The oral microbiota – a mechanistic role for systemic diseases

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Key points

- Provides an overview on basic composition and distribution of oral microbiota.
- Elucidates the underlying mechanisms of endogenous and exogenous factors on oral microbiota and oral health.
- Reviews oral microbiota and its implications for systemic diseases.
- Summarises the improvement of clinical diagnosis and treatment based on microbial community information.

Human oral microbiota is the ecological community of commensal, symbiotic, and pathogenic microorganisms found in the oral cavity. Oral microbiota generally exists in the form of a biofilm and plays a crucial role in maintaining oral homeostasis, protecting the oral cavity and preventing disease development. Human oral microbiota has recently become a new focus research for promoting the progress of disease diagnosis, assisting disease treatment, and developing personalised medicines. In this review, the scientific evidence supporting the association that endogenous and exogenous factors (diet, smoking, drinking, socioeconomic status, antibiotics use and pregnancy) modulate oral microbiota. It provides insights into the mechanistic role in which oral microbiota may influence systemic diseases, and summarises the challenges of clinical diagnosis and treatment based on the microbial community information. It provides information for noninvasive diagnosis and helps develop a new paradigm of personalised medicine. All these benefit human health in the post-metagenomics era.

Introduction

The oral cavity is a connection channel between outside environments and the respiratory tract and digestive tract. It provides an appropriate temperature, humidity, and nutrition for microorganism colonisation. The human oral microbiome has been extensively studied as part of the Human Microbiome Project. The oral microbiome has an essential role in maintaining a normal oral ecological balance and in the development of oral diseases. There is abundant evidence supporting the theory that endogenous and exogenous factors are closely related to oral microbiota and systemic diseases.[1,2] Studies on dietary behaviours demonstrate a fundamental aspect of the oral disease paradigm.[3] Lifestyles and diets including smoking, alcohol drinking and consuming spicy food, and antibiotic treatments can persistently alter commensal microbial communities.[4] The resultant microbial disturbances may increase pathogen susceptibility.[5]

The disturbance of the oral microbiota–ecology balance in the host usually causes a series of oral infectious diseases including dental caries, apical periodontitis, periodontal diseases, pericoronitis, and craniofacial bone osteomyelitis. Oral microbiota is also associated with several systemic diseases, namely cardiovascular disease, pneumonia, heart disease, rheumatoid arthritis, pancreatic cancer, colorectal cancer, oesophageal cancer, stroke, and adverse pregnancy outcomes. Accordingly, oral microbiota has been considered as a potential biomarker for human diseases. Relationships between oral microbiota and systemic diseases are essential and need to be elucidated, in order to provide a reasonable diagnosis basis for disease prevention and treatments.

This article mainly discusses the mechanisms for how endogenous and exogenous factors modulate oral microbiota, provides insights into their roles in the influence of oral microbiota on systemic diseases, and summarises the challenges for clinical diagnosis and treatment.

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Basic composition and distribution of oral microbiota

The oral microbiome can be classified into core microbiome and variable microbiome. The core microbiome is similar for all individuals and comprised of the predominant species at different sites of the healthy body. The variable microbiome is different between individuals in response to unique lifestyles and phenotypic and genotypic determinants.

For newborns, within five minutes of birth, bacterial communities in the oral cavity and other body habitats are very similar to each other.[6] Types of microorganisms are closely decided by the delivery mode.[7] In addition, the mother's oral microbiota is the most important source of infants' and young children's oral microbiota by successful vertical transmission.[7,8] As ageing continues, babies and children form a wide variety of oral microorganisms in response to different diets, lifestyles, environments and so on.[9]

The oral cavity contains over 700 microbial species as well as commensal

and opportunistic bacteria, archaea, fungi, protozoa, and viruses.[10,11] Every species plays its particular role and strongly interacts with the other species and the host.[7] *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* are probably most significant for oral health.[12] The major genera with the largest representation in oral cavities include the following: *Streptococcus*, *Prevotella*, *Haemophilus*, *Rothia*, *Veillonellaceae*, *Neisseria*, *Fusobacterium* and *Porphyrin*.[13] Recently, primer pairs have been developed to make phylum-selective 16S rRNA clone libraries. In the libraries, species (*Chloroflexi, Synergistetes, Chlorobi, Gracilibacteria, Saccharibacteria*, and others) are identified from the lesser known oral phyla or candidate divisions.[14,15]

The salivary microbial groups are stable for a short term, although there are significant differences in the oral microbial groups affected by a variety of factors.[16,17] There are 11 human microbial habitats in the oral cavity, including hard palate, tongue dorsum, saliva, palatine tonsils, throat, buccal mucosa, keratinised gingiva, supra-gingival plaque, subgingival plaque, dentures, and lips. The 11 habitats have been shown to contain different core microorganisms when sampled from more than 200 healthy people with high throughput sequencing methods as listed in Table 1.[7,13,18,19] Microbiomes from the same location on the body are more similar among different individuals than those from different locations on the same individual.[12,20] Within these oral habitats, 13 (tongue dorsum) to 19 (hard palate) bacteria phyla were described, including 185 (tongue dorsum) to 322 (throat) genera.[7] After many years of research, there are still new discoveries due to the microbial diversity.

Table 1 Distribution of dominant microorganisms in oral cavity

Section	Dominant microorganism
Hard palate	*Streptococcus, Uncl.Pasteurellaceae, Veillonella, Prevotella, Uncl.Lactobacillales*
Tongue dorsum	*Streptococcus, Veillonella, Prevotella, Uncl. Pasteurellaceae, Actinomyces*
Saliva	*Prevotella, Streptococcus, Veillonella, Uncl. Pasteurellaceae*
Palatine tonsils	*Streptococcus, Veillonella, Prevotella, Uncl. Pasteurellaceae, Fusobacterium*
Throat	*Streptococcus, Veillonella, Prevotella, Uncl. Pasteurellaceae, Actinomyces, Fusobacterium, Uncl. Lactobacillales*
Buccal mucosa	*Streptococcus, Uncl. Pasteurellaceae, Gemella*
Keratinised ginguva	*Streptococcus, Uncl. Pasteurellaceae*
Supragingvial plaque	*Streptococcus, Capnocytophaga, Corynebacterium, Uncl. Pasteurellaceae, Uncl. Neisseriaceae*
Subgingival plaque	*Streptococcus, Fusobacterium, Capnocytophaga, Prevotella, Corynebacterium*
Dentures	*Staphylococcus epidermidis, Streptococcus*
Lips	*Streptococcus, Candida albicans*

Endogenous and exogenous factors affecting oral microbiota

In healthy individuals, oral microbiome balance is regarded as dynamic because it changes in response to endogenous and exogenous factors. Human lifestyle and experiences can quickly and profoundly change the stability of microbial communities associated with the host.[5] In other words, host lifestyle, physiology, genotype, pathobiology, environment, immune system, transient community members, and socioeconomic status are generally considered as important factors in the multifactorial background of oral diseases and systemic diseases.[1,21–24] However, the underlying mechanisms of these factors on oral microbiota and oral health are not yet fully elucidated.

Diets

The change of dietary macronutrients and diet type can lead to a shift of the oral microbiome and diseases. Nutrients, such as sugars, fats and vitamins, play important roles in the oral microbiome. In severe early childhood caries, sugar-rich diets and frequent snacks show the highest associations with *Streptococcus mutans* (*S. mutans*) and *Fusobacterium nucleatum* (*F. nucleatum*).[25,26] Saturated fatty acids (SFA) and vitamin C intakes are consistently correlated with alpha (within-subjects) diversity indexes in both richness and diversity.[27] The higher the SFA intake, the higher the relative abundance of fusobacteria (*Leptotrichiaceae*) and betaproteobacteria. Vitamin C and the other intake-related vitamins, for example, B vitamins and vitamin E, exhibited positive correlations with the population of *fusobacteria*.[27] Adler and his co-workers also found that cats on dry-food diets showed very high diversity in oral microbiome, especially with a higher abundance of *Porphyromonas spp*.[28]

Dietary modification with increased fibrous foods and dairy products and decreased fatty and sugary foods has been advised to maintain a normal oral ecological balance.[29–31]

Smoking

Cigarettes are rich in bacterial diversity, harbouring a variety of microorganisms from environmental bacteria and commensals to potential oral pathogens. Bacteria presenting in the cigarette could be transferred to the mouths of smokers even before the cigarette is lit.[32] Some of these bacteria including *Bacillus spp*. and *Clostridium spp*. could survive the burning/smoking process, be inhaled by smokers and other exposed individuals, and colonise the oral cavity.[33]

Additionally, several other potential mechanisms also reveal how smoking alters oral microbial ecology, including increasing the acidity of saliva, depleting oxygen, antibiotic effects, influencing oral bacterial adherence to mucosal surfaces, and impairing host immunity.[34,35] For example, the oxygen deprivation hypothesis proves that smoking creates an environment favouring strict or facultative anaerobes over strict aerobes.[36] The genus *Streptococcus, Veillonella* and *Actinomyces* are facultative or obligate anaerobes. Conversely, aerobes such as *Neisseria subflava* and *Corynebacterium* are depleted in smokers,[1,36] consistent with previous studies.[37,38]

Drinking

The influence of red liquor and wine on the oral microbiota is different. Liquor could lead to an increase in the concentration and number of gram-positive bacteria, such as *S. mutans*.[39] Oral bacteria converts ethanol to acetaldehyde, which is a toxin and recognised human carcinogen.[40] The production of acetaldehyde might also directly result in inhibition of fusobacteria.[41] Specific impurities, contaminants, N-nitrosodiethylamine, and polycyclic aromatic hydrocarbons generated in the fermentation, distillation or maturation processes also change the oral environment and affect certain

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species.[42] However, the regular and moderate consumption of red wine does not change the overall diversity and stability of representative bacterial groups of the human saliva.[43] Furthermore, the synthetic mixtures of the organic acids (succinic, malic, lactic, tartaric, citric, and acetic acid) in red and white wines are active against oral streptococci responsible for caries development and *Streptococcus pyogenes* responsible for pharyngitis.[44] These suggest that moderate drinking of red and white wines can enhance oral health.

Antibiotics use

Antibiotics are a mainstay of treatment for bacterial infections worldwide. Antibiotics influence bacterial growth curves and this is why they are used to kill pathogens. Bactericidal antibiotics directly kill the bacteria, while bacteriostatic antibiotics inhibit their growth. Many studies report that antibiotics such as azithromycin, amoxicillin clindamycin and ciprofloxacin affect the amount and diversity of oral microbes.[45,46] Some general changes can be observed such as an immediate decrease in actinobacteria count in throat.[31] The reported data also demonstrate that the oral microbiome functions (microbe metabolic activity, microbial gene expression and protein synthesis) were also drastically changed as a direct consequence of antibiotic treatments. For example, antibiotics might damage and/or destruct the bacterial cells and consequently decrease their enzymatic activity.[47] The extent to which our oral microbiota changes after an antibiotic intervention depends not only on the chemical nature of the antibiotic used to treat specific infections, but also on the type of administration, duration and dose, as well as the level of resistance that each microbiota develops.[48] Therefore, the establishment of new drug-based therapeutic strategies would require multi-variable analysis.

Socioeconomic status

Among the factors affecting oral health, socioeconomic status (SES) is the important factor that should not be neglected. At present, little is known about the influence of differences in SES on the composition of the oral microbiome. Studies report that these differences are reflected by the bacterial profiles of saliva. *Megasphaera micronuciformis, Veillonella atypical, Veillonella parvula, Rothia mucilaginosa, Prevotella histicola, Fusobacterium periodontium, Granulicatella adiacens* and *Tannerella forsythia* were abundant in the high socioeconomic status group, while *Aggregatibacter segnis, Achromobacter xylosoxidans* and *Neisseria cluster 11* were abundant in the low socioeconomic group.[21]

The SES of each family determines the choices of family members' educational attainment, health concepts, hygiene habits, dietary patterns and medical services. Therefore, Chu *et al.* believed that the oral microbiome might vary considerably over time because of SES and the phenomenon of reducing oral microbial diversity was disproportionately prevalent in low-SES neighbourhoods.[49]

Pregnancy

Variations in oral microbiota in pregnancy have been observed. In the early stages of pregnancy, the total number of cultivated microbes in pregnant women increase significantly. For example, the abundance of *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) in the gingival sulcus were significantly higher than that in the non-pregnant group, whereas *Prevotella intermedia* (*P. intermedia*) and *F. nucleatum* did not change.[50] From weeks 12 to 28 of pregnancy, no changes occur. During late pregnancy, Candida species were more frequently detected.

Total bacterial counts decreased postpartum. The most dramatic microbial changes were the decrease of species including *Capnocytophaga ochracea, Capnocytophaga sputigena, Eubacterium saburreum, Fusobacterium nucleatum naviforme, Fusobacterium nucleatum polymorphum, Leptotrichia buccalis, Parvimonas micra, P. intermedia, Prevotella melaninogenica, Staphylococcus aureus (S. aureus), Streptococcus anginosus, Streptococcus intermedius, S. mutans, Streptococcus oralis, Streptococcus sanguinis, Selenomonas noxia*, and *Veillonella parvula*, while the abundance of *Neisseria* mucosa was found to increase significantly over time.[51] Changes in pregnancy (especially physiological condition and female hormones) have a significant impact on the oral microbiota, and may promote the colonisation of various microorganisms, especially periodontal pathogens, that may be a risk factor for the health of pregnant women.[52]

Oral bacteria and systemic diseases

Increasingly, evidence suggests that specific bacterial infections promote development of certain diseases. Accordingly, this section mainly summarises the relationships between oral bacteria and systemic diseases. It also provides a deep insight into the mechanistic role in the influence of oral microbiota on cancers and inflammatory diseases.

Oral bacteria and cancers

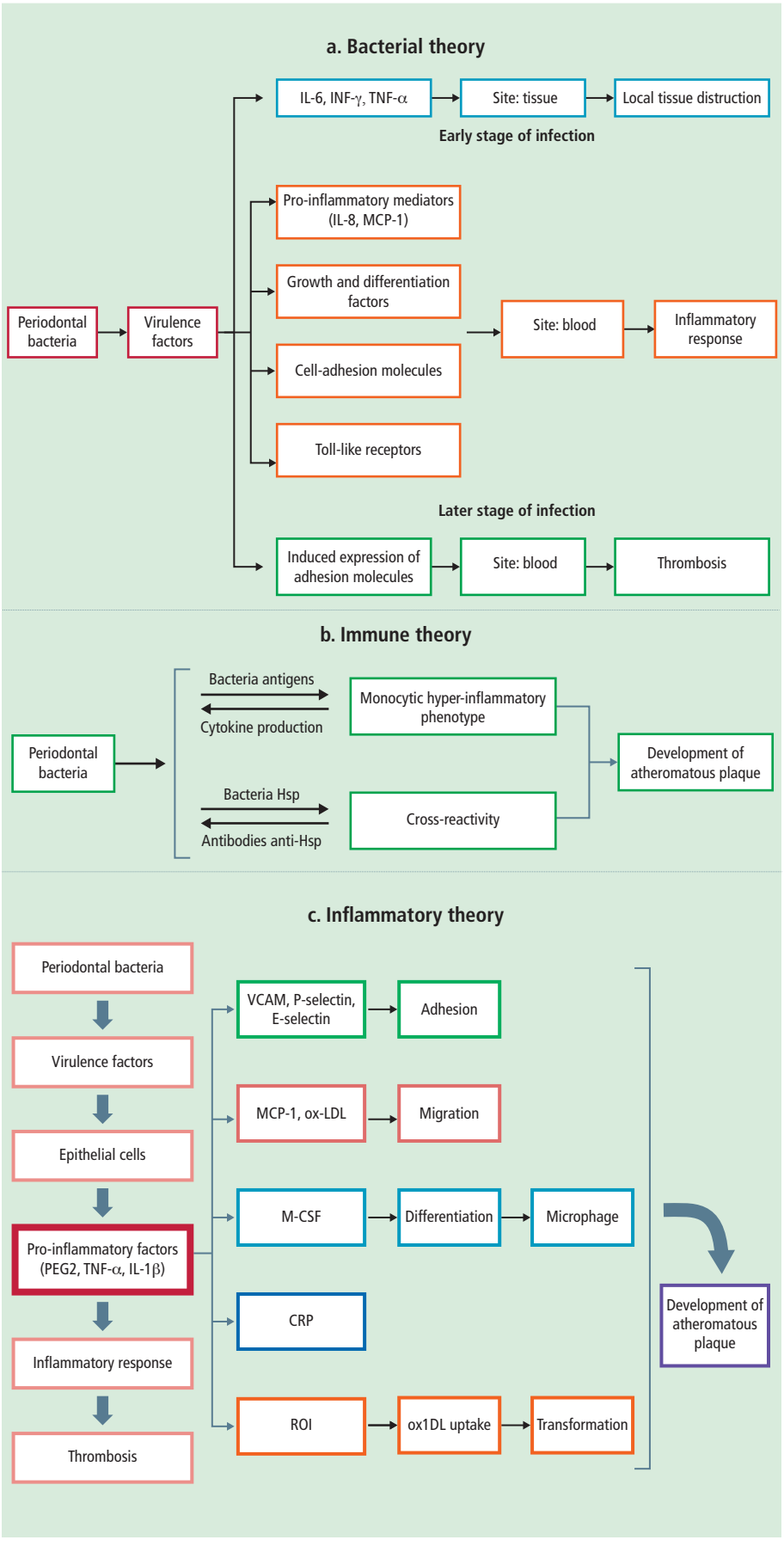
Oesophageal cancer

Oesophageal cancer is the eighth most frequent tumour and sixth leading cause of cancer death worldwide.[55] The latest study showed that oral bacteria might increase the risk of oesophageal cancer. Immunohistochemically, *Porphyromonas gingivalis* (*P. gingivalis*) has been detected in 61% of cancerous tissues, 12% of adjacent tissues, and 0% of normal oesophageal mucosa. In addition, lysine-specific gingipain distribution and *P. gingivalis* 16S rDNA were also researched. These findings were pioneering in proving that *P. gingivalis* infected the oesophageal epithelium of oesophageal cancer patients. The infection was observed in association with the progression of oesophageal cancer, and could be an important biomarker for this disease. Furthermore, eradication of a common oral pathogen might help to reduce the burden of oesophageal cancer.[56]

Colorectal cancer

Fusobacteria, which are from the mouth, cause excessive immune responses and turn on cancer growth genes. The microbes have been linked with colorectal cancer.[57] Fusobacteria gather massively in adenomas – a benign bowel growth that will become cancerous as time goes on. The polymicrobial nature of oral biofilms and the asaccharolytic metabolism of many of these species helps them live well in the microenvironment of colonic lesions.[58] By attracting special immune cells, fusobacteria invade the bowel and set off an inflammatory response that could accelerate the formation of colorectal tumours. Fusobacteria have specific surface molecules assisting them to attach and invade human colorectal cancer cells. In colorectal cancer, *F. nucleatum* has been demonstrated to expand myeloid-derived immune cells, strongly inhibit T-cell proliferation or activation, and induce T-cell apoptosis.[59] Periodontal diseases including tooth loss might increase systemic inflammation, lead to immune dysregulation, and alter gut microbiota, and therefore possibly influence colorectal carcinogenesis.[60] However, oral bacteria *F. nucleatum* could protect all sorts of tumour cells from being killed by immune cells.[61] The Fap2 protein of *F. nucleatum* directly interacts with TIGIT(T-cell

Fig. 1 An association between oral bacteria and cardiovascular diseases. (PGE2: prostaglandin E2; TNF-α: tumour necrosis factor-α; IL-1β: interleukin-1β; IL-6: interleukin-6)



immunoglobulin and ITIM domain), leading to the inhibition on NK cell cytotoxicity and T-cell activities.[61] This was a tumour-based immune evasion mechanism that was bacteria-dependent, wherein *F. nucleatum* bound tumours were protected from NK-mediated killing and immune cell attack due to an interaction between the *Fusobacterial* protein Fap2 with the immune cells inhibitory receptor TIGIT. *F. nucleatum* adhered to various tumour cells. NK cells clustered around *F. nucleatum* coated tumour cells. *F. nucleatum* exerted its inhibitory effect on TIGIT through the immunoreceptor tail tyrosine (ITT)-like and the immunodominant tyrosine-based inhibitory (ITIM) motifs located in TIGIT cytoplasmic tail. TIGIT was expressed in tumour infiltrating lymphocytes (TILs) found within colon adenocarcinoma and *F. nucleatum* inhibited the activity of these TILs in a Fap2-dependent manner. Furthermore, the Fap2 protein of *F. nucleatum* could inhibit the activity of T-cells presenting in the peripheral blood, such as interferon-γ secretion. These discoveries can lead to a better early diagnosis technique and new strategy for the treatment of cancer patients. Furthermore, the correlation of *Fusobacterium* with T-cells and microRNA expressions still needs to be clarified in colorectal cancer.

Pancreatic cancer

Pancreatic cancer is the fourth leading cause of cancer-related death and a serious threat to human health. Oral pathogens, especially *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans)* were associated with a high risk of pancreatic cancer.[62] Microbial cells released from the biofilm through the epithelium and spread systemically via the blood circulation. Some of the bacteria isolated from pancreatic tissues were members of the oral microbiome.[63] *P. gingivalis*, one of the aetiological factors in pancreatic cancer, had the ability to escape host response and impair innate immunity, subsequently strengthening the favourable inherent environment for bacterial overgrowth, which in turn might mediate the microbial community and promote the conversion from a symbiotic state to a dysbiotic state. All above changes might cause high levels of inflammation in pancreatic cancer.[64] Nitrosamines in the oral cavity that raised levels of major oral bacteria (*P. gingivalis*), could induce and promote a rapid development of pancreatic cancer. Therefore, the oral bacteria (*P. gingivalis* and *A. actinomycetemcomitans*) can be

a good candidate as an effective biosensor for early diagnosis of pancreatic cancer.

Oral bacteria and inflammatory diseases

Atherosclerosis

Accurate and early diagnosis of cardiovascular diseases will greatly improve the survival rate of patients. Oral microbiota such as *S. mutans*, *P. gingivalis*, and *Gemella haemolysans* (*G. haemolysans*) may play a role in cardiovascular disease.[65–67]

S. mutans could contribute directly to atherosclerosis by disrupting endothelial cell function, one of the earliest indicators of cardiovascular diseases.[65] *S. mutans* is a major pathogen for dental caries. Oral *S. mutans* induced intracerebral haemorrhage experimentally and affected cerebral microbleeds.[65] A significant correlation of cnm-positive *S. mutans* was observed with hypertensive intracerebral haemorrhage and deep cerebral microbleeds.[68] *G. haemolysans* was simultaneously found in atherosclerotic and oral plaques of the elderly without periodontitis.[66] As shown in Figure 1, there are three theories about bacteriology, inflammation, and immunology to explain the relationships between periodontal diseases and cardiovascular diseases.

In the early stage of infection, epithelial cells responded strongly to *P. gingivalis* by producing IL-6, INF-γ, or TNF-α, causing local tissue destruction. Subsequently, bacteria and virulence factors (for example, gingipains, lipopolysaccharide or fimbriae) entered into the bloodstream through degraded gingival tissues and activated endothelial cells, and produced inflammatory reaction under the action of pro-inflammatory mediators (for example, IL-8, MCP-1), growth factors, differentiation factors, cell-adhesion molecules and toll-like receptors. Eventually, *P. gingivalis* stimulation could shift endothelial cells toward a pro-thrombotic state (Fig. 1a).[69]

Furthermore, virulence factors accelerate the development of atheromatous plaque. Infection of epithelial cells by periodontal bacteria stimulated the production of proinflammatory cytokines (TNF-α, IL-1β, IL-6, and PGE2). These cytokines entered the blood circulation and affected cells in atheromatous plaques, leading to the development of atherosclerosis (Fig. 1c).[67] Especially during developing periodontal diseases, both these monocytic hyper-inflammatory phenotypes amplify the inflammatory process and cross-reactivity inducing destruction of host cells promotes the development of atheromatous plaques (Fig. 1b).[70,71]

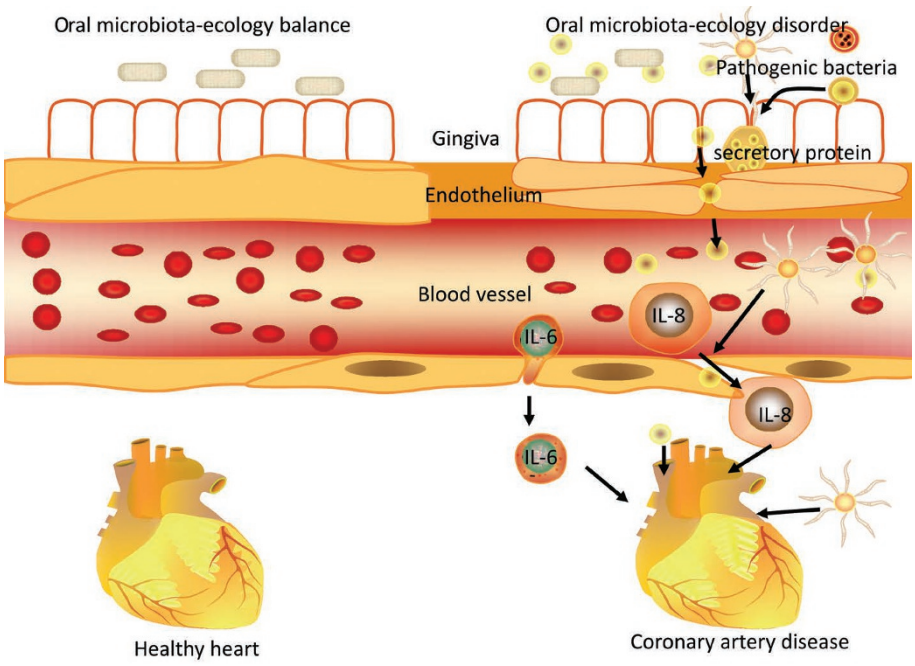


Fig. 2 An association between oral bacteria and coronary artery disease

Pneumonia

Aspiration of bacteria from the oral cavity into the lower airway was possible since the surfaces of oral cavity were contiguous with those of the trachea and lower airway.[72] Oral bacteria continuously flowed into the lungs, and the lungs exhausted the bacteria through ciliary actions and coughing.[73] The lungs are constantly exposed to diverse communities of microbes from the oropharynx, and novel culture-independent techniques of microbial identification have revealed that the lungs, previously considered sterile in health, harbor diverse communities of microbes.

. *Streptococcus*, *Prevotella* and *Veillonella* were the most common bacteria in healthy lungs. The microbial density in lungs was less than 1/1000 of that in the oral cavity, mainly because the lungs had no mucosa suitable for forming bacterial ecology.[74,75] Control of oral biofilm formation could reduce the numbers of potential respiratory pathogens in the oral secretions, which in turn could reduce the risk for pneumonia.[72] Recently, the concept of lung specific microbial groups had been accepted, but oral bacteria were still derived as a risk factor for ventilator-associated pneumonia. Under poor oral hygiene conditions, pathogens were easy to colonise in the oral cavity including methicillin resistant S. aureus, *Pseudomonas aeruginosa*, and ten genera of gram-negative bacilli. Subsequent aspiration would deposit these bacteria (especially anaerobic organisms derived from the gingival crevice) and inflammatory products (associated with periodontal disease) into the lower airway, thereby increasing the risk of lung infection.[76, 77]

Heart disease

To date, the accumulated epidemiological evidence supported an association between oral bacterial diseases (such as periodontal diseases) and coronary artery disease (CAD). The present studies confirmed that five oral commensal bacteria (*Campylobacter* rectus, *P. gingivalis*, *Porphyromonas endodontalis*, *P. intermedia*, *Prevotella nigrescens*) were unique to coronary artery disease when compared with several non-cardiac disorders.[78] And the presence of *A. actinomycetemcomitans* in the subgingival area was associated with an almost two fold risk of angiographically confirmed stable CAD.[79] This suggested a special role for *A. actinomycetemcomitans* in CAD, other than only as a pathogen associated with periodontitis. Studies on the infection mechanisms between oral bacteria and CAD were essential for providing some clues for medicinal treatments in clinic. As depicted in Figure 2, many oral microbes that secreted proteins, peptides and proteases lived in the gingival crevice. These secretory peptides and proteases were likely responsible for altering the host actin cytoskeleton in the gingival

Table 2 Characteristics analysis of different microbiome platforms

Name	Organisation	Taxonomy	Functional annotation	Comparison between samples	Comparison between projects	Advanced search	Connect to public database
MG-RAST	Argonne National Laboratory	Yes	No	Yes	No	No	No
IMG	DOE Joint Genome institute	Yes	Yes	No	No	No	No
iMicrobe	University of Arizona	Yes	No	No	No	No	No
EMG	European Bioinformatics institute	Yes	Yes	Yes	Yes	No	Yes

epithelium leading to oral microbial entry into the bloodstream system. Upon gaining entry into the coronary vasculature, these migratory bacteria could form biofilm structures within atherosclerotic plaques and caused CAD. These secreted proteins could also activate the immune system causing inflammation. For example, cytokine-mediated (IL-6 and IL-8) inflammation was associated with CAD. Furthermore, certain proteases caused an inflammatory response by activating the complement system.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic, inflammatory autoimmune disease. Most clinical studies involving specific oral microorganisms as triggers for RA were only dependent on serological detection methods. Rheumatoid factors had been identified as autoantibodies that reacted to the IgG molecule in the Fc region, and these antibodies could be the IgM, A, G or E epitopes. *P. gingivalis* proteinase was responsible for the epitope development in the RF Fc region. A previous study identified the lysine and arginine amino acid sequences for the Fc region of the IgG molecule; because *P. gingivalis* specifically decomposed lysine and arginine, the IgG3 CH2 and CH3 domains processed by *P. gingivalis* proteinase became powerful targets for the RF produced by rheumatoid cells.[80] It was also found that the microbiome of patients with RA was similar to that of healthy subjects with similar periodontal status with the multiplexed-454-16S rRNA pyrosequencing method; however, specific *Prevotella* and *Leptotrichia* were only found in patients with new-onset RA, and anaeroglobus geminatus was correlated with the presence of peptidylarginine deiminase and rheumatoid factors, and with periodontitis.[23] Another large-scale study using metagenomic shotgun sequencing identified compositional and functional alterations in RA-associated oral microbiomes, which were partly resolved by disease-modifying antirheumatic drugs treatments.[81] Thus, all these data approaches suggested that microbiome composition could be important in the prognosis and diagnosis of RA.

The improvement of clinical diagnosis and treatment based on microbial community information

Databases of bacterial compositions generally identified by using high-throughput sequencing methods of the microbiome will facilitate advanced functional studies on genomics, transcriptomics, and the metabolomics of both host and pathogens. Such analysis can provide deep insights into the activity of the microbes, the relationship of the host and microbes, and potential causative mechanisms. The challenges of clinical diagnosis and treatment based on the microbial community information are still waiting to be conquered.

The standardisation of clinical samples

Standardisation of sampling plans implies that (a) the design elements of the sampling plan must be considered in any standardisation process and (b) the elements are selected to maximise performance.[82] Oral microecosystem is a complex system. Its microbial community species composition and genetic types are significantly different to the ecological sites and are even within the same site. These differences are further governed by a variety of host factors, including gene, health state, age, gender, dentition status, life-style, socioeconomic status, mobile phone use, living area, and religion.

Previous studies using high-throughput analysis techniques had observed that the oral cavity was a highly heterogeneous ecological system containing significantly different microbial communities. The Firmicutes was the dominant bacterium of salivary and dental mucosa, while Proteobacteria, Firmicutes, Bacteroidetes and Fusobacteria were the dominant bacteria of the dental plaque. More importantly, the oral microbial structure varied with age and dentition status.[83] The results indicated that sampling process, sampling parts and the age of the sample objects were crucial to collect the accurate, systematic, and reproducible results.

Therefore, developing a uniform sampling plan to be used by all researchers is extremely important. The factors mentioned above should be taken into account in the standardisation of oral clinical samples so the errors can be effectively reduced. Furthermore, the standardisation of clinical samples also should be that:

- The oral site needs to be delimited before sampling
- Objective sampling should represent the entire oral ecosystem or site
- Sampling should consist of the same small subsamples
- Sampling using large-size samples implies that the selection of the sample site is representative
- The sampling unit should be large enough for efficient statistical processing.

Analysis and processing of big data in an oral microbial community

Microbial big data are generated by high throughput sequencing, for functional prediction, biological classification of species, and gene analysis. They have rapidly developed into a hot topic that attracts extensive attention from academia, industry, and governments around the world. Although enhanced by the contents of the Human Oral Microbiome Database, the explosive growth of data presents us with grand challenges (namely, data complexity, computational complexity, and system complexity). The single data analysis process (Table 2) has some limitations, and does not meet the need for deep mining of microbial big data.

The lack of corresponding bioinformatics tools for reducing sequencing cost, optimising the analysis process, increasing specificity and sensitivity of biological community information and analysis method of sorting and digging large medical data are still the major bottleneck in the era of big data.

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The following work may improve this situation:

- Developing a set of interoperable data analysis tools that can run on different computing platforms. This will effectively improve the reliability and comparability of data analysis
- Combining the data of electronic health records and genome data can help effectively explore the pathogenesis and therapeutic effect of diseases
- Functional studies including genomics, transcriptomics and metabolomics of both host and oral pathogens.

This specific microbial application and analysis is in an exciting phase of research. Such analysis could guide researchers to develop new therapies that target key mechanisms. These are very crucial in advancing the personalised diseases early warning service for personalised diseases based on the oral microbiome.

Further verification of the cross-sectional study

More and more data show that the oral microbiome is related to dental diseases, cardiovascular disease and others. In these cross-sectional studies, various factors such as individual gene and bacterial variation influence the cross-sectional data and reduce reliability and accuracy of flora mapping. More importantly, the pure cross-sectional studies only provide the correlation of microbial community and diseases rather than clear their 'causality'. Host and microbiota have significant heterogeneity in various stages of the disease development. If a large number of data of genome, transcriptome, proteome, and metabolome are associated with clinical data such as clinical manifestations, pathology, biochemical markers and immune indicators, it looks forward to making clear the 'causality' of the core microbial group and disease, and crediting an oral microbiota-based prediction model to develop a new paradigm of personalised medicine.

Trends of treatment without antibiotics

Due to antibiotic overuse, the emergence of drug-resistant strains and frequent recurrence of the disease in affected individuals are increasing challenges in antifungal therapy. Moreover, indiscriminate use of antibiotics affects the delicate balance between normal flora and host. Beneficial bacteria are also eliminated, depriving the host from their beneficial effects. This has prompted the need for an alternative therapeutic and prevention strategy. Antibodies, vaccine, antimicrobial peptides, probiotics, prebiotics, synbiotics, and arginine become alternative therapeutic options, as illustrated in Table 3.

Antimicrobial peptides, probiotics combined with prebiotics and the screening probiotics, and arginine may assist or replace antibiotic treatments for oral microbial problems and in turn prevent systemic diseases. In the near future, a rapidly increasing body of knowledge promises to indicate more targeted applications of probiotics. It still needs to clearly determine which organisms are beneficial and play a preventive or therapeutic role. For those that can duly be termed probiotics, a variety of applications have to be defined more precisely than before.

Summary

Oral microbiota is an important intermediate link, causing different oral and overall health in the body under the influence of changes in a variety of factors. Once the microbiota balance has been disturbed, it may result in oral and even systemic diseases. Although a number of causes including infectious pathogens or use of antibiotics can lead to a disruption of microbial equilibrium, the role of our diet, nutrition, lifestyle and socioeconomic status is crucial.

In addition, observation of oral microbiota is a major indicator for the occurrence, development, and prognosis of disease. It has been verified that the microbiome is related to human physiology and pathology. An oral microbiota-based prediction model can

Table 3 An alternative therapeutic and prevention strategy of oral diseases

Kinds	Mechanism	Typical researches	References
Vaccine and antibodies	1. Stimulates the production of a protective antibody.	1. Mucosal anti-caries DNA vaccine	84,85
	2. Other immune mechanisms.	2. mouth rinse (containing egg yolk antibodies IgY)	
Antimicrobial peptides	1. Inhibit biofilm accumulation via the down-regulation of genes.	1. Chewing gums	86-89
	2. Kill cells by targeting both extracellular and intracellular components.	2. Histatin peptides	
		3. Fusion peptide	
		4. D-Enantiomeric Peptide	
Probiotics, prebiotics, and synbiotics	1. Direct interaction – inhibition of pathogen adhesion, colonisation and biofilm formation.	1. Chewing gums	90-93
	2. Competitive exclusion – competing and intervening with bacterial attachments and engaging in metabolism of substrate.	2. Probiotic mouthwash	
	3. Indirect actions – modulating systemic immune function.	3. Medicine(eg BLIS K12)	
		4. Functional foods	
Arginine	1. Prevent shifts in biofilm flora to acid-producing bacteria.	1. Dentifrice	94-96
	2. Neutralise plaque acids and stabilise the residual plaque biofilm on susceptible tooth surfaces.	2. Toothpaste	
		3. Office desensitising paste	

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provide the basis for noninvasive diagnosis and facilitate the development of a new paradigm of personalised medicine. All these benefit human health in the post-metagenomics era.

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Correction

Research article *Br Dent J* 2018; **224:** 113–115.

When this article was initially published a paragraph on page 115 was incorrect. The corrected paragraph reads as follows:

The culmination of the three-year programme is defined by the satisfactory completion of the Annual Review of Competence Progression (ARCP) process and attainment of the Membership in Restorative Dentistry (MRD) (soon to be replaced with Membership in Periodontology, Membership in Endodontics and Membership in Prosthodontics) within the speciality which enables the individual to register onto the General Dental Council's specialist list relevant to their training.

The author apologises for this error and any inconvenience caused.