Dysbiosis of the Oral Microbiome

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Abstract The oral cavity is influenced by the dietary characteristics of each individual. It is in the oral cavity that food will cause the first impact within the human body and its microbiome, due to its composition and consistency. On the other hand, the oral microbiome will affect food processing and impact the human gut microbiome, since bacterial biofilm that is processed within saliva forms the food bolus, which will then be swallowed. The mouth is one of the most heavily colonized parts of our bodies and its microbiome consists of microorganisms that live in symbiosis with healthy individuals who have adequate dietary and oral hygiene habits. Nevertheless, perturbations in the microbiome due to certain stress factors, such as high carbohydrate intake and biofilm accumulation, can lead to dysbiosis and the development of oral diseases. The most prevalent diseases in the oral cavity are dental caries and periodontal diseases including gingivitis and periodontitis, but endodontic (pulp) and soft tissue infections are also prevalent. Thus, this chapter will describe the influence of dietary habits on the oral microbiome, the development of prevalent oral diseases, and their relation to the gut microbiome.

Keywords Oral microbiome dysbiosis · Dental caries · *Streptococcus* · Endodontic infection · Periodontal disease

Introduction

The famous quote "we are what we eat" suggests the direct impact of dietary habits and lifestyle on our systemic health. As portal of entry for the digestive system, the oral cavity is greatly influenced by the dietary characteristics of each individual: it is in the oral cavity that food will cause the first impact within the human body,

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since it influences the mouth environment by its composition (leading to pH fluctuation) and consistency (sticky, liquid or hard food). Food is first introduced to the oral cavity to initiate the processing pathway and then swallowed to 'travel' though the gastrointestinal tract. For this reason, a two-way relationship exists: the dietary composition can have direct impact on the oral microbiome composition, activity, and local disease development; on the other hand, the oral microbiome, the transient microorganisms and the functional components of the oral cavity will affect food processing and impact the human gut microbiome, since oral biofilm is the major microbial constituent of the saliva that facilitates the formation of the food bolus that is subsequently swallowed (see chapter "Baby's First Microbes: The Microbiome of Human Milk").

The oral cavity provides different oral structures and tissues for bacterial colonization and community development, including saliva, gingival fluid, and keratinized/non-keratinized epithelial or mineralized tooth surfaces, such as the tongue, gingiva and teeth (Kolenbrander 2000; Aas et al. 2005; Simón-Soro et al. 2013a, b). The mouth is one of the most heavily colonized parts of our bodies and, as explained in the first chapter of this section, the microbiome of the oral structures consists of microorganisms that live in symbiosis with healthy individuals who have favorable dietary and oral hygiene habits. This balance is possible due to the diverse microbial communities that prevent the colonization of foreign pathogens and contribute to a healthy host physiology (Hezel and Weitzberg 2015). Nevertheless, perturbations in the microbiome due to certain stress factors, such as high carbohydrate intake, undisturbed biofilm development, and/or saliva alterations in volume or composition, can lead to imbalances in the symbiotic composition of the commensal populations and the development of oral diseases (Marsh 1994, 2016). There are both shifts in species and their functional expressions associated with dysbiosis characteristic of both caries and periodontal diseases (Belda-Ferre et al. 2012; Griffen et al. 2012; Wang et al. 2013; Jorth et al. 2014).

The oral cavity must be recognized as a complex macro ecosystem, composed of different microhabitats with distinct characteristics that either favor or prevent different species from establishing. It has been shown that different microbiome profiles can be verified among different tooth surfaces in the same individual, but very similar microbiome profiles are observed on the same tooth surfaces within different individuals. For example, *Streptococcus* were found at high abundance on the buccal surfaces of teeth and sulci, but were found at lower levels on the lingual surfaces of the same tooth (Simón-Soro et al. 2013a, b). In terms of structure, mucosal surfaces (constantly shedding) will favor microorganisms that can express unique receptors with high affinity to rapidly re-adhere to the newly exposed mucosal cell; whereas, other microorganisms accumulate in dental plaque mainly in protected areas of the tooth, such as the occlusal and the interproximal surfaces. Teeth are the only natural non-shedding surfaces in the human body and provide unique opportunities for undisturbed biofilm formation and sustained fermentation of dietary carbohydrates sufficient to permit accumulation of metabolic end-products such as lactic acid to alter the environmental pH (Marsh and Devine 2011). Teeth can also be recognized as harboring different micro-ecosystems, since they present

differentiated habitats with singularities such as differences in saliva access and flow, oxygen availability, different temperatures, pH and food retention. For example, smooth free surfaces of the tooth (buccal and lingual) are constantly being "washed" by salivary flow, are highly aerated and are subjected to sheer forces from the lips, tong and other teeth during mastication. It leads to a development of biofilm in an ordered fashion, located closely to the gingival margin, markedly with bacteria that have strong adhesins to the pellicle that coats the enamel. On the other hand, protected surfaces, such as that of the pit and fissures on occlusal surfaces, and the interproximal spaces (in between contacting teeth) are characterized by a compacted biofilm formed mostly by short rods and often *Actinomyces* spp. in a condensed inner layer, and a looser biofilm layer is seen with a random arrangement of bacteria, including *S. mitis*, *Veillonella* spp. and *Fusobacterium* spp. (Dige et al. 2014). The most prevalent diseases in the oral cavity are dental caries and periodontal diseases including gingivitis and periodontitis, but endodontic (pulp) and soft tissue infections are also prevalent. These diseases are mainly caused by the oral microbiome imbalance, which may also play an important role in altering the homeostasis of systemic conditions, including gut diseases and its microbiome. Thus, this chapter will describe the influence of dietary habits on the oral microbiome, the development of these diseases, and their relation to the gut microbiome.

Dental Caries as a Dysbiosis

Despite all the knowledge and years of research, dental caries remains the most common chronic disease in the United States (NCHS—National Center for Health Statistics 2017), as well as in the rest of the world (Bagramian et al. 2009; Bourgeois and Llodra 2014). Recent data from the CDC showed that, in the US, the prevalence of untreated cavities among children remains high, affecting 19.5% of children between the ages of 2 and 5 years and 22.9% of children and adolescents aged 6–19 years. Dental caries is four times more common than asthma among adolescents aged 14–17 years, and it also affects 9 out of 10 adults older than 20 years (NCHS—National Center for Health Statistics 2017). Although the percentage of untreated dental caries declined steadily from 39.0% in 1988–1994 to 24.7% in 2011–2014 for children and adolescents aged 5–19 living below the federal poverty level, this percentage was similar in 1988–1994 and 2011–2014 for adults of all income levels (NCHS—National Center for Health Statistics 2017).

Dental caries can be defined as a biofilm-mediated dysbiosis (Fig. 1a). It is characterized by the dissolution of tooth tissues (enamel and dentin) by acid produced by oral bacteria as a result of the fermentation of dietary carbohydrates. When the fermentation process is enhanced by the excessive and/or frequent ingestion of fermentable sugars, the buffering capacity of saliva overwhelmed and the sustained local reduction in pH leads to the demineralization of enamel, cementum, and dentin. Due to the highly dynamic nature of the disease, resulting from continuous physical-chemical interactions between the tooth surface and biofilm that covers the

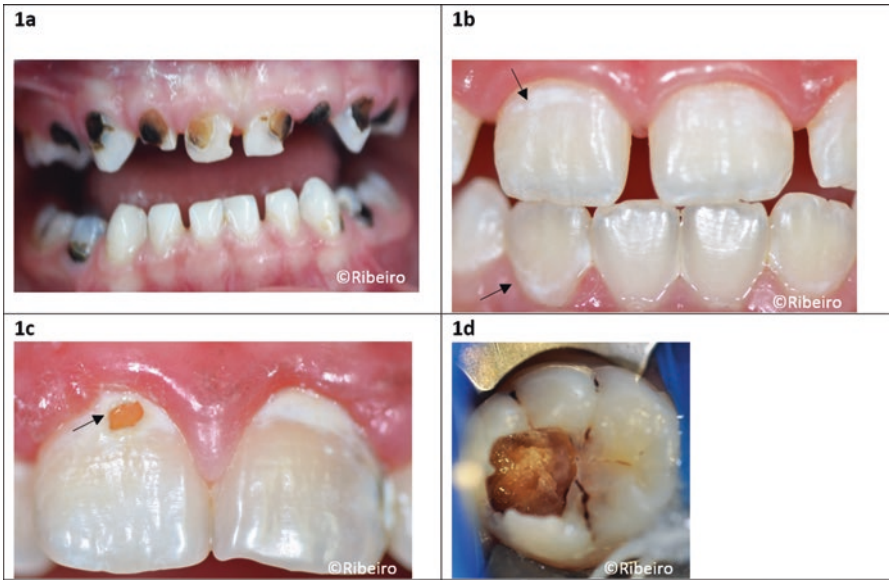

Fig. 1 Clinical pictures of dental caries. (**a**) Patient with multiple lesions caused by dental caries disease; (**b**) Incipient caries lesion, known as active white spot lesion (arrows), is the first clinical sign of caries disease; (**c**) Dentin cavity, a clinical sign of lesion development, from white spot lesion, to cavitation (arrow); (**d**) dentin tissue destruction caused by proteolytic enzymes produced by bacteria from the biofilm

surface, multiple pH fluctuations in the biofilm lead to episodes of mineral loss (demineralization) and mineral gain (remineralization) of the teeth. If equilibrium of these episodes is not achieved over time, demineralization will reach the level when an incipient lesion, known as active white spot lesion (Fig. 1b), can be visually detected by a trained professional (Xu et al. 2014).

Chemically, the lesion is characterized by the dissolution of the calcium and phosphate constituents of enamel. The acid production depends on the carbohydrate intake from dietary sources. If the demineralization episodes exceed the remineralization process, the development of the disease will not be controlled, and the lesion will not be arrested. Clinically, the destruction of the enamel tissue progresses with a breakdown of the superficial layer, leading to a cavity that is more prone to accumulate biofilm (Fig. 1c) and the lesion is more likely to rapidly progress, affecting the underlying tissue, called dentin. At this point, a change in the bacterial composition and metabolic profiles can be observed, with species that produce proteolytic enzymes capable of affecting the collagen fibers that compose dentin (Fig. 1d).

The bacterial microbiome from dental biofilms can harbor more than 720 unique species. 800 to 1000 different oral bacterial taxa (as sharing >98.5% 16S ribosomal RNA (rRNA) sequence identity) can be identified with more modern techniques with differences in abundance and diversity patterns across age, sample quality and origin, and health status (Dewhirst et al. 2010). Since diversity on oral health was

the theme of the previous chapter, the focus now will be towards the core group of phylotypes found under diseased conditions (Becker et al. 2002; Ling et al. 2010; Ribeiro et al. 2017), that are different from those observed in healthy conditions (Bik et al. 2010; Dewhirst et al. 2010; Zaura and Mira 2015). However, it is important to recognize that it is still necessary to find consistent bacterial markers across studies and cohorts.

A key aspect to consider in relation to caries dysbiosis is that the development of carious lesions does not occur in all teeth nor on all surfaces of a tooth at the same time nor with the same intensity. Although many studies compared the microbiome associated with caries activity by using pooled samples (for example, saliva and pooled biofilm from multiple tooth surfaces) (Li et al. 2007; Aas et al. 2008; Bik et al. 2010; Peterson et al. 2013), it is important to recognize the highly local nature of the microhabitats responsible for disease progression and that pooled sampling will compromise the resolution of the microbial composition at the involved sites. A study conducted by Dige et al. (2014) showed the spatial distribution of bacterial taxa *in vivo* at various stages of occlusal caries, applying a molecular methodology involving fluorescence in situ hybridization (FISH) and confocal microscopy. *S. mutans* could be observed on sites with both active and inactive caries, but not on clinically sound enamel; whereas, *Bifidobacterium* spp. were only detected in sites with active caries. *Lactobacillus* spp. was not detected on clinically sound and non-cavitated sites.

By using 16S rRNA amplicon sequencing combined with the BLASTN-based search algorithm for species identification, a recent study compared healthy and caries active occlusal tooth surfaces from 12-year-old children. It was found that the sites varied not only among individuals, but also among caries samples from the same individual. Interestingly, the same levels of members of the genera *Streptococcus*, *Pseudomonas*, *Granulicatella, Actinomyces, Prevotella and Veillonella*, traditionally associated with caries active patients, were found on both sound surfaces and active white spot lesions, but the percentage of *Actinobaculum* and *Porphyromonas* were higher in active white spot lesions. On the other hand, the numbers of *Klebsiella and Acinetobacter* species were higher on sound surfaces. The presence of eight bacterial taxa were observed in active carious sites (*Abiotrophia defectiva, Actinomyces sp._Oral_Taxon_448, Propionibacterium acidifaciens, Actinobaculum sp._Oral_Taxon_183, Streptococcus gordonii, Streptococcus sp._Oral_Taxon_064*, *Streptococcus oralis, Streptococcus pneumoniae* and *Rothia dentocariosa*). Five bacterial taxa (*Lactobacillus johnsonii, Actinomyces gerencseriae, Actinomyces naeslundii, Cardiobacterium hominis* and *Streptococcus sp.*_Oral_Taxon_B66) were present at significantly higher proportions in the biofilm from healthy occlusal surfaces (Ribeiro et al. 2017).

Another oral microbiome investigation, conducted on saliva samples from adults with dental caries, reported that higher levels of two bacterial taxa (*Streptococcus salivarius* and *Solobacterium moorei*) and three bacterial clusters (*Streptococcus parasanguinis I* and *II* and sp. clone BE024_ot057/411/721, *Streptococcus parasanguinis I* and *II* and sinensis_ot411/721/767, *S. salivarius* and sp. clone FO042_ot067/755) were found compared to individuals without caries activity (Belstrøm et al. 2016).

As a recognized complex disease, dental caries has been identified as a two-step process, namely initiation/demineralization of enamel followed by progression through dentin, characterized by a succession of microorganisms (Simón-Soro et al. 2013a, b). Healthy biofilms are characterized by high numbers of species, while mature biofilms express lower bacterial diversity because it requires special abilities from the microorganism to survive and to overcome a hostile environment. For example, *S. mutans*, one of the most studied bacterial specie involved in caries initiation and progression, can harbor different virulence factors such as the ability to form biofilm by the synthesis of adhesive glucans from sucrose by the action of three glucosyltransferases (GtfB, GtfC, GtfD; encoded by *gtfB*, *gtfC* and *gtfD*, respectively) (Merritt and Qi 2012) and glucan-binding protein (Banas and Vickerman 2003). This biofilm-forming capacity varies widely among strains (Merritt and Qi 2012; Banas and Vickerman 2003). Other virulence factors include the cell surface protein antigen c (Pac), responsible for bacterial adherence to the salivary pellicle (Palmer et al. 2013); production and excretion of organic acids, such as lactic acid; and the production of antibacterial bacteriocins, such as mutacins I and IV (Paes Leme et al. 2006). Species other than *S. mutans,* such as *S. sobrinus, Rothia dentocariosa, Actinomyces* species and *S. salivarius* are also related to the early stages of dental caries due to the genetic virulence repertoire that allows these species to set up the environment for more acid-tolerant and acidogenic species, including *Scardovia wiggsiae* and *Actinomyces sp.* HOT 448 (Kressirer et al. 2018).

The cariogenic biofilm is characterized, then, by bacterial species with the ability to: (1) adhere to the tooth surface, (2) produce water-insoluble exopolysaccharides (EPS)-rich matrix, which will limit the diffusion of the carbohydrate fermentation end products (acids) and, (3) survive in this environment with organic acid accumulation due to the presence of a diffusion-limiting EPS-rich matrix. This causes an acid dissolution of the enamel mineral due to the localized acidic pH microenvironments across the biofilm structure and at the tooth-biofilm interface (Ilie et al. 2012; Xiao et al. 2017).

Knowledge obtained through NGS technology has enabled research of traditional bacterial species, which have been investigated for years and are considered the most cariogenic species (Loesche 1986; Lang et al. 1987; Alaluusua et al. 1996; Burt et al. 1998; Harris et al. 2004; Palmer et al. 2010; Kanasi et al. 2010). A recent study showed that both healthy and diseased sites show high relative abundance of *Streptococcus mutans* and low abundance of *Streptococcus sobrinus* and a relationship between these species and the presence of active white spot lesions could not be observed (Ribeiro et al. 2017). Bacterial species other than *S. mutans* and *S. sobrinus*, e.g., species of the genera *Lactobacillus, Prevotella, Propionibacterium*, non-*mutans* streptococci and *Actinomyces* spp., may also play important roles in caries initiation and biofilm community interactions (Aas et al. 2008; Simón-Soro et al. 2014; Ribeiro et al. 2017).

Since initiation and progression of dental caries are carbohydrate-dependent, some studies also investigated the influence of dietary habits on the oral microbiome related to dental caries. The first dietary stimuli have a strong influence on the etiology of caries at later developmental stages. For example, meta-analyses have

shown that early childhood caries (ECC) is more frequent in bottle-fed children. The disease is characterized by a high caries activity and rapid tooth destruction in 3-month-old babies up to the age of 3 years (Avila et al. 2015) due to frequent sucrose intake at an early age, influencing increased colonization with acidogenic (acid producing) and aciduric (acid tolerant) cariogenic bacteria. Many studies also found that *S. mutans* and *S. sobrinus* are the predictive factors for dental caries (Alaluusua et al. 1996; Burt et al. 1998; Kanasi et al. 2010; Harris et al. 2004; Loesche 1986; Palmer et al. 2010). However, bacterial species from genera *Lactobacillus, Prevotella, Propionibacterium* and *Actinomyces* are also related to caries initiation and progression (Aas et al. 2008*;* Simón-Soro et al. 2014; Ribeiro et al. 2017). Additionally, sucrose is a substrate for the production of extracellular and intracellular polysaccharides, two components that determine biofilm formation and structure (Paes Leme et al. 2006). Thus, the constant intake of fermentable sugars in daily diet results in increased carbohydrate fermentation by acidogenic bacteria, which results in lactic acid production, followed by longer periods of low pH and the selection of aciduric bacteria, such as *S. mutans*, Lactobacilli and Bifidobacteria, that survive under these conditions (Marsh 1994, 2016).

Among the highly abundant species observed in biofilm from adolescent patients with high and frequent carbohydrate intake, *Lactobacillus* spp. showed higher counts in dental biofilms *in situ* in the presence of glucose + fructose and sucrose, and correlations were also found between intake of confectionery-eating events and lactobacillus levels among 12-year-old schoolchildren (Beighton et al. 1996). In addition, the association between relative abundance of bacterial species and frequency of carbohydrate intake (high vs low consumption) was shown by Ribeiro et al. (2017): among 12-year-old patients with high frequency of carbohydrate intake (more than two times between meals), statistically significant differences in the increased relative abundance were observed among *Actinomyces gerencseriae, Actinomyces naeslundii, Lactobacillus crispatus* and *Streptococcus vestibulares.*

The relationship between fermentable carbohydrate intake and oral microbiome in adult populations showed that mutans and non-mutans streptococci of several types, including *S. sanguinis* and *S. salivarius*, are known to be extremely abundant in the mouth and present acidogenic and acid-tolerant properties (Guggenheim 1968; Nyvad and Kilian 1990). However, concerning their relation the development of caries, some data suggest an inverse relationship of *S. sanguinis* and abundance of mutans streptococci (Loesche and Straffon 1979). On the other hand, lactobacilli are known as highly acidogenic from carbohydrates as well as being extremely acid tolerant.

As for the influence of dietary habits on the oral bacterial metabolism, a metatranscriptomic approach was used to investigate the active oral microbiota before and after a carbohydrate meal (Benítez-Páez et al. 2014). It was found that the metabolism of the microbiota changed, irrespective of the quality of the diet of the individual. Interestingly, no changes were observed in one individual who had never had dental caries, indicating a strong resilience (that is, a high capacity to overcome stress factors and recover from perturbations). Thus, it is an important factor for oral homeostasis since inadequate resilience can lead to oral diseases when disease drivers are strong or sufficiently persistent.

Fermentable carbohydrates will lead to decreased biofilm pH due to bacterial metabolism. Microbial communities located in acidic pH strata biofilms show low diversity of microbial populations, with *Lactobacillus* species being prominent. In comparison, the distinctive species of a more diverse flora are associated with more neutral pH regions of carious lesions, including *Alloprevotella tanerrae*, *Leptothrix* sp., *Sphingomonas* sp. and *Streptococcus anginosus* (Kianoush et al. 2014). These findings were also observed by a more recent study that showed that the high consumption of fermentable carbohydrates was associated with a reduction in bacterial diversity.

Altogether, these observations highlight the non-specific source, polymicrobial nature, and complex metabolic and community dynamics of dental caries and provide a deeper understanding of the differences in bacterial composition associated with health and initial development of caries, and the influence of the diet in the microbiome composition and metabolism.

Endodontic Infections

If dental caries is left untreated, the lesion can progress through the dentine into the root canal that contains the vasculature and innervation (pulp) that maintains the vitality of the tooth. The pulp becomes infected associated with inflammation and pain and ultimately dies. Infectious agents can also reach the pulp through dentinal tubules when the distance between the approaching border of the carious lesion and the pulp is sufficiently small. There can also be direct pulp exposure resulting from fractures or failing restorations and salivary contamination.

Due to the characteristics of the vasculature of the pulp, infections could potentially result from bacteremia. Infections of the dental pulp are generally polymicrobial in nature with anaerobic proteolytic bacteria dominating (Munson et al. 2002) presumably due to the necrotic nature of the environment. Persistent infection can progress through the foramen of the root tip resulting in an abscess in the alveolar bone presenting as a periapical lesion (periapical periodontitis).

Interestingly, in refractory (persistence following treatment) endodontic infections, the most commonly identified species cultured are the Gram positive enterococci. These are considered commensal inhabitants of the gastrointestinal tract and are not normally found in the mouth in health, resulting in some controversy as to the source of this infection. It is possible that they are introduced through transient bacterium from the gut (Goh et al. 2017). In regard to the fermented food topic of this volume, it has been demonstrated that enterococci are found in both unpasteurized and pasteurized cheeses and can persist in the mouth for some time after their consumption (Razavi et al. 2007). Further studies have demonstrated that enterococci can gain access to the root canal by microleakage through the temporary filling materials used between endodontic treatment visits (Kampfer et al. 2007). It therefore has been suggested that cheese and perhaps other fermented dairy and

meat products might be a source of enterococci infection challenge during the endodontic treatment course and that patients should avoid eating foods known to be colonized by enterococci (Goh et al. 2017; Wade 2013).

Periodontal Diseases: Gingivitis and Periodontitis

As discussed previously, the biofilm that accumulates on the tooth surface exposed to the oral cavity (supragingival plaque) is saliva-bathed, composed mainly of saccharolytic, acidogenic and aciduric populations of bacteria selected by dietary sugar on nutrient poor enamel surfaces of the teeth. In contrast, there are unique ecological niches created by the architecture and dynamics of the supporting structures of the teeth that select for asaccharolytic, nutritionally fastidious, acid-intolerant, proteolytic anaerobes.

The attachment of epithelium to teeth in a healthy dentition occurs at the transition from the enamel surfaces of the crown of the tooth to the cementum of the root (cemento-enamel junction) forming a thin barrier (junctional epithelium) that protects the underlying supporting structures. The teeth are suspended in sockets in the alveolar bone of the jaws by periodontal ligament. The gingiva (gums) create a sulcus (gingival crevice) surrounding teeth that is composed of unique specialized gingival epithelial cells and keratinocytes. This arrangement creates a close association between the non-sloughing hard surfaces of the teeth and the renewable soft tissue of the gingiva that limits accessibility of saliva and provides microbial attachment sites on both mineral and cell surfaces bathed in a protein rich, tissue-derived gingival crevicular fluid (GCF) of this subgingival space. This relatively sequestered site if undisturbed permits a hierarchical development of complex biofilm communities driven by environmental alterations in nutrient availability, oxygen limitations, specific interspecies co-aggregations, synergisms and antagonisms.

As with the supragingival plaque, the early subgingival colonizers are predominated by facultative anaerobes including the saccharolytic streptococci and actinomycetes. If the biofilm is permitted to develop without mechanical disruption (oral hygiene), robust bacterial species such as *Fusobacteria* and *Prevotella* (Ramberg et al. 2003) neutralize the pH of this subgingival environment by nitrogenous metabolism and stimulate increased efflux of GCF further promoting proteolytic activity allowing a shift in the microbial communities toward the establishment of more acid-intolerant, oxygen-sensitive, more diverse, inflammation-promoting and potentially periodontopathic species.

Gingivitis

Gingivitis is arguably the most common bacterial disease of humans with a prevalence greater than 90% in adults (Coventry et al. 2000). Following meticulous cleaning of the teeth, the gingival margins proximal to the gingival crevice are

rapidly repopulated (within hours) with pioneer colonizers predominated by Gram positive, aerotolerant anaerobes including streptococci and actinomycetes (Nyvad and Kilian 1990; Ramberg et al. 2003; Li et al. 2004). This initial adherence is favored by the selective affinity of these bacteria for epitopes of the salivary proteins that specifically adsorb to tooth surfaces (pellicle) and coat the epithelium (Murray et al. 1992). If left undisturbed, the accumulating biofilm of these primary colonizers provides new attachment sites for selected other species through specific co-aggregation interactions (Kolenbrander et al. 2006) and by metabolic reduction in oxygen tension favoring more anaerobic Gram negative species including *Fusobacterium, Treponema* and members of the phylum *Synergistetes* (Zijnge et al. 2010). This increase in the proportions of Gram negative, asaccharolytic and anaerobic bacteria results in the accumulation of endotoxins, metabolic end-products and lytic enzymes that irritate the gingivae activating pro-inflammatory pathways resulting in the clinical signs of gingivitis, including red, swollen and inflamed gums that bleed either spontaneously or on gentle probing. These clinical presentations are entirely reversible with restoration of effective oral hygiene (Loe et al. 1965). It is generally considered that there are no specific pathogens associated with gingivitis, but rather plaque load and especially its level of maturity (transition to Gram negative anaerobes) correlate with disease severity (Socransky 1977). Because established gingivitis is frequently not painful, it can remain undiagnosed in the absence of routine dental care, and thus go untreated for many years without progressing to irreversible periodontitis.

Periodontitis

Periodontitis is a bacterially-induced chronic inflammatory disease of the periodontium that includes not only inflammation of the gingiva, but also destruction of the tissues that surround and support the teeth including the periodontal ligament and the alveolar bone. In susceptible individuals, inflammation in the gingival tissues results in the destruction of the epithelial and connective tissue attachments to the tooth through the activities of neutral proteases, elastases, collagenases and metalloproteinases (Smith et al. 1995; Golub et al. 1997; Hernández et al. 2010). In an attempt to repair, the junctional epithelium responds to the damage by migrating toward the apex of the tooth possibly due to the proteolytic activity of degranulating neutrophils within the gingival environment (Bosshardt and Lang 2005; Eskan et al. 2012). This is measured clinically as attachment loss by calibrated dental probing as a metric of periodontal disease severity. This retreating attachment of the connective tissue results in a deepening of the sulcus forming a periodontal pocket providing an anaerobic environment and neutral pH favoring asaccharolytic, proteolytic anaerobes (Eggert et al. 1991) creating a vicious cycle. The resulting biofilm ultimately provokes a chronic inflammatory response in the surrounding connective tissue that drives the destruction of the alveolar bone that supports the tooth (Armitage 2004).

While accumulation of biofilm triggers gingivitis, the presence of biofilm alone is not sufficient to progress to periodontitis as evidenced by the clinical course observed with untreated chronic gingivitis mentioned above. It is now evident that complex interactions between immune response elements of the host with the biofilm are required for progression to periodontitis. In this scenario, it is proposed that most of the tissue damage is due to a dysbiotic microbial community's subversion of the host response leading to an inappropriate, exaggerated inflammatory response (Darveau 2010; Kilian et al. 2016). The resulting local inflammation provokes an increased flow of the nutrient-rich gingival crevicular fluid possibly associated with bleeding and a reduction in oxygen favoring a shift from a symbiotic microbial population to the more nutritionally fastidious, protein-dependent obligate anaerobic dysbiosis (Marsh et al. 2015). The resulting inflammation damages the sulcular epithelium providing red blood cells for bacterial hemolysis and release of hemoglobin for processing by heme-dependent bacteria such as *Porphyromonas gingivalis*.

Since the 1950s, investigators have sought to identify the microbial species critical for the initiation and progression of periodontitis. From these studies, it was clear that there were profound shifts in the microbial community structures that were associated with the transition from a healthy gingiva to disease and specific organisms were proposed as potential periodontopathogens based on culture biases and virulence properties identified in animal models. In 1994, Socransky and colleagues employed checkerboard DNA-DNA hybridization techniques that permitted enumeration of then relatively large numbers of species in very large numbers of samples. Using 40 species-specific DNA-DNA hybridization probes to quantitate oral bacteria in the subgingival plaque samples from healthy and periodontally diseased sites (Socransky et al. 1998), they first advanced the idea of discrete microorganisms working together to cause disease. They defined five different "complexes" based on their level of association with disease severity. These complexes were color coded with the most highly associated with chronic severe periodontitis identified as the "red complex" that included three species: *P. gingivalis, Tannerella forsythia* and *Treponema denticola*. Although sometimes present in low numbers in healthy subjects (Kumar et al. 2003), the red complex was considered to be responsible for initiation and progression of disease. The disappearance (or significant reduction) of red complex was associated with successful periodontal treatment and again became prominent when inflammation and deep pockets reappeared thus fulfilling a modification of Koch's postulates (Socransky 1977). The "orange complex" demonstrated a less stringent association with disease, but were considered foundational for the subsequent colonization by the "red complex" and included among others *Prevotella* spp., *Fusobacterium* spp. and *Parvimonas micra*. On the other end of the spectrum, members of the "yellow complex" (*Streptococcus gordonii, Streptococcus intermedius, Streptococcus mitis, Streptococcus oralis* and *Streptococcus sanguinis*) and of the "purple complex" (*Actinomyces odontolyticus* and *Veillonella parvula*) were mainly associated with healthy sites. The selection of the probes used in these studies was by necessity based on culture data and were

therefore restricted by the same biases that confounded other culture-dependent studies.

Using more contemporary sequencing approaches, the power to study bacterial community compositions has grown exponentially and has facilitated identification of novel associations between periodontitis and previously uncultivable or previously underappreciated species including the Gram positive *Filifactor alocis* (Griffen et al. 2012) and *Peptostreptococcus stomatis* and species from the genera *Prevotella, Synergistes* (Vartoukian et al. 2009), *Megaspaera, Selenomonas* and *Desulfobulbus* (Kumar et al. 2003; Dewhirst et al. 2010). Many of these species correlate as strongly with disease severity as do the classic "red complex". It is now clear that periodontitis is a polymicrobial infection that arises from the expansion of so-called pathobionts within the microbial community that leads to dysbiosis-associated pathologies (Hajishengallis 2014). This shift in dominance from symbionts to pathobionts appears to be driven by low prominence microorganisms (keystone pathogens) that are capable of modulating the host response and possibly the pathobionts directly (Frias-Lopez and Duran-Pinedo 2012) leading to an alteration in the nutrient foundation of the community through a subverted inflammatory response. *P. gingivalis* has long been associated with human periodontitis and is capable of orchestrating disease in a variety of animal models. Recent studies suggest that its role is more consistent with that of a keystone pathogen in that it is not a potent inducer of inflammation, but rather can impair host innate and adaptive defenses in ways that alter the growth, composition and development of the entire microbial community resulting in homeostatic disruption driving commensals toward pathobionts that deregulate inflammation causing bone loss (reviewed in Hajishengallis 2014; Costalonga and Herzberg 2014). The inflammatory destruction of host tissues provides a nutrient-rich inflammatory exudate (e.g. degraded host proteins and hemin) favoring the growth of asaccharolytic and proteolytic bacteria resulting in a dysbiotic shift in the microbiota further altering the environment to create new niches for sustaining and expanding the periodontopathic communities at the expense of the homeostatic symbionts associated with periodontal health.

Dietary Influences on Periodontal Diseases

There is evidence that periodontal diseases are influenced by diet. For example, vitamin C depletion can lead to profuse gingival bleeding, lower serum magnesium/calcium levels, lower antioxidant micronutrient levels, and lower docohexanoic acid intake have also been shown to significantly correlate with higher levels of periodontal diseases (reviewed in Chapple et al. 2017). Vitamin B12 deficiency was also associated with periodontal disease progression and bone and periodontal ligament destruction (Zong et al. 2016).

In relation to carbohydrates, subjects on a high-carbohydrate diet develop gingivitis because it increases the risk of inflammation and thus gingival bleeding (Hujoel 2009; Sidi and Ashley 1984), whereas a switch to a "Stone Age" diet, based on

whole grains of barley, wheat, herbs, honey, milk, and meat from domestic animals (goats and hens), resulted in a decrease in gingival bleeding (Baumgartner et al. 2009). Thus, fermentable carbohydrates (sugars and starches) are recognized as the most relevant common dietary risk factor for periodontal diseases, because glycemia drives oxidative stress and advanced glycation end-products may also trigger a hyper inflammatory state (reviewed in Chapple et al. 2017).

Thus, there is evidence that together with sugar restriction, functional foods may improve clinical treatment outcomes following the adjunctive ingestion of fruit and vegetable extracts (Chapple et al. 2012) and probiotics (Martin-Cabezas et al. 2016), although evidence is limited and biological mechanisms not fully elucidated (Chapple et al. 2017). There is also an intriguing study that suggests that a diet that includes frequent ingestion of fermented foods might positively influence periodontal health (Takeshita et al. 2014). This study compared the salivary microbiomes of orally healthy adult participants from a representative community in Japan with that of a cohort from South Korea. This selection was based on national surveys that suggested that South Koreans had better periodontal health than that of Japanese, despite their similar inherent backgrounds. The microbiota of the Japanese individuals comprised a more diverse community, with greater proportions of 17 bacterial genera, including *Veillonella*, *Prevotella* and *Fusobacterium*, compared to the higher proportions of *Neisseria* and *Hemophilus* species found in Korean saliva samples. A previous study by this group found that salivary microbiomes with larger proportions of *Prevotella* and *Veillonella* were associated with periodontitis; whereas, larger proportions of *Neisseria*, *Hemophilus* and *Porphyromonas* were associated with periodontal health (Takeshita et al. 2009). Therefore, the salivary microbiome composition of the Korean cohort could be considered healthier than that of the Japanese subjects, even though all of these individuals were orally healthy. The authors noted that there are major differences in the diets of these two cohorts and suggested that the Korean preferences for spicier foods and especially for the fermented vegetables, kimchi, might contribute to these differences in their microbiomes (Takeshita et al. 2014).

The Influence of Oral Health on the Gut Microbiome

To the best of our knowledge, there are no studies that show that caries dysbiosis has a direct impact on the gut microbiome. Thus, any correlation between presence of active dental caries and gut microbiome remains unclear and further investigations are highly recommended.

However, it is well known that the gut microbiome is highly impacted by the quality of food intake. The microorganisms that reside in the human colon fulfill their energy requirements mainly from diet- and host-derived complex carbohydrates. Individual bacterial species exhibit different preferences for the same set of glycans and this maintains a competitive environment, which promotes stable coexistence and shows that predictable changes in the gut microbiota can improve health through diet (Tuncil et al. 2017).

The use of probiotics can contribute to bacterial resilience (bacterial capacity to recover from perturbations caused by disease drivers) and, thus, represents beneficial functions (e.g., preventing biofilm acidification, biofilm accumulation, or harmful inflammation) (See chapter "Microbial Manipulation of Dysbiosis: Prebiotics and Probiotics for the Treatment of Oral Diseases"). A recent systematic review of 50 studies (3247 participants) concluded that current evidence is insufficient for recommending probiotics for managing dental caries (Gruner et al. 2016). However, instead of using general dairy products or gut-associated bacteria, the identification of new probiotic species without acidogenic characteristics and the development of individualized treatments could improve these results in the future (López-López et al. 2017). The idea for the oral cavity would be to obtain indigenous probiotic species or communities with certain beneficial functions (e.g., arginolytic pathways produce ammonia or denitrification pathways produce nitric oxide) to compete for the bacterial sites and food consumption, and, instead of decreasing the biofilm pH, raising it or maintaining it to a neutral level (Rosier et al. 2017).

Another interesting observation was published recently by Yasuda et al. (2017) while investigating the effect of the use of systemic and topical fluoride in the oral and gut microbiome. Topical and systemic fluoride are regularly used products for dental caries prevention and treatment due to their capacity for increasing mineral remineralization of the dental tissues and inhibiting energy harvest in oral cariogenic bacteria (such as *S. mutans* and *S. sanguinis*). Fluoride also inhibits bacterial growth by inhibiting the enzyme enolase, which catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate (the last step of anaerobic glycolysis), thus leading to bacterial depletion (Marquis 1995; Qin et al. 2006). By treating mice with low or high levels of fluoride over a 12-week period (fluoride exposures at levels commonly found in municipal water and dental products), followed by 16S rRNA gene amplicon and shotgun metagenomic sequencing, they found changes in oral microbiome in both the low- and high-fluoride groups. Several operational taxonomic units (OTUs) belonging to acidogenic bacterial genera (such as *Parabacteroides*, *Bacteroides*, and *Bilophila*) were depleted in the oral community. In addition, fluoride-associated changes in oral community composition resulted in depletion of gene families involved in central carbon metabolism and energy harvest (2-oxoglutarate ferredoxin oxidoreductase, succinate dehydrogenase, and the glyoxylate cycle). However, fluoride treatment, exposure at physiological levels, did not induce a significant shift in the overall composition of the oral microbiome, and even on the established gut microbiome or function, possibly due to absorption in the upper gastrointestinal tract. Fluoride-associated perturbations thus appeared to have a selective effect on the composition of the oral, but not on the gut microbial community.

Systemic Consequences of Oral Dysbiosis

Oral bacteria have been proposed to play a role in a number of human systemic diseases, including atherosclerotic cardiovascular disease (Dietrich et al. 2013), stroke, abnormal pregnancy outcomes, rheumatoid arthritis, respiratory tract

infections including pneumonia, meningitis or brain abscesses, inflammatory bowel disease and colorectal cancer (Scannapieco and Binkley 2012; Dewhirst et al. 2010; Han and Wang 2013; Chapple and Genco 2013; de Pablo et al. 2009) and even links to Alzheimer's disease (Shoemark and Allen 2015). Dysbiosis in periodontal disease likely triggers bacteremia facilitating systemic dissemination of oral bacteria (Forner et al. 2006). Dissemination has been demonstrated for select strains (but not all) of *P. gingivalis* through modifications in vascular permeability and septicemia from sequestered sites in animal models (Genco et al. 1991). Oral administration of *P. gingivalis* in a mouse model had direct effect on the gut microbiome and provokes inflammatory changes in various tissues and organs (Arimatsu et al. 2014). Is it possible that *P. gingivalis* can play a role as a keystone pathogen in the gut? It is well known that severe periodontitis negatively impacts glycemic control, not only in diabetes, but in subjects without diabetes. Severe periodontitis is an established risk factor for the onset of type 2 diabetes, and periodontal disease severity correlates with diabetic complications (Chapple and Genco 2013). It therefore follows that good oral hygiene is important not only to dental health maintenance, but should also be considered for controlling total microbial load that disseminates to or influences extra-oral infections and inflammation (Han and Wang 2013).

Summary

In dental caries, biofilm microbiome stability is disturbed by the high and frequent consumption of fermentable carbohydrates, thus this dietary habit is considered a disease driver. The indigenous microbiota ferment these carbohydrates into organic acids and the local pH will drop from 7.0 to below 5.5 when the acid surpasses the buffering capacity of the biofilm and saliva. Acidogenic and aciduric species that are adapted to the acidic conditions in the biofilm environment will gain a selective advantage. Over time, dysbiosis is characterized by a shift in the microbiota, leading to a less diverse and more cariogenic community that is more efficient at fermenting carbohydrates (i.e., saccharolytic) and more adapted to growth and metabolism in low pH (i.e., aciduric). These include, but are not limited to, aciduric representatives of *Lactobacillus*, *Streptococcus*, *Veillonella*, *Bifidobacterium* and *Actinomyces*. During the low pH period, enamel demineralization (mineral loss) exceeds remineralization (mineral gain). If the acidic conditions persist or are repeated frequently without sufficient time for remineralization, then a caries lesion may develop. Thus, a dietary habit defined by frequent carbohydrate intake can lead to a positive feedback loop, causing a shift to a saccharolytic, acidogenic and aciduric microbiota that can cause irreversible dental carious lesions over time.

In periodontal diseases, a microbiome composed of asaccharolytic, nutritionally fastidious, acid-intolerant, proteolytic anaerobes is observed, and is related to a chronic inflammatory response in the surrounding connective tissue that drives the destruction of the alveolar bone that supports the tooth. The microorganisms highly associated with chronic severe periodontitis are identified as the "red complex",

which includes three species: *P. gingivalis, T. forsythia* and *T. denticola*. These species and a growing list of others are considered responsible for driving the host compatible commensal microbiome toward dysbiosis leading to the rise of pathobionts to dominance and the initiation and progression of disease. The possibility of specific species orchestrating the shift to dysbiosis resulting in pathology has identified the role of such species as a keystone pathogen. *P. gingivalis* seems especially equipped to play such a role. The disappearance (or significant reduction) of red complex and probably more specifically keystone pathogens was associated with successful periodontal treatment.

References

Aas, J. A., Paster, B. J., Stokes, L. N., Olsen, I., & Dewhirst, F. E. (2005). Defining the normal bacterial flora of the oral cavity. *Journal of Clinical Microbiology, 43*, 5721–5732.

Aas, J. A., Griffen, A. L., Dardis, S. R., Lee, A. M., Olsen, I., Dewhirst, F. E., Leys, E. J., & Paster, B. J. (2008). Bacteria of dental caries in primary and permanent teeth in children and young adults. *Journal of Clinical Microbiology, 46*, 1407–1417. https://doi.org/10.1128/JCM.01410-07.

Alaluusua, S., Mättö, J., Grönroos, L., et al. (1996). Oral colonization by more than one clonal type of mutans streptococcus in children with nursing-bottle dental caries. *Archives of Oral Biology, 41*, 167–173.

Arimatsu, K., Yamada, H., Miyazawa, H., et al. (2014). Oral pathobiont induces systemic inflammation and metabolic changes associated with alteration of gut microbiota. *Scientific Reports, 4*, 4828.

Armitage, G. C. (2004). Periodontal diagnoses and classification of periodontal diseases. *Periodontology 2000, 34*, 9–21.

Avila, W. M., Pordeus, I. A., Paiva, S. M., & Martins, C. C. (2015). Breast and bottle feeding as risk factors for dental caries: A systematic review and meta-analysis. *PLoS One, 10*, e0142922.

Bagramian, R. A., Garcia-Godoy, F., & Volpe, A. R. (2009). The global increase in dental caries. A pending public health crisis. *American Journal of Dentistry, 22*, 3–8.

Banas, J. A., & Vickerman, M. M. (2003). Glucan-binding proteins of the oral streptococci. *Critical Reviews in Oral Biology and Medicine, 14*, 89–99. PMID: 12764072.

Baumgartner, S., Imfeld, T., Schicht, O., Rath, C., Persson, R. E., & Persson, G. R. (2009). The impact of the stone age diet on gingival conditions in the absence of oral hygiene. *Journal of Periodontology, 80*, 759–768.

Becker, M. R., Paster, B. J., Leys, E. J., et al. (2002). Molecular analysis of bacterial species associated with childhood caries. *Journal of Clinical Microbiology, 40*, 1001–1009.

Beighton, D., Adamson, A., & Rugg-Gunn, A. (1996). Associations between dietary intake, dental caries experience and salivary bacterial levels in 12-year-oldEnglish schoolchildren. *Archives of Oral Biology, 41*(3), 271–280.

Belda-Ferre, P., Alcaraz, L. D., Cabrera-Rubio, R., Romero, H., Simón-Soro, A., Pignatelli, M., & Mira, A. (2012). The oral metagenome in health and disease. *ISME Journal, 6*(1), 46–56.

Belstrøm, D., Paster, B. J., Fiehn, N.-E., Bardow, A., & Holmstrup, P. (2016). Salivary bacterial fingerprints of established oral disease revealed by the Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS) technique. *Journal of Oral Microbiology, 8*, 30170. https://doi.org/10.3402/jom.v8.30170.

Benítez-Páez, A., Belda-Ferre, P., Simón-Soro, A., & Mira, A. (2014). Microbiota diversity and gene expression dynamics in human oral biofilms. *BMC Genomics, 15*, 311.

Bik, E. M., Long, C. D., Armitage, G. C., et al. (2010). Bacterial diversity in the oral cavity of 10 healthy individuals. *ISME Journal, 4*, 962–974.

Bosshardt, D. D., & Lang, N. P. (2005). The junctional epithelium: From health to disease. *Journal of Dental Research, 84*, 9–20.

Bourgeois, D. M., & Llodra, J. C. (2014). Global burden of dental condition among children in nine countries participating in an international oral health promotion programme, 2012-2013. *International Dental Journal, 64*(Suppl 2), 27–34. https://doi.org/10.1111/idj.12129.

Burt, B. A., Eklund, S. A., Morgan, K. J., et al. (1998). The effects of sugars intake and frequency of ingestion on dental caries increment in a three-year longitudinal study. *Journal of Dental Research, 67*, 1422–1429.

Chapple, I. L., & Genco, R. (2013). Diabetes and periodontal diseases: Consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *Journal of Clinical Periodontology, 40*(Suppl 14), S106–S112.

Chapple, I. L., Milward, M. R., Ling-Mount-ford, N., Weston, P., Carter, K., Askey, K., Dallal, G. E., De Spirt, S., Sies, H., Patel, D., & Matthews, J. B. (2012). Adjunctive daily supplementation with encapsulated fruit, vegetable and berry juice powder concentrates and clinical periodontal outcomes: A double-blind RCT. *Journal of Clinical Periodontology, 39*, 62–72.

Chapple, I. L., Bouchard, P., Cagetti, M. G., Campus, G., Carra, M. C., Cocco, F., et al. (2017). Interaction of lifestyle, behaviour or systemic diseases with dental caries and periodontal diseases: Consensus report of group 2 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. *Journal of Clinical Periodontology, 44*(Suppl 18), S39–S51. https://doi.org/10.1111/jcpe.12685.

Costalonga M., Herzberg M.C. (2014). The oral microbiome and the immunobiology of periodontal disease and caries. Immunol Lett. 62(2 Pt A):22–38. https://doi.org/10.1016/j.imlet.2014.08.017.

Coventry, J., Griffiths, G., Scully, C., & Tonetti, M. (2000). ABC of oral health: Periodontal disease. *British Medical Journal, 321*(7252), 36–39.

Darveau, R. P. (2010). Periodontitis: A polymicrobial disruption of host homeostasis. *Nature Reviews. Microbiology, 8*(7), 481–490. https://doi.org/10.1038/nrmicro2337.

de Pablo, P., Chapple, I. L., Buckley, C. D., & Dietrich, T. (2009). Periodontitis in systemic rheumatic diseases. *Nature Reviews Rheumatology, 5*, 218–224.

Dewhirst, F. E., Chen, T., Izard, J., et al. (2010). The human oral microbiome. *Journal of Bacteriology, 192*, 5002–5017.

Dietrich, T., Sharma, P., Walter, C., Weston, P., & Beck, J. (2013). The epidemiological evidence behind the association between periodontitis and incident atherosclerotic cardiovascular disease. *Journal of Periodontology, 84*, S70–S84.

Dige, I., Grønkjær, L., & Nyvad, B. (2014). Molecular studies of the structural ecology of natural occlusal caries. *Caries Research, 48*, 451–460. https://doi.org/10.1159/000357920.

Eggert, F. M., Drewell, L., Bigelow, J. A., Speck, J. E., & Goldner, M. (1991). The pH of gingival crevices and periodontal pockets in children, teenagers and adults. *Archives of Oral Biology, 36*, 233–238.

Eskan, M. A., Jorwani, R., Abe, T., Chmelar, J., Lim, J. H., Lian, S., et al. (2012). The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. *Nature Immunology, 13*, 465–473.

Forner, L., Larsen, T., Kilian, M., & Holmstrup, P. (2006). Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. *Journal of Clinical Periodontology, 33*, 401–407.

Frias-Lopez, J., & Duran-Pinedo, A. (2012). Effect of periodontal pathogens on the metatranscriptome of a healthy multispecies biofilm model. *Journal of Bacteriology, 194*, 2082–2095. https://doi.org/10.1128/JB.06328-11.

Genco, C. A., Cutler, C. W., Kapczynski, D., Maloney, K., & Arnold, R. R. (1991). A novel mouse model to study the virulence of and host response to *Porphyromonas* (*Bacteroides*) *gingivalis*. *Infection and Immunity, 59*, 1255–1263.

Goh, H. M. S., Yong, M. H. A., Chong, K. K. L., & Kline, K. A. (2017). Model systems for the study of Enterococcal colonization and infection. *Virulence, 8*, 1525–1562.

Golub, L. M., Lee, H. M., Greenwald, R. A., et al. (1997). A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. *Inflammation Research, 46*, 310–319.

Griffen, A. L., Beall, C. J., Campbell, J. H., Firestone, N. D., Kumar, P. S., Yang, Z. K., Podar, M., & Leys, E. J. (2012). Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. *The ISME Journal, 6*, 1176–1185.

Gruner, D., Paris, S., & Schwendicke, F. (2016). Probiotics for managing caries and periodontitis: Systematic review and meta-analysis. *Journal of Dentistry, 48*, 16–25.

Guggenheim, B. (1968). Streptococci of dental plaques. *Caries Research, 2*(2), 147–163.

Hajishengallis, G. (2014). The inflammophilic character of the periodontitis-associated microbiota. *Molecular Oral Microbiology, 29*, 248–257. https://doi.org/10.1111/omi.12065.

Han, Y. W., & Wang, X. (2013). Mobile microbiome: Oral bacteria in extra-oral infections and inflammation. *Journal of Dental Research, 92*, 485–491.

Harris, R., Nicoll, A. D., Adair, P. M., & Pine, C. M. (2004). Risk factors for dental caries in young children: A systematic review of the literature. *Community Dental Health, 21*, 71–85.

Hernández, M., Gamonal, J., Tervahartiala, T., Mäntylä, P., Rivera, O., Dezerega, A., Dutzan, N., & Sorsa, T. (2010). Associations between matrix metalloproteinase-8 and -14 and myeloperoxidase in gingival crevicular fluid from subjects with progressive chronic periodontitis: A longitudinal study. *Journal of Periodontology, 81*, 1644–1652. https://doi.org/10.1902/jop.2010.100196.

Hezel, M. P., & Weitzberg, E. (2015). The oral microbiome and nitric oxide homoeostasis. *Oral Diseases, 21*(1), 7–16.

Hujoel, P. (2009). Dietary carbohydrates and dental-systemic diseases. *Journal of Dental Research, 88*, 490–502.

Ilie, O., van Loosdrecht, M. C., & Picioreanu, C. (2012). Mathematical modelling of tooth demineralisation and pH profiles in dental plaque. *Journal of Theoretical Biology, 309*, 159–175.

Jorth, P., Turner, K. H., Gumus, P., Nizam, N., Buduneli, N., & Whiteley, M. (2014). Metatranscriptomics of the human oral microbiome during health and disease. *MBio, 5*(2), e01012–e01014.

Kampfer, J., Göhring, T. N., Attin, T., & Zehnder, M. (2007). Leakage of food-borne Enterococcus faecalis through temporary fillings in a simulated oral environment. *International Endodontic Journal, 40*, 471–477.

Kanasi, E., Dewhirst, F. E., Chalmers, N. I., et al. (2010). Clonal analysis of the microbiota of severe early childhood caries. *Caries Research, 44*, 485–497.

Kianoush, N., Adler, C. J., Nguyen, K.-A. T., Browne, G. V., Simonian, M., & Hunter, N. (2014). Bacterial profile of dentine caries and the impact of pH on bacterial population diversity. *PLoS One, 9*, e92940. https://doi.org/10.1371/journal.pone.0092940.

Kilian, M., Chapple, I. L., Hannig, M., Marsh, P. D., Meuric, V., Pedersen, A. M., Tonetti, M. S., Wade, W. G., & Zaura, E. (2016). The oral microbiome—An update for oral healthcare professionals. *British Dental Journal, 221*(10), 657–666. https://doi.org/10.1038/sj.bdj.2016.865.

Kolenbrander, P. E. (2000). Oral microbial communities: Biofilms, interactions, and genetic systems. *Annual Review of Microbiology, 54*, 413–437. PMID: 11018133.

Kolenbrander, P. E., Palmer, R. J., Jr., Rickard, A. H., Jakubovics, N. S., Chalmers, N. I., & Diaz, P. I. (2006). Bacterial interactions and successions during plaque development. *Periodontology 2000, 42*, 47–79.

Kressirer, C. A., Chen, T., Harriman, K. L., Frias-Lopez, J., Dewhirst, F. E., Tavares, M. A., & Tanner, A. C. R. (2018). Functional profiles of coronal and dentin caries in children. *Journal of Oral Microbiology, 10*, 1495976. PMCID: PMC6052428.

Kumar, P. S., Griffen, A. L., Barton, J. A., Paster, B. J., Moeschberger, M. L., & Leys, E. J. (2003). New bacterial species associated with chronic periodontitis. *Journal of Dental Research, 82*, 338–344.

Lang, N. P., Hotz, P. R., Gusberti, F. A., & Joss, A. (1987). Longitudinal clinical and microbiological study on the relationship between infection with Streptococcus mutans and the development of caries in humans. *Oral Microbiology and Immunology, 2*, 39–47.

Li, J., Helmerhorst, E. J., Leone, C. W., Troxler, R. F., Yaskell, T., Haffajee, A. D., Socransky, S. S., & Oppenheim, F. G. (2004). Identification of early microbial colonizers in human dental biofilm. *Journal of Applied Microbiology, 97*(6), 1311–1318.

Li, Y., Ge, Y., Saxena, D., & Caufield, P. W. (2007). Genetic profiling of the oral microbiota associated with severe early-childhood caries. *Journal of Clinical Microbiology, 45*(1), 81–87.

Ling, Z., Kong, J., Jia, P., et al. (2010). Analysis of oral microbiota in children with dental caries by PCR-DGGE and barcoded pyrosequencing. *Microbial Ecology, 60*, 677–690.

Loe, H., Theilade, F., & Jensen, S. B. (1965). Experimental gingivitis in man. *Journal of Periodontology, 36*, 177–187.

Loesche, W. J. (1986). Role of Streptococcus mutans in human dental decay. *Microbiological Reviews, 50*(4), 353–380.

Loesche, W. J., & Straffon, L. H. (1979). Longitudinal investigation of the role of Streptococcus mutans in human fissure decay. *Infection and Immunity, 26*(2), 498–507.

López-López, A., Camelo-Castillo, A. J., Ferrer, M. D., Simón-Soro, A., & Mira, A. (2017). Health-associated niche inhabitants as oral probiotics: The case of Streptococcus dentisani. *Frontiers in Microbiology, 8*, 379.

Marquis, R. E. (1995). Antimicrobial actions of fluoride for oral bacteria. *Canadian Journal of Microbiology, 41*, 955–964. https://doi.org/10.1139/m95-133.

Marsh, P. D. (1994). Microbial ecology of dental plaque and its significance in health and disease. *Advances in Dental Research, 8*, 263–271.

Marsh, P. D. (2016). Dental biofilms in health and disease. In M. Goldberg (Ed.), *Understanding dental caries* (pp. 41–52). Berlin: Springer.

Marsh, P. D., & Devine, D. A. (2011). How is the development of dental biofilms influenced by the host? *Journal of Clinical Periodontology, 38*(Suppl 11), 28–35.

Marsh, P. D., Head, D. A., & Devine, D. A. (2015). Ecological approaches to oral biofilms: Control without killing. *Caries Research, 49*(Suppl 1), 46–54.

Martin-Cabezas, R., Davideau, J. L., Tenenbaum, H., & Huck, O. (2016). Clinical efficacy of probiotics as an adjunctive therapy to non-surgical periodontal treatment of chronic periodontitis: A systematic review and meta-analysis. *Journal of Clinical Periodontology, 43*, 520–530.

Merritt, J., & Qi, F. (2012). The mutacins of Streptococcus mutans: regulation and ecology. *Molecular Oral Microbiology, 27*, 57–69. PMCID: PMC3296966.

Munson, M. A., Pitt-Ford, T., Chong, B., Weightman, A., & Wade, W. G. (2002). Molecular and cultural analysis of the microflora associated with endodontic infections. *Journal of Dental Research, 81*, 761–766. Erratum in: Journal of Dental Research. 2003;82:247. Journal of Dental Research. 2003;82:69.

Murray, P. A., Prakobphol, A., Lee, T., Hoover, C. I., & Fisher, S. J. (1992). Adherence of oral streptococci to salivary glycoproteins. *Infection and Immunity, 60*, 31–38.

NCHS—National Center for Health Statistics. (2017). *Health, United States, 2016: With chartbook on long-term trends in health*. Hyattsville, MD.

Nyvad, B., & Kilian, M. (1990). Comparison of the initial streptococcal microflora on dental enamel in caries-active and in caries-inactive individuals. *Caries Research, 24*(4), 267–272.

Paes Leme, A. F., Koo, H., Bellato, C. M., Bedi, G., & Cury, J. A. (2006). The role of sucrose in cariogenic dental biofilm formation—New insight. *Journal of Dental Research, 85*, 878–887. PMCID: PMC2257872.

Palmer, C. A., Kent, R., Jr., Loo, C. Y., Hughes, C. V., Stutius, E., Pradhan, N., Dahlan, M., Kanasi, E., Arevalo Vasquez, S. S., & Tanner, A. C. (2010). Diet and caries-associated bacteria in severe early childhood caries. *Journal of Dental Research, 89*(11), 1224–1229. https://doi.org/10.1177/0022034510376543.

Palmer, S. R., Miller, J. H., Abranches, J., Zeng, L., Lefebure, T., Richards, V. P., Lemos, J. A., Stanhope, M. J., & Burne, R. A. (2013). Phenotypic heterogeneity of genomically-diverse isolates of Streptococcus mutans. *PLoS One, 8*, e61358. PMCID: PMC3628994.

Peterson, S. N., Snesrud, E., Liu, J., Ong, A. C., Kilian, M., Schork, N. J., & Bretz, W. (2013). The dental plaque microbiome in health and disease. *PLoS One, 8*(3), e58487. https://doi.org/10.1371/journal.pone.0058487.

Qin, J., Chai, G., Brewer, J. M., Lovelace, L. L., & Lebioda, L. (2006). Fluoride inhibition of enolase: crystal structure and thermodynamics. *Biochemistry, 45*, 793–800. https://doi.org/10.1021/bi051558s.

Ramberg, P., Sekino, S., Uzel, N. G., Socransky, S., & Lindhe, J. (2003). Bacterial colonization during de novo plaque formation. *Journal of Clinical Periodontology, 30*, 990–995.

Razavi, A., Gmür, R., Imfeld, T., & Zehnder, M. (2007). Recovery of Enterococcus faecalis from cheese in the oral cavity of healthy subjects. *Oral Microbiology and Immunology, 22*(4), 248–251.

Ribeiro, A. A., Azcarate-Peril, M. A., Cadenas, M. B., Butz, N., Paster, B. J., Chen, T., et al. (2017). The oral bacterial microbiome of occlusal surfaces in children and its association with diet and caries. *PLoS One, 12*(7), e0180621.

Rosier, B. T., Marsh, P. D., & Mira, A. (2017). Resilience of the oral microbiota in health: Mechanisms that prevent dysbiosis. *Journal of Dental Research, 97*(4), 371–380. https://doi.org/10.1177/0022034517742139.

Scannapieco, F. A., & Binkley, C. J. (2012). Modest reduction in risk for ventilator-associated pneumonia in critically ill patients receiving mechanical ventilation following topical oral chlorhexidine. *The Journal of Evidence-Based Dental Practice, 12*, 103–106. https://doi.org/10.1016/j.jebdp.2012.03.010.

Shoemark, D. K., & Allen, S. J. (2015). The microbiome and disease: Reviewing the links between the oral microbiome, aging and Alzheimer's disease. *Journal of Alzheimer's Disease, 43*, 725–738.

Sidi, A. D., & Ashley, F. P. (1984). Influence of frequent sugar intakes on experimental gingivitis. *Journal of Periodontology, 55*, 419–423.

Simón-Soro, A., Belda-Ferre, P., Cabrera-Rubio, R., Alcaraz, L. D., & Mira, A. (2013a). A tissue-dependent hypothesis of dental caries. *Caries Research, 47*, 591–600.

Simón-Soro, A., Tomás, I., Cabrera-Rubio, R., Catalan, M. D., Nyvad, B., & Mira, A. (2013b). Microbial geography of the oral cavity. *Journal of Dental Research, 92*, 616–621. https://doi.org/10.1177/0022034513488119.

Simón-Soro, A., Guillen-Navarro, M., & Mira, A. (2014). Metatranscriptomics reveals overall active bacterial composition in caries lesions. *Journal of Oral Microbiology, 6*, 25443. https://doi.org/10.3402/jom.v6.25443.

Smith, G. L. F., Cross, D. L., & Wray, D. (1995). Comparison of periodontal disease in HIV seropositive subjects and controls (1). Clinical features. *Journal of Clinical Periodontology, 22*, 558–568.

Socransky, S. S. (1977). Microbiology of periodontal disease—Present status and future considerations. *Journal of Periodontology, 48*(9), 497–504.

Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C., & Kent, R. L., Jr. (1998). Microbial complexes in subgingival plaque. *Journal of Clinical Periodontology, 25*(2), 134–144.

Takeshita, T., Nakano, Y., Kumagai, T., Yasui, M., Kamio, N., Shibata, Y., Shiota, S., & Yamashita, Y. (2009). The ecological proportion of indigenous bacterial populations in saliva is correlated with oral health status. *The ISME Journal, 3*, 65–78.

Takeshita, T., Matsuo, K., Furuta, M., Shibata, Y., Fukami, K., Shimazaki, Y., Akifusa, S., Han, D.-H., Kim, H.-D., Yokoyama, T., Ninomiya, T., Kiyohara, Y., & Yamashita, Y. (2014). Distinct composition of the oral indigenous microbiota in South Korean and Japanese adults. *Scientific Reports, 4*, 6990. https://doi.org/10.1038/srep06990.

Tuncil, Y. E., Xiao, Y., Porter, N. T., Reuhs, B. L., Martens, E. C., & Hamaker, B. R. (2017). Reciprocal prioritization to dietary glycans by gut bacteria in a competitive environment promotes stable coexistence. *MBio, 8*(5), pii: e01068-17. https://doi.org/10.1128/mBio.01068-17.

Vartoukian, S. R., Palmer, R. M., & Wade, W. G. (2009). Diversity and morphology of members of the phylum "synergistetes" in periodontal health and disease. *Applied and Environmental Microbiology, 75*, 3777–3786. https://doi.org/10.1128/AEM.02763-08.

Wade, W. G. (2013). The oral microbiome in health and disease. *Pharmacological Research, 69*, 137–143. https://doi.org/10.1016/j.phrs.2012.11.006.

Wang, J., Qi, J., Zhao, H., He, S., Zhang, Y., Wei, S., & Zhao, F. (2013). Metagenomic sequencing reveals microbiota and its functional potential associated with periodontal disease. *Scientific Reports, 3*, 1843.

Xiao, J., Hara, A. T., Kim, D., Zero, D. T., Koo, H., & Hwang, G. (2017). Biofilm three-dimensional architecture influences in situ pH distribution pattern on the human enamel surface. *International Journal of Oral Science, 9*(2), 74–79.

Xu, H., Hao, W., Zhou, Q., Wang, W., Xia, Z., Liu, C., Chen, X., Qin, M., & Chen, F. (2014). Plaque bacterial microbiome diversity in children younger than 30 months with or without caries prior to eruption of second primary molars. *PLoS One, 9*(2), e89269. https://doi.org/10.1371/journal.pone.0089269. eCollection 2014.

Yasuda, K., Hsu, T., Gallini, C. A., et al. (2017). Fluoride depletes acidogenic taxa in oral but not gut microbial communities in mice. *mSystems, 2*(4), pii: e00047-17.

Zaura, E., & Mira, A. (2015). Editorial: The oral microbiome in an ecological perspective. *Frontiers in Cellular and Infection Microbiology, 5*, 39. https://doi.org/10.3389/fcimb.2015.00039.

Zijnge, V., van Leeuwen, M. B., Degener, J. E., Abbas, F., Thurnheer, T., Gmür, R., & Harmsen, H. J. (2010 Feb 24). Oral biofilm architecture on natural teeth. *PLoS One, 5*(2), e9321. https://doi.org/10.1371/journal.pone.0009321.

Zong, G., Holtfreter, B., Scott, A. E., Volzke, H., Petersmann, A., Dietrich, T., Newson, R. S., & Kocher, T. (2016). Serum vitamin B12 is inversely associated with periodontal progression and risk of tooth loss: A prospective cohort study. *Journal of Clinical Periodontology, 43*, 2–9.