



Anaerobic Bacteria in Oral Cavities and Dental Health

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ABSTRACT

Oral cavity is a very suitable habitat for a wide range of bacteria of which a significant proportion is facultative or strict anaerobes. In healthy individuals, specific sites of the oral cavity are colonized by specific microbial communities, and a balance of the species within the community, known as “microbial homeostasis”, is maintained. When this balance is disrupted by ecological perturbations, the biofilm composition changes leading to the initiation of local infections that may ultimately lead to tooth loss. At the onset of the infections, Gram-positive bacteria dominate the biofilm composition, but if left undisturbed, a more complex biofilm builds up where Gram-negative anaerobic and proteolytic rods become dominant.

Keywords: Oral anaerobic bacteria, Dental caries, Periodontal diseases

INTRODUCTION

Oral cavity, one of the routes of entry into the human body, consists of several distinct microbial habitats, such as teeth, gingiva, tongue, cheek, lip, hard palate, and soft palate, which have favorable physicochemical conditions like warmth and moistness for the growth of a diverse range of microorganisms including bacteria, archaea, mycoplasma, virus, protozoa, and fungi. Of them, bacteria are the most numerous, and it has been estimated from different culture-dependent and independent studies that more than 600 resident bacterial species or phylotypes are present in the oral cavity, and approximately half of them can be cultivated in the laboratory (1). A significant proportion of this resident microflora is facultatively or strictly anaerobic. Usually, the composition of oral microbiota not only differs from one person to another, but also from one niche of the oral cavity to another in the same individual. In healthy individuals, a unique consortium of resident microflora colonizes the tooth surface or gingival sulcus in a specific site of the oral cavity and form dental biofilm (popularly known as plaque). Generally, Gram-positive aerobic (e.g. *Neisseria* spp.) and facultatively anaerobic bacteria

(e.g. *Streptococcus*, and *Actinomyces* spp.) initiate the dental biofilm formation. They rapidly consume the available oxygen, and produce different reduced compounds and gases (e.g. CO₂ and H₂) creating an environment suitable for obligate anaerobes. As a result, a spatial organization of different bacteria in the dental biofilm develops. Based on molecular biology studies, a core dental microbiome has been proposed, which includes *Streptococcus*, *Actinomyces*, *Veillonella*, *Granulicatella*, *Neisseria*, *Haemophilus*, *Corynebacterium*, *Rothia*, *Prevotella*, *Capnocytophaga*, *Porphyromonas*, and *Fusobacterium* spp. (2-5). Like normal microflora in other habitats within the human body, resident oral microbiota also provides benefits for the host by preventing the colonization of foreign microorganisms or pathogens and interacting with the host immune system to an extent well-suited to health. Notably, the oral health depends on a delicate balance of the resident microbial species within biofilms at specific niches of the oral cavity. When stress factors like changes in diet, insufficient oral hygiene, medication influencing salivary flow, and changes in host immune response disrupt the homeostasis, a more complex biofilm including Gram-negative anaerobic

bacteria is built up allowing more virulent species to be dominant. Consequently, local infections such as dental caries and periodontal diseases are initiated. Recent studies also reported the association of oral microbiota with different systemic diseases including cardiovascular disease, stroke, preterm birth, diabetes, and pneumonia (2,4-6). In this mini-review, the association of anaerobic bacteria in two most common types of oral diseases, dental caries and periodontal diseases will be discussed.

ORAL ANAEROBIC BACTERIA AND LOCAL INFECTIONS

A dental biofilm is a structurally and functionally organized multispecies microbial community

comprising of up to 100 different microbial species embedded in a matrix of self-produced exopolymers (e.g. polysaccharides, proteins, or DNA). It is first established on the protective areas of a tooth, i.e., in fissures of the occlusal surfaces, approximately between adjacent teeth, and gingiva along the gingival margin. Biofilm on the tooth surface, if not removed regularly, may initiate dental caries followed by pulpitis and apical periodontitis. On the other hand, supra- and sub-gingival biofilms may cause periodontal diseases- initially gingivitis followed by periodontitis and tooth loss (Figure 1). If a tooth is replaced by an implant, periimplantitis- inflammation of the surrounding tissues of the implant- may also occur (2,4,7-8).

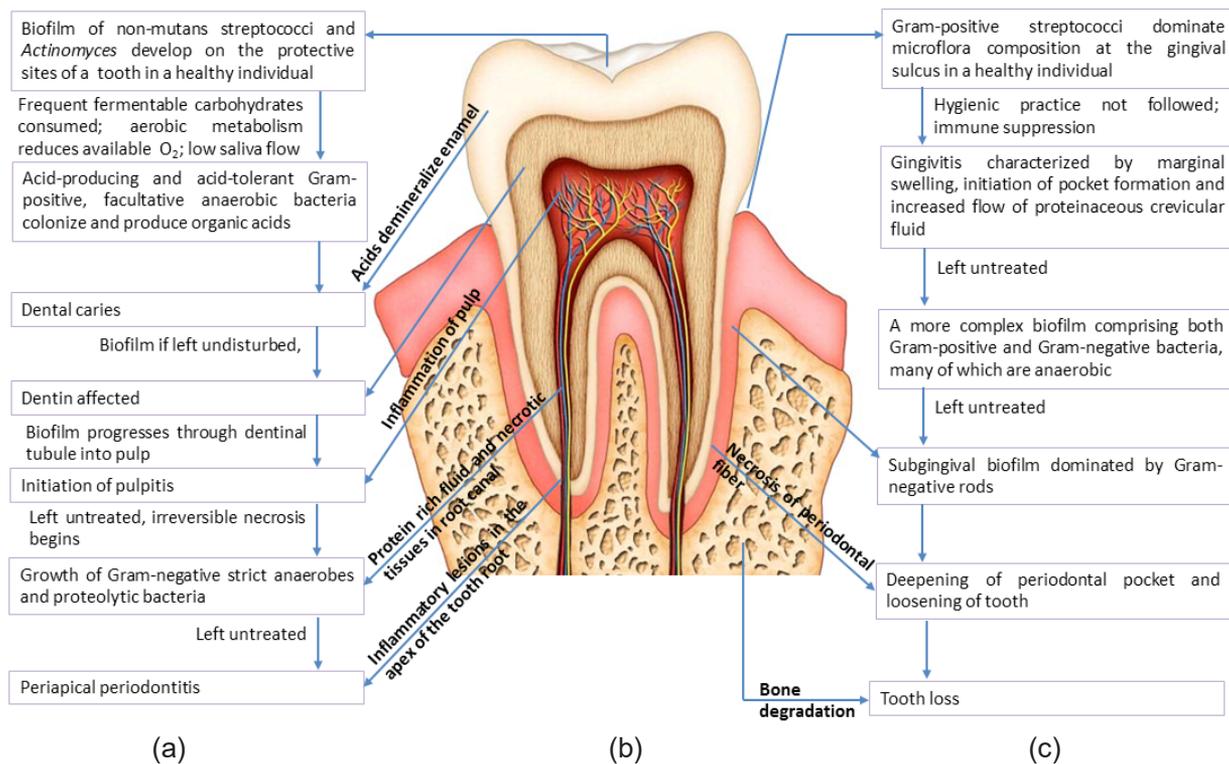


Figure 1: A schematic representation of ecological shifts affecting the dental biofilm composition and dental health. (a) Development of dental caries, pulpitis, and periapical periodontitis, (b) cross-section of a molar teeth (courtesy: <https://goo.gl/Lgmifc>), and (c) development of periodontal diseases leading to tooth loss.

ORAL ANAEROBIC BACTERIA AND DENTAL CARIES

Dental caries, also known as tooth decay, develops initially as the demineralized enamel or white spot lesions which may progress into enamel cavities and finally into dentin. There are three major risk factors for dental caries: cariogenic bacteria, susceptible hosts, and frequent consumption of fermentable

carbohydrates (9). Apart from these, several local risk factors such as form and arrangement of teeth, salivary flow, and oral hygiene and general risk factors such as age, sex, race, geographic location and social class are also involved (10). Cariogenic bacteria primarily form a biofilm on the tooth surface and an anaerobic environment develops as oxygen is quickly used up. Consequently, bacteria begin

anaerobic metabolism and produce low-molecular-weight organic acids (of which lactate is the most important) by breaking down carbohydrates that cause the tooth decay when pH in the biofilm falls below 5.5, the critical pH. In the progression of dental caries, a sequence of bacterial colonization occurs. Initially, non-mutans *Streptococcus* spp. and *Actinomyces* spp. colonize the tooth surface and result in a mild acidification which is followed by a second acidogenic stage, characterized by the colonization by low pH species. At the last stage, *Streptococcus mutans*, *Lactobacillus* spp. and *Bifidobacterium* spp. which are tolerant to a high concentration of acids begin colonization (4-5, 9, 11-15). In a recent study, a new bacterium, *Scardovia wiggsiae* has been reported to be significantly associated with severe early childhood caries (9, 16). Moreover, recent molecular biology studies have added *Dialister*, *Eubacterium*, *Olsenella*, *Atopobium*, *Propionibacterium*, *Abitrophia*, *Selenomonas*, and *Veillonella* spp. into the list of caries-associated microflora, although many of them are uncultivable and their cariogenic potential has not been determined yet (4, 11, 17).

If remain untreated, bacteria from dental caries lesion and saliva can attack dental pulp, usually sterile in a healthy individual, via dentinal tubules resulting in the initiation of pulpitis. At the onset of pulpitis, the biofilm composition is similar to that of dental caries. However, as the biofilm progresses through the dentinal tubules, the growth of proteolytic bacteria such as Gram-negative anaerobic rods: *Prevotella*, *Porphyromonas*, *Eubacterium*, *Parvimonas*, and *Campylobacter* spp. is favored by the protein-rich fluid and thus the complexity of the root canal microflora increases with the dominance of Gram-negative anaerobic rods and proteolytic bacteria. However, when bacteria reach the pulp via extra radicular root canals from periodontal lesions, the composition of the biofilm will be almost similar to that of chronic periodontitis (discussed in the next section). At the final stage, when the dental pulp is fully necrotic, the biofilm may get access to the outer wall of the apical part of the tooth through the apical foramen of the root resulting in periapical periodontitis. As for dental caries, recent molecular biology studies also suggested the association of a more complex biofilm with pulpitis and apical periodontitis and included *Treponema*, *Dialister*, *Olsenella*, *Synergistes* and *Enterococcus faecalis* to the above-mentioned list of root canal microflora (18-21).

ORAL ANAEROBIC BACTERIA AND PERIODONTAL DISEASES

Periodontal diseases result from the infections of tooth-supporting structures which include the gingiva (gum), periodontal ligament, and alveolar bone. The initial stage of the periodontal diseases is gingivitis or the gingival inflammation where the infection affects the gum and results in frequent spontaneous bleeding. Gram-positive oral streptococci initiate the establishment of supragingival biofilm resulting in marginal swelling, periodontal pocket formation, and increased flow of proteinaceous gingival crevicular fluid (6). In 1–2 weeks, these changes in the host environment induce the formation of a more complex biofilm containing both Gram-positive cocci and rods, and Gram-negative rods of different sizes of which many members are anaerobic.

If gingivitis left untreated, the supragingival biofilm will extend into the periodontal pocket and form a subgingival biofilm which will cause gradual deepening of the periodontal pockets, loosening of teeth, degradation of the bone, and ultimately loss of teeth. It is generally agreed that the bacterial biofilm initiates the disease, but the host immune response is responsible for main tissue destruction. The composition of the periodontitis biofilm differs from that of the gingivitis, as it is dominated by different Gram-negative rods most of which are anaerobic, proteolytic bacteria. Their growth is favored by the anaerobic condition in periodontal pockets and protein-rich gingival crevicular fluid (4,6-7).

Based on the clinical features and the composition of associated biofilm, periodontitis has been differentiated into three categories-aggressive periodontitis, post-antibiotics refractory periodontitis, and chronic periodontitis. Aggressive periodontitis has been associated with a microbial community dominated by the *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*; refractory periodontitis with a microbial community dominated by *Prevotella intermedia*, *Eikenella corrodens*, and *Tannerella forsythia*; while chronic periodontitis is controlled by a complex of *P. gingivalis*, *Fusobacterium nucleatum* and *T. forsythia* (9). Several new anaerobic species have been recently reported to be significantly associated with periodontitis, which included *Selenomonas noxia*, *S. flueggei*, *Actinomyces georgiae*, *Actinomyces gerencseriae*, *Olsenella uli*, *Lactobacillus rimae*, *Oribaculum cationiae*, *Dialister pneumosintes*, *Prevotella tanneriae*, *P. enoeca*, *Filifactor alocis*, and

Eubacterium sulci (9). Furthermore, a recent study showed that the presence of non-oral Gram-negative facultative rods like *Bordetella bronchispetica*, *Pasteurella* spp. and *Neisseria zoodegmatis* in the subgingival biofilm along with *P. gingivalis* and *T. forsythia* in patients who had received mechanical debridement therapy (22). Therefore, the possibility of the association of exogenous bacteria with the periodontal diseases cannot be nullified completely.

As for periodontitis, peri-implantitis can also lead to the loss of the "implant tooth". The microbiotas of peri-implantitis are usually similar to that of periodontitis.

ANTIBIOTIC RESISTANCE

Dental diseases are caused by polymicrobial biofilms and with the progression of the infection, anaerobes become dominant. If prompt and proper treatments are not undertaken, it may lead to tooth loss and systemic infection. To limit the local infection and systemic spread, appropriate regimen of antimicrobial drugs should be selected, that will be effective against both aerobic and anaerobic bacteria in the biofilm. Otherwise, infection may persist and result in more serious complications. Some of the most common antibiotics that have been shown to be effective in the treatment of dental diseases are beta-lactams such as penicillin, amoxicillin, and cephalosporin; beta-lactams along with a beta-lactamase inhibitor such as clavulanic acid; erythromycin; metronidazole; and tetracyclines (23). However, misuse and overuse of antibiotics has led to the emergence of antibiotic resistance among bacteria. Dental infections are caused by biofilms which are intrinsically more resistant to many antibiotics because bacteria in biofilms are different in phenotypes than their planktonically grown counterparts. Bacteria in a biofilm usually produce extracellular polymeric substances (EPS) which become deposited surrounding the bacterial cells and prevent the antibiotics from getting access to them. Similarly, some bacteria in a biofilm may secrete beta-lactamase which in turn may render beta-lactam antibiotics, the first line of drugs for the treatment of dental infections, inactive. Moreover, deep within the biofilm, bacteria are metabolically less active and therefore, antibiotics that interfere with protein synthesis such as tetracyclines or erythromycin may not exert their best effect (24). In addition, recent reports of the abundance of genetic determinants for the bacterial resistance to these widely used drugs in oral biofilm specimens have raised concerns about appropriate empirical therapy for the treatment of dental caries and periodontal diseases. Many beta-lactamase genes belonging

to class A of the Ambler classification (CepA, CblA, CfxA, CSP-1 and TEM), class B (CfiA), or class D in *Fusobacterium nucleatum* (FUS-1) have been found in oral Gram-negative bacilli. Similarly, several genetic determinants for tetracycline resistance have been detected in oral biofilm samples of both healthy and diseased individuals, of which *tetM*, *tetQ*, *tetO*, *tetW*, *tetO/32/O*, and *tet37* are the most common. Similarly, *ermB*, *ermV*, and *ermE* have been detected to be the most common erythromycin resistance determinants in oral biofilm samples. Macrolide resistant bacteria from oral cavity have also been characterized (25-32). When these above-mentioned determinants are present in the oral cavity, it might affect the efficacy of the administered drugs. It has also been observed that these determinants are often carried in mobile genetic elements such as plasmids and transposons (24, 33). As a result, proximity of bacteria in the oral biofilm facilitate the transmission of antibiotic resistance genes by transformation and conjugation, especially to strictly anaerobic Gram-negative bacilli.

CONCLUSION

The oral cavity is colonized by a wide range of resident bacteria which are beneficial to the host. However, if the composition of dental biofilm changed because of disrupted homeostasis, it may result in caries and/or periodontal diseases. To prevent dental infections, proper hygienic practices should be followed regularly, for example, brushing teeth twice with fluoride-containing toothpaste. The treatment of dental caries and gingivitis should reestablish the microbial community that is compatible with health. However, if an otherwise sterile site, such as root canal or apex of the root, is infected, the treatment should remove that biofilm by mechanical debridement followed by an appropriate antibiotic prophylaxis.

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