA et al. A new view of the tree of life. Nat. Microbiol 1, 16048 (2016). [PubMed: 27572647]
16. Parks DH et al. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat. Biotechnol 36, 996–1004 (2018). [PubMed: 30148503]
17. Shaiber A et al. Functional and genetic markers of niche partitioning among enigmatic members of the human oral microbiome. Genome Biol. 21, 292 (2020). [PubMed: 33323122]
18. Jaffe AL et al. Patterns of gene content and co-occurrence constrain the evolutionary path toward animal association in candidate phyla radiation bacteria. mBio 12, e0052121 (2021). [PubMed: 34253055]
19. Hugenholtz P, Tyson GW, Webb RI, Wagner AM & Blackall LL Investigation of candidate division TM7, a recently recognized major lineage of the domain Bacteria with no known pure-culture representatives. Appl. Environ. Microbiol 67, 411–419 (2001). [PubMed: 11133473]
20. Abusleme L et al. The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. ISME J. 7, 1016–1025 (2013). [PubMed: 23303375]
21. McLean JS et al. Acquisition and adaptation of ultra-small parasitic reduced genome bacteria to mammalian hosts. Cell Rep. 32, 107939 (2020). [PubMed: 32698001]
22. He X et al. Cultivation of a human-associated TM7 phylotype reveals a reduced genome and epibiotic parasitic lifestyle. Proc. Natl Acad. Sci. USA 112, 244–249 (2015). [PubMed: 25535390]
23. Bor B et al. Rapid evolution of decreased host susceptibility drives a stable relationship between ultrasmall parasite TM7x and its bacterial host. Proc. Natl Acad. Sci. USA 115, 12277–12282 (2018). [PubMed: 30442671]
24. Bor B, Bedree JK, Shi W, McLean JS & He X Saccharibacteria (TM7) in the human oral microbiome. J. Dent. Res 98, 500–509 (2019). [PubMed: 30894042]
25. Chipashvili O et al. Episymbiotic Saccharibacteria suppresses gingival inflammation and bone loss in mice through host bacterial modulation. Cell Host Microbe 29, 1649–1662.e7 (2021). [PubMed: 34637779]
26. Tian J et al. Acquisition of the arginine deiminase system benefits epiparasitic Saccharibacteria and their host bacteria in a mammalian niche environment. Proc. Natl Acad. Sci. USA 119, e2114909119 (2022). [PubMed: 34992141]
27. Baker JL Complete genomes of clade G6 suggest a divergent ecological niche and lifestyle. mSphere 6, e0053021 (2021). [PubMed: 34378983]
28. Dunn DW et al. A role for parasites in stabilising the fig-pollinator mutualism. PLoS Biol. 6, e59 (2008). [PubMed: 18336072]
29. Ghannoum MA et al. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog. 6, e1000713 (2010). [PubMed: 20072605]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

30. Diaz PI, Hong B-Y, Dupuy AK & Strausbaugh LD Mining the oral mycobiome: methods, components, and meaning. Virulence 8, 313–323 (2017). [PubMed: 27791473]
31. Diaz PI & Dongari-Bagtzoglou A Critically appraising the significance of the oral mycobiome. J. Dent. Res 100, 133–140 (2021). [PubMed: 32924741]
32. Gabaldón T et al. Comparative genomics of emerging pathogens in the *Candida glabrata* clade. BMC Genomics 14, 623 (2013). [PubMed: 24034898]
33. Turner SA & Butler G The *Candida* pathogenic species complex. Cold Spring Harb. Perspect. Med 4, a019778 (2014). [PubMed: 25183855]
34. Ahmad KM et al. Genome structure and dynamics of the yeast pathogen *Candida glabrata*. FEMS Yeast Res. 14, 529–535 (2014). [PubMed: 24528571]
35. Hong BY et al. The salivary mycobiome contains 2 ecologically distinct mycotypes. J. Dent. Res 99, 730–738 (2020). [PubMed: 32315566]
36. Dupuy AK et al. Redefining the human oral mycobiome with improved practices in amplicon-based taxonomy: discovery of *Malassezia* as a prominent commensal. PLoS ONE 9, e90899 (2014). [PubMed: 24614173]
37. Montelongo-Jauregui D & Lopez-Ribot JL *Candida* interactions with the oral bacterial microbiota. J. Fungi 4, 122 (2018).
38. Salvatori O et al. Bacteria modify *Candida albicans* hypha formation, microcolony properties, and survival within macrophages. mSphere 5, e00689–20 (2020). [PubMed: 32759336]
39. Ellepola K et al. Multi-omics analyses reveal synergistic carbohydrate metabolism in *Streptococcus mutans–Candida albicans* mixed-species biofilms. Infect. Immun 87, e00339–19 (2019). [PubMed: 31383746]
40. Fultz R, Ticer T, Glover J, Stripe L & Engevik MA Select Streptococci can degrade *Candida* mannan to facilitate growth. Appl. Environ. Microbiol 88, e0223721 (2022). [PubMed: 34936835]
41. Xiao J et al. Association between oral *Candida* and bacteriome in children with severe ECC. J. Dent. Res 97, 1468–1476 (2018). [PubMed: 30049240]
42. Wan SX et al. Cross-kingdom cell-to-cell interactions in cariogenic biofilm initiation. J. Dent. Res 100, 74–81 (2021). [PubMed: 32853527]
43. Kim H-E et al. Synergism of *Streptococcus mutans* and *Candida albicans* reinforces biofilm maturation and acidogenicity in saliva: an in vitro study. Front. Cell. Infect. Microbiol 10, 623980 (2020). [PubMed: 33680985]
44. Ren Z et al. Interkingdom assemblages in human saliva display group-level surface mobility and disease-promoting emergent functions. Proc. Natl Acad. Sci. USA 119, e2209699119 (2022). [PubMed: 36191236]
45. Bisson C, Dridi SM & Machouart M Assessment of the role of *Trichomonas tenax* in the etiopathogenesis of human periodontitis: a systematic review. PLoS ONE 14, e0226266 (2019). [PubMed: 31846467]
46. Lepp PW et al. Methanogenic Archaea and human periodontal disease. Proc. Natl Acad. Sci. USA 101, 6176–6181 (2004). [PubMed: 15067114]
47. Belmok A, de Cena JA, Kyaw CM & Damé-Teixeira N The oral archaeome: a scoping review. J. Dent. Res 99, 630–643 (2020). [PubMed: 32167855]
48. Bonner M, Fresno M, Gironès N, Guillén N & Santi-Rocca J Reassessing the role of *Entamoeba gingivalis* in periodontitis. Front. Cell. Infect. Microbiol 8, 379 (2018). [PubMed: 30420943]
49. Bao X, Wiehe R, Dommisch H & Schaefer AS *Entamoeba gingivalis* causes oral inflammation and tissue destruction. J. Dent. Res 99, 561–567 (2020). [PubMed: 32023135]
50. Bao X, Weiner J III, Meckes O, Dommisch H & Schaefer AS *Entamoeba gingivalis* exerts severe pathogenic effects on the oral mucosa. J. Dent. Res 100, 771–776 (2021). [PubMed: 33792418]
51. García G et al. A new subtype of *Entamoeba gingivalis*: '*E. gingivalis* ST2, kamaktli variant'. Parasitol. Res 117, 1277–1284 (2018). [PubMed: 29429011]
52. Benabdelkader S et al. Specific clones of *Trichomonas tenax* are associated with periodontitis. PLoS ONE 14, e0213338 (2019). [PubMed: 30856220]
53. de Cena JA et al. Meta-analyses on the periodontal archaeome. Adv. Exp. Med. Biol 1373, 69–93 (2022). [PubMed: 35612793]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

54. Vianna ME, Holtgraewe S, Seyfarth I, Conrads G & Horz HP Quantitative analysis of three hydrogenotrophic microbial groups, methanogenic archaea, sulfate-reducing bacteria, and acetogenic bacteria, within plaque biofilms associated with human periodontal disease. J. Bacteriol 190, 3779–3785 (2008). [PubMed: 18326571]
55. Deng Z-L, Szafrański SP, Jarek M, Bhuju S & Wagner-Döbler I Dysbiosis in chronic periodontitis: key microbial players and interactions with the human host. Sci. Rep 7, 3703 (2017). [PubMed: 28623321]
56. Djemai K, Drancourt M & Tidjani Alou M Bacteria and methanogens in the human microbiome: a review of syntrophic interactions. Microb. Ecol 83, 536–554 (2022). [PubMed: 34169332]
57. Grine G et al. Tobacco-smoking-related prevalence of methanogens in the oral fluid microbiota. Sci. Rep 8, 9197 (2018). [PubMed: 29907776]
58. Hitch G, Pratten J & Taylor PW Isolation of bacteriophages from the oral cavity. Lett. Appl. Microbiol 39, 215–219 (2004). [PubMed: 15242464]
59. Camargo AP et al. IMG/VR v4: an expanded database of uncultivated virus genomes within a framework of extensive functional, taxonomic, and ecological metadata. Nucleic Acids Res 10.1093/nar/gkac1037 (2022).
60. Yahara K et al. Long-read metagenomics using PromethION uncovers oral bacteriophages and their interaction with host bacteria. Nat. Commun 12, 27 (2021). [PubMed: 33397904]
61. Münch PC, Franzosa EA, Stecher B, McHardy AC & Huttenhower C Identification of natural CRISPR systems and targets in the human microbiome. Cell Host Microbe 29, 94–106.e4 (2021). [PubMed: 33217332]
62. Lum AG et al. Global transcription of CRISPR loci in the human oral cavity. BMC Genom. 16, 401 (2015).
63. Tisza MJ & Buck CB A catalog of tens of thousands of viruses from human metagenomes reveals hidden associations with chronic diseases. Proc. Natl Acad. Sci. USA 118, e2023202118 (2021). [PubMed: 34083435]
64. Li S et al. A catalog of 48,425 nonredundant viruses from oral metagenomes expands the horizon of the human oral virome. iScience 25, 104418 (2022). [PubMed: 35663034]
65. Matrishin CB et al. Phages are unrecognized players in the ecology of the oral pathogen *Porphyromonas gingivalis*. Microbiome 11, 161 (2023). [PubMed: 37491415]
66. Tylenda CA, Enriquez E, Kolenbrander PE & Delisle AL Simultaneous loss of bacteriophage receptor and coaggregation mediator activities in *Actinomyces viscosus* MG-1. Infect. Immun 48, 228–233 (1985). [PubMed: 3980085]
67. Jahn MT et al. A phage protein aids bacterial symbionts in eukaryote immune evasion. Cell Host Microbe 26, 542–550.e5 (2019). [PubMed: 31561965]
68. Szafrański SP, Slots J & Stiesch M The human oral phageome. Periodontol 2000 86, 79–96 (2021). [PubMed: 33690937]
69. Kauffman KM et al. Viruses of the Nahant Collection, characterization of 251 marine Vibrionaceae viruses. Sci. Data 5, 180114 (2018). [PubMed: 29969110]
70. Bernheim A & Sorek R The pan-immune system of bacteria: antiviral defence as a community resource. Nat. Rev. Microbiol 18, 113–119 (2020). [PubMed: 31695182]
71. Hussain FA et al. Rapid evolutionary turnover of mobile genetic elements drives bacterial resistance to phages. Science 374, 488–492 (2021). [PubMed: 34672730]
72. Armau E, Bousque JL, Boue D & Tiraby G Isolation of lytic bacteriophages for *Streptococcus mutans* and *Streptococcus sobrinus*. J. Dent. Res 67 (Suppl. 1), 121 (1988).
73. Guerin E et al. Isolation and characterisation of ΦcrAss002, a crAss-like phage from the human gut that infects *Bacteroides xylanisolvens*. Microbiome 9, 89 (2021). [PubMed: 33845877]
74. Chibani CM et al. A catalogue of 1,167 genomes from the human gut archaeome. Nat. Microbiol 7, 48–61 (2022). [PubMed: 34969981]
75. Kinsella CM et al. *Entamoeba* and *Giardia* parasites implicated as hosts of CRESS viruses. Nat. Commun 11, 4620 (2020). [PubMed: 32934242]
76. Graves KJ, Ghosh AP, Kissinger PJ & Muzny CA *Trichomonas vaginalis* virus: a review of the literature. Int. J. STD AIDS 30, 496–504 (2019). [PubMed: 30626281]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

77. Rada P et al. Double-stranded RNA viruses are released from *Trichomonas vaginalis* inside small extracellular vesicles and modulate the exosomal cargo. Front. Microbiol 13, 893692 (2022). [PubMed: 35602021]

78. Park M et al. A novel virus alters gene expression and vacuolar morphology in *Malassezia* cells and induces a TLR3-mediated inflammatory immune response. mBio 11, e01521–20 (2020). [PubMed: 32873759]

79. Abbas AA et al. Redondoviridae, a family of small, circular DNA viruses of the human oro-respiratory tract associated with periodontitis and critical illness. Cell Host Microbe 25, 719–729.e4 (2019). [PubMed: 31071295]

80. Keeler EL et al. Widespread, human-associated redondoviruses infect the commensal protozoan *Entamoeba gingivalis*. Cell Host Microbe 31, 58–68.e5 (2023). [PubMed: 36459997]

81. Diaz PI Subgingival fungi, Archaea, and viruses under the omics loupe. Periodontol 2000 85, 82–89 (2021).

82. Liang G & Bushman FD The human virome: assembly, composition and host interactions. Nat. Rev. Microbiol 19, 514–527 (2021). [PubMed: 33785903]

83. Virgin HW, Wherry EJ & Ahmed R Redefining chronic viral infection. Cell 138, 30–50 (2009). [PubMed: 19596234]

84. Kaczorowska J & van der Hoek L Human anelloviruses: diverse, omnipresent and commensal members of the virome. FEMS Microbiol. Rev 44, 305–313 (2020). [PubMed: 32188999]

85. Slots J Human viruses in periodontitis. Periodontol 2000 53, 89–110 (2010). [PubMed: 20403107]

86. Edlund A, Santiago-Rodriguez TM, Boehm TK & Pride DT Bacteriophage and their potential roles in the human oral cavity. J. Oral Microbiol 7, 27423 (2015). [PubMed: 25861745]

87. Mark Welch JL, Dewhirst FE & Borisy GG Biogeography of the oral microbiome: the site-specialist hypothesis. Annu. Rev. Microbiol 73, 335–358 (2019). [PubMed: 31180804]

88. Eren AM, Borisy GG, Huse SM & Mark Welch JL Oligotyping analysis of the human oral microbiome. Proc. Natl Acad. Sci. USA 111, E2875–E2884 (2014). [PubMed: 24965363]

89. Proctor DM & Relman DA The landscape ecology and microbiota of the human nose, mouth, and throat. Cell Host Microbe 21, 421–432 (2017). [PubMed: 28407480]

90. Mark Welch JL, Ramírez-Puebla ST & Borisy GG Oral microbiome geography: micron-scale habitat and niche. Cell Host Microbe 28, 160–168 (2020). [PubMed: 32791109]

91. Dal Co A, van Vliet S, Kiviet DJ, Schlegel S & Ackermann M Short-range interactions govern the dynamics and functions of microbial communities. Nat. Ecol. Evol 4, 366–375 (2020). [PubMed: 32042125]

92. Diaz PI & Valm AM Microbial interactions in oral communities mediate emergent biofilm properties. J. Dent. Res 99, 18–25 (2020). [PubMed: 31590609]

93. 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 113, E791–E800 (2016). [PubMed: 26811460]

94. Palmer RJ Jr et al. Interbacterial adhesion networks within early oral biofilms of single human hosts. Appl. Environ. Microbiol 83, e00407–17 (2017). [PubMed: 28341674]

95. Wilbert SA, Mark Welch JL & Borisy GG Spatial ecology of the human tongue dorsum microbiome. Cell Rep. 30, 4003–4015.e3 (2020). [PubMed: 32209464]

96. Kim D et al. Spatial mapping of polymicrobial communities reveals a precise biogeography associated with human dental caries. Proc. Natl Acad. Sci. USA 117, 12375–12386 (2020). [PubMed: 32424080]

97. Perera D et al. Mechanisms underlying interactions between two abundant oral commensal bacteria. ISME J. 16, 948–957 (2022). [PubMed: 34732850]

98. Morillo-Lopez V, Sjaarda A, Islam I, Borisy GG & Mark Welch JL Corncob structures in dental plaque reveal microhabitat taxon specificity. Microbiome 10, 145 (2022). [PubMed: 36064650]

99. Kolenbrander PE et al. Bacterial interactions and successions during plaque development. Periodontol 2000 42, 47–79 (2006).

100. Sakanaka A et al. Fusobacterium nucleatum metabolically integrates commensals and pathogens in oral biofilms. mSystems 10.1128/msystems.00170-22 (2022).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

101. McLean AR, Torres-Morales J, Dewhirst FE, Borisy GG & Mark Welch JL Site-tropism of streptococci in the oral microbiome. Mol. Oral Microbiol 10.1111/omi.12387 (2022).
102. Simon-Soro A et al. Polymicrobial aggregates in human saliva build the oral biofilm. mBio 13, e0013122 (2022). [PubMed: 35189700]
103. Pitts NB et al. Dental caries. Nat. Rev. Dis. Primers 3, 17030 (2017). [PubMed: 28540937]
104. Banas JA & Drake DR Are the mutans streptococci still considered relevant to understanding the microbial etiology of dental caries? BMC Oral Health 18, 129 (2018). [PubMed: 30064426]
105. Baker JL et al. Deep metagenomics examines the oral microbiome during dental caries, revealing novel taxa and co-occurrences with host molecules. Genome Res. 31, 64–74 (2021). [PubMed: 33239396]
106. Simón-Soro A & Mira A Solving the etiology of dental caries. Trends Microbiol. 23, 76–82 (2015). [PubMed: 25435135]
107. Teng F et al. Prediction of early childhood caries via spatial-temporal variations of oral microbiota. Cell Host Microbe 18, 296–306 (2015). [PubMed: 26355216]
108. Agnello M et al. Microbiome associated with severe caries in Canadian first nations children. J. Dent. Res 96, 1378–1385 (2017). [PubMed: 28709393]
109. Havsed K et al. Bacterial composition and metabolomics of dental plaque from adolescents. Front. Cell. Infect. Microbiol 11, 716493 (2021). [PubMed: 34395316]
110. Rosier BT, Buetas E, Moya-Gonzalvez EM, Artacho A & Mira A Nitrate as a potential prebiotic for the oral microbiome. Sci. Rep 10, 12895 (2020). [PubMed: 32732931]
111. Rosier BT et al. The importance of nitrate reduction for oral health. J. Dent. Res 10.1177/00220345221080982 (2022).
112. Eick S & Lussi A Arginine: a weapon against cariogenic biofilm? Monogr. Oral Sci 29, 80–90 (2021). [PubMed: 33427222]
113. Baker JL & Edlund A Exploiting the oral microbiome to prevent tooth decay: has evolution already provided the best tools? Front. Microbiol 9, 3323 (2018). [PubMed: 30687294]
114. Darveau RP Periodontitis: a polymicrobial disruption of host homeostasis. Nat. Rev. Microbiol 8, 481–490 (2010). [PubMed: 20514045]
115. Caton JG et al. A new classification scheme for periodontal and peri-implant diseases and conditions — introduction and key changes from the 1999 classification. J. Periodontol 89, S1–S8 (2018). [PubMed: 29926946]
116. Kinane DF, Stathopoulou PG & Papapanou PN Periodontal diseases. Nat. Rev. Dis. Primers 3, 17038 (2017). [PubMed: 28805207]
117. Lamont RJ, Koo H & Hajishengallis G The oral microbiota: dynamic communities and host interactions. Nat. Rev. Microbiol 16, 745–759 (2018). [PubMed: 30301974]
118. Williams DW et al. Human oral mucosa cell atlas reveals a stromal-neutrophil axis regulating tissue immunity. Cell 184, 4090–4104.e15 (2021). [PubMed: 34129837]
119. Chen C et al. Oral microbiota of periodontal health and disease and their changes after nonsurgical periodontal therapy. ISME J. 12, 1210–1224 (2018). [PubMed: 29339824]
120. 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. 7, 27 (2015). [PubMed: 25918553]
121. Miralda I & Uriarte SM Periodontal pathogens' strategies disarm neutrophils to promote dysregulated inflammation. Mol. Oral Microbiol 36, 103–120 (2021). [PubMed: 33128827]
122. Abusleme L, Hoare A, Hong B-Y & Diaz PI Microbial signatures of health, gingivitis, and periodontitis. Periodontol 2000 86, 57–78 (2021).
123. Cai Z, Lin S, Hu S & Zhao L Structure and function of oral microbial community in periodontitis based on integrated data. Front. Cell. Infect. Microbiol 11, 663756 (2021). [PubMed: 34222038]
124. Martínez A, Kuraji R & Kapila YL The human oral virome: shedding light on the dark matter. Periodontol 2000 87, 282–298 (2021).
125. Bamashmous S et al. Human variation in gingival inflammation. Proc. Natl Acad. Sci. USA 118, e2012578118 (2021). [PubMed: 34193520]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

126. Tatakis DN & Trombelli L Modulation of clinical expression of plaque-induced gingivitis. I. Background review and rationale. J. Clin. Periodontol 31, 229–238 (2004). [PubMed: 15016250]

127. Nascimento GG, Danielsen B, Baelum V & Lopez R Identification of inflammatory response patterns in experimental gingivitis studies. Eur. J. Oral Sci 127, 33–39 (2019). [PubMed: 30412312]

128. Tsao SW, Tsang CM & Lo KW Epstein–Barr virus infection and nasopharyngeal carcinoma. Philos. Trans. R. Soc. Lond. B Biol. Sci 372, 20160270 (2017). [PubMed: 28893937]

129. Economopoulou P, Kotsantis I & Psyrri A Special issue about head and neck cancers: HPV positive cancers. Int. J. Mol. Sci 21, 3398 (2020). [PubMed: 32403399]

130. Perera M et al. A dysbiotic mycobiome dominated by Candida albicans is identified within oral squamous-cell carcinomas. J. Oral Microbiol 9, 1385369 (2017). [PubMed: 29152157]

131. Mohamed N et al. Analysis of salivary mycobiome in a cohort of oral squamous cell carcinoma patients from Sudan identifies higher salivary carriage of as an independent and favorable predictor of overall survival. Front. Cell. Infect. Microbiol 11, 673465 (2021). [PubMed: 34712619]

132. Zong Y et al. The interaction between the microbiome and tumors. Front. Cell. Infect. Microbiol 11, 673724 (2021). [PubMed: 34532297]

133. Baraniya D et al. Screening of health-associated oral bacteria for anticancer properties. Front. Cell. Infect. Microbiol 10, 575656 (2020). [PubMed: 33123499]

134. Hajishengallis G & Chavakis T Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. Nat. Rev. Immunol 21, 426–440 (2021). [PubMed: 33510490]

135. Kelly L & Khan S Oral microbes are a signature of disease in the gut. Preprint at Res. Sq 10.21203/rs.3.rs-1631596/v1 (2022).

136. Kennedy KM et al. Questioning the fetal microbiome illustrates pitfalls of low-biomass microbial studies. Nature 613, 639–649 (2023). [PubMed: 36697862]

137. Xu B & Han YW Oral bacteria, oral health, and adverse pregnancy outcomes. Periodontol 2000 89, 181–189 (2022). [PubMed: 35244963]

138. Mammen MJ, Scannapieco FA & Sethi S Oral–lung microbiome interactions in lung diseases. Periodontol 2000 83, 234–241 (2020).

139. Qin N et al. Alterations of the human gut microbiome in liver cirrhosis. Nature 513, 59–64 (2014). [PubMed: 25079328]

140. Acharya C, Sahingur SE & Bajaj JS Microbiota, cirrhosis, and the emerging oral-gut-liver axis. JCI Insight 2, e94416 (2017). [PubMed: 28978799]

141. Atarashi K et al. Ectopic colonization of oral bacteria in the intestine drives T_H1 cell induction and inflammation. Science 358, 359–365 (2017). [PubMed: 29051379]

142. Kitamoto S et al. The intermucosal connection between the mouth and gut in commensal pathobiont-driven colitis. Cell 182, 447–462.e14 (2020). [PubMed: 32758418]

143. Abed J et al. Fap2 mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. Cell Host Microbe 20, 215–225 (2016). [PubMed: 27512904]

144. Abed J et al. Colon cancer-associated *Fusobacterium nucleatum* may originate from the oral cavity and reach colon tumors via the circulatory system. Front. Cell. Infect. Microbiol 10, 400 (2020). [PubMed: 32850497]

145. Rubinstein MR et al. *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. Cell Host Microbe 14, 195–206 (2013). [PubMed: 23954158]

146. Meng Q et al. *Fusobacterium nucleatum* secretes amyloid-like FadA to enhance pathogenicity. EMBO Rep. 22, e52891 (2021). [PubMed: 34184813]

147. Gur C et al. Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. Immunity 42, 344–355 (2015). [PubMed: 25680274]

148. Gur C et al. *Fusobacterium nucleatum* supresses anti-tumor immunity by activating CEACAM1. Oncoimmunology 8, e1581531 (2019). [PubMed: 31069151]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

149. Teles R & Wang C-Y Mechanisms involved in the association between periodontal diseases and cardiovascular disease. Oral Dis. 17, 450–461 (2011). [PubMed: 21223455]
150. Genco RJ & Sanz M Clinical and public health implications of periodontal and systemic diseases: an overview. Periodontol 2000 83, 7–13 (2020).
151. Farrugia C et al. Mechanisms of vascular damage by systemic dissemination of the oral pathogen *Porphyromonas gingivalis*. FEBS J. 288, 1479–1495 (2021). [PubMed: 32681704]
152. Dominy SS et al. *Porphyromonas gingivalis* in Alzheimer's disease brains: evidence for disease causation and treatment with small-molecule inhibitors. Sci. Adv 5, eaau3333 (2019). [PubMed: 30746447]
153. Zhao Y et al. Characterization and regulation of osteoclast precursors following chronic infection. J. Leukoc. Biol 108, 1037–1050 (2020).
154. Herrera BS et al. Peripheral blood mononuclear phagocytes from patients with chronic periodontitis are primed for osteoclast formation. J. Periodontol 85, e72–e81 (2014). [PubMed: 24059638]
155. Dioguardi M et al. The role of periodontitis and periodontal bacteria in the onset and progression of Alzheimer's disease: a systematic review. J. Clin. Med. Res 9, 495 (2020).
156. Miles B et al. Secondary lymphoid organ homing phenotype of human myeloid dendritic cells disrupted by an intracellular oral pathogen. Infect. Immun 82, 101–111 (2014). [PubMed: 24126519]
157. Sainz J & Sata M CXCR4, a key modulator of vascular progenitor cells. Arterioscler. Thromb. Vasc. Biol 27, 263–265 (2007). [PubMed: 17229969]
158. Rojas-Tapias DF et al. Inflammation-associated nitrate facilitates ectopic colonization of oral bacterium *Veillonella parvula* in the intestine. Nat. Microbiol 7, 1673–1685 (2022). [PubMed: 36138166]
159. Konig MF et al. *Aggregatibacter actinomycetemcomitans*-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. Sci. Transl Med 8, 369ra176 (2016).
160. Gully N et al. *Porphyromonas gingivalis* peptidylarginine deiminase, a key contributor in the pathogenesis of experimental periodontal disease and experimental arthritis. PLoS ONE 9, e100838 (2014). [PubMed: 24959715]
161. Calderón-Gómez E et al. Commensal-specific CD4(+) cells from patients with Crohn's disease have a T-helper 17 inflammatory profile. Gastroenterology 151, 489–500.e3 (2016). [PubMed: 27267052]
162. Li X et al. Maladaptive innate immune training of myelopoiesis links inflammatory comorbidities. Cell 185, 1709–1727.e18 (2022). [PubMed: 35483374]
163. Xiao E et al. Diabetes enhances IL-17 expression and alters the oral microbiome to increase its pathogenicity. Cell Host Microbe 22, 120–128.e4 (2017). [PubMed: 28704648]
164. Teles F, Wang Y, Hajishengallis G, Hasturk H & Marchesan JT Impact of systemic factors in shaping the periodontal microbiome. Periodontol 2000 85, 126–160 (2021). [PubMed: 33226693]
165. Balachandran M, Cross KL & Podar M Single-cell genomics and the oral microbiome. J. Dent. Res 99, 613–620 (2020). [PubMed: 32091935]
166. Cross KL et al. Targeted isolation and cultivation of uncultivated bacteria by reverse genomics. Nat. Biotechnol 37, 1314–1321 (2019). [PubMed: 31570900]
167. Huber H & Stetter KO in The Prokaryotes Vol. 3 (eds Dworkin M, Falkow S, Rosenberg E, Schleifer K-H & Stackebrandt E) 101–112 (Springer New York, 2006).
168. Germano F, Testi D, Campagnolo L, Scimeca M & Arcuri C Cell-wall-deficient bacteria in oral biofilm: association with periodontitis. Sci. Repos 10.31487/j.DOBCR.2020.06.03 (2020).
169. Collins AJ, Murugkar PP & Dewhirst FE Establishing stable binary cultures of symbiotic Saccharibacteria from the oral cavity. J. Vis. Exp 10.3791/62484 (2021).
170. Thurber RV, Haynes M, Breitbart M, Wegley L & Rohwer F Laboratory procedures to generate viral metagenomes. Nat. Protoc 4, 470–483 (2009). [PubMed: 19300441]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

171. Kauffman KM et al. A major lineage of non-tailed dsDNA viruses as unrecognized killers of marine bacteria. Nature 554, 118–122 (2018). [PubMed: 29364876]

172. Richter Ł et al. Adsorption of bacteriophages on polypropylene labware affects the reproducibility of phage research. Sci. Rep 11, 7387 (2021). [PubMed: 33795704]

173. Vartoukian SR et al. In vitro cultivation of 'Unculturable' oral bacteria, facilitated by community culture and media supplementation with siderophores. PLoS ONE 11, e0146926 (2016). [PubMed: 26764907]

174. Vartoukian SR, Moazzez RV, Paster BJ, Dewhirst FE & Wade WG First cultivation of health-associated *Tannerella* sp. HOT-286 (BU063). J. Dent. Res 95, 1308–1313 (2016). [PubMed: 27193146]

175. Kearney SM, Coe A, Castro KG & Chisholm SW Filter plating method for rendering picocyanobacteria cultures free of heterotrophic bacterial contaminants and clonal. Front. Microbiol 13, 821803 (2022). [PubMed: 35250937]

176. Ruscitto A et al. Regulation and molecular basis of environmental muropeptide uptake and utilization in fastidious oral anaerobe *Tannerella forsythia*. Front. Microbiol 8, 648 (2017). [PubMed: 28446907]

177. Tanaka T et al. A hidden pitfall in the preparation of agar media undermines microorganism cultivability. Appl. Environ. Microbiol 80, 7659–7666 (2014). [PubMed: 25281372]

178. Khelaifia S et al. Aerobic culture of methanogenic archaea without an external source of hydrogen. Eur. J. Clin. Microbiol. Infect. Dis 35, 985–991 (2016). [PubMed: 27010812]

179. Lagier J-C et al. Culture of previously uncultured members of the human gut microbiota by culturomics. Nat. Microbiol 1, 16203 (2016). [PubMed: 27819657]

180. Guindo CO et al. Culture of salivary methanogens assisted by chemically produced hydrogen. Anaerobe 61, 102128 (2020). [PubMed: 31759176]

181. García-Bayona L et al. Nanaerobic growth enables direct visualization of dynamic cellular processes in human gut symbionts. Proc. Natl Acad. Sci. USA 117, 24484–24493 (2020). [PubMed: 32938803]

182. Dridi B, Henry M, El Khéchine A, Raoult D & Drancourt M High prevalence of *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* detected in the human gut using an improved DNA detection protocol. PLoS ONE 4, e7063 (2009). [PubMed: 19759898]

183. Horz H-P & Conrads G Methanogenic Archaea and oral infections — ways to unravel the black box. J. Oral Microbiol 3, 5940 (2011).

184. Roux S et al. IMG/VR v3: an integrated ecological and evolutionary framework for interrogating genomes of uncultivated viruses. Nucleic Acids Res. 49, D764–D775 (2021). [PubMed: 33137183]

185. Bor B, Cen L, Agnello M, Shi W & He X Morphological and physiological changes induced by contact-dependent interaction between *Candida albicans* and *Fusobacterium nucleatum*. Sci. Rep 6, 27956 (2016). [PubMed: 27295972]

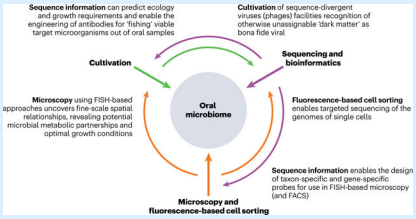
186. Bedree JK et al. Quorum sensing modulates the epibiotic–parasitic relationship between *Actinomyces odontolyticus* and its Saccharibacteria epibiont, a *Nanosynbacter lyticus* strain, TM7x. Front. Microbiol 9, 2049 (2018). [PubMed: 30319555]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Box 1

Strategies and factors that may influence detection of oral microorganisms

- Detection of oral microorganisms is facilitated by synergy between key methodologies[165] including cultivation, sequencing and bioinformatics, and microscopy and fluorescence-based cell sorting (see the figure). For example, in an approach deemed ‘reversegenomics isolation’, sequence information can be used to engineer antibodies for recovering specific microorganisms from complex samples for subsequent cultivation[166]
- Examples of factors that may influence detection of oral microorganisms



Sample collection and fractionation

Cells that are small, narrow or lacking cell walls (for example, *Mycoplasma*, *Thermoplasmata*[167], L-forms[168] and candidate phyla radiation (CPR) bacteria[169]) will pass through 0.2-μm filters commonly used to collect bacteria; those without cell walls, and amoebae and flagellates, may be killed by osmotic shock. Viruses associated with large particles are lost if using sub-0.2 μm fractions (common for viromes), and cells and viruses inside microeukaryotes and human cells are lost if excluding larger size fractions. Small or richly encapsulated bacteria may not pellet readily, and lipid-containing virions partition differently from tailed phages in ultra-centrifugal density gradients[170,171]. Some viruses (including phages) are damaged by chloroform[170,171] and bind to plastic consumables[172].

Dependence on interactions

Removed from the comforts of their natural niches, microorganisms may require or benefit from co-culture with syntrophic microorganisms[173–175] that provide necessary unknown growth factors. The need for co-cultivation reaches extremes with microorganisms that rely on specific hosts for replication (CPR and predatory bacteria, and viruses).

Cultivation conditions

For culture experiments, the following needs to be considered: temperature (35–39 °C); pH (2–8); electron donors and acceptors; osmolarity; vitamins; iron (and perhaps siderophores[173]); suitable nutrient and carbon sources (for example, dietary and human-derived or microorganisms-derived, such as muropeptides for *Tannerella*[176]); presence of reactive oxygen species produced during autoclaving of agar and phosphate-containing media[177]; and oxygen toxicity — new chemical-based and co-cultivation-based approaches are reported for oxic cultivation of anaerobes[178–180]. Recent work[181]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

on gut anaerobes shows that they grow well at low oxygen concentrations (0.10–0.14%, ‘anaerobic’ growth), which enables the use of traditional oxygen-requiring fluorescent protein tags and opening the doors for use of these methods with oral anaerobes.

Nucleic acid extraction, sequencing and bioinformatics

Microorganisms differ in susceptibility to extraction protocols (for example, proteinase K-resistant cell walls[182], requirement for bead beating[30] and requirement for protease treatment for some viruses[171]). Common primers may have mismatches or lack specificity (for example, divergence of 16S ribosomal DNA sequences in some CPR phyla[14] and off-target amplification with high cycle number[183]). Finally, rarity or unusual genome features (for example, DNA modifications, repeats and viral genome length) and lack of appropriate reference databases affect sequence-based detection and classification, especially for fungi[30] and viruses (identification of oral phages for incorporation into the Human Oral Microbiome Database is underway, and IMG/VR[184] identifies and clusters phage contigs predicted in oral metagenomes).

FACS, fluorescence-activated cell sorting; FISH, fluorescence in situ hybridization.

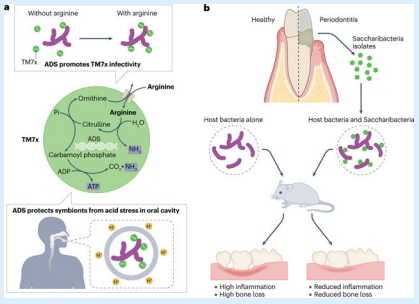
Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Box 2

Challenges to early hypotheses in Saccharibacteria ecology

Ideas about the ecological roles of oral Saccharibacteria are evolving. A recent study identified an arginine deiminase system (ADS) that was found in the genomes of the mammal-associated G1 group Saccharibacteria, but was lacking in their environmental counterparts[26]. TM7x used the ADS to maintain membrane integrity and higher levels of infectivity in the presence of arginine (see the figure, part **a**). ADSs are known in the context of the dental plaque microbiome to be part of the bacterial acid tolerance response, as the metabolism of arginine to ATP and ammonia (an alkaline molecule) both directly buffers the acidic environment and can increase ATPase-mediated proton extrusion. Interestingly, the ADS activity not only increased the viability of TM7x in the presence of acid stress but also increased survival of the *Schaalia odontolytica* host (see the figure, part **a**). Although TM7x was initially regarded as a parasite that negatively affects the growth of its host bacterium under laboratory conditions, and even kills its host under starvation[22,23,185], these findings, along with others that showed that TM7x promoted biofilm formation by the *S. odontolytica* host[186], indicate that association with TM7x can provide advantages for the host bacteria to persist within the oral cavity under certain conditions.

Another study[25] used a mouse ligature-induced periodontitis model to investigate the pathogenicity of Saccharibacteria (see the figure, part **b**). Interestingly, although host Actinobacteria alone induced severe periodontal tissue loss, association with the Saccharibacteria that were isolated from periodontal pocket reduced inflammation and consequential bone loss by modulating host Actinobacteria pathogenicity, challenging the hypothesis that TM7 are overtly pathogenic bacteria[7,20,125]. A possible explanation for these observations is that although Saccharibacteria are inflammophilic and can efficiently utilize nutrients from the inflammatory destruction of tissues, they may not necessarily induce a further inflammatory response[25]. Furthermore, the role of Saccharibacteria in human periodontal disease is likely to be more complicated than what was demonstrated in this simplified animal model, in which only Saccharibacteria and their host bacteria were introduced. On the basis of the current view of the importance of polymicrobial metabolic synergy in the disease aetiology, the presence of other human periodontal pathobionts may have an impact on the interaction between Saccharibacteria and its host bacteria, thus modulating their role in the disease initiation or progression.



Pi, inorganic phosphate.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

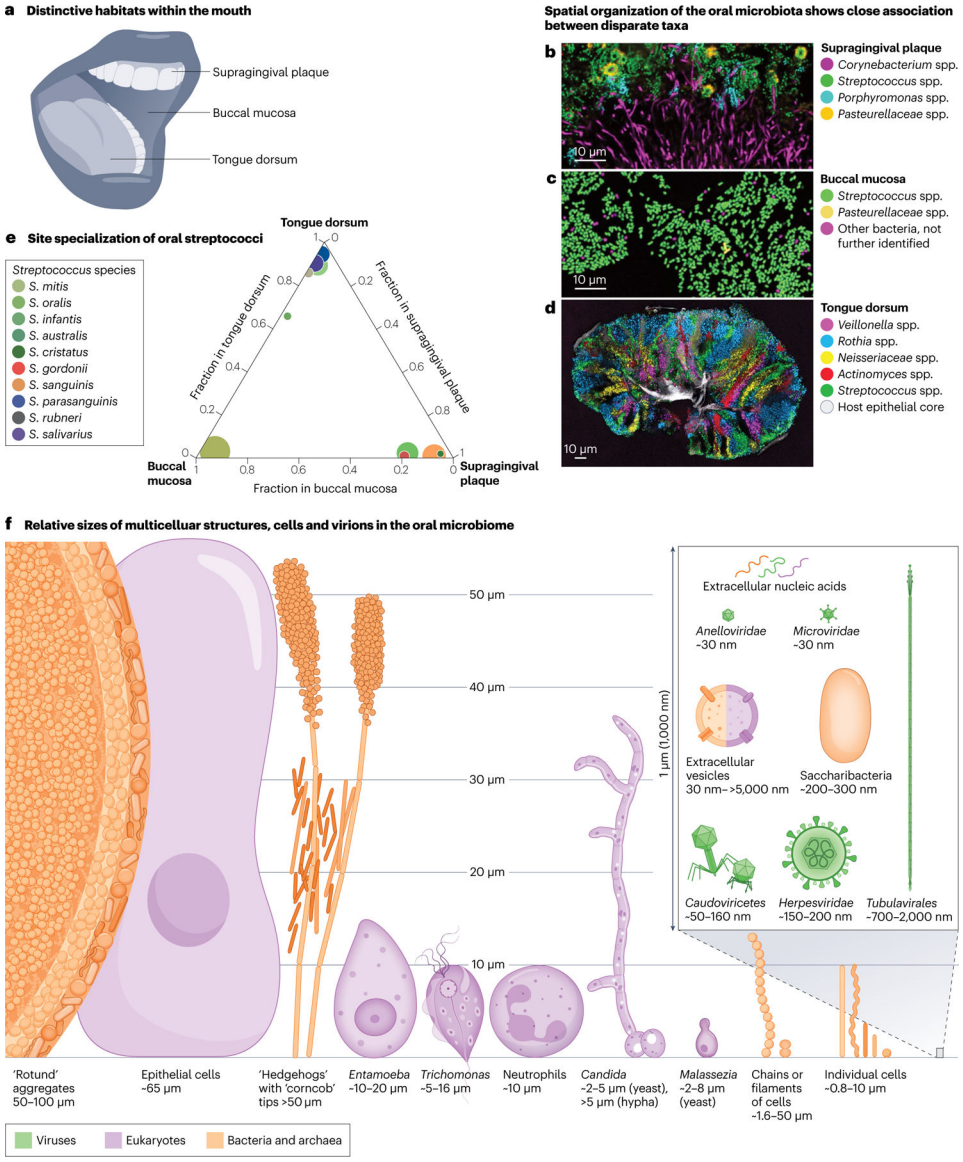


Fig. 1 |. Biogeography of the oral microbiome and relative sizes of its members.

a–d, Microbial communities with disparate structure and composition colonize different surfaces in the mouth. **a**, Distinctive habitats within the oral cavity host a diversity of resident taxa, whose biogeography can be visualized via combinatorial labelling and spectral imaging fluorescence in situ hybridization microscopy of the supragingival plaque (part **b**), the buccal mucosa (part **c**) and the dorsum of the tongue (part **d**). Shown are bacterial members of the oral microbiome and the characteristic structures they form at each site. Until recently, most human microbiome studies analysed the bacterial distribution at the genus level or even at the phylum level. With the increasing availability of whole-genome metagenomic sequence data, distribution patterns of closely related species can be distinguished. **e**, A recent study analysed the distribution of species of the abundant oral genus *Streptococcus* using short-read metagenomic sequence data from the human mouth and found that each species was found primarily at one oral site. In the figure, species

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

are indicated by coloured dots corresponding to the colours in the legend; the size of species dots corresponds to their abundance. These whole-genome data could differentiate closely related taxa such as *Streptococcus mitis*, found primarily on the buccal mucosa; *Streptococcus oralis* found in dental plaque; and *Streptococcus infantis*, found on the tongue dorsum[101]. **f**, Together, the sizes of oral microorganisms and microbial structures span four orders of magnitude, from nanoscale viruses to bacterial aggregates of hundreds of microns. Bacterial cells range in size from 200–300 nm (the diminutive Saccharibacteria) up to 10 μm (such as the long spirilliform *Treponema*), with the majority around 1 μm (for example, 0.8-μm diameter *Streptococcus* spp.). Bacterial aggregates and consortia comprise the largest component of oral microbial biomass (up to hundreds of microns) and include ordered polymicrobial structures, distinctively named to suggest their features. 'Hedgehog' aggregates are found abundantly in healthy oral microbiomes and are composed of long filaments of multiple cells of *Corynebacterium* decorated with *Streptococcus* and other cocci at their periphery (forming 'corncobs' up to 50 μm in length) and creating densely packed environments that facilitate the growth of anaerobe species including *Leptotrichia* spp., *Fusobacterium* spp. and *Actinomyces spp*. within. 'Rotund' aggregates have been identified in association with caries and comprise an inner mass of *Streptococcus mutans* and associated exopolysaccharide matrix, a surrounding layer of *S. oralis* or other non-mutans streptococci and an outer layer of non-streptococci. Oral eukaryotes include the rare motile protists *Trichomonas* and *Entamoeba*, as well as the non-motile fungal genera *Candida* and *Malassezia*, and they are all around the same order of magnitude in size as human neutrophils (around 5–15 μm), which are abundant in the gingival crevicular fluid during inflammation. The smallest members of the oral microbiome are the viruses, which are known to include human-infecting viruses (for example, the abundant anelloviruses), viruses infecting bacteria (bacteriophages) and likely also viruses infecting oral micro-eukaryotes. Part **b** reprinted with permission from ref. 93, PNAS. Photos in parts **c** and **d** courtesy of J.M.W. Part **e** adapted with permission from ref. 101, Wiley.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

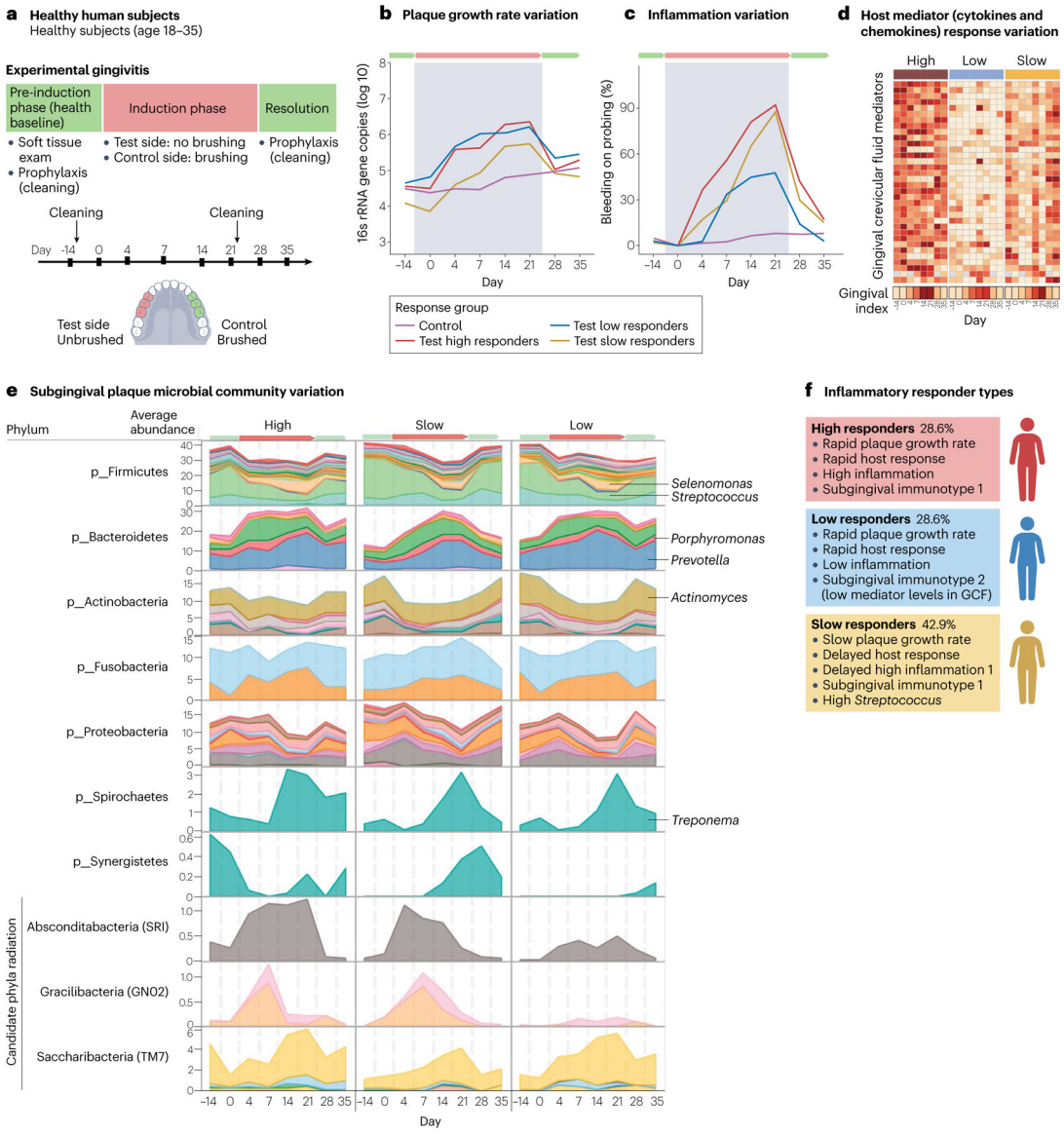


Fig. 2 |. Experimental gingivitis in humans reveals three distinct response types.

a, Human experimental gingivitis model study design (Supplementary Box 1). A typical experimental gingivitis model with healthy subjects includes the following phases: hygiene phase for 2 weeks before baseline (days −14 to 0), gingivitis induction phase lasting for 3 weeks (days 0–21) and resolution phase for 2 weeks (days 21–35). Subgingival plaque and gingival crevicular fluid (GCF) are taken from unbrushed test teeth (test teeth) as well as the teeth that had maintained oral hygiene (control teeth). This model has allowed tracking of changes in the oral microbiome as well as variation in the inflammatory response of the subjects. Although all subjects typically respond to oral plaque accumulation in experimental gingivitis studies, the rate and severity of the inflammatory response has been shown to vary. A recent study investigating healthy subjects (age 18–35) has stratified these responses on the basis of the combined analyses and clustering of temporal trajectories in clinical measures of inflammation. This analysis resulted in three inflammatory responder

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

types[125]: high and low responders (previously recognized in the literature) and a novel slow responder each with distinct microbial and host signatures. **b**, Plaque growth rate variation between inflammatory responder types. Slow responders display a delayed plaque growth rate, whereas high and low responders have the same growth rate. **c**, Inflammation variation over time measured by the percent of unbrushed test sites with bleeding on probing. The low responders do not reach a high level of inflammation and the newly described slow responders have delayed inflammation. **d**, Heatmap illustrating variation in a panel of host mediators (cytokines and chemokines) in GCF. When comparing mean values for chemokine expression over time for responders (row normalized data, red represents high values, white is low value and each column is a visit), the low responder phenotype exhibits lower overall mediator concentrations than the other types. Relative inflammation changes measured by the gingival index (a standard clinical measure of gingivitis severity based on tissue redness) shown as a row-normalized heatmap across the bottom of the plot. **e**, Dynamic changes in relative abundance across the seven major phyla and three candidate phyla radiation group members in responder type across the phases. Variation is observed across subgingival plaque microbial compositions as well as the rate of change in the relative abundance of certain genera over the induction phase. Coloured areas represent the different genera that make up each phylum, and the plots show the mean relative abundance of different genera by responder group. Consistently across experimental gingivitis studies that have relied on culturing or culture-independent methods, the two most abundant Gram-positive phyla, Firmicutes and Actinobacteria, decrease in relative abundance as the plaque community grows and matures. With 16S rRNA sequencing across early and late time points, additional resolution has been gained recently. For example, *Selenomonas* becomes a higher proportion of the total Firmicutes by day 21 across responders. By contrast, members of the *Prevotella* genus predominantly contribute to this increase of the Bacteroidetes. *Porphyromonas* also increase over time but represent a smaller relative proportion of this total. Within the phylum Spirochaetes, *Treponema* genus members in gingivitis here tend to show a large enrichment that occurs after a week or more of plaque growth. A defining feature of the slow responder phenotype is the higher abundance of *Streptococcus* at the time of inclusion in the study (day −14), which is then restored after the induction phase inflammation is resolved. In addition, the newly recognized ultrasmall reduced genome epibionts belonging to the CPR, '*Candidatus* Absconditabacteria', '*Candidatus* Gracilibacteria' and Saccharibacteria also show increases during gingival inflammation with variation across responder types. **f**, Overview of the prevalence and defining features of the newly characterized responder types. Data in parts **b**–**d** from ref. 125. Parts **b**–**d** adapted from ref. 125, PNAS.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

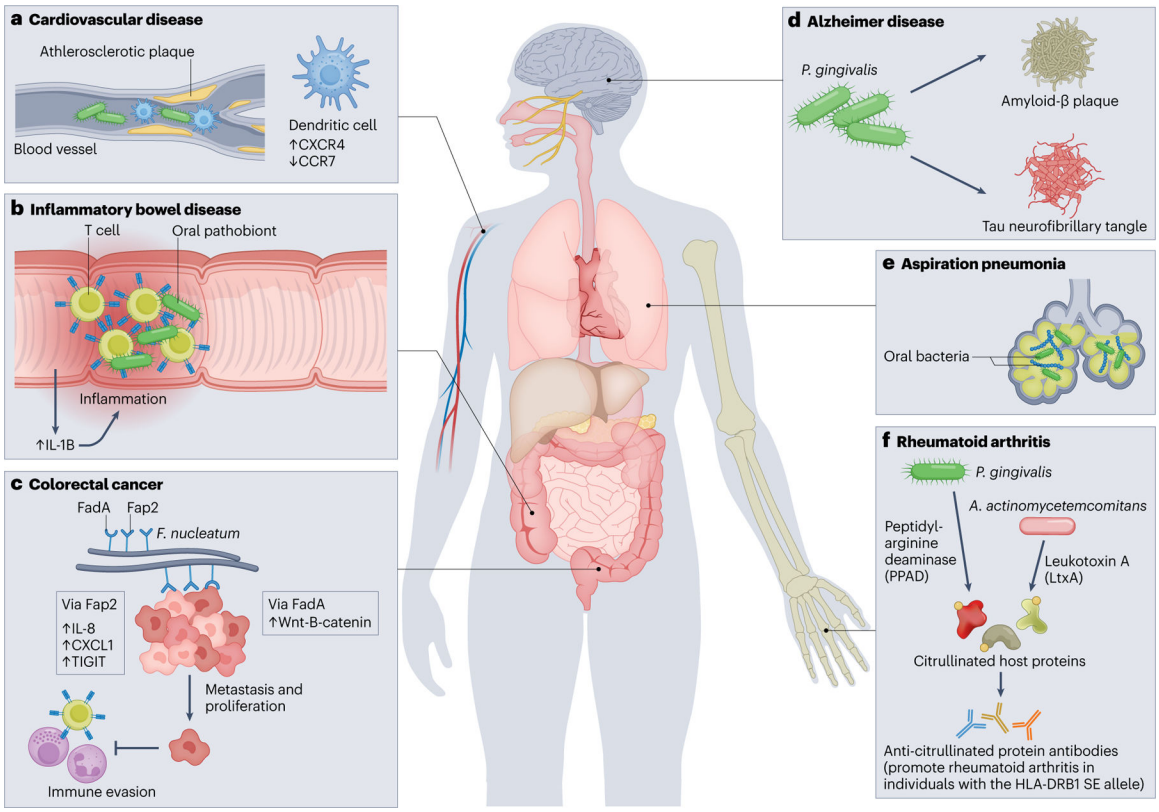


Fig. 3 |. Links between the oral microbiota and systemic diseases.

The oral microbiota, and particularly periodontal pathogens, have been increasingly linked to a number of systemic diseases, either directly through translocation of oral pathogens to other sites or indirectly through modulation of the host immune system and inflammatory response. This illustration highlights a number of these links with the insets highlighting the mechanism(s). **a**, Cardiovascular disease: in vitro and ex vivo studies have suggested that *Porphyromonas gingivalis* bacteraemia alters dendritic cell behaviour, causing pro-inflammatory accumulations at atherosclerotic plaques[156]. **b**, Inflammatory bowel disease: animal studies have illustrated that when T cells primed and reactive to oral pathobionts encounter ectopic periodontal pathogens in the gut, they increase inflammation (particularly via IL-1B) and contribute to colitis[141,142]. **c**, Colorectal cancer: mouse models and human studies have established that *Fusobacterium nucleatum* outgrowth synergizes with tumour growth via multiple mechanisms. The *F. nucleatum* surface protein Fab2 binds to cancer cells, inducing pro-metastatic chemokines, and activates TIGIT, allowing for evasion of immune surveillance by the tumour[143,147]. Another *F. nucleatum* surface protein, FadA, stimulates cancer cell proliferation by inducing E-cadherin-mediated Wnt-β-catenin signalling[145]. **d**, Alzheimer disease: animal models and observational studies in humans have shown that *P. gingivalis*, on reaching the brain via bacteraemia, causes neuronal inflammation, degrades tau protein causing neurofibrillary tangles and contributes to the formation of amyloid-β plaques, reviewed elsewhere[155]. **e**, Aspiration pneumonia: human studies have shown that the translocation of oral bacteria to the lung can lead to pneumonia, reviewed elsewhere[138]. **f**, Rheumatoid arthritis: in mice, *P. gingivalis* that reaches the bone marrow skews immune cell development via IL-6, promoting production of osteoclasts and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

contributing to bone loss[153]. Meanwhile, animal and human studies have illustrated that *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* can citrullinate host proteins, which triggers autoimmunity[159,160].