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The oral microbiome diversity and its relation to human diseases

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Abstract As one of the most clinically relevant human habitats, the human mouth is colonized by a set of microorganisms, including bacteria, archaea, fungi, and viruses. Increasing evidence has supported that these microbiota contribute to the two commonest oral diseases of man (dental caries and periodontal diseases), presenting significant risk factors to human health conditions, such as tumor, diabetes mellitus, cardiovascular diseases, bacteremia, preterm birth, and low birth weight in infants. It is widely accepted that oral microorganisms cause diseases mainly by a synergistic or cooperative way, and the interspecies interactions within the oral community play a crucial role in determining whether oral microbiota elicit diseases or not. Since a comprehensive understanding of the complex interspecies interactions within a community needs the knowledge of its endogenous residents, a plenty of research have been carried out to explore the oral microbial diversity. In this review, we focus on the recent progress in this field, including the oral microbiome composition and its association with human diseases.

Introduction

Only about 10 % of cells in our bodies are truly from the human host, and the rest are from human microbiota (Savage 1977; Wilson 2008). These commensal microorganisms help us resist pathogens, educate immune system, and provide some traits humans do not originally evolve with the body (Dethlefsen et al. 2007; Gill et al. 2006; Turnbaugh et al. 2007). For instance, the plant polysaccharides commonly consumed in the diet are rich in xylan-, pectin-, and arabinose-containing carbohydrate structures. Although the human genome lacks most of the enzymes required for degrading these compounds, the distal gut microbiota provides us with this capacity (Gill et al. 2006). In fact, the human genetic landscape is a blend of the human genome and the metagenome of microorganisms colonizing in/on the human bodies (Turnbaugh et al. 2007). Therefore, the genetic diversity of humans resides not only in the allele frequencies of shared *Homo sapiens* genes but also in the genes within our microbial communities (Bäckhed et al. 2005; Li et al. 2008). To fully understand the human genetic and physiological variations, the composition and structure of human microbiota in major parts (e.g., mouth, skin, and gut) of the body and their influencing factors must be characterized (Gill et al. 2006; Heijtz et al. 2011).

As one of the most clinically relevant microbial habitats, the oral cavity is colonized by a personalized set of microorganisms, including bacteria, archaea, fungi, and viruses. If the term "human microbiome" is used to describe the sum of microbes that live in symbiosis or commensalism with us and elicit various human diseases under certain conditions (Lederberg and McCray 2001), the "oral microbiome" is suitable to refer specifically to the microorganisms inhabiting the human mouth (Dewhirst et al. 2010). The oral microbiome not only greatly contributes to the two commonest human oral diseases (i.e., dental caries and periodontal diseases) but also has been proven to present a significant risk factor to human health, such as tumor (Farrell et al. 2011), diabetes mellitus (Löe 1993), cardiovascular diseases (Figuero et al. 2011), bacteremia (Bahrani-Mougeot et al. 2008), and preterm birth and low birth weight in infants (Mitchell-Lewis et al. 2001;

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Offenbacher et al. 2006). It is widely accepted that oral microorganisms cause diseases mainly by a synergistic or cooperative fashion (Darveau 2010; Griffen et al. 2012; Hajishengallis and Lamont 2012), which means, in addition to the host immune response, it is the dynamic balance of both synergistic and antagonistic interspecies interactions rather than the presence/absence of specific bacteria within the community that plays a crucial role in determining whether diseases occur or not (Kleinberg 2002; Marsh 2005; Medzhitov 2007; Socransky et al. 1998). Since a comprehensive understanding of the microbial interspecies interactions of a given community requires the full knowledge of its endogenous residents, a slew of investigations have been carried out to explore the microbial diversity, composition, and structure of oral microbial communities. For a long time, this research field has been impeded by the intrinsic limitation of the conventional culture-dependent methods. However, more than 50 % of the oral microorganisms are unable to be cultivated (Paster et al. 2006); culture-independent methods, such as reverse-capture checkerboard hybridization (Mager et al. 2003), fluorescence in situ hybridization (Gersdorf et al. 1993), terminal restriction fragment length polymorphism (Takeshita et al. 2008), denaturing/thermal gradient gel electrophoresis (Alves et al. 2009), microarrays (Lif Holgerson et al. 2011), and *16S rRNA* clone library analysis (Aas et al. 2005), have be used to refine and redefine the knowledge of the microbial diversity in the different oral sites, substantially expanding the list of candidate pathogens associated with oral diseases. More importantly, in this decade, high-throughput DNA sequencing technologies, such as 454 pyrosequencing (Roche Applied Science, Basel, Switzerland), Illumina/Solexa Genome Analyzer (Illumina, San Diego, CA, USA), and SOLiD (Applied Biosystems, Foster City, CA, USA), have been used, dramatically increasing the resolution at which microbial communities can be analyzed. Here, we describe the recent progress in the field of oral microbiome. Since the majority of the research is concentrated on the domain Bacteria, we first discuss the bacterial diversity in different oral niches under health and disease conditions and the evidence confirming relationships between oral bacterial community shifts and some systemic diseases and health risk conditions, and then, a brief introduction of oral viruses, fungi, and archaea and their relationships with human health and diseases is presented.

Oral bacterial microbiome

Over 700 bacterial species have been identified by culture-independent approaches in the human mouth, and more than 250 have been isolated, cultivated, and named (Paster et al. 2006). Undoubtedly, more novel species are expected to be identified (Belda-Ferre et al. 2012; Bik et al. 2010; Griffen et al. 2012; Keijser et al. 2008). Two types of surfaces are available in the human mouth for microbial colonization, including shedding (mucosa), and solid surfaces (teeth). In addition, microflora attaching to surfaces continuously shed into the saliva, making salivary microbiota the "fingerprint" of the oral microbiome inhabiting in the oral surface (Fábián et al. 2008). Since it has been well established that microorganisms colonizing the oral cavity display significant tropism for different inhabiting environments (Mager et al. 2003), we discuss the microbial composition of saliva, biofilms formed on the tooth surface, and mucosa separately.

Saliva

Each milliliter of saliva contains an average of 1.4×10^8 CFU bacteria, most of which belong to one of seven major phyla: *Actinobacteria*, *Bacteroides*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Spirochaetes*, and TM7 (The Human Microbiome Consortium 2012; Zaura et al. 2009). Although members of the population shared similar salivary organisms (The Human Microbiome Consortium 2012), there is an inter-individual difference in the salivary composition, although it is stable within a certain period for a given individual within a certain time frame (Lazarevic et al. 2010). However, salivary microbes do not show obvious geographical distribution characteristics, that is, the microbial composition does not indicate any strong influence of geography (Nasidze et al. 2009). The salivary microbiota may be used as biomarkers of disease diagnosis. For example, patients with dental caries (Yang et al. 2011a), periodontitis (Sakamoto et al. 2000), oral squamous cell carcinoma (Pushalkar et al. 2011), and pancreatic cancer (Farrell et al. 2011) show a different salivary bacterial composition and/or distribution from healthy populations.

Dental plaque

Dental plaque is a kind of biofilm building on the tooth surfaces (Yang et al. 2011b). It could be classified into two categories according to the location: supragingival dental plaque above the gingival margin and subgingival plaque below the gingival margin (Filoche et al. 2010). The supragingival plaque of healthy populations contains a rich variety of bacteria. Approximately 6,888 bacterial taxa in the supragingival ecology of 98 healthy adult subjects were identified with *Firmicutes* and *Actinobacteria* as the dominant groups (Keijser et al. 2008). The composition of supragingival plaque on the dental surfaces varies with the balance between health and disease conditions. Taking the development of dental caries as an example, the microbial composition of plaque on the surface of healthy enamel, white spot, and caries cavity was significantly different, and as caries progressed, the microbial diversity gradually decreased and dominant bacteria changed (Aas et al. 2008; Gross et al. 2010). Similar results

were obtained from studies on root surfaces (Preza et al. 2008). The subgingival plaque is closely associated with the periodontal destruction. Microbiological studies of this niche mainly focus on changes during the development of periodontal diseases, which are described in the section "Periodontal diseases" of this review.

Oral mucosa

Compared with other oral niches, the microbes colonizing the oral mucosa are relatively limited. The tongue has attracted great attention due to its colonization by microbes associated with halitosis (i.e., unpleasant odor exhaled in breathing). The tongue dorsum of healthy populations is colonized by large amounts of *Streptococcus salivarius*, *Rothia mucilaginosa*, and an uncharacterized cultivable species of *Eubacterium* strain FTB41 (Kazor et al. 2003).

Oral bacteria related to oral diseases

Dental caries

Dental caries is one of the most prevalent oral diseases, and humans are susceptible to it during the whole life (Selwitz et al. 2007). Dental caries not only leads to tooth destruction but also causes pulp and periapical infection (Balakrishnan et al. 2000). When investigating the microbial diversity of dental caries, specimens are usually collected from supragingival plaque, saliva, and infected dentin of children with severe early childhood caries (S-ECC), caries-active adult patients, and elders suffering from root caries.

S-ECC is a kind of rampant caries involving multiple primary teeth, especially the maxillary anterior teeth (Drury et al. 1999). *Streptococcus mutans* has been studied intensively for its cariogenic properties and has even been regarded as a specific pathogen of caries. However, other bacteria, including species of *Streptococcus*, *Veillonella*, *Actinomyces*, *Granulicatella*, *Leptotrichia*, *Thiomonas*, *Bifidobacterium*, and *Prevotella* were also detected at higher frequencies in the plaque of children with S-ECC than that of caries-free children, and these bacteria are believed to be S-ECC associated (Becker et al. 2002; Kanasi et al. 2010; Ling et al. 2010; Tanner et al. 2011). Follow-up observation of S-ECC patients who underwent systemic treatments showed that children affected with S-ECC again had higher abundance of *Prevotella nigrescens* and *Capnocytophaga* in pre-treatment plaque compared with relapse-free patients (Tanner et al. 2011). In addition, if S-ECC is effectively controlled, the percentage of *S. mutans* in supragingival dental plaque would be reduced (Tanner et al. 2011).

The genera *Streptococcus*, *Lactobacillus*, *Actinomycetes*, *Propionibacterium*, and *Veillonella* were also detected with a high relative abundance in the plaque of caries-active adults (Belda-Ferre et al. 2012). In addition, *Prevotella* species including *Prevotella* sp., *Prevotella histicola*, and *Prevotella shahii* were not similarly distributed between healthy and caries-active hosts (Yang et al. 2011a). More significantly, genes related to acid production, DNA uptake, and stress responses were highly expressed in the plaque of caries-active patients (Belda-Ferre et al. 2012).

Root caries (RC) often occurs in elders with gingival recession. The genera *Atopobium*, *Olsenella*, *Pseudoramibacter*, *Propionibacterium*, and *Selenomonas* were found to be involved in the occurrence and development of root caries (Preza et al. 2008). A large number of *Lactobacillus* were detected in the biofilms formed on the healthy root surface of RC patients; however, no *Lactobacillus* were found in plaques formed on the root surface of caries-free populations (Preza et al. 2008, 2009).

From the clinical perspective, dental caries can be manifested as white spot with intact tooth structure and cavity. During the development of dental caries, microbial composition undergoes dynamic changes: the microbial diversity gradually decreases in the order of plaque from healthy enamel surfaces, white spot, plaque from cavity surfaces, and infected dentin (Gross et al. 2010). The predominant bacteria also changes during the progression, gradually transitioning from non-*S. mutans*, *Streptococci*, and *Actinomycetes* to *S. mutans*, *Lactobacillus*, and *Bifidobacterium* (Takahashi and Nyvad 2011). Interestingly, even at the same caries developmental stages, predominant bacteria vary between primary and permanent dentition. For example, the dominant bacteria in the white spot of both primary and permanent teeth are *S. salivarius* and *Streptococcus parasanguis*, whereas the bacteria most frequently detected from cavity surfaces are *S. salivarius*, *S. parasanguis*, *Corynebacterium*, and *Actinomyces gerencseriae* in primary dentition and *S. salivarius*, *S. parasanguis*, *Campylobacter*, and *Selenomonas* in permanent dentition. Furthermore, infected dentin contains a large number of acid-producing bacteria, such as *S. mutans*, *Lactobacillus*, and *Propionibacterium* (Aas et al. 2008).

Periapical infection

Periapical periodontitis is an infectious disease occurring on the tooth periapical tissue and it is the result of the interaction between the microbial community and the host immune response (José 2002; Siqueira and Rôças 2009a, b).

As demonstrated above, microbial colonization in the root canal system is the major pathogenic factor of periapical periodontitis (Siqueira 2011). A long ago, researchers found that root periapical infection was a multi-bacterial infection, by using culture-dependent technologies (Byström et al. 1987;

Fabricious et al. 1982), which has been confirmed by studies with culture-independent molecular methods (Siqueira and Rôças 2009a, b). The most represented, abundant, and prevalent phyla in infected root canal were *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, and *Actinobacteria* at the phylum level (Siqueira 2011) and *Olsenella uli*, *Prevotella baroniae*, *Porphyromonas endodontalis*, *Fusobacterium nucleatum*, and *Tannerella forsythia* at the species level (Rôças et al. 2010). There are inter-individual variations in the microbial spectrum of infected root canals, that is, the microbial communities in infected root canals of different individuals are not exactly the same (Machado de Oliveira et al. 2007; Santos et al. 2011; Siqueira et al. 2008; Siqueira 2011). The microbial communities associated with root canal infection also differ according to the location of the affected tooth (Alves et al. 2009; Rôças et al. 2010). Even within a single infected root canal, the microbial composition in the apical and coronal regions differs: the former typically has a higher level of microbial diversity than the latter (Özok et al. 2012), and the dominant microbes are different (the apical area mainly contains obligate anaerobes) (Siqueira and Rôças 2005).

Although most of the periapical periodontitis can be controlled with root canal therapy, in some cases, the infection could be persistent or the filled canal could be re-infected. By using PCR-DGGE, Chugal et al. (2011) compared the microbiota residing at the apical portion of primary infected canals and root canals with failed treatments and found that the apical bacterial communities in primary infections were significantly more diverse, and different roots of the same teeth with primary infections contained almost identical bacterial composition while an equivalent sample collected from unsuccessfully treated tooth displayed low similarity.

In a small proportion of cases, bacteria colonized in the root canals could also develop communities on root surfaces outside the apical foramen and cause extraradicular infections, which is associated with refractory periapical periodontitis. Bacterial taxa detected in extraradicular infections belong to six phyla (Siqueira and Rôças 2009b). Species reported include *Propionibacterium propionicum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella oralis*, *Parvimonas micra*, *P. endodontalis*, *F. nucleatum*, and *T. forsythia* (Noguchi et al. 2005; Siqueira and Rôças 2009b; Su et al. 2010).

The extensive application of molecular methods has not only dramatically increased the amount of available information related to root canal infection but has also, to some extent, led to changes in our understanding of the etiology of root canal infection (Siqueira and Rôças 2009a). For a long time, some Gram-negative bacteria were believed to be associated with specific symptoms of periapical periodontitis (Gomes et al. 1994). Because these Gram-negative bacteria were detected at similar frequencies in infected root canals without symptoms (Baumgartner et al. 1999; Rôças and Siqueira 2008; Siqueira et al. 2000), the overall structure of the microbial community are supposed to be closely associated with the symptoms instead (Sakamoto et al. 2006; Siqueira et al. 2004). For example, a much more diverse root canal microbial community was observed among periapical periodontitis patients with acute infection symptoms than these without clinic symptoms (Santos et al. 2011).

Periodontal diseases

Gingivitis is an inflammation limited in the gingiva. It is believed to result from the accumulation of plaque and the associated interactions between bacteria in dental plaque and gingival tissues (Moore et al. 1987). With the development of gingivitis, the dominant bacteria in subgingival plaque gradually shift from *Streptococcus* to *Actinomycetes*, *Capnocytophaga*, *Campylobacter*, *Eikenella*, *Fusobacterium*, and *Prevotella* (Zaura et al. 2009). Besides, the saliva microbiome of people with and without gingivitis showed significant differences (Huang et al. 2011).

Periodontitis is also a chronic inflammatory disease of the tooth-supporting tissues and the leading cause of tooth loss worldwide (Pihlstrom et al. 2005). Compared with gingivitis, it causes not only the progressive destruction of the gum but also periodontal membrane and alveolar bone. In contrast to previous results obtained using bacterial cultures, studies with methods based on *16S rRNA* gene PCR/cloning/sequencing techniques have demonstrated that most dominant bacteria in sites affected by periodontitis are not Gram-negative species (Wade 2011). The reasons for the different findings might be that staining characteristics of Gram-positive anaerobic bacteria colonizing the subgingival plaque could vary and that excessively long periods of culture could also lead to negative gram staining (Kononen and Wade 2007). Another factor is that the culture technique has bias, and it only detects what is specific for the culturing conditions.

The subgingival microbial composition in patients with periodontitis undergoes extremely complex changes. More than 400 phenotypes have been detected from periodontal pockets (Wade 2011). Griffen et al. (2012) found that the relative abundances of 123 phenotypes in the subgingival plaque microbiome were increased in periodontitis, whereas the abundances of 53 others decreased. In addition to *P. gingivalis*, *Treponema denticola*, and *T. forsythia*, bacteria including *Bacteroidetes* species, *Eubacterium saphenum*, *P. endodontalis*, *Prevotella denticola*, *Parvimonas micra*, *Peptostreptococcus* species, *Filifactor alocis*, *Desulfobulbus* species, *Dialister* species, and *Synergistetes* species are closely related to periodontitis (Dahlén and Leonhardt 2006; Kumar et al. 2003, 2005; Paster et al. 2006). *Streptococcus*, *Veillonella*, *Abiotrophia*, *Campylobacter*, *Capnocytophaga*, *Gemella*, and *Neisseria* are considered as beneficial bacteria

(Kumar et al. 2005). Recently, a study focusing on microorganisms associated with aggressive periodontitis yielded surprising results: *Selenomonas* was the dominant microbiota in subgingival plaque whereas *Aggregatibacter actinomycetemcomitans*, which was formerly believed to be closely associated with this disease (Schacher et al. 2007), was not detected (Faveri et al. 2008). However, this finding should be taken with cautious: one possibility is that *A. actinomycetemcomitans* is not present in aggressive periodontitis samples, and another possibility is that *A. actinomycetemcomitans* is below the detection of *16S rRNA* gene clones. The subgingival plaque of refractory periodontitis patients contains a greater number of *Parvimonas micra*, *Campylobacter gracilis*, *Eubacterium nodatum*, *Selenomonas noxia*, *T. forsythia*, *P. gingivalis*, *Prevotella* species, *Treponema* species, and *Eikenella corrodens* compared to healthy subjects (Colombo et al. 2012, 2009).

As an important risk factor for periodontitis, smoking could affect the subgingival microbiota composition. In the subgingival environment, greater abundances of species belonging to *Bacteroides*, *Campylobacter*, *Fusobacterium*, *Parvimonas*, and *Porphyromonas*, and lower abundances of *Veillonella*, *Neisseria*, and *Streptococcus* have been detected in smokers with periodontitis than in never smokers (Shchipkova et al. 2010). Moreover, tobacco smoking also causes changes in the symbiotic and commensalistic relationships among the subgingival microbes. For example, never-smoking patients with high levels of *Streptococci* exhibited low levels *Parvimonas* while current smokers with high levels of *Streptococci* demonstrated high levels of *Parvimonas* (Shchipkova et al. 2010). Also, recent evidence suggests that *Streptococci* play an essential role in preventing colonization of periodontal ecology by pathogens (Stingu et al. 2008; Van Hoogmoed et al. 2008). It is possible that the protective function of *Streptococci* is impaired by tobacco, leading to a co-colonization pattern alteration. In addition, smoking cessation results in a decrease in the prevalence of *P. endodontalis* and *Dialister pneumosintes* and in the relative abundances of *Parvimonas micra*, *Filifactor alocis*, and *Treponema denticola* (Delima et al. 2010). An increase in the proportion of beneficial bacteria *Veillonella parvula* was also observed after quitting tobacco (Delima et al. 2010).

Halitosis

Halitosis (oral malodor) refers to unpleasant odor exhaled in breathing; it can be classified into intra-oral halitosis and extra-oral halitosis (Murata et al. 2002). Volatile sulfur compounds and malodorous fatty acids produced from the decomposition of sulfur-containing amino acids, peptides, and proteins by oral bacteria are considered to be the direct cause of intra-oral halitosis (Murata et al. 2002).

It has been gradually recognized that bacteria on the tongue dorsum, especially the posterior dorsum, are the main factor leading to intra-oral halitosis in people with complete dentition and healthy periodontal tissues (Allaker et al. 2008; Porter and Scully 2006). There are differences in the microbial diversity of the tongue dorsum between patients with intra-oral halitosis and healthy controls. The dominant bacteria found in the tongue dorsum of patients with intra-oral halitosis include *Solobacterium moorei*, *Atopobium parvulum*, and *Eubacterium sulci*, whereas *S. salivarius*, *Rothia mucilaginosa*, and an uncharacterized cultivable *Eubacterium* species are abundant in healthy controls (Kazor et al. 2003). It was also shown that *Solobacterium moorei* was only found in specimens from patients with halitosis (Haraszthy et al. 2007); these bacteria were once called the "arch-criminal of halitosis." The oral distributions of *S. salivarius* in patients with halitosis and in healthy populations remain controversial. For example, Kazor et al. (2003) suggested that *S. salivarius* was the most dominant species on the tongue dorsum of healthy controls and was relatively rare or even absent on the tongue dorsum of halitosis patients. In contrast, Riggio et al. (2008) found that it was the dominant species in both halitosis patients and healthy persons with the same method (i.e., *16S rRNA* gene cloning). Until today, there are still researchers who believe that *S. salivarius* represents as a benign commensal probiotic for the reduction of oral malodor (Masdea et al. 2012).

Oral bacteria and systemic diseases

Tumor

A tumor is a mass of tissue as a result of abnormal growth or division of cells (Cooper 1992). Microbe-induced inflammation is involved in 15–20 % of human tumors (Allavena et al. 2008). Researches in this area have focused on the correlation between the microbiome and tumor occurrence, microbiome shifts in cancer patients, the feasibility of identifying early diagnostic markers from the microbiome, and the effects of tumor treatments on the microbiome (Meurman 2010).

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor in the oral cavity (Bagan et al. 2010). Levels of *Capnocytophaga gingivalis*, *Prevotella melaninogenica*, and *Streptococcus mitis* in the saliva of patients with OSCC significantly increased (Mager et al. 2005), and the salivary microbiomes of cancer patients were more similar to each other than the microbiomes of individuals in healthy populations (Pushalkar et al. 2011). The OSCC surface biofilm harbors increased aerobes and anaerobes, including *Veillonella*, *Fusobacterium*, *Prevotella*, *Porphyromonas*, *Actinomyces*, *Clostridium*, *Haemophilus*, *Enterobacteriaceae*,

and *Streptococcus* species (Nagy et al. 1998). In addition, bacteria could be detected within the OSCC tissues. Hooper et al. (2007) found 52 different bacterial phylotypes from tumorous tissues, and the majority of species were saccharolytic and aciduric, including *Proteobacteria*, *Fusobacterium*, *Streptococcus*, *Prevotella*, and *Veillonella*, which is consistent with a previous study indicating the acidic and hypoxic microenvironment within tumor tissues (Raghunand et al. 2003).

The oral microbiome is also involved in or affected by tumors of distant organs (Ahn et al. 2012; Farrell et al. 2011). For example, the proportions of *S. mitis* and *Neisseria elongata* in the salivary microbiome were significantly lower in patients with pancreatic cancer than in healthy people (Farrell et al. 2011). As the authors discussed, the cross-sectional nature of this study has not enabled us to understand the mechanisms or the association, and whether these two types of bacteria can be called markers for early diagnosis of pancreatic cancer has yet to be investigated.

Surgery, radiotherapy, and chemotherapy are three primary approaches to cancer treatments. The changes of oral microbiome undergoing chemoradiotherapy have been confirmed by many approaches, including high-throughput sequencing (Hu et al. 2013; Meurman 2010; Napeñas et al. 2010; Shao et al. 2011; Xu et al. 2014). These studies suggested a shift to a more complex oral bacterial profile in patients undergoing cancer chemotherapy by a host-specific manner.

Diabetes mellitus

Diabetes mellitus (DM) is a clinical syndrome characterized by hyperglycemia due to a deficiency in the secretion of insulin and/or reduced insulin action (Alberti and Zimmet 1998). There are two main types: type 1 DM resulting from the body's failure to produce insulin and type 2 DM resulting from insulin resistance. Both type 1 and type 2 DM show a three- to four-fold increased risk of periodontitis which is regarded as the sixth complication of DM (Löe 1993).

Some studies have explored the composition of subgingival dental plaque in diabetics compared with non-diabetics; however, no agreement has been reached regarding the effect of DM on periodontal subgingival microbiota. Hintao et al. (2007) found increased frequency of *Treponema denticola*, *Streptococcus sanguinis*, *Prevotella nigrescens*, *Staphylococcus intermedius*, and *Streptococcus oralis* in the supragingival plaque of type 2 diabetics compared with non-diabetics while no significant differences were found in subgingival plaque samples. Similar subgingival infection patterns were also observed between type 1 diabetics and non-diabetics after controlling of the periodontal severity (Lalla et al. 2006). In contrast, Ebersole et al. (2008) demonstrated that the periodontitis sites in type 1 DM patients showed a higher frequency of *P. gingivalis*, *A. actinomycetemcomitans*, and *Campylobacter* spp. A higher prevalence of *P. gingivalis*, *Candida* spp. (mainly *Candida albicans* and *Candida dubliniensis*), as well as a lower frequency of *T. forsythia* was also demonstrated in type 2 diabetics (Campus et al. 2005; Sardi et al. 2011). In a much more recent study using *16S rRNA* gene sequencing, significant differences were observed in subgingival microbiota between type-2 DM and non-diabetic subjects: diabetic subjects presented higher abundance of total clones of TM7, *Aggregatibacter*, *Neisseria*, *Actinomyces*, *Capnocytophaga*, *Gemella*, *Eikenella*, *Selenomonas*, *Fusobacterium*, *Veillonella*, and *Streptococcus* and lower percentages of *Synergistetes*, *Tannerella*, *Porphyromonas*, *Filifactor*, *Eubacterium*, and *Treponema*s; moreover, some species, such as *F. nucleatum*, *V. parvula*, *Veillonella dispar*, and *E. corrodens* were detected significantly more often in diabetics (Casarin et al. 2013). Considering the elevated glucose content in subgingival microenvironment and altered or impeded immune responses of hosts (Ohlrich et al. 2010), there may indeed be differences in the subgingival microbiome in diabetic patients compared with non-diabetics.

Cardiovascular disease

Cardiovascular diseases are a set of diseases that include congestive heart failure, cardiac arrhythmias, valvular heart disease, and stroke and coronary artery disease (including atherosclerosis and myocardial infarction) (Ross 1999).

Atherosclerosis, a major component of cardiovascular diseases, is a condition caused by abnormal lipid metabolism and neurovascular dysfunction, in which yellow substances containing cholesterol and fat appear in the intima of large and medium arteries, often leading to thrombosis and ischemia (Ross 1999). Oral microbiota, including *Streptococcus*, *Veillonella*, *P. gingivalis*, *F. nucleatum*, *T. forsythia*, and *Neisseria* were detected from atherosclerotic plaques (Figuero et al. 2011; Ford et al. 2005; Koren et al. 2011; Pucar et al. 2007). The levels of *Fusobacterium*, *Streptococcus*, and *Neisseria* were found to be related to the risk factors for the disease, such as the plasma cholesterol level (Koren et al. 2011). These findings also indirectly support one of the pathogenic models of cardiovascular disease, namely, the infection model (the bacteria invade the bloodstream and subsequently get into the endothelium, resulting in endothelial dysfunction, inflammation, and atherosclerosis) (Seymour et al. 2007).

The atherosclerotic plaque rupture participating in thrombus formation and chronic inflammation may cause plaque instability (Libby et al. 2002). Chiu (1999) found *P. gingivalis* and *S. sanguinis* in unstable atherosclerotic plaques, and Ohki et al. (2012) detected *A. actinomycetemcomitans*, *P. gingivalis*, and *Treponema denticola* in thrombi of patients with acute myocardial infarction by PCR. These studies not only

confirmed the presence of oral bacteria but also suggested that oral microbiota might have a role in plaque inflammation and instability.

Bacteremia

Bacteremia is an invasion of the bloodstream by bacteria. Invasive dental manipulation (tooth extraction for instance), as well as daily oral hygiene activities (brushing the teeth for example), can produce bacteremia (Poveda-Roda et al. 2008). According to the study of Bahrani-Mougeot et al. (2008), the most predominant species in the blood of following dental procedures were *Streptococcus* spp., followed by *Peptostreptococcus micros*, *Veillonella dispar* or *V. parvula*, and *D. pneumosintes*. Another research also confirmed that the most predominant *Streptococcus* species were *S. mitis*, *S. oralis*, and *S. sanguinis* (Forner et al. 2006). Although majority of such bacteremia is transient, it might also be a risk factor of distant site infections. For example, oral streptococci's ability to aggregate platelets is a potential pathogenic factor in the development of endocarditis and formation of thrombi (Herzberg and Meyer 1996). A relationship has been reported between bacteremia caused by tooth brushing and the risk of cardiovascular diseases (Roberts 1999).

Other systemic diseases

The application of molecular biological methods continues to expand our understanding of the relationship between oral microorganisms and systemic diseases. For example, Docktor et al. (2012) utilized the human oral microbiome chip to reveal that tongue microbial diversity was lower in pediatric patients with Crohn's disease than in healthy children and to show that changes in *Fusobacteria* and *Firmicutes* were among the most significant. Han et al. (2010) identified *F. nucleatum* with an oral origin in the uteri of miscarried women and preterm birth and low birth weight has been associated with high levels of *T. forsythi*a, *Campylobacter rectus*, *Prevotella intermedia*, *Prevotella nigrescens*, and *P. gingivalis* (Mitchell-Lewis et al. 2001; Offenbacher et al. 2006). Goodson et al. (2009) even proposed that oral microorganisms might be involved in obesity.

Oral fungal, viral, and archaeal microbiome

Oral fungal microbiome

Fungi comprise a minor component of the oral microbiome, and they are collectively named oral fungal microbiome or mycobiome (Ghannoum et al. 2010). In a recent study aiming to obtain a comprehensive profile of the oral mycobiome, researchers identified 74 cultivable and 11 uncultivable fungal genera from oral rinse of 20 healthy individuals with pyrosequencing (Ghannoum et al. 2010). It was also found that each individual carried 9~23 fungal species, and the detection rate of *Candida* in the subjects was the highest, followed by *Cladosporium*, *Aureobasidium*, *Saccharomyces*, *Aspergillus*, *Fusarium*, and *Cryptococcus* (Ghannoum et al. 2010). The composition of fungal microbiome is, to some extent, associated with gender and race; it differs between white males and Asian males, but it is similar between white females and Asian females (Ghannoum et al. 2010).

Candida are the most frequently detected oral fungi (Ghannoum et al. 2010). The most common *Candida* species is *C. albicans* and less common species include *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, *Candida stellatoidea*, *Candida kefyr*, and *C. dubliniensis* (Krishnan 2012). The frequency, intensity, species, and strains of oral *Candida* varied with age (Kleinegger et al. 1996), and the frequency of *C. albicans* decreased with increasing age (Qi et al. 2005). More interestingly, elders with high *Candida* load harbored a lower diverse salivary microbiome and had a distinct microbial composition toward dominance by *Streptococci* (Kraneveld et al. 2012). Candidiasis is the commonest infection caused by *Candida* (Cannon et al. 1995). In addition, this genus is considered to be involved in some other oral diseases. Recent evidence implies that the occurrence of caries in children was positively correlated with the frequency of oral candidal carriage (Raja et al. 2010; Yang et al. 2012), and that *C. albicans* is able to cause occlusal caries in rats at a high rate (Klinke et al. 2011), indicating the role of *Candida* in the cariogenic development. Actually, in vitro studies have proven that *S. mutans* could enhance the adherence of *C. albicans* and excrete lactate as a carbon source for yeast growth while the growth of yeast reduces oxygen tension to levels preferred by streptococci and provide growth stimulatory factors for the bacteria (Brogden and Guthmiller 2008; Metwalli et al. 2013). Moreover, *Candida* was also detected in periodontal pockets and *C. albicans* was observed to be highly associated with the severity of chronic periodontitis (Canabarro et al. 2013). Although still in its infancy, the research thus far strongly warrants that investigations on the microbiology of periodontitis should include yeasts.

Oral virome

The virome is the collective of viruses that populate an organism or ecosystem at any given time (Haynes and Rohwer 2011). The oral virome contains a range of viruses, the vast majority of which present homology with bacteriophages and their presence may be closely related to oral microbial diversity (Pride et al. 2012; Robles-Sikisaka et al. 2013). Comparisons of the salivary virome with respiratory and gut virome

revealed that the habitat is an important selection factor for the human virome composition (Pride et al. 2012). The living environment of hosts has a role in shaping oral viral ecology, as demonstrated by Robles-Sikisaka et al. (2013) that a significantly greater fraction of shared viral homologous reads were observed among subjects from the same family than those from separate households.

Oral virome is primarily disease-associated, such as herpes simplex virus (pathogen of herpetic gingiva-stomatitis and herpes labialis), varicella zoster virus (pathogen of herpes zoster), and human papilloma virus (pathogen of papillomas) (Kumaraswamy and Vidhya 2011; Whitley and Roizman 2001). Herpes virus, including human cytomegalovirus (HCMV), Epstein–Barr virus (EBV) type 1–2, herpes simplex virus (HSV) type 1, and human herpes virus types 6–8, could also be detected in periodontal pockets of patients with chronic periodontitis (Imbronito et al. 2008), localized and generalized juvenile periodontitis (Imbronito et al. 2008), Papillon–Lefèvre syndrome periodontitis (Velazco et al. 1999), Down's syndrome periodontitis (Hanookai et al. 2000), HIV-associated periodontitis (Contreras et al. 2001), and acute necrotizing ulcerative gingivitis (Contreras et al. 1997). Moreover, EBV-1 and HCMV are positively associated with the subgingival presence of some periodontal pathogens (Contreras et al. 1999) and the severity of periodontitis (Ling et al. 2004; Saygun et al. 2002). So, some oral microbiologists postulate that virus might play an important role in the pathogenesis of human periodontitis (Kubar et al. 2005; Saygun et al. 2002; Slots 2010) and herpesviruses may promote the process of periodontitis through releasing tissue-destructive cytokines, initiating cytotoxic or immunopathogenic event and boosting pathogenic periodontal bacteria growth (Slots and Contreras 2000). However, the role of oral virus in the periodontal pathogenesis is under debate. For instance, although an obvious association of the viruses with clinical samples was observed, Sunde et al. (2008) still believed that their presence might reflect that clinical samples contain more saliva or blood compared to healthy controls or an accumulation of lymphoid cells harboring virus in the inflamed tissue.

Oral archaeal microbiome

The *Archaea* are non-bacterial prokaryotes, and they are restricted to a small number of species/phylotypes. Human *Archaea* are mainly found in the gut and the oral cavity, majority of which are methanogens with few exceptions (Dridi et al. 2011). The diversity of oral *Archaea* is limited compared to bacteria domain and the reported oral *Archaea* include the genera *Thermoplasmatales*, *Methanobrevibacter*, *Methanobacterium*, *Methanosarcina*, and *Methanosphaera* (Dridi et al. 2011; Eckburg et al. 2003; Lepp et al. 2004; Nguyen-Hieu et al. 2013).

Studies have primarily focused on the role of oral *Archaea* in periodontal disease (Lepp et al. 2004; Li et al. 2009; Matarazzo et al. 2012; Vianna et al. 2008) and endodontic infections (Jiang et al. 2009; Vianna et al. 2006, 2009; Vickerman et al. 2007). These reports demonstrated a higher detection frequency of *Archaea* was observed in the infected population. The relative abundance of *Archaea* in subgingival plaque increased as the severity of chronic periodontitis increased (Lepp et al. 2004), and treated periodontitis sites showed a decrease in *Archaea* when the treatment was followed by improvement of lesions (Lepp et al. 2004; Lira et al. 2013). Although methanogenic archaea could promote periodontal tissue destruction, the archaea are not involved in the initiation of periodontal infection (Farrell et al. 2011). Furthermore, the presence of *Archaea* in infected root canal might be associated with clinical symptoms (Jiang et al. 2009). Research on *Archaea* has begun to extend to other oral infectious diseases, such as peri-implantitis (Faveri et al. 2011) and pericoronitis (Mansfield et al. 2012). However, the role of *Archaea* in oral pathologies remains controversial and further studies are required to explore the potential mechanisms of these microorganisms (Nguyen-Hieu et al. 2013).

Conclusions

Although we can only partially annotate a portion of the completely sequenced oral microbiome, the vast amounts of data present an encrypted book available to researchers interested in the oral microbiome. Interpretation of the coded information within this encrypted book is sure to encourage and facilitate the investigation of microbial pathogenic mechanisms, drug development, and the identification of new diagnostic markers.

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