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Diversity of the Oral Microbiome and Dental Health and Disease

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Abstract

The oral microbiome is extremely diverse, and consists of potentially over 1000 different microorganisms, including viruses, protozoa, fungi, archaea, and bacteria. In conditions of oral health, biofilms are in a state of microbial homeostasis, with the vast majority of the microbiota being commensal or mutualistic in nature. In conditions of oral disease, the composition, quantity, and stability of the oral microbiota become disrupted. Factors that influence microbial composition include genetics, host defenses, microbial interactions, receptors used for attachment, acidogenicity, and salivary flow. The interplay of these factors determines the balance between oral health and disease, and when microbial homeostasis becomes disrupted, oral diseases including dental caries, endodontic disease, and periodontal disease may occur. As the aims of this review article, we will discuss the microorganisms that have been identified as the key players in dental caries, endodontic disease and periodontal disease, and propose as a hypothesis that microbial homeostasis and imbalance will have an impact on oral health and disease. Identifying the factors that influence oral health and disease may help develop preventive and therapeutic strategies for dental care provided by oral health practitioners.

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Introduction of the Oral Microbiome

The term 'microbiome' was introduced to underscore the importance of trillions of symbiotic, commensal and pathogenic bacteria that occupy the human body [1]. Microbes that occupy the body outnumber human cells by at least several-fold, further underscoring the importance of the microbiome and leading some to refer to humans as 'supraorganisms' [2]. In 2008, the Human Microbiome Project was founded to document the profound diversity of microbes in the human body associated with health and disease [3]. This recent interest in the microbiome has spawned a promising field of research, microbiomics, which targets the microbiome for therapeutic purposes [4]. From the viewpoint of the clinician, the health of the individual is intrinsically linked to the composition of the various microbiomes of the human body, and the study of the oral microbiome may represent a new frontier in human genetics and lead to new therapeutic tools for the oral health practitioner [5].

The oral microbiome is extremely diverse, and includes viruses, protozoa, fungi, archaea, and bacteria [6]. There are potentially over 1000 different microorganisms, including 700 bacterial species, in the oral cavity [7-8]. Approximately 96% of the bacteria in the oral microbiome belong to the six phyla Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes, and Fusobacteria; the remaining 4% belong to the phyla Euryarchaeota, Chlamydia, Chloroflexi, Synergistetes, and Tenericutes, and candidate phyla or divisions SR1 and TM7 (TM7 also known as *Candidatus* Saccharibacteria) [7].

The oral microbiome is not a uniform microbial niche, but includes numerous distinct habitats, including the teeth, attached gingiva, gingival sulcus, lips, hard and soft palate, and tongue. This is also continuous with several other structures, including the nasopharynx, tonsils, middle ear, esophagus, and lungs [7]. The various structures of the oral cavity are preferentially colonized by different microorganisms, and are largely determined by the fastidiousness and oxygen demands of the bacteria [8-9]. For example, periodontal pockets that are largely shielded from the atmosphere are preferentially colonized by anaerobic species. The oral microbiome is also particularly dynamic because of its continuity with the external environment, leading to considerable variation among individuals due to dietary and hygiene habits [10].

Bacteria are deposited within the teeth microbiome as dental plaque. These biofilms are extremely complex and sophisticated bacterial communities embedded in a protective matrix composed primarily of water and a mix of host and microbial-secreted extracellular polymeric substances (EPS) [11-13]. As a result of their unique structure and composition, biofilms have several essential functions that enhance the ability of bacterial communities to colonize the oral cavity. Primarily, the biofilm provides protection from the host immune response [14] and antibiotic and antimicrobial resistance [15-19]. One potential mechanism of antibiotic resistance is known as 'indirect pathogenicity,' where antibiotic-resistant bacteria capable of producing defensive enzymes exert a protective effect on antibiotic-sensitive bacteria within the same biofilm [20]. This form of antibiotic resistance can be illustrated in animal models, demonstrating the protective effect of *Moraxella catarrhalis,* capable of producing ß-lactamase, on the penicillin-sensitive bacteria *Streptococcus pyogenes* [21-22]. Biofilms also expand the range of habitable areas for certain bacteria; for example, aerobic, oxygen-consuming bacteria

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can create environmental conditions suitable for obligate anaerobes and microaerophiles [13]. Furthermore, the association of bacteria into the structured community of the biofilm allows for integration, and thus increased efficiency of metabolic function [23]. Finally, biofilms enhance bacterial virulence due to pathogenic synergy, which infers that individual microorganisms may lack the necessary virulence factors to cause disease, but bacterial communities within biofilms may collectively express the virulence necessary to induce disease [24]. As a result of these properties, biofilms have been described as the most successful structure of life in the world [25].

Oral Microbiome in Health and Disease

In health, biofilms are relatively stable in a state of microbial homeostasis [26], and the vast majority of the microorganisms of the oral microbiome are commensal or mutualistic in nature [6]. The host provides an environment–the hard and soft tissues of the oral cavity–in which the microorganisms are able to survive and flourish. In turn, the microorganisms prevent or reduce colonization by potential pathogens in the oral cavity (colonization resistance) [26]. For example, *Candida albicans* is known to be asymptomatically present in healthy mouths [9], with the normal flora of the oral microbiome preventing the overgrowth of the fungus and the development of Candidiasis. However, broad-spectrum antibiotics may reduce the quantity and diversity of the oral flora, leading to yeast over-growth and potential pathology [27-28]. In addition, recent research suggests that oral bacteria may be involved in the normal development of the immune system [29].

There are myriad host factors that may influence the composition, quantity, and stability of the oral flora, and impact microbial homeostasis. Proper oral hygiene practices are essential for the constant disruption and prevention of overgrowth of bacterial biofilms on the oral tissues [30]. Furthermore, the ingestion of excess sugars and carbohydrates is associated with an increase in acidogenic bacteria, often leading to dental caries [31]. Other factors that may influence microbial composition include genetics, host defenses, microbial interactions, receptors for attachment, temperature, atmosphere, pH, and salivary flow [26]. The interplay of these factors is responsible for the development of the microbial composition of the oral microbiome and is central in the determination of oral health and disease. When microbial homeostasis is disrupted, oral diseases, including dental caries, endodontic infections, and periodontal disease, may frequently occur.

Demographics and Biological Mechanisms of Dental Caries

Dental caries is one of the most common chronic diseases of adults and children and has an enormous global health burden [32]. The World Health Organization estimates global caries prevalence as 60-90% in children and nearly universal in adults [33]. Furthermore, as stated by the U.S. Surgeon General, dental caries in children is five times more common than asthma and seven times more common than hay fever [34]. In light of these statistics, dental caries has been delegated as a global health pandemic [33]. According to the National Institute of Dental and Craniofacial Research, 1 out of 5 children between ages 2-5 years have developed caries in their primary dentition. Furthermore, at least 92% of adults over the past 20 years exhibited caries at some point in their lives [35].

In dental caries, tooth structure is dissolved by acid produced from the fermentation of carbohydrates by acidogenic bacteria [6]. Sucrose is widely considered the most cariogenic carbohydrate because it is not only fermentable, but also capable of being metabolized into extracellular polysaccharides that can be incorporated into the biofilm matrix [36]. The mechanism of dental caries begins with (1) acidogenic bacteria in the oral biofilms fermenting dietary carbohydrates into organic acids, leading to (2) acid diffusion into the hard tissues of the teeth (enamel, dentin, cementum), causing (3) demineralization of the tooth, where calcium and phosphate dissolve out of the tooth structure, and (4) eventually leading to the development of carious lesions [37]. An increase in dietary carbohydrates allows cariogenic bacteria such as *Streptococcus mutans*, Lactobacilli, and *Streptococcus sobrinus* to ferment the sugar and tightly adhere to tooth surfaces. As a result, there is an increase in the acidity of the biofilm, which allows these bacteria to increase in proportion and outcompete resident flora, such as *S. sanguis* and *S. gordonii* [38-39]. Traditionally, *S. mutans*, Lactobacilli, and *S. sobrinus* have been considered the primary cariogenic microorganisms involved in caries. However, more recent research have identified many other microbes associated with caries such as Bifidobacterium, *Scardovia wiggsiae, Slackia exigua*, Veillonella, Propionibacterium, Actinomyces spp., and Atopobium spp [40-41].

During conditions of health or disease, the oral environment is constantly undergoing cycles of demineralization and remineralization on tooth surfaces. Remineralization maintains the integrity of the enamel surface and prevents loss of inorganic minerals [31, 42-44]. However, when the demineralization and remineralization equilibrium shifts to a net loss of hydroxyapatite, tooth decay occurs. Fluoride is proposed to arrest and reverse the caries process in three ways: the inhibition of demineralization, enhancement of remineralization, and inhibition of destructive bacterial enzymes [45].

Microarray/New Generation Sequencing Analyses in Dental Caries

Utilizing next-generation sequencing, studies have shown that the three most prevalent phyla in patients with caries are Firmicutes (most abundant), Actinobacteria, and Proteobacteria [46-48]. As described in Lee et al. [48], Lactobacillus, *Catonella morbi*, Enterococcus, Atopobium, Clostridiales, and Eubacterium were the major microorganisms contained in the supragingival plaque of caries active children. On the other hand, Streptobacillus, *Moryella indoligenes*, Johnsonella, Propionibacterium, Corynebacterium, Selenomonas, Prevotella, Pseudomonas, and Megasphaera were found preferentially in caries-free children [48].

In dentinal caries, the most prevalent microorganisms included Lactobacillus sp., Prevotella sp., Atopobium sp., Olsenella sp. and Actinomyces sp. At an acidic pH, Lactobacillus species, including *L. fermentum, L. rhamnosus* and *L. crispatus*, were present at the highest levels [49]. In deep dentinal caries with irreversible pulpitis, the major genera included Lactobacillus (42.3%), Olsenella (13.7%), Pseudoramibacter (10.7%) and Streptococcus (5.5%) [46-47]. However, in another study by Jagathrakshakan et al. [47] researchers observed that the predominant microorganisms in dentinal caries include Streptococcus, Rothia, Granulicatella, Gemella, Actinomyces, Selenomonas, Haemophilus and Veillonella [47].

Streptococcus Mutans and *Streptococcus Sobrinus*

For many years, *S. mutans* has been identified as the major pathogen involved in dental caries and has been strongly associated with the development of white spot lesions. However, a more recent study has indicated that *S. mutans* may not be as significant in caries progression

or deep caries [50]. *S. mutans* possesses three prominent virulence factors: 1) adherence within plaque biofilms of tooth surfaces, 2) lowering the pH of the oral environment through carbohydrate fermentation, and 3) production of bacteriocins. With sucrose as a carbohydrate source, *S. mutans* can produce extracellular glucan, fructan, and mutan that helps the bacteria adhere to the biofilm. Simultaneously, lactic acid is produced from carbohydrate metabolism and increases the acidity of the environment [51-52]. In addition, *S. mutans* can produce two bacteriocins, lantibiotic and non-lantibiotic mutacins, to inhibit the growth of resident flora such as *S. sanguinis, S. gordonii, S. mitis, S. pneumoniae, S. cristatus, S. oralis,* and *S. parasanguinis* [53, 54]. *S. sobrinus* shares the same pathogenic traits with *S. mutans* and has been closely associated with smooth-surface caries of children ages 3-5 years [55-57].

Johansson et al. [58] found that increased levels of *S. mutans* and *S. sobrinus* were associated with individuals with higher caries rate and lack of proper oral hygiene. Interestingly, the levels of mutans streptococci as a primary etiologic agent were less pronounced in communities with prevention programs. Instead, species in the Actinomyces, Selenomonas, Prevotella, and Capnocytophaga genera were more dominant in patients with caries. It was hypothesized that the difference in bacteria levels could be due to the maturity of the accumulated biofilm [58].

Lactobacilli

Lactobacilli are oxygen-tolerant or anaerobic Gram-positive bacilli. Similar to *S. mutans*, Lactobacilli species are capable of producing lactic acid and have aciduric properties. Also, *L. reuteri, L. fermentum, L. parabuchneri*, and *L. sakeican* all produce glucans to facilitate adherance to tooth surfaces [59-60]. In addition, many Lactobacilli species, such as *L. paracasei* can secrete bacteriocins that perforate the cytoplasmic membranes of *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, S. salivarius* and *S. sanguinis* [54]. Lactobacilli are typically found in coronal caries in children, and on root caries in adults [61, 62]. Lactobacilli significantly increase in proportion as decay progresses from enamel to dentin lesions. Specifically, levels of *L. gasseri, L. johnsonii, L. casei,* and *L. paracasei* were dominant in dentinal caries [50, 63].

Bifidobacteria

Lactobacilli have been the primary agents in the progression of deep caries. However, Bifidobacterium outnumbered *Lactobacillus fermentum* in deep caries [64, 65]. Furthermore, *Bifidobacterium dentium*, *Parascardovia denticolens*, *Scardovia inopinata*, *Bifidobacterium longum*, and *Bifidobacterium breve* were strongly associated with occlusal caries that penetrated the dentin in both the primary and secondary dentition [66]. Elevated levels of *B. dentium* were only found in cavitated lesions [67].

Scardovia Wiggsiae

S. wiggsiae is an anaerobic Gram-positive bacillus that has recently been characterized as a cariogenic pathogen [41]. Although *S. wiggsiae* is also found in conditions of oral health, its levels significantly increase in tooth decay, particularly in early childhood caries [67-68]. Several studies have suggested that *S. wiggsiae* may participate in initial caries development, dental plaque, white spot lesions, and dentinal caries, in the presence or absence of *S. mutans* [41, 68-70]. Further investigation in the virulence factors of *S. wiggsiae* may provide therapeutic targets for reducing caries especially in children.

Other Microorganisms in Dental Caries

Several studies have found a myriad of additional microorganisms involved in caries: Veillonella spp. (present in both primary and secondary dentition in health and disease), *Actinomyces gerencseriae,* Selenomonas, Neisseria, Propionibacterium FMA5 (associated with caries initiation), *S. parasanguinis* and *S. salivarius* (prevalent in white spot lesions and dentin lesions), Prevotella sp. (carious dentin), *Slackia exigua* (advanced caries in severe early childhood disease),and *Actinomyces naeslundii* (commonly associated with periodontal disease, but also found in root caries) [40, 50, 54, 71, 72].

Oral Microbiome and Endodontic Disease

The intact root canal is a unique environment in the oral cavity because it is sterile, devoid of normal microbiota [73]. While fungi or arachae have been found in endodontic infections, by far the most common infectious agents are bacteria [73]. Bacteria are introduced into the root canal as sequela to trauma, cracks, periodontal disease, caries, or iatrogenic introduction. The result of infection may be pulpitis, necrosis, or periapical involvement [73].

The American Association of Endodontists (AAE) classifies pulpal and periapical diagnoses based on subjective findings and clinical examination [74]. Pulpal disease may be classified as irreversible pulpitis or necrosis. Periapical disease may be apical periodontitis or acute or chronic apical abscess. The microbiota associated with these diagnoses are distinctive and key to understanding how to effectively treat endodontic disease.

Oral Microbiome and Pulpal Disease

Deep carious lesions are the most common source of initial exposure of the pulp to microbes. While reversible pulpitis may be relieved by removal of the causative agent, irreversible pulpitis requires intervention from a dental professional. Irreversible pulpitis is associated with deep dentinal caries, rather than frank enamel caries. The bacteria that cause the two are distinctly different, with anaerobic, proteolytic bacteria primarily associated with dentinal caries [75]. Rocaset et al. (2015) noted a shift in microorganisms that initially cause deep carious lesions, with those that proceed to infect the pulp, suggesting a change in environment. While *Atopbium genomo species C1, P. alactolyticus,* Streptococcus species*, S. mutans, P. micra, F. nucleatum*, and Veillonella species were found to be the most prevalent microbes associated with pulpitis, the first three were associated with deep dentinal caries while the last three were associated with endodontic pulpitis [76]. *P. micra* was also found in high amounts in infected root canals, in addition to *E. faecalis, S. constellatus, T. forsythia,* and Parvimonas species [75]. Also, Prevotella species, particularly, *Prevotella endodontalis*, have been found to be associated with dentinal caries and pulpitis [77]. While the microbial etiology of pulpitis may be diverse, Streptococcus, Veillonella, Prevotella species, and *P. micra* are identified most consistently in the literature.

Oral Microbiome and Periapical Disease

Periapical or apical disease consists of apical periodontitis (either symptomatic or asymptomatic) or apical abscess (either acute or chronic). The microbiota that cause periapical disease are distinct from those that are associated with pulpitis alone. Gram-negative saccharolytic rods, specifically Fusobacterium or Bacteroides, are predominantly found in infected root canal spaces associated with periapical disease [78, 79]. *F. nucleatum* is one of the most prevalent

microorganisms isolated from root canals and periodontal infections [78, 80]. Additionally, spirochetes, specifically Treponema, an obligatory anaerobe, have been associated with endodontic abscesses and apical periodontitis [78, 81], and may represent 50-89% of the total amount of bacteria in these endodontic infections [82]. Streptococcus has also been implicated because of its ability to co-aggregate and also rapidly adapt to new environments. Actinomyces is associated with apical abscesses and endodontic failure. Its virulence is derived from its fimbriae, which enhance its abilities to cause periapical pathosis. Lactobacillus may also readily adapt to environment changes, making it particularly virulent and contributing to its role in apical periodontitis. Candida species have also been isolated from persistent apical periodontitis infections, with *C. albicans* being the most frequently isolated [81, 83]. *Enterococcus faecalis* may readily colonize root canals and also has the capabilities of invading dental tubules, making this microorganism particularly pathogenic in apical abscesses and persistent endodontic infections [83, 84]. Finally, Dialister species have been implicated in symptomatic and asymptomatic periodontitis along with acute apical abscesses [78, 85].

Oral Microbiome and Periodontal Disease

Periodontal diseases are inflammatory diseases of the periodontium caused by bacterial accumulation in oral biofilms [86], and are broadly classified by the severity and symptoms of the disease into gingivitis and periodontitis. Gingivitis, like dental caries, are among the most common chronic infections of humans worldwide [87]. The etiology of gingivitis has been extensively detailed, with a lack of proper oral hygiene being a primary factor in the occurrence of the disease. In conditions of health, the gingival sulcus is primarily colonized by Gram-positive microorganisms, which include various Streptococcus and facultative Actinomyces species [88]. However, in the absence of proper oral hygiene, there is a dramatic increase in the proportion of Gram-negative bacteria, which activate the host inflammatory response through lipopolysaccharide and other destructive enzymes [89]. The clinical signs of gingivitis include gingival inflammation, bleeding on probing, and spontaneous bleeding.

Periodontitis, a more severe form of periodontal disease, is characterized by attachment loss leading to the generation of periodontal pockets and resorption of alveolar bone and tooth loss [6]. The disease is broadly divided into two main features: the rate of periodontal destruction (chronic versus aggressive) and the extent of destruction (localized versus generalized) [90]. A recent task force modification by the American Academy of Periodontology has further dictated that aggressive periodontitis is associated with a circumpubertal onset and familial pattern [91]. The etiology of periodontitis is extremely complex and poorly understood. Dental plaque, especially that containing *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* (the 'red complex'), is necessary but not sufficient for disease; host susceptibility is also necessary [92-93]. Risk factors for the development of periodontitis include genetics, smoking, diabetes, microbial composition, and stress [94].

Porphyromonas Gingivalis

P. gingivalis is an opportunistic Gram-negative obligate anaerobe present in normal host flora, with the capability of transforming into a disease-causing pathogen when dysbiosis occurs within the oral microbiome [63, 95]. Although present at minimum numbers within the oral microbiome, *P. gingivalis* continually appears as a keystone pathogen when considering the etiology, prognosis and unsuccessful treatment outcomes of periodontitis [63]. *P. gingivalis* has several requirements for growth and destruction of periodontal tissues. First, since it is considered a late colonizer, it requires attachment sites within previously established biofilms found on tooth surfaces. The ability for *P. gingivalis* to adhere and attach is largely due to fimbriae, which facilitates the adherence to epithelial cells, salivary molecules, and other components within the biofilm. Once adhered and colonized on supragingival surfaces, *P. gingivalis* will subsequently migrate towards the subgingival area where the mechanisms of periodontal disease occur [95]. *P. gingivalis* has the additional capability of adhering and invading epithelial cells of the oral microenvironment, entering by way of membrane invagination endocytosis. Secondly, it requires a proper nutrient supply including peptides and hemin. *P. gingivalis* does not ferment free amino acids and thus uses the peptides available in the environment to nurture growth [95]. Since *P. gingivalis* has an obligate need for iron in order to survive, it utilizes the iron derivative, hemin. Although levels of hemin in the oral cavity vary, for patients with periodontal disease, bleeding due to the inflammatory response increases subgingival hemin levels substantially, thus enhancing the chances of *P. gingivalis* colonization [95]. Third, as an obligate anaerobe, *P. gingivalis* requires an environment with a reduced oxygen tension in order to thrive. Hemin also has the added ability of helping to maintain the anaerobic environment in which *P. gingivalis* thrives [95]. Once all three requirements have been met, *P. gingivalis* can initiate the mechanisms of periodontitis through a combination of dampening the immune host response, expression of key proteases, and via the activation of *P. gingivalis*' lipoprotein. Through the expression of specific lysine and arginine proteases, *P. gingivalis* has the ability to dampen the host immune response while utilizing the degradation of proteins for energy [95]. *P. gingivalis* releases lipoprotein, which hinders the cAMP response that would normally induce the nitric oxide synthase-dependent bacterial defense mechanism. In addition to lysine and arginine proteases, the production of collagen proteases, laminin, keratin, fibronectin, and fibrinogen all contribute to the deterioration of gingival crevice constituents [95]. Although *P. gingivalis* continues to be considered as a key causative microbe of periodontitis, this microorganism is necessary but not sufficient for disease [63, 95]. For example, monocolonization of *P. gingivalis* in mice failed to result in bone destruction associated with periodontitis, while a combination of *P. gingivalis* and other microbiota acted in concert to produce the symptoms characteristic of periodontal disease [63].

Tannerella Forsythia

Tannerella forsythia, another component of the "Red Complex" associated with periodontal disease, is often isolated with *P. gingivalis*, and the combination of the two pathogens is thought to synergistically enhance the virulence potential of each microorganism. For example, animal models have indicated that a combination of *T. forsythia* and *P. gingivalis* resulted in the formation of abscesses [96], while others found that *T. forsythia* had a direct dose-dependent relationship with the growth of *P. gingivalis* [96]. In Zhu et al. [96], *T. forsythia* was found within the hemagglutination surface layer, which plays a significant role in the invasion of epithelial cells and subsequent abscess development in mice. The surface layer serves as a key mediator in coaggregating the two pathogens, and there is evidence that surface layer-deficient mutants of *T. forsythia* are correlated with a reduced presence of the two pathogen-combination [96].

Treponema Denticola

The third member of the Red Complex is *Treponema denticola*, an anaerobic and opportunistic microbe found to be a key component in periodontal plaque biofilms. Like *T. forsythia, T. denticola* works synergistically with *P. gingivalis* subgingivally and in the manifestations of periodontal disease. Recent evidence has emerged that *P. gingivalis* may even be required for the growth and appearance of *T. denticola*, further strengthening the relationship between the two microbes [63, 97, 98]. *T. denticola* has the capability of adhering, invading, and causing direct harm to the periodontium [98]. Found within close proximity of the junctional epithelium, *T. denticola* utilizes its adhesins to bind to several structures including matrix proteins, fibroblasts, host epithelial cells, collagen binding proteins, and other bacteria. Upon binding first to gingival fibroblasts, *T. denticola* adheres to extracellular matrix proteins and suppresses the proliferation of fibroblasts through the interruption of proper filamentous actin arrangement [98-99]. *T. denticola* also contains an outer sheath layer that contributes to forming pores, and has been shown to cause the production of extensive vacuoles in epithelial cells [98, 100]. Similar to *P. gingivalis, T. denticola* acts to down regulate and alter the host immune response, including obstruction of the production and activity of superoxides, neutrophils, and other central immune mediators. For instance, superoxide production has been found to decrease by as much as 56% by *T. denticola*. At the same time, however, *T. denticola* also works to upregulate the production of critical inflammatory mediators including TNF-alpha, IL-1, and nitric oxide [98, 101], the latter two found in significantly increased levels in periodontal patients and implicated in bone resorptive activity [98]. The microbe also produces hemolysin, which lyses red blood cells, provides heme-containing molecules essential for agglutination, and promotes its ultimate survival in the subgingival anaerobic environment [98].

Localized Aggressive Periodontitis (LAP) and *Aggregatibacter Actinomycetemcomitans*

Unlike chronic periodontitis, localized aggressive periodontitis is unique in that it results in accelerated bone resorption from the onset, and typically affects adolescents of specific ethnic groups (African American and Hispanic children) and those with limited access to care [102, 103]. However, like chronic periodontitis, LAP involves a shift in the microbial equilibrium resulting in opportunistic infections by periopathogens [104]. Left untreated, loss of periodontal structures can result in mobile teeth and damage to oral tissues [103]. *Aggregatibacter actinomycetemcomitans*, the key microorganism implicated in LAP, belongs to the HACEK group of anaerobic and facultative Gram-negative bacteria [102]. The HACEK group includes members of the Haemophilus species (*Haemophilus parainfluenzae*, *Haemophilus aphrophilus*, *Haemophilus paraphrophilus*), and other microorganisms including *Aggregatibacter actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*. The presence of *A. actinomycetmcomitans* may have a causal relationship with the initiation of localized aggressive periodontitis, and upon removal of the microbe from affected sites, disease remission ensues [104]. Fine et al. [104] identified the buccal mucosa to be the site most prone to containing *A. actinomycetemcomitans* [103], with upwards of 75% of subjects containing this microbe [102]. *A. actinomycetemcomitans* has two major virulence factors: 1) over activation of osteoclasts and RANKL leading to rapid bone resorption, and 2) production of both leukotoxins and cytolethal-distending toxin which both play a role in evading host defense [102]. The over activation of osteoclastogenesis and production of RANKL is largely due to *A. actinomycetemcomitans* ability to target and alter the cells of monocytic linkage, including macrophages and osteoclast precursors. The leukotoxin obliterates polymorphonuclear leukocytes and monocytes, thereby serving as a defense mechanism from the host immune response [103]. The cytolethal-distending toxin belonging to *A. actinomycetemcomitans* has a large impact on arresting cells during various stages of the cell cycle through its DNA cleavage mechanism on the cells' plasma membranes, while simultaneously remaining toxic to immune cells including lymphocytes and defensive epithelial cell layers [102].

Other Microorganisms Implicated in Periodontal Disease

In recent years, several novel microbial agents have been implicated in the development of periodontal disease. Using next generation sequencing strategies, the Fusobacterium species, specifically *F. alocis* and reclassified as *Filifactor alocis*, has been found to potentially influence the development of periodontal-associated subgingival biofilm. *F. alocis* has been found to be closely associated with members of the Red complex, and may have the highest prevalence among the periopathogens [105, 106]. *F. alocis*, a late colonizing bacterial species in periodontal pockets and a key biodiagnostic marker of periodontal biofilms, is a fastidious gram-positive anaerobic rod that plays a key role in periodontal pathogenesis [105]. Similar to the microbes discussed, *F. alocis* engages in a symbiotic relationship with *P. gingivalis*, with vesicle-mediated endocytosis and increased adherence and invasive virulence properties when combined than monocultured [105]. Although synergistic, it is likely that *F. alocis* and *P. gingivalis* have different mechanisms of destruction. One of the major virulence properties of *F. alocis* includes the activation of neutrophil activating protein A, which is a chemotactic activator for neutrophils; and with the activation of neutrophils, elevated levels of enzymes and oxidative radicals are induced which promotes an oxidative stress environment to outcompete microbes that are more oxidation-sensitive [104-105]. Through neutrophil interactions, there is also a reduction of the host response to neutralize bacteria [104]. *F. alocis* also contributes to an increased release of proinflammatory cytokines from gingival epithelial cells including IL-6, TNF-alpha, and IL-beta. *F. alocis* also uses amino acids arginine and lysine as an energy source and growth, produces proteases including sialoglycoproteases that degrade the extracellular matrix, and contains sialidase activity which then further contributes to reducing the oxidative stress in the environment leading to the survival capabilities of the microbe [105].

Fine et al. [103] have determined that the harmonious activity of *F. alocis, A. actinomycetemcomitans*, and *S. parasanguinis* may serve as an indicator for future bone loss at sites from patients suffering with localized aggressive periodontitis. *Streptococcus parasanguinis* is a viridans group streptococci species, which contributes to the formation of the oral biofilm [103]. While both *A. actinomycetemcomitans* and *F. alocis* are found to colonize the subgingival area, they tend to be contained in the deeper portions of the periodontal pocket, while *S. parasanguinis* is typically found in the more superficial areas of the pocket [103]. *A. actinomycetemcomitans* was present at all targeted sites of bone resorption, although some sites did not undergo bone loss while containing *A. actinomycetemcomitans* [103].

Conclusion

In conditions of oral health, biofilms are in a state of microbial homeostasis, while in conditions of oral disease, homeostasis becomes

disrupted. Under these conditions of microbial imbalance, several oral diseases including dental caries, endodontic disease, and periodontal disease, may occur. In this review article, we discussed the key microbial players in cariogenic, endodontic, and periodontal disease, and have validated the premise that the dynamic interplay between microbial homeostasis and imbalance has a dramatic impact on oral health and disease. The identification of factors that influence oral health and disease may help elucidate important preventive and therapeutic strategies for dental care provided by oral health practitioners.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

BN, CC, EF, JC and NK all contributed to the primary writing of this review article and in the compilation and development of reference citations, and are noted as equal contributors to this work. CC is the OHSU representative for the 2016 ADA/Dentsply Sirona Student Clinician Research Program and the recipient of the 2016 Dean's Student Research Fellowship Award from the OHSU School of Dentistry. EF is a recipient of the 2015 Dean's Research Fellowship Award. BN, CC, EF, JC and NK are enrolled in the DMD program at the OHSU School of Dentistry. TM and CM contributed in the development of revised and final versions of this manuscript. SS is a Clinical Study Coordinator for our research program at the OHSU School of Dentistry. TM and CM are OHSU faculty members and receive support from the OHSU School of Dentistry. CM is the Principal Investigator of NIH DE024317 and DE024317 Supplement, and is supported in part by both grant awards.

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