The Oral Host–Microbial Interactome: An Ecological Chronometer of Health?

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An increasing number of studies reveal that host–microbial interactome networks are coordinated, impacting human health and disease. Recently, several lines of evidence have revealed associations between the acquisition of a complex microbiota and adaptive immunity, supporting that host–microbiota symbiotic relationships have evolved as a means to maintain homeostasis where the role of the microbiota is to promote and educate the immune system. Here, we hypothesize an oral host–microbial interactome that could serve as an ecological chronometer of health and disease, with specific focus on caries, periodontal diseases, and cancer. We also review the current state of the art on the human oral microbiome and its correlations with host innate immunity, and host cytokine control, with the goal of using this information for disease prediction and designing novel treatments for local and systemic dysbiosis. In addition, we discuss new insights into the role of novel host–microbial signals as potential biomarkers, and their relevance for the future of precision dentistry and medicine.

Introduction
The oral cavity is of major importance for human health and well-being. Not only does it provide entry to the digestive and respiratory systems, but it also provides immunity, and a protective barrier from invading pathogens (Box 1). Approximately 700 oral bacterial species have been identified to date, with evidence of 296 species-level taxa in a typical individual’s mouth [1]. These species are known collectively as the human oral microbiota. This microbiota colonizes five main, physically distinct niches: saliva, tongue, oral mucosa, mineralized tooth surfaces, and periodontal tissues – each niche harboring a distinct microbial community [2]. Some bacterial community members are considered pathobionts and cause diseases including dental caries (tooth decay) [3–5], periodontal diseases (gingival inflammation) [6,7], comprising gingivitis and periodontitis, and have been implicated in oral cancers [8]. Oral bacteria have also been linked to systemic diseases such as type 2 diabetes, cardiovascular disease, cancer, preterm labor and Alzheimer’s disease [8–10]. However, molecular mechanisms supporting any direct role for the oral microbiota in systemic diseases have yet to be determined.

This review highlights evidence and gaps in knowledge related to the oral host–microbiota interactome across global human populations and in healthy and disease states. Note that the term dysbiosis does not necessarily implicate disease, and that authors apply different meanings to the concept, which has been discussed thoroughly in a review by Hooks and O’Malley [11]. In this review, we explain dysbiosis as a process that impacts the bacterial community, and/or immune cells, resulting in differences in composition and consequently microbial metabolism, which either can promote health or disease. We further explore individual microbiota differences and survey the latest frontiers in diagnostics and therapeutics to prevent and treat oral diseases, including caries, periodontal diseases and oral squamous cell carcinoma. Lastly, we explore how functional molecular signatures of the oral microbiome are key in interacting with the host, thereby defining health.

Highlights
Dynamic interactions between the human microbiome and the host immunity shape health and disease.

Global human populations are major carriers of streptococci and Prevotella bacteria.

Lifestyle habits such as a high-sugar diet, alcohol consumption, and smoking can impact oral microbial diversity, and interactions between the microbiota and the host.

Members belonging to the Haemophilus genus are associated with oral health in populations of hunter-gatherers.

Oral host–microbial interactome provides signals able to impact both local and systemic dysbiosis.

Monitoring oral, dental, and craniofacial systems can reveal novel biomarkers for diagnostics and targeted therapies.
The Oral Microbiome Landscape

Most of the initial studies of oral microbes focused on addressing Koch’s postulates and generating aerobic isolates in pure cultures. Culture-based approaches, as was common in the first half of the 20th century, enabled a focus on key pathogens thought to be associated with oral diseases. By 1924, Clarke and colleagues had described the caries causing pathogen, *Streptococcus mutans* [12]. Later, in the 1960s, the introduction of anaerobic cultivation approaches expanded the number of known microbial species that could be cultivated from the human body, in particular the gut and the oral cavity. DNA technology in the 1970s allowed for the application of recombinant and genetic approaches to elucidate cellular mechanisms present in several oral species and by the 1980s and 1990s, DNA mapping was being used routinely to characterize the 16S rDNA gene (16S) sequence [13] of many oral pathogens and commensals. *Porphyromonas gingivalis* was the first oral microbe to have its genome completely sequenced in 2003 [14] using Sanger sequencing which was the typical sequencing technology of that time. Shotgun sequencing was also first applied to evaluate the metagenomes in oral populations starting in the late 2000s [15]. As recently as 2010, Bik and colleagues used Sanger sequencing to describe the oral microbiota in ten healthy individuals and revealed 247 species-level phylotypes and nine bacterial phyla [16]. Following the advent of 454 and Illumina sequencing, oral metagenomic studies became more common and were included in the pivotal Human Microbiome Project [15], which began in the late 2000s. Metagenomic surveys are essential to reveal the complete genetic profile of oral consortia. Additional assays that are being used to describe the oral microbiome (see below) include metatranscriptomics to evaluate gene expression [17], metabolomics to evaluate small molecules that are produced by community members [18], proteomics [19] and other ‘omic’ technologies which are applied to gain a deeper knowledge of functional mechanisms of the oral microbiota. Today the Human Oral Microbiome Database (eHOMD; www.homd.org) is the most comprehensive repository of microbial species that inhabit both the oral and nasal cavities [101].

**Bacterial Signatures and Anthropology of the Oral Microbiome**

Multiple research efforts have been made to obtain microbial signatures of human populations spanning the globe [20] as well as signatures across human body sites [15]. In an excellent review by Gupta et al. [21], biogeographic microbiome patterns across global populations were explored, which included data from the gut, skin, oral cavity, and urogenital tract. The authors identified multiple patterns associated with lifestyle, diet, ethnicity, age, gender, parasitic load, and exposure to modern therapeutics. However, it was not possible to deduce the individual effects of each of these factors. A major finding was that *Haemophilus* were more prevalent in dental plaque from contemporary hunter-gatherer groups with low incidences of caries as compared with agricultural groups. This is noteworthy since *Haemophilus* species are known to keep pH neutral in dental plaque and prevent enamel erosion because of their capacity to hydrolyze urea to alkaline ammonia [22]. Their findings also show that both tooth and root morphology may play a role in oral microbiome composition as well as innate immune responses to infectious agents.

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**Box 1. Metabolites of the Oral Microbiota**

The human microbiota is estimated to harbor ~14 000 biosynthetic gene clusters (BGCs) encoding small molecules with a broad range of activities such as antimicrobial and anti-inflammatory activities [29,30]. A typical oral microbiome of an individual harbors ~1601 BGCs [29]. Most of this diversity is unexplored for the oral microbiota but a handful of studies of the caries model organism *Streptococcus mutans* have revealed molecules such as mutacins, which are antifungal bacteriocin compounds [31], the anti-inflammatory mutanocyclin [32,33], and the antibacterial reutericyclins [33]. These findings emphasize the importance of exploring the rich repertoire of oral BGCs further and reminds us that the human microbiota is a treasure trove of therapeutic drug leads. A metabolomics study focusing on peptidic small molecules (PSMs) secreted from in vitro multi-species oral biofilms, and from mono-cultures representing 27 oral bacterial species, identified between 400 and 900 PSMs signatures per bacterial isolate and per time point of growth [18]. Some PSMs overlapped between cultures but many were unique showing an incredible richness with regards to this particular class of metabolites.
By learning about the evolutionary forces that have led to the selection of our modern oral microbiota, we can gain a deeper understanding of the role of host genetics, immune selection, and dietary influences. Evolutionary microbiome studies have revealed compositional differences in microbiota among great apes, archaic hominins, and modern humans, with a marked reduction in microbial diversity observed within modern humans [23]. The loss of microbial diversity in modern humans is proposed to be a result of lifestyle changes, such as dietary shifts towards the consumption of high-energy foods, and away from plant-based foods [24]. Unlike other human body cavities, the oral cavity can provide evidence of the ancient human microbiome as well as contemporary dietary habits because DNA is relatively preserved in dental calculus formation on teeth. By analyzing bacterial composition in calculus from different time periods, it was recently suggested that dietary changes brought about by agriculture altered the human microbiota considerably over 7500 years ago, and changed again with the recent movement toward animal-based and fat-rich western diets [25]. This reduction in diversity is thought to be partially responsible for dysbiosis, a process that impacts bacterial community composition and consequently microbial metabolism, which either promotes health or disease [11]. In the human microbiotas of the modern world, dysbiosis is often associated with various metabolic, inflammatory, and autoimmune diseases [26].

To disentangle the complex web of interactions between the human host, their microbiota and the environment, new approaches with data-driven informatics and computational modeling have been developed. One pioneering genome-wide association study (GWAS) by our own group examined oral microbiota–host genome interactions, and reported that signatures of heritability were lost over time for oral microbes when studying caries-associated microbiome in twins [27]. However, before the signal was lost, health-associated Prevotella pallens was highly heritable in a group representing 5–7-year-old children, suggesting that interactions between the host and this taxon may be of significance to explore further in younger age groups among children. Furthermore, initial evidence from GWAS and microbiome studies of ancient and modern bacterial communities preserved in dental plaque has shown that host genomics, age, sex, and various environmental factors are important selective forces that impact these associations [28]. As with most of the microbiome field, the GWAS research area is still in its infancy and begs further investigation to identify the forces that shape interactions between the host and the oral microbiota (Box 1), which in the future can serve as targets for early disease detection, diagnosis, and treatment.

Saliva and Oral Mucosal Immunity
As the first entry port to the gastrointestinal tract, the oral mucosal epithelium comprises stratified squamous epithelium divided by both the masticatory epithelium and lining mucosa [34]. The overall permeability of oral tissues is heterogeneous; keratinized areas present lower cell permeability (gingival tissue and palatal mucosa, lips), while non-keratinized areas which are more permeable (long junctional epithelium, vestibule, buccal mucosa) present a more diverse immune repertoire. The oral mucosa is predominantly populated by antigen presenting cells (APCs) and neutrophils that form a network of innate immune cells, which signal activation of lymphocytes including T- and B-cells. In healthy connective tissue, the associated microbiota stimulates the activity of adaptive immune cells, which are responsible for maintaining homeostasis and preventing tissue loss. However, when pathogenic microbial species are present in disease (e.g., P. gingivalis in deep sulcular pockets), heterogeneous APC subsets become activated [35]. Recently, we have developed a novel protocol to evaluate salivary immunity reliably [36]. In contrast to oral mucosal tissues, saliva is a biofluid that has been shown to be composed of mostly epithelial cells and immune cells with an increased level of neutrophil heterogeneity, followed by lymphocytes and other myeloid cells. Our machine learning strategy for single cell RNA sequencing and flow cytometry markers demonstrated novel salivary neutrophil populations.
with different levels of maturation by chemokine expression. The heterogeneity of cells derived from oral fluids is vast, yet functional and mechanistic assays are needed to determine the function of this wide cell repertoire.

Of all immune cells, neutrophils constitute 95% of the total leukocytes present in oral tissues [37]. Transmigration of 30,000 neutrophils through the highly permeable epithelium, namely the junctional epithelium, and oral mucosa allow immune cells to enter the oral cavity. These neutrophil estimates are increased when polymicrobial and dysbiotic biofilms transition to periodontal diseases [70]. Although few in number, other important immune cells reside in the gingival tissues; these include resident T- and B-cells, innate lymphoid cells, macrophages, and dendritic cells [39,40]. As compared with the conventional stratified lining oral epithelia, the gingival tissues lack a submucosa, thus establishing a more intimate interaction of the lamina propria with the outer membrane of the alveolar bone. With a well-defined and bountiful blood supply, this arrangement is thought to readily facilitate the access of immune cells into the periodontal apparatus in the presence of noxious products, for instance microbial metabolites that damage host tissue.

In addition to mucosal immunity, salivary content provides remarkable value to oral immunity. Saliva plays an important role in the maintenance of oral health and regulation of the oral microbiome. It is involved in digestion, clearance of microbes, speech, and lubrication of hard and soft tissues. First, the salivary pellicle is composed of proteins required for the early microbial colonizers mainly consisting of members belonging to the Actinomyces, Streptococcus, Haemophilus, Capnocytophaga, Veillonella, and Neisseria genera followed quickly by certain Gram-negative rods and filamentous bacteria [41,42]. Secondly, salivary mucins, antimicrobial peptides and proline-rich proteins produced by salivary neutrophils help initiate selection of bacterial colonization and biofilm formation [43,44]. Saliva also provides antimicrobial and anti-viral activity via lactoferrins, lactoperoxidase, lysozyme, statherin, and histatins produced by the host and microbial cells. A comprehensive repository of the human saliva proteome has been recently launched (www.salivaryproteome.nidcr.nih.gov) to further investigations of health and disease. Under normal conditions, the unstimulated whole saliva flow rate is on average of 0.3–0.4 ml/min and stimulated saliva flow rate is ~1.5–2.0 ml/min [45,46]. Loss of salivary flow, caused by systemic diseases, medications, and environmental factors, is known cause progressive caries and infections.

Microbes are introduced to saliva via multiple pathways, for example our diet, and shedding biofilms from oral mucosa, dental surface, tongue, and buccal mucosa [47]. Several groups of bacteria belonging to the Streptococcus, Gemella, Granulicatella, Neisseria, Prevotella, and Veillonella genera are shared among intraoral sites. However, some taxa are site-specific showing unique niche adaptations [48]. While mono-species models were ideal to investigate microbial mechanistic functions in previous studies [49], and also host–microbial communications, a keystone pathogen hypothesis [50] does not likely represent the complexity of chronic conditions. Bacteria recovered from patients with aggressive periodontitis including Aggregatibacter actinomycetemcomitans, Eikenella corrodians, and other oral bacteria (S. mutans, Streptococcus gordonii, Haemophilus aphrophilus) were able to activate dendritic cells and produce a 1000-fold increase in chemokine production, while P. gingivalis, which has been postulated as a keystone pathogen, did not show the immune phenotype [51]. A polymicrobial homeostatic stimulation model (i.e., when multiple bacteria stimulate the host tissue to self-regulate), which is highly interactive with the immune system, could therefore provide a stronger scientific support than single pathogens [6].

Host–Microbiota Interactions in Health and Disease
Various systemic diseases are influenced by microbial metabolism and host interactions. In humans, chronic and/or infectious diseases such as periodontal diseases, rheumatoid arthritis,
lupus, ulcerative colitis, diabetes, Alzheimer’s disease, cancer, and others present low-grade unresolved inflammation as a common link. ‘How’ and ‘why’ the imbalance of immune cells fails to control chronic oral inflammation, which in turn lead to diseases remains an active area of investigation. For many conditions, the challenge is to discover whether there is a causal link between the microbiome and the pathology. Examples of associations of human conditions with particular microbiota characteristics include: (i) obesity, which was associated with a reduced ratio of Firmicutes to Bacteroidetes; (ii) inflammatory bowel disease presented increased Enterobacteriaceae; (iii) cardiovascular diseases promoted by phosphatidylcholine derived from the gut microbiota; (iv) psoriasis showed increased ratio of Firmicutes to Actinobacteria; (v) colon cancer association with Fusobacteria. Microbiome analysis in humans has been largely based on observations, with associations of disease phenotypes and microbial constituents. For understanding causation and pathogenesis, model organisms provide important approaches to map mechanisms and can help validate omics findings. The use of mouse models in microbiome studies provide important methods to reproduce human diseases. The use of gnotobiotic mouse models has well-controlled microbial variability, allowing for better understanding of microbe interaction, and the genetically homogenous environment allows mechanistic studies. However, many challenges such as the cage effect, coprophagy, and mouse strain genetics are worth considering when planning for host–microbiome interaction studies, which are further discussed in a review by Nguyen and colleagues and in an article by Kim and colleagues. Humanized models, however, provide more relevant human disease states and direct modulation of the immune system. The scale of the oral interface suggests that microbiota-host interactions have important influence on disease susceptibility, microbial metabolic activities, and immunity. Factors influencing pathological transitions from health to oral disease including caries, gingivitis, periodontal diseases, and oral cancers remain important questions to pursue. An overall view of the three most common oral diseases and examples of known immune–microbiome interactions and bacterial pathogens associated with each disease are presented in Table 1.

Periodontal Diseases

Within the oral ecosystem, specific niches select for microbial communities that vary considerably in composition according to their exact location. A highly innervated tissue that supports and surrounds the tooth is called periodontium, and it is derived from multi-tissue layers including ectoderm, mesoderm, and endoderm primitive tissues. The eruption of teeth corresponds to a major change in the oral microbiome and formation of periodontal niches (sulcular, gingival fluid, and periodontal pocket). There are differences in the microbiome even millimeters apart; supragingival and subgingival dental plaque are evolutionarily selected according to distinct compositions, niche anatomy, antigen and immune exposure, and nutritional backgrounds. Periodontitis is characterized by microbially-associated, host-mediated inflammation resulting in loss of periodontal attachment. The pathophysiology of the disease includes host-derived proteinases, cell heterogeneity, and marginal periodontal loss of ligament fibers and junctional epithelium. The initiation and progression of the lesion is modulated by ecological dysbiosis of the biofilm, attached to the tooth/root surface, and salivary planktonic ecology. In order to control the growth and infiltration of the oral microbes, oral neutrophils patrol healthy gingival tissues which are primed by the molecules and inhabitants of the oral microbiome. Local microbial dysbiosis may lead to a tenfold higher immune cell migration out of the permeable sulcus as compared with the homeostatic control, presenting potential impact to the individual’s health. When unresolved, chronicity of the local lesion alters molecular, cellular, and overall tissue structures.

Studies have reported differences in salivary microbiota diversity between subjects with periodontal disease and healthy subjects. By using metagenomics and metatranscriptomics approaches,
traditional periodontal pathogens such as P. gingivalis, Tannerella forsythia, Parvimonas micra, and Filifactor alocis were identified as transcriptionally active in saliva from patients with periodontal disease [65]. Moreover, members of the elusive Saccharibacteria (the former TM7 phylum) have been implicated both in association with host inflammatory mucosal diseases as well as caries [64,66,67]. In some patients with periodontal disease, a surprisingly high abundance, 21%, of Saccharibacteria was observed [76,77]. Collectively, these findings suggest that facultative anaerobic and anaerobic periodontal pathogens, whose natural habitat is in the gingival pocket, also have the capacity to disseminate distal to the lesions including major blood vessels, which can potentially disseminate systemically. The organization of the oral microbiome is complex and while studies have shown that the oral microbiota is specific to oral niches, host-dependent and distinct in health and disease, much less is known regarding the role of immune activity (e.g., defensins, cytokines, or neutrophil extracellular traps) in shaping the oral microbiome in biofilm states or in planktonic states. Hard-to-reach niches, such as the subgingival milieu, provide technical and biological difficulties related to the understanding of fundamental organizational changes during gingivitis, and periodontitis transitions.
Dental Caries

Dental decay (caries) is one of the most prevalent diseases in the world. Not only does caries disease stem from poor oral hygiene practices, a sugary diet, immunological, and genetic factors but also from the presence of pathobionts such as mutans streptococci and members belonging to the acid-producing Lactobacilli and Bilbobacteria groups [78]. Strains of S. mutans, such as UA159 have been studied for their known cariogenicity and ability to secrete hundreds of millimolar of acidic lactate in just a few minutes resulting in rapid demineralization of tooth enamel and a feedforward selection of acidogenic (acid producing) and aciduric (acid tolerant) community members, which worsen the condition further [79]. S. mutans also produces an insoluble glucan matrix (from sucrose) that allows it to stick to the tooth enamel and prevent diffusion of the acidic

Table 1. Examples of Potential Taxonomic Biomarkers and Studies of Oral Health and Disease

<table>
<thead>
<tr>
<th>Organism Type of study (design/country of investigation)</th>
<th>Refs</th>
</tr>
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<tbody>
<tr>
<td>Periodontal diseases</td>
<td></td>
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<tr>
<td>Porphyromonas gingivalis</td>
<td>[62]</td>
</tr>
<tr>
<td>Bacteroides forsythus</td>
<td></td>
</tr>
<tr>
<td>Treponema denticola</td>
<td></td>
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<tr>
<td>Tannerella forsythiens</td>
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<tr>
<td>Filifactor alocis</td>
<td></td>
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<tr>
<td>Fusobacterium nucleatum</td>
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<tr>
<td>Aggregatibacter actinomycetemcomitans</td>
<td></td>
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<tr>
<td>Prevotella intermedia</td>
<td></td>
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<tr>
<td>Eikenella corrodens</td>
<td></td>
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<tr>
<td>TM7 (various groups)</td>
<td></td>
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<tr>
<td>• Saliva collected from adults in Denmark. Metagenomics and metatranscriptomics sequencing.</td>
<td>[62]</td>
</tr>
<tr>
<td>• Subgingival plaque collected from adults of mixed ethnicity in the USA. Identification by checkerboard DNA–DNA hybridization.</td>
<td>[7]</td>
</tr>
<tr>
<td>• Subgingival and supragingival plaque collected from young adults of Indian/Alaskan natives, adults from the American population in the USA, adults of mixed ethnicity in the USA. Metagenomics analysis and supervised machine learning.</td>
<td>[63]</td>
</tr>
<tr>
<td>• Subgingival plaque collected from adults of mixed ethnicity in the USA. Identification with TM7-specific 16S rDNA primers and sequencing as well as FISH probes.</td>
<td>[63,64]</td>
</tr>
<tr>
<td>Caries</td>
<td></td>
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<tr>
<td>Streptococcus mutans</td>
<td>[65]</td>
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<tr>
<td>Streptococcus sobrinus</td>
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<tr>
<td>Streptococcus parasanguinis</td>
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<tr>
<td>Lactobacillus sp.</td>
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<tr>
<td>Bilbobacterium sp.</td>
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<tr>
<td>Prevotella sp.</td>
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<tr>
<td>Actinomycetes sp.</td>
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<tr>
<td>Propionibacterium sp.</td>
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<tr>
<td>Veillonella sp.</td>
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<td>Granulicatella sp.</td>
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<td>Catonella sp.</td>
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<td>Olsenella sp.</td>
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<tr>
<td>Selenomonas sp.</td>
<td>[63]</td>
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<tr>
<td>Neisseria sp.</td>
<td></td>
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<tr>
<td>TM7 (various groups)</td>
<td></td>
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<tr>
<td>• Dentine collected from adult patients of European, Asian or African descent residing in Sydney, Australia. 16S and 454 sequencing.</td>
<td>[65]</td>
</tr>
<tr>
<td>• Saliva collected from adults in Denmark. Metagenomics and metatranscriptomics sequencing.</td>
<td>[62]</td>
</tr>
<tr>
<td>• Dental plaque collected from adults of mixed ethnicity (Hispanic and Caucasian) in the USA.16S sequencing.</td>
<td>[3]</td>
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<tr>
<td>• Dental plaque and dentin collected from children of mixed ethnicity in the USA. 16S sequencing.</td>
<td>[66]</td>
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<tr>
<td>• Dental plaque collected from children of mixed ethnicity in Australia. Metagenomics sequencing.</td>
<td>[65,67]</td>
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<tr>
<td>Oral cancer</td>
<td>[68,69]</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td></td>
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<tr>
<td>Peptostreptococcus sp.</td>
<td></td>
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<tr>
<td>Prevotella sp.</td>
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<tr>
<td>Fusobacterium sp.</td>
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<tr>
<td>Porphyromonas gingivalis</td>
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<tr>
<td>Capnocytophaga gingivalis</td>
<td>[68,69]</td>
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<tr>
<td>Veillonella sp.</td>
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<tr>
<td>Actinomycetes sp.</td>
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<tr>
<td>Clostridiurn sp.</td>
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<tr>
<td>Haemophilus sp.</td>
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<tr>
<td>Enterobacteriaceae (various genera) Human papillomavirus</td>
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<tr>
<td>• Mucosal tissue collected from adults of mixed ethnicity. Aerobic and anaerobic cultivation, 16S sequencing, gene expression analysis.</td>
<td>[68,69]</td>
</tr>
<tr>
<td>• Oral wash samples collected from adults of mixed ethnicity in the USA. 16S and metatranscriptomics.</td>
<td>[70]</td>
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</tbody>
</table>

Abbreviation: FISH, fluorescence in situ hybridization.

Dental Caries

Dental decay (caries) is one of the most prevalent diseases in the world. Not only does caries disease stem from poor oral hygiene practices, a sugary diet, immunological, and genetic factors but also from the presence of pathobionts such as mutans streptococci and members belonging to the acid-producing Lactobacilli and Bilbobacteria groups [78]. Strains of S. mutans, such as UA159 have been studied for their known cariogenicity and ability to secrete hundreds of millimolar of acidic lactate in just a few minutes resulting in rapid demineralization of tooth enamel and a feedforward selection of acidogenic (acid producing) and aciduric (acid tolerant) community members, which worsen the condition further [79]. S. mutans also produces an insoluble glucan matrix (from sucrose) that allows it to stick to the tooth enamel and prevent diffusion of the acidic
metabolites, which further contributes to its virulence. In the 1990s, Marsh hypothesized that selection of cariogenic bacteria is directly coupled to alterations in the environment that shift the balance of the community [80,81]. If the pH remains below the ‘critical pH’ (value of 5.5) for demineralization for extended time periods (typically after a carbohydrate pulse), a shift in the bacterial populations to more cariogenic organisms that are acidogenic and acid-tolerant (aciduric) can occur [80,82]. Shortly thereafter Marsh developed this concept further and stated the ecological plaque hypothesis, which proposes a homeostasis mechanism that is responsible for regulating the pH and returning the temporary drop in pH back to neutral [4]. Implicit in these concepts is that disease can be prevented, not only by directly inhibiting acidogenic and aciduric caries-associated pathogens, but also by interfering with the environmental factors driving the selection and enrichment of these bacteria [82,83]. After the introduction of deep sequencing technologies, it became clear that caries is a polymicrobial disease where different species are associated with different stages of disease [84,102]. A diverse array of bacteria including non-mutans streptococci and members of the genera Actinomyces, Bilisobacterium, Lactobacillus, Propionibacterium, Veillonella, Selenomonas, and Atopobium have been associated with different stages of carious lesions [84]. Other caries-associated taxa are members belonging to the Granulicatella, Catonella, Prevotella, and Olsenella genera [65].

Oral Cancer
Development of cancer has been involved with specific microbiotas through several mechanisms, especially with the production of toxins, loss of hormonal homeostasis and immune tolerance, induction of chronic inflammatory signals, and induction of carcinogenic metabolites. Several pathogens, especially viruses, have been implicated in human carcinogenesis through well-described genetic mechanisms [68]. Positive associations between specific microorganisms and cancer have been established [85,86]. Helicobacter pylori, for example, has been highly implicated in gastric cancers. Other pathogens have been involved with specific types of cancers, such as Streptococcus bovis in colon cancer, Salmonella typhi in hepatobiliary cancer. Also, human papillomavirus has been implicated in the pathogenesis of both cervical cancer and head and neck cancers (HNCs) [87,88]. HNC includes any cancer that affects the head and neck tissues including, oral cancer, throat cancer, salivary glands, tongue cancer, nose, tonsil cancer, or other areas of the head and neck. An altered oral microbiome environment is now considered a risk factor for HNC. For example, the risk ratio for cancer with subjects with periodontal diseases as a result of microbial dysbiosis was 2.63 (95% confidence interval: 1.68, 414).

Current evidence suggests that multiple human diseases are attributable to global changes in the microbiome [89,90]. In studies of the human gut and esophagus dysbiosis-related inflammation the generation of chemical carcinogens such as acetaldehyde and N-nitroso compounds are among several mechanisms that have been proposed through the microbiota to impact cell transformation to malignancy [91,92]. The likelihood that similar microbiota–host relationships exist in the oral cavity are high, but these still remain unexplored. The gut and oral microbiome compositions differ greatly, thus the metabolism from both niches could be different. While the gut is predominantly influenced by the phylum Bacteroidetes, saliva and oropharyngeal swabs showed that the phylum Firmicutes predominated in the mouth [73]. Gut microbiome studies related to HNCs have expanded quickly, but only limited studies have looked at the oral microbiota. Beyond the microbiome, classic environmental and risk factors such as tobacco and alcohol consumption are also known to modulate the bacteria inhabiting the oral tissues. Neisseria has been associated with high alcohol dehydrogenase activity converting ethanol to acetaldehyde, which is a carcinogen [93]. Similarly, increased salivary levels of acetaldehyde were found in heavy drinkers and smokers [94]. The observed changes influence lipids of inflammation which in turn increases the risk for oncogenesis [95]. Diverse metabolites are affected globally, and whether individuals
vary in their microbial content or not, a hypothesis that metabolic dysbiosis could lead to carcinogenesis is emerging [96]. Future studies investigating the microbial-associated carcinogenic mechanisms, will yield a clearer view to what extent these interactions can provide novel avenues for the development of HNC diagnostics and therapeutics. Several prebiotics and probiotics therapies have been developed to prevent and treat oral diseases and the most common ones are presented in Box 2.

Box 2. Existing Prebiotics to Prevent Oral Disease
Prebiotics support the metabolism of a health-associated microbiota. Detailed discussions of prebiotics developments with regards to caries prevention can be found in the Review articles by Chen and Wang [97] and by Baker [98]. In brief, these reviews present that: (i) the most commonly used oral prebiotics are pectin, gums, and arginine-containing toothpaste; (ii) pectin increases saliva flow resulting in the delivery of ions for tooth remineralization and the clearance of bacteria from the tooth surface; (iii) arginine is a substrate for the microbial production of ammonia, which serves as an alkalizing agent in the dental plaque environment and deactivated metabolism of low-pH thriving bacteria. They also mention that recent studies have identified several other compounds; Met-Pro, succinic acid, beta-methyl-D-galactoside and N-acetyl-D-mannosamine as prebiotic candidates for caries prevention [99]. A broader market for these products has developed over time but clearly avoiding a diet consisting of refined sugars is the best prevention for the production of glucans and formation of cariogenic biofilms on teeth.

Concluding Remarks and Outlook
The era when dental and oral sciences were separated from medicine has passed, and dental medicine has become an important field in health care. Classic and recent studies show that the human body is a complex ecosystem in which microbes and host cells interact continuously through molecular processes and health outcomes. Although recent findings were capable of demonstrating bacterial taxonomic shifts both at the individual and populational levels we have barely revealed the tip of an iceberg with regards to the complexity of host–microbiota interactions. By intensifying studies in these areas of research, it will likely be possible to implement host–microbiome signatures as a clinical chronometer that can track early onset of disease as well as facilitate the development of microbiota-targeted therapies.

References

Outstanding Questions
Which bacterial taxa have we missed that are important for both healthy and diseased states globally?
What are the molecular mechanisms of the oral host–microbiome that allow the transitions between oral health and disease?
How important is bacterial strain variability in oral and systemic virulence?
What has been lost and gained in the microbial composition of disease?
How is the microbiome affecting pharmacological responses to medications, drug resistance, and drug interactions?
What are the mechanistic interactions of the native microbiome and its environment within a dynamic host?
Could we harness knowledge from microbial communities to improve diagnostics of oral and systemic health, pre-disease and disease staging?
Could the host–microbial interactome guide novel targeted therapeutic designs including small molecules, monoclonal antibodies, pre/probiotics and antibiotics?
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