 doi:10.20944/preprints202106.0529.v1

Review

Progress in Oral Microbiome related to Oral and Systemic Diseases: An Update

Yeon-Hee Lee[1,*], Sang Wan Chung[2], Q-Schick Auh[1], Seung-Jae Hong[2], Yeon-Ah Lee[2], Junho Jung[3], Gi-Ja Lee[4], Hae Jeong Park[5], Seung-Il Shin[6], Ji-Youn Hong[6,*]

[1] Department of Orofacial Pain and Oral Medicine, Kyung Hee University Dental Hospital, #613 Hoegi-dong, Dongdaemun-gu, Seoul 02447, Korea; omod0209@gmail.com, dental21@khu.ac.kr
[2] Division of Rheumatology, Department of Internal Medicine, School of Medicine, Kyung Hee University, Seoul, Korea; wanyworld83@gmail.com, aprildaum@hanmail.net, hsj718@hanmail.net
[3] Department of Oral and Maxillofacial Surgery, School of Dentistry, Kyung Hee University, Seoul 02447, Korea; ssa204@khu.ac.kr
[4] Department of Biomedical Engineering, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Korea; gjlee@khu.ac.kr
[5] Department of Pharmacology, School of Medicine, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Korea; hjpark17@khu.ac.kr
[6] Department of Periodontology, Periodontal-Implant Clinical Research Institute, School of Dentistry, Kyung Hee University 26, Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Korea; shin.dmd@khu.ac.kr, jkama7@naver.com
* Correspondence: omod0209@gmail.com; Tel.: 82-2-958-9454; Fax: 82-2-968-0588, jkama7@naver.com; Tel.: 82-2-958-9409. Two authors contributed equally to this work as a corresponding author.

Abstract: The human oral microbiome refers to an ecological community of symbiotic and pathogenic microorganisms found in the oral cavity. The oral cavity is a suitable environment that provides various kinds of biological niches such as teeth, tongue, and oral mucosa. The oral cavity is the gateway between the external environment and the human body, maintaining oral homeostasis, protecting the mouth, and preventing disease. On the flip side, the oral microbiome plays an important role in triggering, development, and progression of oral and systemic diseases. Currently, disease diagnosis through the analysis of the human oral microbiome has been realized with the recent development of innovative detection technology, and is overwhelmingly promising compared to the previous era. It has been found that patients with oral diseases and systemic diseases have variations in the oral microbiome compared to normal subjects. This narrative review provides insight into the pathophysiological role that oral microbiome plays in influencing oral and systemic diseases, and updates the knowledge related to the oral microbiome over the past 30 years. A wide range of updates was provided with the latest knowledge of the oral microbiome to help researchers and clinicians in both academic and clinical aspects. The microbial community information can be utilized in non-invasive diagnosis and help develop a new paradigm in precision medicine, which will benefit human health in the era of post-metagenomics.

Keywords: Oral microbiome; oral cavity; dysbiosis; oral diseases; systemic diseases

1. Introduction

Human bodies are spatially shared by oral microbiome and temporally serve as messengers or carriers of their genomes. That is, the human brain and nervous system recognize and control our body as an organism, but our body space is a biological recruitment unit that contains numerous microorganisms and their genomes. The number of complex microbial components is largely outnumbered by that of the cells of the human body [1]. The

 doi:10.20944/preprints202106.0529.v1

microbial community is referred to as the microbiome, a term used to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms [1, 2].

Various efforts have been done to identify and understand the role of the microbiome in human health and disease over the past decade. With the advent of innovative genomics technologies, represented by next-generation sequencing (NGS) and bioinformatic tools, which deviate from the conventional culture-based detection method, understanding of the contribution of the human microbiome to health is deepening [3]. In addition, studies on the microbiomes in and on our bodies forming a functional organ that is fundamental to our health and physiology are still ongoing.

As the oral cavity is ideal for microbiome residents, it is one of the most heavily colonized parts of the body. In addition, several distinct habitats within the oral cavity support complex and heterogeneous microbial communities. The interaction among oral microorganisms protects the human body against invasion and attacks [4]. Conversely, an imbalance of the oral microbiome can contribute to oral diseases such as dental caries, periodontitis, oral mucosal diseases, and/or systemic disease [5]. Furthermore, the relationship between the oral microbiome and host is dynamic, and influenced by many aspects of lifestyle, such as diet, tobacco consumption, stress, and systemic conditions, which can alter the composition and its properties, and induce a state in which this finely tuned ecosystem is no longer in balance [6]. The oral microbiome constitutes an important link between oral and general health.

We know that oral microbiomes are not colonized at random, but that our microbial residents have coevolved with us over millions of years. To address microbial homogeneity or divergence and maintain a harmonious state to maintain health and prevent disease, we must pay attention to our microbiomes, and consider our body a superorganism. The purpose of this review is to update oral and general healthcare practitioners on the current knowledge of the oral microbiome in health and disease, to review how molecular methods of microbial characterization have advanced our understanding, and to discuss potential implications for clinical practice. For these purposes, we searched the literature via PubMed and Google scholar search engines, and we selected the top 147 articles that matched the theme for a period of 30 years prior to January 2021 and did massive systematic review.

 doi:10.20944/preprints202106.0529.v1

1. What is the oral microbiome?

The microbiome refers to the community of microbial residents in the body. The term "microbiome" was coined by the 2001 Nobel Prize laureate Joshua Lederberg, who is credited for the Human Genome Project. The original meaning of the term is an ecosystem of symbiotic, commensal, and pathogenic microorganisms that reside in the human body [7]. Microorganisms in our microbiome share our body. The oral microbiome, oral microbiota, or oral microflora refer to microorganisms found in the human oral cavity, constituting bacteria, eukaryotes, and viruses [4]. The genome is the genetic material of an organism—the complete set of DNA including all of its genes. In terms of the genome, the oral microbiome is defined as the collective genome of microorganisms that reside in the oral cavity. The oral microbiome comprises diverse groups, including bacteria, fungi, and viruses (Figure 1). The oral microbiome reaches homeostasis with regard to this composition, which is a dynamic balance in inter-bacterial and host-bacterial interactions [8].

2. The uniqueness of the oral cavity as a microbial niche

The oral cavity has most diverse microbiome in the digestive system. The oral cavity is constantly exposed to both inhaled and ingested microbes, comprising more than 700 species of bacteria, fungi, viruses, archaea, and protozoans. Furthermore, saliva contains up to 10^9 commensal bacteria per milliliter [9]. The oral cavity is an exceptionally complex habitat in which numerous microorganisms exist.

Within the oral cavity, there are distinct microenvironments, such as the hard surfaces of the teeth and epithelial surfaces of the mucosal membranes. The oral cavity consists of other various habitats, such as the tongue, gingival sulcus, tonsils, hard palate, and soft palate, which provide a rich environment in which microorganisms can flourish [4]. These niches are mainly exposed to the fluid phase of the saliva or gingival crevicular fluid. It keeps the bacteria hydrated and serves as a medium for the transportation of nutrients to microorganisms [10]. As the initiation point of digestion, the oral cavity provides the nutrients necessary for the formation and maintenance of the oral microbiome.

Stable temperature and pH also provide an ideal environment for the growth of microorganisms. The human oral cavity is maintained at a relatively stable temperature of 35–37 °C. This temperature, without significant changes, is vital for the growth and survival of various microorganisms. Saliva has a stable pH of 6.5–7, which is favorable for most species of bacteria. The main component involved in maintaining the neutral pH of the oral cavity is the saliva; however, it has been found that different areas of the oral cavity have different pHs [11]. The salivary microbiome has been shown to be a conglomerate of bacteria shed from oral surfaces with the throat, tongue, and tonsils as the main sites of origin [12]. In addition, the oral microbiome composition differs between various habitats and has its own microbial identity, consisting of its own unique microbial population [1].

3. The salivary microbiome in health

 doi:10.20944/preprints202106.0529.v1

The commensal microbiome plays an important role in the maintenance of oral and systemic health (Figure 2). The salivary microbiome has been shown to be individualized and temporarily stable in orally healthy individuals. However, limited information is available on the normal microflora of healthy individuals [2, 13]. The healthy human oral microbiome is predominantly composed of members of the phyla *Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes*, and *Fusobacter*, with *Spirochaeteae* present in lower numbers [4]. The taxonomic profiles of spit, drool, and oral rinse samples based on the proportion of bacterial sequences determined at the genus level [14]. The five major genera found in all three saliva fractions were *Streptococcus* (17.5%), *Prevotella* (15.5%), *Veillonella* (15.3%), *Neisseria* (12.7%), and *Haemophilus* (10%). The patterns of global diversity in any human microbiome based on analyses of partial 16S ribosomal RNA (rRNA) sequences from diverse locations around the world, the most frequent genus was *Streptococcus*, which accounted for 22.7% of the 101 bacterial genera, and 39 genera had not been previously described in the human oral cavity. As a result of limitations in the ability to discriminate species using 16S ribosomal RNA analysis, the vast majority of studies have only identified oral bacteria at the genus level [7]. As there is significant variation in the structure and composition of the genomes of the same species, further research with both advanced sequencing and bioinformatics analysis is needed to characterize the oral microbiome at the strain level.

3-1 Oral fungal and protozoal microbiota

There are very few reports on the fungal microbiome and other microorganisms. However, the oral microbiome includes eukaryotic microbes such as fungi and protozoa, which are important non-bacterial components. Fungi have been reported very often as members of healthy oral microbiota, where up to 101 species have been described, including *Candida* spp., followed by *Cladosporium, Aureobasidium, Saccharomyces, Aspergillus, Fusarium*, and *Cryptococcus* spp. [9, 10]. Among the protozoa, *Entamoeba gingivalis* and *Trichomonas tenax* are most common and are mainly saprophytic [15]. However, the detected *Candida* species are generally not associated with invasive human infections. In contrast, replacement with more virulent microbial species, including *Aspergillus* spp., *Fusarium* spp., and *Cryptococcus*, as part of an individual's oral microbiome could potentially serve as a marker of increased risk of infection [16]. Archaea have also been detected, although they represent a minor proportion and are generally elevated in subjects with periodontitis [12]. We should recognize the critical contribution of the mycobiome as a trigger for immune responses in diseases.

3-2 Oral viral microbiota

Viruses are the most abundant infectious agents in different habitats, including other human body parts. The assemblage of viruses, the virome, has rarely been described in the human oral cavity [15, 16], compared to their bacterial counterparts. Studies on oral viruses have focused mostly on unstimulated saliva, dental plaque, or oral swabs from healthy individuals [17, 18]. These viruses are predominantly bacteriophages and differ from viromes described in other habitats. Approximately 10^8 virus-like particles per milliliter of fluid from saliva swabs have been reported [17], and 10^7 of them exist per milligram of dental plaque [18]. The most abundant reads

 doi:10.20944/preprints202106.0529.v1

among eukaryotic viruses belonged to herpesviruses (74%), particularly human herpesvirus (66%), followed by retroviruses (24%), and papillomaviruses (1.2%), with occasional counts of coronaviruses, poxviruses,, and others (< 1% of all reads) [19]. In general, viral infections focus on viruses that are found in and/or are transmitted via the oral cavity: norovirus, human papillomavirus, Epstein-Barr virus, herpes simplex viruses, hepatitis C virus, and HIV. Determining the virulence factor homologs of oral viromes may support the notion that viruses can serve as reservoirs for pathogenic gene functions [20]. Although little is known about the role or constituents of viruses as members of the human microbiome, viruses could play a role as drivers of ecosystem diversity and are important contributors to the human oral microbiome in health and disease.

4. Effective and rapid detection of oral microbiota diversity

In the past, analysis of the human oral microbiota has been hindered by the limitations of conventional methods. With conventional culture-based methods, many abundant oral microflora species remain unculturable. However, with the advancement of culture-independent approaches, including gel-based techniques, DNA microarrays, polymerase chain reaction (PCR)-based methods, and next-generation sequencing (NGS) technology, diverse unculturable flora have been identified [21, 22]. The development of cost-effective high-throughput NGS technology allowed researchers to easily obtain large amounts of DNA fingerprint data in a single instrumental run to decipher the complex oral microbial community in clinical samples such as saliva and subgingival plaque.

With the increasing clinical importance of oral pathogens, it is necessary to develop simple, rapid, and sensitive detection methods for point-of-care testing. The 2019 coronavirus disease (COVID-19) pandemic is currently our biggest challenge and a global health emergency [23]. Rapid and accurate diagnosis of COVID-19 is crucial in controlling the outbreak. Currently, reverse RT-PCR (rRT-PCR) is the gold standard for SARS-CoV-2 detection [24]. Saliva can have a significant role in human-to-human transmission, and salivary diagnostics may provide a comfortable and easy point-of-care platform for early and quick diagnosis of COVID-19 [25]. Detection of SARS-CoV-2 using saliva samples has been proposed as an alternative to standard swab diagnostic methods for the nose and pharynx [26].

NGS has represented the standard for studying the composition of microbial communities, allowing the differentiation of bacteria by sequencing the variable regions of the gene coding for the 16S ribosomal RNA (rRNA) (amplicon sequencing) [27, 28]. Although 16S rRNA sequencing greatly improved our knowledge of the bacterial component of the oral microbiome, it only determines the presence or abundance of bacterial species. It usually does not provide sufficient information to resolve communities at the subspecies level, nor can it detect eukaryotic microorganisms [29]. With high-throughput NGS technologies, the genome of the entire community (metagenome) can be sequenced without the targeting step and without the bias of amplicon sequencing in PCR (shotgun whole metagenome sequencing). Furthermore, metagenomic analysis is improving our knowledge on host-pathogen interactions by revealing the genes that potentially allow microbes to influence their hosts in unexpected ways [3]. Identification of viruses requires metagenomic sequencing, which refers to the direct sequencing of the total DNA extracted from a microbial community, due to the lack of the phylogenetic marker gene 16S.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 June 2021 doi:10.20944/preprints202106.0529.v1

To rapidly detect specific oral pathogens, methods based on the detection of specific bacterial DNA sequences have recently become invaluable in basic dental science and translational research [30]. In particular, multiplex real-time PCR offers a sensitive method for detecting and quantifying a small number of bacteria in clinical samples. Real-time PCR offers a number of advantages over conventional PCR, including high sensitivity, improved accuracy, and evaluation of data without post-PCR detection procedures [31, 32].

5. Factors influencing the oral microbiome

Changes in oral and systemic environments can disrupt the normal symbiotic relationship between the host and its resident microorganisms and increase the risk of disease. Various endogenous and exogenous factors, including smoking, alcohol consumption, socioeconomic status, antibiotic use, diet, and pregnancy affect oral microbiota (Figure 3). Disruption of the host-microbial mutualism, or dysbiosis, can occur due to significant changes in the oral environment or an individual's lifestyle that favor the colonization of disease-associated microbiota [33].

Smoking is a major factor that affects the oral microbiota composition and orodental pathophysiology [34]. Toxic components in cigarettes can induce loss of beneficial oral species and pathogen colonization, and eventually disease, directly or indirectly through immunosuppression, oxygen deprivation, or biofilm formation [35]. In smokers, poor commensals such as *Streptococcus sanguinis* and *S. parasanguinis*, and abundant anaerobic microbiomes such as *Fusobacterium nucleatum* and *F. naviforme* were observed together with a high taxonomic diversity and richness, which was closely aligned with a disease-associated microbiota composition in clinically healthy individuals. Wu et al. [36] reported that the relative abundance of the phylum *Proteobacteria* was lower in current smokers than in never-smokers, with no difference between former and never-smokers. In addition, the *Capnocytophaga, Peptostreptococcus*, and *Leptotrichia* genera were depleted in current smokers compared with never-smokers, whereas the *Atopobium* and *Streptococcus* genera were enriched. Kumar et al. [37] reported differences between smokers and non-smokers in the formation of marginal and subgingival biofilms. In the biofilms of smokers, high taxonomic diversity and relatively unstable initial colonization were observed, with lower niche saturation than that seen in non-smokers. In particular, periodontal pathogens belonging to the *Fusobacterium, Cardiobacterium, Synergistes*, and *Selenomonas* genera, as well as respiratory pathogens belonging to the genera *Haemophilus* and *Pseudomonas* colonized the early biofilms of smokers [37].

An alteration in the oral microbiota composition is expected during pregnancy. There have been reports suggesting an increased risk of periodontal disease during pregnancy and alteration of the composition of oral microbiota [38-40]. *Porphyromonas gingivalis* in periodontal pockets has been linked to microbial invasion of the amniotic cavity in association with preterm labor [41]. Moreover, in a study that examined alterations in the oral microbiota between the non-pregnant and pregnant states, increases in the total cultivable microbial counts and of periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* during pregnancy were observed in the gingival sulcus compared to that in non-pregnant women [42]. In particular, *Neisseria*, *Porphyromonas*, and *Treponema* were more abundant during pregnancy, while *Streptococcus* and *Veillonella* were less abundant [43].

 doi:10.20944/preprints202106.0529.v1

Antimicrobial use has also been suggested as an important factor affecting the composition of oral microbiota. Amoxicillin treatment reduced species richness and diversity and shifted the relative abundance of 35 taxa [44]. They reported a substantial but incomplete recovery of the salivary microbiota composition from the antibiotic approximately 3 weeks after the end of treatment. At the phylum level, the abundance of *Actinobacteria* was markedly decreased by amoxicillin, which was administered for approximately 10 days. This was recovered at the level of the pretreatment, approximately 3 weeks after amoxicillin treatment. In comparison, the abundance of *Proteobacteria* was increased in saliva by amoxicillin and also showed higher abundance, about 3 weeks after the amoxicillin treatment, than the pre-treatment saliva [44]. Raju et al. [45] reported the impact of antimicrobial use on saliva microbiota diversity and composition in preadolescents who systemically used amoxicillin, azithromycin amoxicillin-clavulanate, or phenoxymethylpenicillin. In addition, amoxicillin and amoxicillin-clavulanate potently decreased the abundance of *Rikenellaceae*. In children using azithromycin, a linear inverse association was observed between the use of azithromycin and the Shannon index [45].

Diet intake also influences oral microbes. For instance, frequent sugar intake increases acid production, which dissolves tooth structure and increases the risk of dental caries, via the fermentation of dietary carbohydrates by oral bacteria [46]. Subsequent acid production by repeated intake of high levels of carbohydrates led to sustained reductions in pH, along with the low buffering capacity of saliva. In turn, this can change the oral microbiota composition and up-regulate aciduric species [47]. Aciduric species, including *Streptococcus mutans* and *Lactobacilli*, which are considered to be caries-associated microbiota, also produce acid under acidic conditions [48]. The change to acidic pH can lead to altered gene expression in sub-gingival bacteria, which favors the growth of pathogenic anaerobes such as *P. gingivalis*, which have an optimum pH for growth of approximately 7.5 [49].

There is controversy regarding whether alcohol consumption is a protective factor against or a risk factor for periodontitis. However, low socioeconomic status was reported to be associated with periodontitis [50] and dental caries [51]. Alcohol consumption and socioeconomic status may also affect the oral microbiome composition, considering reports on the accusations of oral microbes in periodontitis and dental caries. Alterations in host immune competence can lead to an increase in the production of virulence factors, which can affect the community composition and meta-transcriptional landscape.

6. The oral microbiome within common oral diseases

The oral microbiome associated with health is considered more general, whereas the disease-associated microorganism is a specialist that elevates virulence potential that is largely absent in healthy individuals. As a community shifts to dysbiosis, it ultimately facilitates the over-representation or overgrowth of microorganisms associated with dysbiosis.

6-1 Role of oral microbiome on Periodontitis

Periodontal disease is one of the most prevalent oral diseases worldwide. Periodontitis is characterized by the extension of inflammation into the supporting tissues of the teeth, causing loss of attachment and bone. Progression of the disease may

 doi:10.20944/preprints202106.0529.v1

involve local, systemic, or environmental factors, and subsequent immune-inflammatory responses can occur in both hypo- and hyper-responsive manners [52]. It is a chronic non-communicable disease (NCD) that has common risk factors with other NCDs, including cardiovascular disease, chronic obstructive pulmonary disease, diabetes, and cancer, which contribute to an increase of the global burden [53-55]. Gingivitis is an inflammation within the gingival tissue induced by the accumulation of bacterial deposits at the gingival crevice which can be resolved by removal of the deposits [56]. Despite the complex multifactorial features of the host response, the essential role of bacteria in the etiopathogenesis of gingivitis and periodontitis has long been identified [57-59].

The crevicular epithelium and gingival crevice are oral microbial habitats that are critical for the initiation and development of gingivitis and periodontitis. The microbial community on the root surface can be protected from shear forces; the microenvironment is nourished by the gingival crevicular fluid (GCF), a serum-like exudate from the adjacent tissue, and a low redox potential to maintain anaerobic conditions. It has been estimated that more than 500 species exist in the subgingival plaque [60], and the current methods of high-throughput molecular technologies provide extended knowledge and understanding of the highly diverse microbial community in the oral cavity. The healthy subgingival microbiome is characterized by the dominant gram-positive cocci and rods, *Actinomyces* spp. and *Streptococcus* spp., as early colonizers that co-aggregate and form early dental plaque [60]. The gram-negative rod *Fusobacterium nucleatum* is the second most abundant species that acts as a secondary colonizer to bridge multiple bacteria as the plaque matures. Other gram-negative species, such as *Veillonella* spp. and *Capnocytophaga* spp., are also important components of biofilms. Recently, gram-positive *Rothia* spp. and *Corynebacteria* have emerged for their roles in spatial arrangements [61]. With the development of gingivitis, the subgingival microbiome shifts to an increase in gram-negative bacteria, including *Prevotella* spp., *Selenomonas* spp., and *F. nucleatum* ss. *polymorphum* along with a decrease in gram-positive species and is involved in the elevation of inflammatory cytokines in GCF [62-64]. The total biomass of the bacteria, as well as the compositional changes, increased 3-log.

The periodontitis-associated subgingival microbiome can be described as the enrichment of diverse groups of gram-negative species. In the classic fundamental study of Socransky et al., red complex bacteria comprising three species, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, were strongly associated with diseased sites and possessed virulence factors such as gingipain, dentilisin, and PrtH, respectively, which showed high protease activity [6]. *Aggregatibacter actinomycetemcomitans* appears to be associated with aggressive periodontitis rather than chronic periodontitis and secretes leukotoxin to damage host cells. In terms of dysbiosis, *P. gingivalis* can subvert or avoid host immune components such as Toll-like receptors and complements, which triggers an imbalance in host-bacterial interaction and relative abundance of other bacteria compared with that in the healthy state [58]. Several keystone pathogens, including red complex triad orchestrated inflammatory disease by alteration of microbiota associated with disease state and optimization of the acquisition of nutrition from the host, and further facilitate the growth of pathobionts that stimulate host immune responses, resulting in bone loss [65, 66] (Figure 4).

6-2 Role of oral microbiome and dental caries

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 June 2021 doi:10.20944/preprints202106.0529.v1

Dental caries is characterized by the demineralization of susceptible dental hard tissues by acidic bacterial by-products from sugar metabolism that leads to cavitation [67], and a chronic continuous process from sub-clinical decay to dentinal involvement [68]. Dental caries results from an ecological imbalance towards acidogenic and aciduric bacterial shifts and environmental acidification within the dental biofilm, and can therefore be explained as a dysbiosis-associated disease [69]. Microbiota adapt to the reduced pH microenvironment along with the prolonged maturation of the biofilms; the cavitated lesions allow advanced ecological niches [70].

Bacterial diversity was considerably different according to the disease conditions, as the supragingival plaque on the sound surface included 500–600 species, which was reduced to 200 in dentin caries, and approximately 125 in non-cavitated enamel lesions [71]. In addition, bacterial composition was variable among the different carious sites and lesions within the individual or among individuals [72, 73]. Despite the complex microbial variability, bacterial diversity of the caries-associated microbiome decreases due to the competitiveness of the microorganisms; the characterization of the cariogenic consortia should be further identified [74]. *Streptococcus mutans* are the initial colonizers of the supragingival biofilm, they produce water-insoluble glucans that promote bacterial adhesion and only comprise <1% of the total community [75]. In addition, *mutans streptococci*, especially *Streptococcus mutans* and *Streptococcus sobrinus*, were first identified as major pathogens due to their extensive acidogenic and aciduric properties [76, 77]. The mutans-centric paradigm in caries-etiopathogenesis has been challenged by the identification of other acidogenic species, including *Bifidobacterium* [78], *Lactobacillus* [79] and *Scardovia wiggsiae* [80], which showed a strong association with caries. It was also revealed that a complex community associated with caries included other prominent species such as *Atopobium*, *Prevotella*, *Corynebacterium*, non-*mutans streptococci*, *Veillonella,* and *Capnocytophaga* [71, 75]. These species produce weak organic acids after carbohydrate fermentation and decrease the local pH to demineralize the tooth tissues. Nevertheless, its potential role in triggering dysbiosis should not be underestimated.

6-3 Role of oral microbiome on oral lichen planus

Oral lichen planus (OLP) is a chronic inflammatory mucocutaneous disease that mainly involves the oral mucosa. The etiology and pathogenesis of OLP are not clearly understood. However, OLP has been linked to multiple disease processes and agents, such as autoimmune diseases, allergic reactions to dental restorative materials, viral and bacterial infections, vaccinations, and medications [81]. So far, there has been little research on the oral microbiome in OLP. Bornstein et al. [82] reported higher bacterial counts of *C. sputigena, E. corrodens, L. crispatus, M. curtisii, N. mucosa, P. bivia, P. intermedia, and S. agalactiae S. haemolyticus* at the sites of OLP lesions. Ertugrul et al. also reported that OLP patients had higher levels of infection with *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia*, and *T. denticola* than non-OLP patients [83]. Choi et al. found a decrease in *Streptococcus* and an increase in gingivitis/periodontitis-associated bacteria in OLP lesions [84]. Furthermore, they demonstrated that intracellular bacteria in the tissue and bacterial LPS may induce the production of T cell chemokines C-X-C motif chemokine ligand 10 and C-C motif chemokine ligand [85]. To date, the association between OLP and HCV viral infection appears to be dependent on geographical heterogeneity. It was first suggested by Mokni et al. in 1991, and Carrozzo et al. demonstrated a strong association between

 doi:10.20944/preprints202106.0529.v1

hepatitis C viral infection and OLP [86, 87]. However, the changes in the oral microbiome in OLP remain unclear.

6-4 Role of the oral microbiome on pre-malignancy and oral cancers

Oral cancer is a cancer of the lips, mouth, or oropharynx. In an effort to elucidate the pathogenesis of oral cancers, oral microbiota has come into the spotlight and have been suggested to be involved through three possible mechanisms [88]. The provoked chronic inflammatory responses by bacteria could be responsible for this, since chronic inflammatory mediators facilitate cell proliferation, mutagenesis, and oncogene activation.

Anaerobic oral bacteria are known to cause chronic inflammatory processes in periodontal tissue by increasing interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α, and matrix metalloproteinases MMP-8 and MMP-9 [89]. The bacterial effector proteins using type 3 or type 4 secretion systems might influence cell proliferation, cytoskeletal rearrangements, activation of NF-κB, and inhibition of cellular apoptosis [14, 88]. In particular, *P. gingivalis* has been suggested to be anti-apoptotic. In addition, the purinergic receptor $P2X_7$ receptors activated by ATP are involved in cell death and apoptosis, and are affected by a nucleoside-diphosphate-kinase homolog, which is an ATP-consuming enzyme in *P. gingivalis* [90, 91].

Carcinogenic substances such as acetaldehyde converted from ethanol, reactive oxygen species, reactive nitrogen species, and volatile sulfur compounds by bacteria might facilitate carcinogenic processes [92]. *S. gordonii, S. mitis, S. oralis, S. salivarius, and S. sanguinis* [94], and Candida can metabolize alcohol to acetaldehyde using the enzyme alcohol dehydrogenase [93]. ROS and RNS produced by peroxigenic oral bacteria, including *S. mitis, S. gordonii, S. sanguinis, S. oralis, L. fermentum*, and *Ljensenii*, have been identified in various oral cancers. VSCs have genotoxic effects and cause genomic mutations [94]. *P. gingivalis, Pr. intermedia, A. actinomycetemcomitans*, and *F. nucleatum* are mainly responsible for the production of hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide ($(CH_3)_2S$) [95, 96].

More studies investigating the pathophysiology of oral cancers are required to confirm the association between oral cancer and oral microbiota; however, evidence advocating for the association is accumulating, and oral cancer surfaces harbor significantly higher numbers of oral aerobes and anaerobes compared to the healthy mucosa surface [97]. Therefore, the importance of detecting and managing the oral microenvironment is important for the control of oral malignancy.

7. Oral microbiome and systemic diseases

The oral microbiome can also be a pathogenic factor in systemic diseases (Figure 5). Because the mouth is the entrance connecting the external environment and the inside of the body, the association between the oral microbiome and systemic diseases has been reported. There may be a bidirectional relationship between the oral microbial community and systemic diseases [98].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 June 2021 doi:10.20944/preprints202106.0529.v1

7-1 Autoimmune disease

Dysbiosis of the oral microbiome plays a prominent role in several autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and primary Sjogren syndrome (SS) [99]. In autoimmune diseases, the most studied connection between the oral microbiome and disease is RA. The RA disease activity scores, DAS 28, were higher in patients with RA with more serum antibodies of *P. gingivalis*, an oral anaerobe involved in the development of periodontitis [100-102]. *P. gingivalis* produces gingipains and peptidylarginine deiminase, which enable protein citrullination, an important trigger for RA anticitrullinated peptide antibody [103, 104]. In addition, many epidemiological studies have shown a correlation between RA and periodontitis [105, 106]. In a recent meta-analysis of 21 studies, periodontitis was more frequent in RA patients than in healthy controls, with a risk ratio of 1.13 [107].

Dysbiosis of the oral microbiome in SS has been reported in several studies [108-114]. A decrease in salivary secretion is the most important factor in oral dysbiosis in SS [112, 113]. However, the impact of the oral microbiome on SS pathogenesis remains unclear. van der Meulen et al. reported higher *Firmicutes*/*Proteobacteria* ratios compared to those of healthy controls, and higher abundances of 19 genera in SS patients. SLE is also associated with changes in the oral microbiome. In 2017, Corrêa et al. reported the influence of SLE on the subgingival microbiota, and the *Lachnospiraceae* family was increased compared to controls at healthy gingival sites, and the proportions of *Prevotella oulorum* and *Prevotella pleuritidis*, *Pseudomonas* spp., *Treponema maltophilum*, and *Actinomyces* were those in healthy people [115]. Bacteria were elevated at periodontal sites compared to controls. Periodontal disease is also associated with SLE, which increases the risk or severity of SLE [116, 117].

7-2 Systemic malignancies

In a recent longitudinal study of hospitalized cancer patients, increased variability of the oral microbiome was associated with adverse clinical outcomes [94]. In colorectal cancer (CRC), the role of microbiota in carcinogenesis and its clinical significance have been reported in many studies. The identification of the *Fusobacterium* genus in about 30% of CRC cases by 16S rRNA sequencing has initiated research on the impact of oral microbiota in CRC [118-120]. *Fusobacterium nucleatum*, an oral commensal species, is more frequently identified in CRC than in colorectal adenoma, suggesting that *Fusobacterium* may contribute to later progression rather than an earlier stage along the colorectal adenoma-carcinoma sequence [121, 122]. However, Kato et al. reported no association between *Fusobacterium* and CRC; instead, they observed associations with the genera *Lactobacillus* and *Rothia* by an NGS-based study [123]. They explained that *Lactobacillus* and *Rothia* were related to oral hygiene, and poor oral hygiene was related to CRC. Other microbes of oral microbiota, such as *Porphyromonas, Peptostreptococcus, Prevotella, Parvimonas,* and *Gemella* are often reported to be associated with CRC. The carcinogenic potential and virulence factors of these genera are unknown [122].

Oral microbiota variation was associated with pancreatic cancer, and the combination of decreased *Neisseria elongata* with *Streptococcus mitis* was suggested to distinguish factors for pancreatic cancer [124]. Michaud et al. reported that the risk for pancreatic cancer was significantly increased in the presence of elevated serum

 doi:10.20944/preprints202106.0529.v1

antibodies to *P. gingivalis* [125]. Both *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* have the potential to initiate Toll-like receptor pathways, which have been shown to be a driver of pancreatic carcinogenesis [126]. In 2016, a prospective large cohort study supported that *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* may contribute to a higher risk of pancreatic cancer [127].

Esophageal cancer has also been reported to have relations with *Tannerella forsythia* and *P. gingivalis* [128]. The study showed that the genus *Neisseria* was associated with a lower risk of esophageal cancer, as was the carotenoid biosynthesis pathway, to which a number of *Neisseria* species can potentially contribute.

Some studies have shown that periodontal diseases are associated with lung cancer risk [129-131]; however, it was difficult to analyze the association between oral microbiota and lung cancer, as smoking, one of the biggest risk factors for lung cancer, can also affect oral microbiota. In a small cross-sectional study, the abundance of *Capnocytophaga* and *Veillonella* was elevated together with a reduced number of *Neisseria* by16S rRNA gene sequencing [132]. Thus, the current data are insufficient to conclude that oral microbial variations contribute to lung cancer.

7-3 Other systemic diseases

There is still conflict regarding the relationship between chronic inflammatory conditions, such as diabetes, and changes in the oral microbiome. In 2013, Chapple et al. reported that there is no compelling evidence that diabetes has a significant impact on the oral microbiota [133]. However, several studies have reported the impact of diabetes on changes in the oral microbiome. da Cruz et al. reported an increase in *P. gingivalis* and *Tannerella forsythia* in diabetes [134]. Ganesan et al. reported increased levels of *Capnocytophaga, Pseudomonas, Bergeyella, Sphingomonas, Corynebacterium, Propionibacterium,* and *Neisseria* in hyperglycemic individuals [135]. One NGS study reported that diabetes reduces *P. gingivalis, Tannerella forsythia*, and *Treponema denticola* [136]. To obtain clearer conclusion, large-scale longitudinal studies are needed in the future.

The direct effect of oral bacteria on cardiovascular disease (CVD) is even less known, and the involvement of bacteria in atherosclerotic plaque in atherogenesis is not clear. However, since periodontitis and oral dysbiosis are related, oral dysbiosis is thought to be related to a systemic inflammation. In a number of studies, the oral microbiota affected the outcome of CVD. The periodontal pathogen burden has been linked with acute coronary syndrome (ACS) and subclinical atherosclerosis [137-139], and Fak et al. reported that *Anaeroglobus* was more abundant in patients with symptomatic atherosclerosis than in controls [140]. Lipopolysaccharide (LPS), endotoxin, and virulence factors of bacteria are considered a molecular link between the microbiome and cardiometabolic disorders [141]. Serum LPS activity correlates with the levels of the *P. gingivalis* antibody [142]. In addition, the proteins secreted by *P. gingivalis*, such as gingipains, are implicated in their pathogenicity [143] and subsequently activate cytokine production [144]. There appear to be fewer studies using NGS techniques of oral cavity samples to determine the associations between the oral microbiome composition and cardiovascular disease.

Neurodegenerative disorders, such as Alzheimer's disease [145] and Parkinson's disease [146] have also been reported to be associated with the oral microbiome. The

 doi:10.20944/preprints202106.0529.v1

most complete study in this regard is the association between *P. gingivalis* and Alzheimer's disease [145]. *P. gingivalis* infection in mice resulted in brain colonization and that gingipain proteases produced by *P. gingivalis* are revealed to have neurotoxic character. It has also been shown that typical oral species of the phylum Spirochaetes, including multiple species of the genus *Treponema*, often comprise amyloid plaques [147]. The potential mechanisms of their action by oral taxa will make this another attractive area for investigation.

8. Conclusions

There is a dynamic interaction between the oral and systemic environments and the composition of the resident oral microbiome. Accumulated evidence suggests that the oral microbiome is individualized and relatively stable over time, as long as oral and general health is maintained. Substantial changes in key environmental parameters that affect microbial growth can disrupt the natural balance of the oral microbiome and select potentially pathogenic organisms. In addition, the presence of systemic diseases and oral microbiota seems to have a bidirectional effect. Thus, the oral microbiome reflects the oral and general health status of individuals. However, future studies are needed to determine if changes in the oral microbiome precede clinical signs of disease, which would enable the use of the oral microbiome in the prediction of future disease risk. In our systematic review, there may be a bias for the selection of papers based on keywords, and only for papers written in English. Accordingly, follow-up studies including meta-analysis will be needed to reach a clearer conclusion. In addition, prospective longitudinal studies are urgently needed to reveal the full potential of using the oral microbiome in the field of precision medicine.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 June 2021 doi:10.20944/preprints202106.0529.v1

Table 1. Various factors affecting the alteration of oral microbiome composition: analysis using 16S rRNA gene sequencing

Factors	Samples	Major finding
Smoking	Subgingival plaque	A high taxonomic diversity and richness in smokers Higher abundances of anaerobes in smokers: F*usobacterium nucleatum, F. naviforme, Filifactor alocis, Dialister microaerophilus, Desulfobulbus sp. clone R004, Megasphaera sueciensis, M. geminatus, M. elsdenii, M. micronuciformis, Acinetobacter johnsonii, A. guillouiae, A. schindleri, A. baumannii, A. haemolyticus, Pseudomonas pseudoalcaligenes,* and *Pseudoramibacter alactolyticus*
	Saliva	Lower abundances of commensal microbes in smokers: *Streptococcus sanguinis, S. parasanguinis, S. oralis, Granulicatella elegans, G. adiacens, Actinomyces viscosus, A. israelii, A. dentalis, Neisseria subflava* and *Hemophilus parainfluenzae*
	Buccal mucosa	A lower taxonomic diversity in smokers
	Marginal and subgingival plaque & gingival crevicular fluid	High diversity in smokers Relatively unstable initial colonization of marginal and subgingival biofilms in smokers Periodontal pathogens belonging to the genera *Fusobacterium, Cardiobacterium, Synergistes*, and *Selenomonas*, as well as respiratory pathogens belonging to the genera *Haemophilus* and *Pseudomonas*, colonized the early biofilms of smokers and continued to persist over the observation period
	Oral wash samples	Lower abundance of the Proteobacteria phylum and genera belong to the Proteobacteria phylum in smokers Taxa not belonging to Proteobacteria: - increase: the *Atopobium* and *Streptococcus* genera in current smokers - decrease: the *Capnocytophaga*, *Peptostreptococcus* and *Leptotrichia* genera in current smokers
	Saliva	Increase: *Streptococcus sobrinus* and *Eubacterium brachy* in smokers
Antimicrobial agent	Saliva	Reduced species richness and diversity At the phylum level: - increase: Proteobacteria at post-treatment - decrease: Actinobacteria at the end of treatment
Antibiotics	Saliva	A linear inverse association between the use of azithromycin and Shannon index Amoxicillin and amoxicillin-clavulanate use was associated with the largest decrease in abundance of *Rikenellaceae* Phenoxymethylpenicillin were associated with a decrease of *Paludibacter*
Pregnancy	Supragingival plaques	Significantly higher Shannon diversity in pregnant women More abundant: *Neisseria, Porphyromonas,* and *Treponema* in pregnant women Less abundant: *Streptococcus* and *Veillonella* in pregnant women

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 June 2021 doi:10.20944/preprints202106.0529.v1

Figure legends

Figure 1. Oral cavity and the composition of oral microbiome

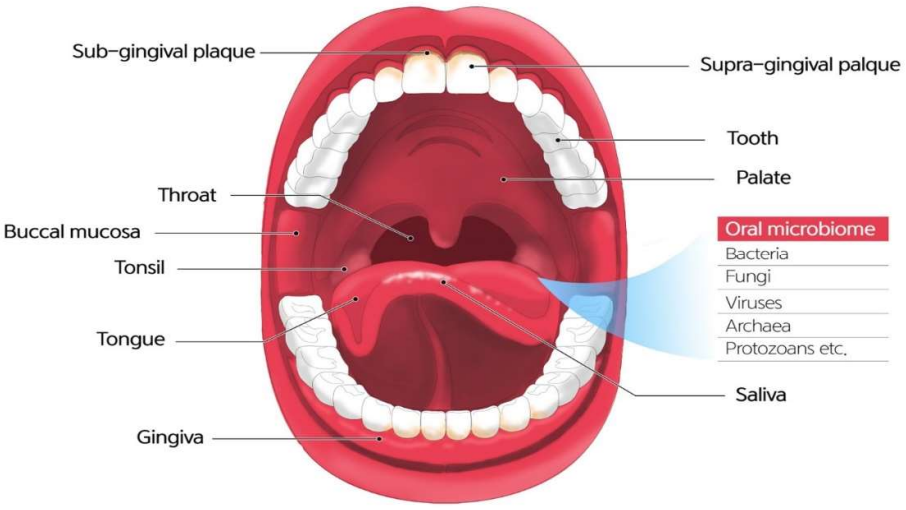


Figure 2. Oral microbiome is crucial factor for systemic health

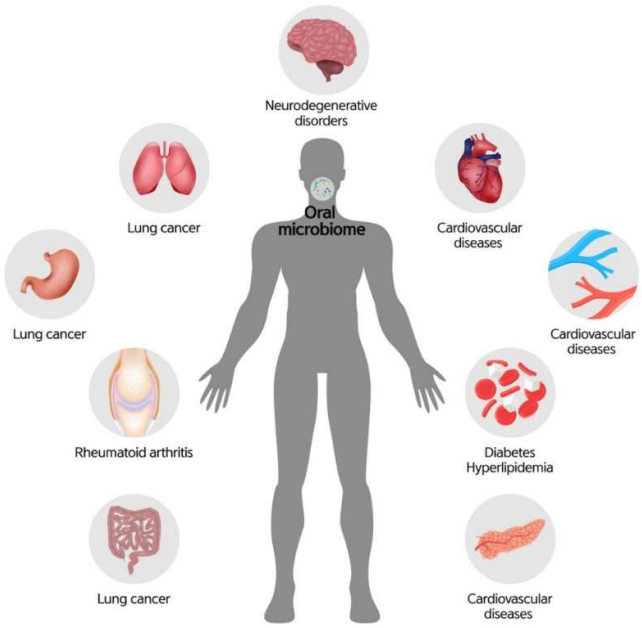


Figure 3. Various factors affecting oral microbiome

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 June 2021 doi:10.20944/preprints202106.0529.v1

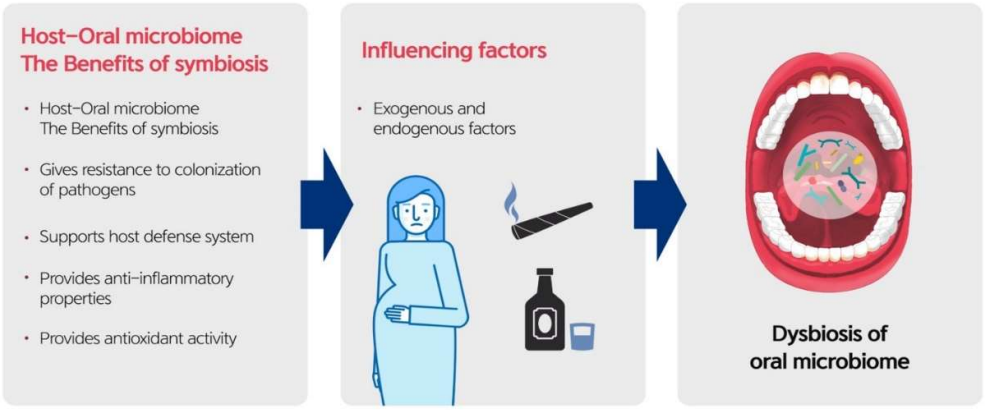


Figure 4. Bacterial colonizers related to periodontitis

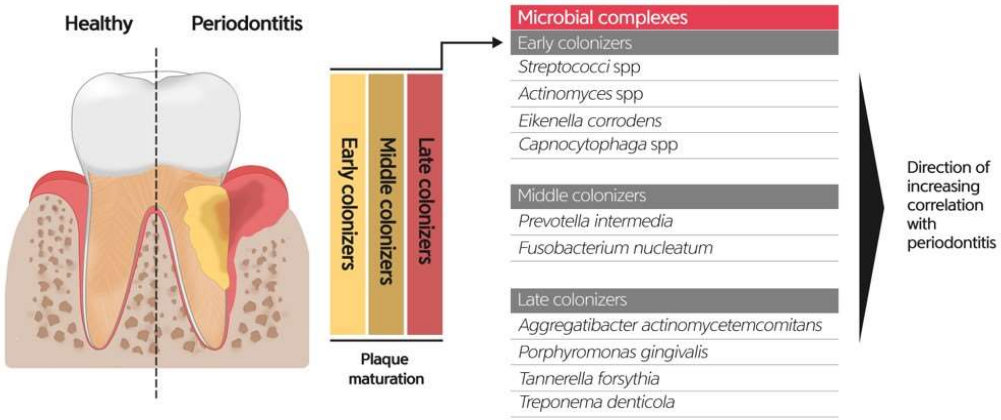


Figure 5. Exacerbation of oral microbial symbiosis to dysbiosis

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 June 2021 doi:10.20944/preprints202106.0529.v1

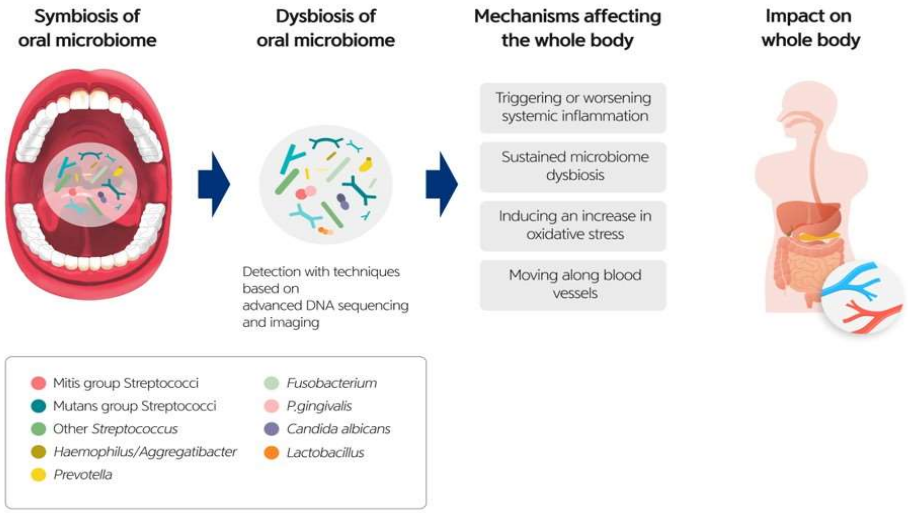
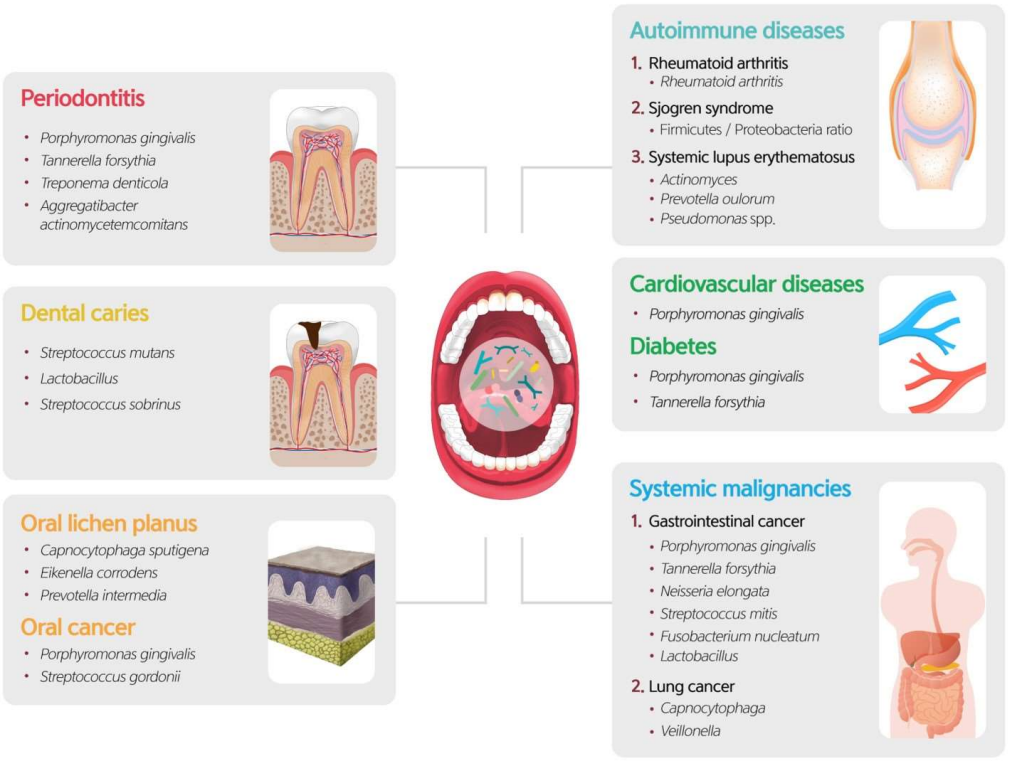


Figure 6. Types of systemic diseases related to dysbiosis of the oral microbiome



Supplementary Materials: None.

Author Contributions: All authors including Y.-H.L., S.-W.C, Q.-S.A., S.-J.H., Y.-A.L., J. J., G.-J. L., H.-J.P., S.-I.S., and J-Y.H. participated in paper writing—original draft preparation. Conceptualization, Y.-H.L.; methodology, Y.-H.L. and J-Y.H.; software, Y.-H.L.; validation, Q.-S.A., S.-J.H., Y.-A.L., J. J., G.-J. L., and H.-J.P.; formal analysis, Y.-H.L., and J-Y.H.; investigation, Y.-H.L., S.-W.C, Q.-S.A., S.-J.H., Y.-A.L., J. J., G.-J. L., H.-J.P., S.-I.S., and J-Y.H.; resources, Y.-H.L., S.-W.C, Q.-S.A., S.-J.H., Y.-A.L., J. J., G.-J. L., H.-J.P., S.-I.S., and J-Y.H.; data curation, Y.-H.L.; writing—review and editing, Y.-H.L.; visualization, Y.-H.L. and J-Y.H.; supervision, Y.-H.L.; project administration, Y.-H.L.; funding acquisition, Y.-H.L.

Funding: This work was supported by the Korea Medical Device Development Fund grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Trade, Industry and Energy, the Ministry of Health & Welfare, Republic of Korea, the Ministry of Food and Drug Safety) (Project Number: KMDF_PR_20200901_0023, 9991006696).

Institutional Review Board Statement: Ethical review and approval were waived for this study because it is a review article, not a study on animals or humans.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: None.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sender R, Fuchs S, Milo R: **Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans**. *Cell* 2016, **164**(3):337-340.
2. Delgado S, Cabrera-Rubio R, Mira A, Suárez A, Mayo B: **Microbiological survey of the human gastric ecosystem using culturing and pyrosequencing methods**. *Microb Ecol* 2013, **65**(3):763-772.
3. Malla MA, Dubey A, Kumar A, Yadav S, Hashem A, Abd Allah EF: **Exploring the Human Microbiome: The Potential Future Role of Next-Generation Sequencing in Disease Diagnosis and Treatment**. *Front Immunol* 2019, **9**:2868-2868.
4. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, Lakshmanan A, Wade WG: **The human oral microbiome**. *J Bacteriol* 2010, **192**(19):5002-5017.
5. Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F: **Oral microbiomes: more and more importance in oral cavity and whole body**. *Protein Cell* 2018, **9**(5):488-500.
6. Kilian M, Chapple ILC, Hannig M, Marsh PD, Meuric V, Pedersen AML, Tonetti MS, Wade WG, Zaura E: **The oral microbiome – an update for oral healthcare professionals**. *British Dental Journal* 2016, **221**(10):657-666.
7. Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, Tonetti MS, Wade WG, Zaura E: **The oral microbiome - an update for oral healthcare professionals**. *Br Dent J* 2016, **221**(10):657-666.
8. Marsh PD: **Are dental diseases examples of ecological catastrophes?** *Microbiology (Reading)* 2003, **149**(Pt 2):279-294.
9. Maukonen J, Mättö J, Suihko ML, Saarela M: **Intra-individual diversity and similarity of salivary and faecal microbiota**. *J Med Microbiol* 2008, **57**(Pt 12):1560-1568.

10. Faran Ali SM, Tanwir F: **Oral microbial habitat a dynamic entity**. *J Oral Biol Craniofac Res* 2012, **2**(3):181-187.
11. Baliga S, Muglikar S, Kale R: **Salivary pH: A diagnostic biomarker**. *J Indian Soc Periodontol* 2013, **17**(4):461-465.
12. Dickinson BC, Moffatt CE, Hagerty D, Whitmore SE, Brown TA, Graves DT, Lamont RJ: **Interaction of oral bacteria with gingival epithelial cell multilayers**. *Mol Oral Microbiol* 2011, **26**(3):210-220.
13. Malla MA, Dubey A, Kumar A, Yadav S, Hashem A, Abd Allah EF: **Exploring the Human Microbiome: The Potential Future Role of Next-Generation Sequencing in Disease Diagnosis and Treatment**. *Front Immunol* 2018, **9**:2868.
14. Lim Y, Totsika M, Morrison M, Punyadeera C: **Oral Microbiome: A New Biomarker Reservoir for Oral and Oropharyngeal Cancers**. *Theranostics* 2017, **7**(17):4313-4321.
15. Bachrach G, Leizerovici-Zigmond M, Zlotkin A, Naor R, Steinberg D: **Bacteriophage isolation from human saliva**. *Lett Appl Microbiol* 2003, **36**(1):50-53.
16. Ly M, Abeles SR, Boehm TK, Robles-Sikisaka R, Naidu M, Santiago-Rodriguez T, Pride DT: **Altered oral viral ecology in association with periodontal disease**. *mBio* 2014, **5**(3):e01133-01114.
17. Pride DT, Salzman J, Haynes M, Rohwer F, Davis-Long C, White RA, 3rd, Loomer P, Armitage GC, Relman DA: **Evidence of a robust resident bacteriophage population revealed through analysis of the human salivary virome**. *Isme j* 2012, **6**(5):915-926.
18. Naidu M, Robles-Sikisaka R, Abeles SR, Boehm TK, Pride DT: **Characterization of bacteriophage communities and CRISPR profiles from dental plaque**. *BMC Microbiol* 2014, **14**:175.
19. Pérez-Brocal V, Moya A: **The analysis of the oral DNA virome reveals which viruses are widespread and rare among healthy young adults in Valencia (Spain)**. *PloS one* 2018, **13**(2):e0191867-e0191867.
20. Edlund A, Santiago-Rodriguez TM, Boehm TK, Pride DT: **Bacteriophage and their potential roles in the human oral cavity**. *J Oral Microbiol* 2015, **7**:27423.
21. Deo PN, Deshmukh R: **Oral microbiome: Unveiling the fundamentals**. *J Oral Maxillofac Pathol* 2019, **23**(1):122-128.
22. Sharma N, Bhatia S, Sodhi AS, Batra N: **Oral microbiome and health**. *AIMS Microbiol* 2018, **4**(1):42-66.
23. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR: **Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges**. *Int J Antimicrob Agents* 2020, **55**(3):105924.
24. Wang Y, Kang H, Liu X, Tong Z: **Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak**. *J Med Virol* 2020, **92**(6):538-539.
25. Sabino-Silva R, Jardim ACG, Siqueira WL: **Coronavirus COVID-19 impacts to dentistry and potential salivary diagnosis**. *Clin Oral Investig* 2020, **24**(4):1619-1621.
26. Medeiros da Silva RC, Nogueira Marinho LC, de Araújo Silva DN, Costa de Lima K, Pirih FQ, Luz de Aquino Martins AR: **Saliva as a possible tool for the SARS-CoV-2 detection: A review**. *Travel Med Infect Dis* 2020, **38**:101920.
27. Zaura E: **Next-generation sequencing approaches to understanding the oral microbiome**. *Adv Dent Res* 2012, **24**(2):81-85.
28. Caselli E, Fabbri C, D'Accolti M, Soffritti I, Bassi C, Mazzacane S, Franchi M: **Defining the oral microbiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture**. *BMC Microbiol* 2020, **20**(1):120.
29. Clarridge JE, 3rd: **Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases**. *Clin Microbiol Rev* 2004, **17**(4):840-862.
30. Lochman J, Zapletalova M, Poskerova H, Izakovicova Holla L, Borilova Linhartova P: **Rapid Multiplex Real-Time PCR Method for the Detection and Quantification of Selected Cariogenic and Periodontal Bacteria**. *Diagnostics (Basel)* 2019, **10**(1).
31. Melendez JH, Frankel YM, An AT, Williams L, Price LB, Wang NY, Lazarus GS, Zenilman JM: **Real-time PCR assays compared to culture-based approaches for identification of aerobic bacteria in chronic wounds**. *Clin Microbiol Infect* 2010, **16**(12):1762-1769.
32. Sakamoto M, Umeda M, Benno Y: **Molecular analysis of human oral microbiota**. *J Periodontal Res* 2005, **40**(3):277-285.

33. Marsh PD, Percival RS: **The oral microflora--friend or foe? Can we decide?** *Int Dent J* 2006, **56**(4 Suppl 1):233-239.
34. Lönnroth I, Falsen E, Westin J, Lindholm L: **Two cases of "heavy chain disease"**. *Acta pathologica et microbiologica Scandinavica Section B: Microbiology and immunology* 1971, **79**(3):443.
35. Nociti FH, Jr., Casati MZ, Duarte PM: **Current perspective of the impact of smoking on the progression and treatment of periodontitis**. *Periodontology 2000* 2015, **67**(1):187-210.
36. Wu J, Peters BA: **Cigarette smoking and the oral microbiome in a large study of American adults**. 2016, **10**(10):2435-2446.
37. Kumar PS, Matthews CR, Joshi V, de Jager M, Aspiras M: **Tobacco smoking affects bacterial acquisition and colonization in oral biofilms**. *Infection and immunity* 2011, **79**(11):4730-4738.
38. Tettamanti L, Lauritano D, Nardone M, Gargari M, Silvestre-Rangil J, Gavoglio P, Tagliabue A: **Pregnancy and periodontal disease: does exist a two-way relationship?** *ORAL & implantology* 2017, **10**(2):112-118.
39. Barak S, Oettinger-Barak O, Oettinger M, Machtei EE, Peled M, Ohel G: **Common oral manifestations during pregnancy: a review**. *Obstetrical & gynecological survey* 2003, **58**(9):624-628.
40. Madianos PN, Bobetsis YA, Offenbacher S: **Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms**. *Journal of periodontology* 2013, **84**(4 Suppl):S170-180.
41. León R, Silva N, Ovalle A, Chaparro A, Ahumada A, Gajardo M, Martinez M, Gamonal J: **Detection of Porphyromonas gingivalis in the amniotic fluid in pregnant women with a diagnosis of threatened premature labor**. *Journal of periodontology* 2007, **78**(7):1249-1255.
42. Fujiwara N, Tsuruda K, Iwamoto Y, Kato F, Odaki T, Yamane N, Hori Y, Harashima Y, Sakoda A, Tagaya A *et al*: **Significant increase of oral bacteria in the early pregnancy period in Japanese women**. *Journal of investigative and clinical dentistry* 2017, **8**(1).
43. Lin W, Jiang W, Hu X, Gao L, Ai D, Pan H, Niu C, Yuan K, Zhou X, Xu C *et al*: **Ecological Shifts of Supragingival Microbiota in Association with Pregnancy**. *Frontiers in cellular and infection microbiology* 2018, **8**:24.
44. Lazarevic V, Manzano S, Gaïa N, Girard M, Whiteson K, Hibbs J, François P, Gervaix A, Schrenzel J: **Effects of amoxicillin treatment on the salivary microbiota in children with acute otitis media**. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2013, **19**(8):E335-342.
45. Raju SC, Viljakainen H, Figueiredo RAO, Neuvonen PJ, Eriksson JG, Weiderpass E, Rounge TB: **Antimicrobial drug use in the first decade of life influences saliva microbiota diversity and composition**. *Microbiome* 2020, **8**(1):121.
46. Gupta P, Gupta N, Pawar AP, Birajdar SS, Natt AS, Singh HP: **Role of sugar and sugar substitutes in dental caries: a review**. *ISRN dentistry* 2013, **2013**:519421.
47. Takahashi N, Nyvad B: **The role of bacteria in the caries process: ecological perspectives**. *Journal of dental research* 2011, **90**(3):294-303.
48. Gross EL, Leys EJ, Gasparovich SR, Firestone ND, Schwartzbaum JA, Janies DA, Asnani K, Griffen AL: **Bacterial 16S sequence analysis of severe caries in young permanent teeth**. *Journal of clinical microbiology* 2010, **48**(11):4121-4128.
49. Faran Ali SM, Tanwir F: **Oral microbial habitat a dynamic entity**. *Journal of oral biology and craniofacial research* 2012, **2**(3):181-187.
50. Han SJ, Bae KH, Lee HJ, Kim SJ, Cho HJ: **Association between regular walking and periodontitis according to socioeconomic status: a cross-sectional study**. *Scientific reports* 2019, **9**(1):12969.
51. Schwendicke F, Dörfer CE, Schlattmann P, Foster Page L, Thomson WM, Paris S: **Socioeconomic inequality and caries: a systematic review and meta-analysis**. *Journal of dental research* 2015, **94**(1):10-18.
52. Pihlstrom BL, Michalowicz BS, Johnson NW: **Periodontal diseases**. *Lancet* 2005, **366**(9499):1809-1820.
53. Federation FDIWD: **FDI policy statement on oral health and the social determinants of health. Adopted by the FDI General Assembly: 30 August 2013 - Istanbul, Turkey**. *Int Dent J* 2013, **63**(6):287-288.

54. Kassebaum NJ, Smith AGC, Bernabe E, Fleming TD, Reynolds AE, Vos T, Murray CJL, Marcenes W, Collaborators GBDOH: **Global, Regional, and National Prevalence, Incidence, and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990-2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors**. *J Dent Res* 2017, **96**(4):380-387.
55. Tonetti MS, Jepsen S, Jin L, Otomo-Corgel J: **Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action**. *J Clin Periodontol* 2017, **44**(5):456-462.
56. Loe H, Theilade E, Jensen SB: **Experimental Gingivitis in Man**. *J Periodontol* 1965, **36**:177-187.
57. Darveau RP: **Periodontitis: a polymicrobial disruption of host homeostasis**. *Nat Rev Microbiol* 2010, **8**(7):481-490.
58. Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA, McIntosh ML, Alsam A, Kirkwood KL, Lambris JD *et al*: **Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement**. *Cell Host Microbe* 2011, **10**(5):497-506.
59. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL, Jr.: **Microbial complexes in subgingival plaque**. *J Clin Periodontol* 1998, **25**(2):134-144.
60. Moore WE, Moore LV: **The bacteria of periodontal diseases**. *Periodontol 2000* 1994, **5**:66-77.
61. Abusleme L, Dupuy AK, Dutzan N, Silva N, Burleson JA, Strausbaugh LD, Gamonal J, Diaz PI: **The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation**. *ISME J* 2013, **7**(5):1016-1025.
62. Curtis MA, Diaz PI, Van Dyke TE: **The role of the microbiota in periodontal disease**. *Periodontol 2000* 2020, **83**(1):14-25.
63. Kistler JO, Booth V, Bradshaw DJ, Wade WG: **Bacterial community development in experimental gingivitis**. *PLoS One* 2013, **8**(8):e71227.
64. Schincaglia GP, Hong BY, Rosania A, Barasz J, Thompson A, Sobue T, Panagakos F, Burleson JA, Dongari-Bagtzoglou A, Diaz PI: **Clinical, Immune, and Microbiome Traits of Gingivitis and Peri-implant Mucositis**. *J Dent Res* 2017, **96**(1):47-55.
65. Hajishengallis G, Darveau RP, Curtis MA: **The keystone-pathogen hypothesis**. *Nat Rev Microbiol* 2012, **10**(10):717-725.
66. Jiao Y, Hasegawa M, Inohara N: **The Role of Oral Pathobionts in Dysbiosis during Periodontitis Development**. *J Dent Res* 2014, **93**(6):539-546.
67. Selwitz RH, Ismail AI, Pitts NB: **Dental caries**. *Lancet* 2007, **369**(9555):51-59.
68. Featherstone JD: **The continuum of dental caries--evidence for a dynamic disease process**. *J Dent Res* 2004, **83 Spec No C**:C39-42.
69. Takahashi N, Nyvad B: **Caries ecology revisited: microbial dynamics and the caries process**. *Caries Res* 2008, **42**(6):409-418.
70. Kidd EA, Fejerskov O: **What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms**. *J Dent Res* 2004, **83 Spec No C**:C35-38.
71. Simon-Soro A, Tomas I, Cabrera-Rubio R, Catalan MD, Nyvad B, Mira A: **Microbial geography of the oral cavity**. *J Dent Res* 2013, **92**(7):616-621.
72. Simon-Soro A, Guillen-Navarro M, Mira A: **Metatranscriptomics reveals overall active bacterial composition in caries lesions**. *J Oral Microbiol* 2014, **6**:25443.
73. Simon-Soro A, Mira A: **Solving the etiology of dental caries**. *Trends Microbiol* 2015, **23**(2):76-82.
74. Bowen WH, Burne RA, Wu H, Koo H: **Oral Biofilms: Pathogens, Matrix, and Polymicrobial Interactions in Microenvironments**. *Trends Microbiol* 2018, **26**(3):229-242.
75. Gross EL, Beall CJ, Kutsch SR, Firestone ND, Leys EJ, Griffen AL: **Beyond Streptococcus mutans: dental caries onset linked to multiple species by 16S rRNA community analysis**. *PLoS One* 2012, **7**(10):e47722.
76. Loesche WJ: **Role of Streptococcus mutans in human dental decay**. *Microbiol Rev* 1986, **50**(4):353-380.
77. Tanzer JM, Livingston J, Thompson AM: **The microbiology of primary dental caries in humans**. *J Dent Educ* 2001, **65**(10):1028-1037.

78. Mantzourani M, Fenlon M, Beighton D: **Association between Bifidobacteriaceae and the clinical severity of root caries lesions**. *Oral Microbiol Immunol* 2009, **24**(1):32-37.

79. Badet C, Thebaud NB: **Ecology of lactobacilli in the oral cavity: a review of literature**. *Open Microbiol J* 2008, **2**:38-48.

80. Tanner AC, Mathney JM, Kent RL, Chalmers NI, Hughes CV, Loo CY, Pradhan N, Kanasi E, Hwang J, Dahlan MA *et al*: **Cultivable anaerobic microbiota of severe early childhood caries**. *J Clin Microbiol* 2011, **49**(4):1464-1474.

81. Boorghani M, Gholizadeh N, Taghavi Zenouz A, Vatankhah M, Mehdipour M: **Oral lichen planus: clinical features, etiology, treatment and management; a review of literature**. *Journal of dental research, dental clinics, dental prospects* 2010, **4**(1):3-9.

82. Bornstein MM, Hakimi B, Persson GRJJop: **Microbiological findings in subjects with asymptomatic oral lichen planus: A cross-sectional comparative study**. 2008, **79**(12):2347-2355.

83. Ertugrul AS, Arslan U, Dursun R, Hakki SSJIjoos: **Periodontopathogen profile of healthy and oral lichen planus patients with gingivitis or periodontitis**. 2013, **5**(2):92-97.

84. Choi YS, Kim Y, Yoon H-J, Baek KJ, Alam J, Park HK, Choi YJSr: **The presence of bacteria within tissue provides insights into the pathogenesis of oral lichen planus**. 2016, **6**(1):1-13.

85. Metzemaekers M, Vanheule V, Janssens R, Struyf S, Proost P: **Overview of the Mechanisms that May Contribute to the Non-Redundant Activities of Interferon-Inducible CXC Chemokine Receptor 3 Ligands**. *Front Immunol* 2018, **8**:1970-1970.

86. Carrozzo M, Gandolfo S, Carbone M, Colombatto P, Broccoletti R, Garzino-Demo P, Ghisetti V: **Hepatitis C virus infection in Italian patients with oral lichen planus: a prospective case-control study**. *J Oral Pathol Med* 1996, **25**(10):527-533.

87. Mokni M, Rybojad M, Puppin D, Jr., Catala S, Venezia F, Djian R, Morel P: **Lichen planus and hepatitis C virus**. *J Am Acad Dermatol* 1991, **24**(5 Pt 1):792.

88. Zhang Y, Wang X, Li H, Ni C, Du Z, Yan F: **Human oral microbiota and its modulation for oral health**. *Biomed Pharmacother* 2018, **99**:883-893.

89. Herrero ER, Fernandes S, Verspecht T, Ugarte-Berzal E, Boon N, Proost P, Bernaerts K, Quirynen M, Teughels W: **Dysbiotic Biofilms Deregulate the Periodontal Inflammatory Response**. *J Dent Res* 2018, **97**(5):547-555.

90. Lee J, Roberts JS, Atanasova KR, Chowdhury N, Yilmaz O: **A novel kinase function of a nucleoside-diphosphate-kinase homologue in Porphyromonas gingivalis is critical in subversion of host cell apoptosis by targeting heat-shock protein 27**. *Cell Microbiol* 2018, **20**(5):e12825.

91. Yilmaz O, Yao L, Maeda K, Rose TM, Lewis EL, Duman M, Lamont RJ, Ojcius DM: **ATP scavenging by the intracellular pathogen Porphyromonas gingivalis inhibits P2X7-mediated host-cell apoptosis**. *Cell Microbiol* 2008, **10**(4):863-875.

92. Meurman JH, Uittamo J: **Oral micro-organisms in the etiology of cancer**. *Acta Odontol Scand* 2008, **66**(6):321-326.

93. Marttila E, Bowyer P, Sanglard D, Uittamo J, Kaihovaara P, Salaspuro M, Richardson M, Rautemaa R: **Fermentative 2-carbon metabolism produces carcinogenic levels of acetaldehyde in Candida albicans**. *Mol Oral Microbiol* 2013, **28**(4):281-291.

94. Attene-Ramos MS, Wagner ED, Plewa MJ, Gaskins HR: **Evidence that hydrogen sulfide is a genotoxic agent**. *Mol Cancer Res* 2006, **4**(1):9-14.

95. Suzuki N, Yoneda M, Takeshita T, Hirofuji T, Hanioka T: **Induction and inhibition of oral malodor**. *Mol Oral Microbiol* 2019, **34**(3):85-96.

96. Nakamura S, Shioya K, Hiraoka BY, Suzuki N, Hoshino T, Fujiwara T, Yoshinari N, Ansai T, Yoshida A: **Porphyromonas gingivalis hydrogen sulfide enhances methyl mercaptan-induced pathogenicity in mouse abscess formation**. *Microbiology (Reading)* 2018, **164**(4):529-539.

97. Nagy KN, Sonkodi I, Szoke I, Nagy E, Newman HN: **The microflora associated with human oral carcinomas**. *Oral Oncol* 1998, **34**(4):304-308.

98. Willis JR, Gabaldón T: **The human oral microbiome in health and disease: from sequences to ecosystems**. *Microorganisms* 2020, **8**(2):308.

99. Rosenbaum JT, Asquith MJ: **The microbiome: a revolution in treatment for rheumatic diseases?** *Current rheumatology reports* 2016, **18**(10):62.

100. Bawadekar M, De Andrea M, Gariglio M, Landolfo S: **Mislocalization of the interferon inducible protein IFI16 by environmental insults: implications in autoimmunity**. *Cytokine & growth factor reviews* 2015, **26**(2):213-219.

101. Eriksson K, Fei G, Lundmark A, Benchimol D, Lee L, Hu YO, Kats A, Saevarsdottir S, Catrina AI, Klinge B: **Periodontal health and oral microbiota in patients with rheumatoid arthritis**. *Journal of clinical medicine* 2019, **8**(5):630.

102. Arvikar SL, Collier DS, Fisher MC, Unizony S, Cohen GL, McHugh G, Kawai T, Strle K, Steere AC: **Clinical correlations with Porphyromonas gingivalis antibody responses in patients with early rheumatoid arthritis**. *Arthritis research & therapy* 2013, **15**(5):R109.

103. Hitchon CA, Chandad F, Ferucci ED, Willemze A, Ioan-Facsinay A, van der Woude D, Markland J, Robinson D, Elias B, Newkirk M: **Antibodies to Porphyromonas gingivalis are associated with anticitrullinated protein antibodies in patients with rheumatoid arthritis and their relatives**. *The Journal of rheumatology* 2010, **37**(6):1105-1112.

104. Mikuls TR, Payne JB, Reinhardt RA, Thiele GM, Maziarz E, Cannella AC, Holers VM, Kuhn KA, O'Dell JR: **Antibody responses to Porphyromonas gingivalis (P. gingivalis) in subjects with rheumatoid arthritis and periodontitis**. *International immunopharmacology* 2009, **9**(1):38-42.

105. Cheng Z, Meade J, Mankia K, Emery P, Devine DA: **Periodontal disease and periodontal bacteria as triggers for rheumatoid arthritis**. *Best Practice & Research Clinical Rheumatology* 2017, **31**(1):19-30.

106. Li R, Tian C, Postlethwaite A, Jiao Y, Garcia-Godoy F, Pattanaik D, Wei D, Gu W, Li J: **Rheumatoid arthritis and periodontal disease: What are the similarities and differences?** *International Journal of Rheumatic Diseases* 2017, **20**(12):1887-1901.

107. Fuggle NR, Smith TO, Kaul A, Sofat N: **Hand to mouth: a systematic review and meta-analysis of the association between rheumatoid arthritis and periodontitis**. *Frontiers in immunology* 2016, **7**:80.

108. Li M, Zou Y, Jiang Q, Jiang L, Yu Q, Ding X, Yu Y: **A preliminary study of the oral microbiota in Chinese patients with Sjögren's syndrome**. *Archives of Oral Biology* 2016, **70**:143-148.

109. Rusthen S, Kristoffersen AK, Young A, Galtung H, Petrovski B, Palm Ø, Enersen M, Jensen JL: **Dysbiotic salivary microbiota in dry mouth and primary Sjögren's syndrome patients**. *PloS one* 2019, **14**(6):e0218319.

110. Sharma D, Sandhya P, Vellarikkal SK, Surin AK, Jayarajan R, Verma A, Kumar A, Ravi R, Danda D, Sivasubbu S: **Saliva microbiome in primary Sjögren's syndrome reveals distinct set of disease-associated microbes**. *Oral diseases* 2020, **26**(2):295-301.

111. Siddiqui H, Chen T, Aliko A, Mydel PM, Jonsson R, Olsen I: **Microbiological and bioinformatics analysis of primary Sjögren's syndrome patients with normal salivation §**. *Journal of oral microbiology* 2016, **8**(1):31119.

112. Van der Meulen TA, Harmsen HJ, Bootsma H, Liefers SC, Vich Vila A, Zhernakova A, Fu J, Wijmenga C, Spijkervet FK, Kroese FG: **Dysbiosis of the buccal mucosa microbiome in primary Sjögren's syndrome patients**. *Rheumatology* 2018, **57**(12):2225-2234.

113. van der Meulen TA, Harmsen HJ, Bootsma H, Liefers SC, Vila AV, Zhernakova A, Weersma RK, Spijkervet FK, Kroese FG, Vissink A: **Reduced salivary secretion contributes more to changes in the oral microbiome of patients with primary Sjögren's syndrome than underlying disease**. *Annals of the rheumatic diseases* 2018, **77**(10):1542-1544.

114. De Paiva CS, Jones DB, Stern ME, Bian F, Moore QL, Corbiere S, Streckfus CF, Hutchinson DS, Ajami NJ, Petrosino JF: **Altered mucosal microbiome diversity and disease severity in Sjögren syndrome**. *Scientific reports* 2016, **6**(1):1-11.

115. Corrêa JD, Calderaro DC, Ferreira GA, Mendonça SMS, Fernandes GR, Xiao E, Teixeira AL, Leys EJ, Graves DT, Silva TA: **Subgingival microbiota dysbiosis in systemic lupus erythematosus: association with periodontal status**. *Microbiome* 2017, **5**(1):34.

116. Fabbri C, Fuller R, Bonfá E, Guedes LK, D'Alleva PSR, Borba EF: **Periodontitis treatment improves systemic lupus erythematosus response to immunosuppressive therapy**. *Clinical rheumatology* 2014, **33**(4):505-509.

117. Kobayashi T, Ito S, Yasuda K, Kuroda T, Yamamoto K, Sugita N, Tai H, Narita I, Gejyo F, Yoshie H: **The combined genotypes of stimulatory and inhibitory Fcγ receptors associated with systemic lupus erythematosus and periodontitis in Japanese adults**. *Journal of periodontology* 2007, **78**(3):467-474.

118. Ahn J, Sinha R, Pei Z, Dominianni C, Wu J, Shi J, Goedert JJ, Hayes RB, Yang L: **Human gut microbiome and risk for colorectal cancer**. *Journal of the National Cancer Institute* 2013, **105**(24):1907-1911.

119. Castellarin M, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, Barnes R, Watson P, Allen-Vercoe E, Moore RA: **Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma**. *Genome research* 2012, **22**(2):299-306.

120. Kostic AD, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, Ojesina AI, Jung J, Bass AJ, Tabernero J: **Genomic analysis identifies association of Fusobacterium with colorectal carcinoma**. *Genome research* 2012, **22**(2):292-298.

121. Feng Q, Liang S, Jia H, Stadlmayr A, Tang L, Lan Z, Zhang D, Xia H, Xu X, Jie Z: **Gut microbiome development along the colorectal adenoma–carcinoma sequence**. *Nature communications* 2015, **6**:6528.

122. Zackular JP, Rogers MA, Ruffin MT, Schloss PD: **The human gut microbiome as a screening tool for colorectal cancer**. *Cancer prevention research* 2014, **7**(11):1112-1121.

123. Kato I, Vasquez AA, Moyerbrailean G, Land S, Sun J, Lin H-S, Ram JL: **Oral microbiome and history of smoking and colorectal cancer**. *Journal of epidemiological research* 2016, **2**(2):92.

124. Farrell JJ, Zhang L, Zhou H, Chia D, Elashoff D, Akin D, Paster BJ, Joshipura K, Wong DT: **Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer**. *Gut* 2012, **61**(4):582-588.

125. Michaud DS, Izard J, Wilhelm-Benartzi CS, You D-H, Grote VA, Tjønneland A, Dahm CC, Overvad K, Jenab M, Fedirko V: **Plasma antibodies to oral bacteria and risk of pancreatic cancer in a large European prospective cohort study**. *Gut* 2013, **62**(12):1764-1770.

126. Zambirinis CP, Levie E, Nguy S, Avanzi A, Barilla R, Xu Y, Seifert L, Daley D, Greco SH, Deutsch M: **TLR9 ligation in pancreatic stellate cells promotes tumorigenesis**. *Journal of Experimental Medicine* 2015, **212**(12):2077-2094.

127. Fan X, Alekseyenko AV, Wu J, Peters BA, Jacobs EJ, Gapstur SM, Purdue MP, Abnet CC, Stolzenberg-Solomon R, Miller G: **Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study**. *Gut* 2018, **67**(1):120-127.

128. Peters BA, Wu J, Pei Z, Yang L, Purdue MP, Freedman ND, Jacobs EJ, Gapstur SM, Hayes RB, Ahn J: **Oral microbiome composition reflects prospective risk for esophageal cancers**. *Cancer research* 2017, **77**(23):6777-6787.

129. Michaud DS, Liu Y, Meyer M, Giovannucci E, Joshipura K: **Periodontal disease, tooth loss, and cancer risk in male health professionals: a prospective cohort study**. *The lancet oncology* 2008, **9**(6):550-558.

130. Vogtmann E, Goedert JJ: **Epidemiologic studies of the human microbiome and cancer**. *British journal of cancer* 2016, **114**(3):237-242.

131. Zeng XT, Xia LY, Zhang YG, Li S, Leng WD, Kwong JS: **Periodontal disease and incident lung cancer risk: A meta-analysis of cohort studies**. *Journal of periodontology* 2016, **87**(10):1158-1164.

132. Yan X, Yang M, Liu J, Gao R, Hu J, Li J, Zhang L, Shi Y, Guo H, Cheng J: **Discovery and validation of potential bacterial biomarkers for lung cancer**. *American journal of cancer research* 2015, **5**(10):3111.

133. Chapple IL, Genco R, workshop* wgotjEA: **Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases**. *Journal of Periodontology* 2013, **84**:S106-S112.

134. da Cruz GA, de Toledo S, Sallum EA, Sallum AW, Ambrosano GM, de Cassia Orlandi Sardi J, da Cruz SE, Goncalves RB: **Clinical and laboratory evaluations of non-surgical periodontal treatment in subjects with diabetes mellitus**. *J Periodontol* 2008, **79**(7):1150-1157.

135. Ganesan SM, Joshi V, Fellows M, Dabdoub SM, Nagaraja HN, O'Donnell B, Deshpande NR, Kumar PS: **A tale of two risks: smoking, diabetes and the subgingival microbiome**. *The ISME journal* 2017, **11**(9):2075-2089.

136. Casarin R, Barbagallo A, Meulman T, Santos V, Sallum E, Nociti F, Duarte P, Casati M, Gonçalves R: **Subgingival biodiversity in subjects with uncontrolled type-2 diabetes and chronic periodontitis**. *Journal of periodontal research* 2013, **48**(1):30-36.

137. Spahr A, Klein E, Khuseyinova N, Boeckh C, Muche R, Kunze M, Rothenbacher D, Pezeshki G, Hoffmeister A, Koenig W: **Periodontal infections and coronary heart disease: role of periodontal bacteria and importance of total pathogen burden in the Coronary Event and Periodontal Disease (CORODONT) study**. *Archives of Internal Medicine* 2006, **166**(5):554-559.

138. Renvert S, Pettersson T, Ohlsson O, Persson GR: **Bacterial profile and burden of periodontal infection in subjects with a diagnosis of acute coronary syndrome**. *Journal of periodontology* 2006, **77**(7):1110-1119.

139. Andriankaja O, Trevisan M, Falkner K, Dorn J, Hovey K, Sarikonda S, Mendoza T, Genco R: **Association between periodontal pathogens and risk of nonfatal myocardial infarction**. *Community dentistry and oral epidemiology* 2011, **39**(2):177-185.

140. Fåk F, Tremaroli V, Bergström G, Bäckhed F: **Oral microbiota in patients with atherosclerosis**. *Atherosclerosis* 2015, **243**(2):573-578.

141. Kallio E: **Lipopolysaccharide: a link between periodontitis and cardiometabolic disorders**. 2014.

142. Pussinen PJ, Tuomisto K, Jousilahti P, Havulinna AS, Sundvall J, Salomaa V: **Endotoxemia, immune response to periodontal pathogens, and systemic inflammation associate with incident cardiovascular disease events**. *Arteriosclerosis, thrombosis, and vascular biology* 2007, **27**(6):1433-1439.

143. Haraguchi A, Miura M, Fujise O, Hamachi T, Nishimura F: **Porphyromonas gingivalis gingipain is involved in the detachment and aggregation of Aggregatibacter actinomycetemcomitans biofilm**. *Molecular oral microbiology* 2014, **29**(3):131-143.

144. Jayaprakash K, Khalaf H, Bengtsson T: **Gingipains from Porphyromonas gingivalis play a significant role in induction and regulation of CXCL8 in THP-1 cells**. *BMC microbiology* 2014, **14**(1):193.

145. Dominy SS, Lynch C, Ermini F, Benedyk M, Marczyk A, Konradi A, Nguyen M, Haditsch U, Raha D, Griffin C: **Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors**. *Science advances* 2019, **5**(1):eaau3333.

146. Mihaila D, Donegan J, Barns S, LaRocca D, Du Q, Zheng D, Vidal M, Neville C, Uhlig R, Middleton FA: **The oral microbiome of early stage Parkinson's disease and its relationship with functional measures of motor and non-motor function**. *PloS one* 2019, **14**(6):e0218252.

147. Miklossy J: **Bacterial amyloid and DNA are important constituents of senile plaques: further evidence of the spirochetal and biofilm nature of senile plaques**. *Journal of Alzheimer's Disease* 2016, **53**(4):1459-1473.