Oral Microbiome and Host Health: Review on Current Advances in Genome-Wide Analysis

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Abstract: The oral microbiome is an important part of the human microbiome. The oral cavity has the second largest microbiota after the intestines, and its open structure creates a special environment. With the development of technology such as next-generation sequencing and bioinformatics, extensive in-depth microbiome studies have become possible. They can also be applied in the clinical field in terms of diagnosis and treatment. Many microbiome studies have been performed on oral and systemic diseases, showing a close association between the two. Understanding the oral microbiome and host interaction is expected to provide future directions to explore the functional and metabolic changes in diseases, and to uncover the molecular mechanisms for drug development and treatment that facilitate personalized medicine. The aim of this review was to provide comprehension regarding research trends in oral microbiome studies and establish the link between oral microbiomes and systemic diseases based on the latest technique of genome-wide analysis.

Keywords: oral microbiome; oral disease; systemic disease; genome-wide analysis; personalized medicine

1. Introduction

The human oral cavity is the second largest microbial habitat comprising fungi, bacteria, and viruses, after the intestines, and has a special niche consisting of soft tissue of the gingiva and oral mucosa and the hard tissue of the teeth [1]. Although the oral cavity and intestines are a distance apart, they are connected and show a marvelous diversity of microorganisms. The oral microbiome refers to the collective genetic materials of oral microorganisms. The microorganisms in the oral cavity are referred to as oral microbiota, oral microflora, and oral microbiome [2]. Microorganisms coexist in our bodies and live through symbiosis, dysbiosis, and pathogenic relations. The microbiome not only refers to the microorganisms involved, but also includes areas of activity that result in the formation of specific ecological niches. Significant changes in the local environment can disrupt the microbe–microbe interaction, which can alter the host–microbe equilibrium, increasing the risk of disease [3]. The oral cavity is the most accessible habitat for studying the relationship between the host and microorganisms. Different oral structures such as the teeth, the gingiva, the palate, the cheek, and the lips are colonized by distinct microbial communities, and the surfaces of the oral cavity are covered with bacterial biofilm [4]. The special environment of the oral cavity, with its stable pH of 6.5–7.0 of saliva, moisture, and its temperature of an average of 37 °C, creates the favorable conditions necessary for the growth of microorganisms [5]. For this reason, various kinds of microorganisms are well distributed in the mouth. The oral cavity is one of the best-studied microbiomes to date, with research focusing on its role as a part of human microbiome development and how it influences systemic health and disease. About 700 species of prokaryotes have been identified [6], and 392 taxa with at least one reference genome exist [7]. In the near future, the advances in our understanding of oral microbiomes may change our way of life through...
in-depth principles of oral biology and novel therapeutics. In this review, we provide a general understanding of the oral microbiome, establish the link between oral microbiomes and general health based on a genome-wide analysis through the latest technique, and discuss the perspective and future directions for both oral and systemic health.

2. Paradigm Shift: Microorganisms to Microbiomes

The term “microorganism” simply refers to very small living things such as archaea, bacteria, protists, fungi, and viruses, while “microbiome” is a collective and comprehensive term for microorganisms. The community of microbial residents is referred to as the microbiome, and recent research on microbiomes has demonstrated an important role of the communities of microorganisms in human homeostasis (Figure 1A) [8]. The term “microbiome” was coined by Joshua Lederberg, a Nobel Prize laureate, “to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease” [9]. As a collection of information, the microbiome includes the microorganisms’ genomic data, structural elements, metabolites, and environmental conditions (Figure 1A) [10]. With the emergence of new technology, including next-generation sequencing (NGS), and in-depth information, such as sequence profiles of microbial communities, numerous insights on the relation between the human microbiome and disease have been obtained [11].

| A | Microorganism | Genomic data | Microbiome | Environmental condition |
|---|---------------|--------------|------------|------------------------|
|   | Archa | DNA, RNA | Polysaccharides, Lipids, Proteins, signaling molecule | Residence type, Temperature, Humidity |
|   | Bacteria | DNA | Toxin, Signaling molecule | |
|   | Protist | Protein | | |
|   | Fungi | | | |
|   | Virus | | | |

| B | 1600s | 1800s | 1980s | 1990-2003 | 2008-2012 | 2010 | 2013-2015 |
|---|-------|-------|-------|-----------|-----------|------|-----------|
| Microscopic observation | Cultivation | Staining | Genomic sequencing | HGP | HMP1 | HOMD | HMP2 |

HGP: Human Genome Project
HMP1: Human Microbiome Project, Phase I
HMP2: Human Microbiome Project, Phase II
HOMD: Human Oral Microbiome Database

Figure 1. From microorganism to microbiome; a paradigm shift with advanced technology. (A) The term, microbiome, refers not only to the microorganisms but also to their theatre of activity. (B) Timeline of microbiome research.

In the 1670s, the study of human microbiology was initiated with the invention of the microscope, and the consequent observation of bacteria in pond water and dental plaque, by Antoni Philips van Leeuwenhoek [12,13] (Figure 1B). He was the first to observe microorganisms and determine their sizes. After that revolutionary invention, a culture-based method that is dependent on the growth of viable and culturable microbes was introduced to identify microbes with biochemical subtyping in the 1800s [14]. After the 1980s, the emergence of new technology, such as DNA sequencing, which enables acquisition of more than 1 trillion sequences of genetic information, provided a powerful unculturable method for understanding human health and disease [15]. Based on this advance in sequencing technology, the Human Genome Project (HGP) was started in 1990 and completed in 2003. The first sequence of the human genome was published in 2001 and constituted about 90% of the genome [16]. With the HGP, the elucidation of the human
genome initiated a new era in “precision medicine”, which is the ideal concept of health care [17]. The basis of precision medicine includes the genomes of humans and of the microorganisms, such as fungi, viruses, and bacteria, that reside in the human body. As the extension of the HGP, the Human Microbiome Project (HMP) was launched in 2007 with the goal of establishing 3000 microbial genomes from the representative six regions of the body (the mouth, the esophagus, the stomach, the colon, the vagina, and the skin) as reference genomes [18].

3. Methodology of the Microbiome Research

Completion of the HGP was accompanied by parallel and ongoing development of commercially available high-throughput DNA sequencing tools, which eventually facilitated the acquisition of genomic information from human samples. High-throughput techniques such as NGS have replaced the culture-based traditional methods for identifying and characterizing microbes [15]. Past studies without NGS and relying on the laboratory culture system were limited to small parts, such as the biochemical or physiological properties of microbiomes, called opportunistic pathogens [19]. In the beginning, microbiology research had focused on identifying pathogens within the commensal microbiota and deciphering their virulence in relation to health and disease. In the 1980s, a sequencing technique was introduced that enabled nucleotide sequences to be determined. This remarkable advance provided new and diverse information about microbial taxonomic profiling regardless of cultivability [19]. In taxonomic profiling, two methods, amplicon sequencing (16S rRNA sequencing) and direct shotgun sequencing (metagenomics), are used [19]. In the oral microbiome studies, samples were taken from dental plaque and crevicular fluid from the periodontal pocket. This technology has allowed the characterization of microbial diversity in the human microbiome with unprecedented depth and coverage [20]. These oral microbiome studies originated from HMP’s 16S rRNA sequencing data, published in the Human Oral Microbiome Database (HOMD), which identified oral bacteria from specific locations in the mouth such as the teeth, the gingival sulcus, the tongue, the cheek, the tonsils, and the soft and hard palates [2]. These early sequencing studies focused on the 16S rRNA sequence, and this conventional sequencing method was performed using a cultivated clonal culture; however, there were many microorganisms that were unculturable and thus could not be identified with this conventional method (Figure 2) [21]. This cultivation-based method could find < 1% of microbial species; therefore, metagenomics was introduced to identify non-isolated organisms via DNA sequencing without the need for cultivation and isolation [21]. In addition to metagenomics, DNA barcoding or metabarcoding is also used for the identification of species, using a short section of DNA from a reference library database [22]. The term “metabarcoding” is used when DNA barcoding is used to identify organisms from samples containing DNA from more than one organism [23]. Metagenomics also enables the metabolic and functional diversity of microbial communities to be accessed via metatranscriptomics, metaproteomics, and metabolomics [24]. Metaproteomics has emerged as a complementary approach to identify the functions of microbial communities [25]. Mass spectrometry-based proteomics has been widely used for studying the composition of proteins in oral microbial communities and has been applied to characterize oral biofilms [26]. Many metaproteomic studies have been performed in periodontal disease and endodontic infections associated with apical periodontitis using saliva and crevicular fluids [27]. Metabolomics is the study of metabolites within a biologic system to discover what happens in cells. Several studies have been conducted on the salivary metabolome; however, these have offered less specific explanations due to difficulties regarding disentangling host reactions and microbial contributions [27]. As such, metaproteomics and metabolomics complement the foundations established by metagenomics and metatranscriptomics in the comprehensive understanding of microbiomes.
4. Core, Pathologic, and Healthy Microbiomes

Microbiomes are largely divided into two types: core and variable microbiomes. The core microbiome is common to all individuals, while the variable microbiome is unique to individuals according to their lifestyle [28]. One of the goals of the HMP was to identify “core microbiomes” defined as microbial taxa or genes shared by most people [29]. The core microbiomes are described via five types: common core, temporal core, ecological core, functional core, and host-adapted core [28]. In order to diagnose and treat disease at an early stage, it is necessary to explain the commensal microbiome associated with health [30]. The commensal human microbiome is estimated to be 10 times greater in number than the quantity of human cells. These microbial communities are normal residents of the oral cavity, the skin, and the intestinal mucosa, and have a wide range of functions that are essential to the survival of the host. When the symbiotic balance between the host and the microbes is disrupted and disease is obvious, the presence of pathologic microbes stands out [31]. On the other hand, if the healthy microbiome is dominated by a “core microbiome”, the homeostasis of health would be maintained [32]. Zaura et al. defined the healthy core microbiome of oral microbial communities from several intraoral niches using 454 pyrosequencing, showing a major proportion of oral bacterial sequences of healthy individuals as identical, which supports the concept that there are key microbiomes in health [30]. In addition, Bao et al., identified the microbiome and proteomic profiles in the gingival tissue of healthy individuals and individuals with periodontitis by a pressure cycling technology-assisted workflow, which is an emerging platform for tissue homogenization and sequence retrieval coverage [33]. The results showed that 69 proteins were differentially expressed in periodontitis, and Treponema sp. HMT253 and Fusobacterium naviforme were strongly associated with disease sites, indicating the existence of a tissue-specific microbiome signature.

5. Human Microbiome Project

The omics era, with its innovative sequencing techniques, accelerated all aspects of biological research, and its effects were particularly evident in studies on microbial communities and the human microbiome. Genomic sequencing has revealed the diversity of microbial communities in the body, and more information has been obtained as the National Institute of Health Human Microbiome Project was launched in 2007 [18] and completed its work in 2018. The HMP was conducted in two phases over a decade to provide resources, methods, and discoveries linking human-to-microbiome interactions with health- and disease-related outcomes. The first phase of HMP (HMP1) focused on the characterization of the microbial community of numerous targeted body parts, including the oral cavity, the nose, the gut, the skin, and the vagina, from healthy adult subjects,
and demonstration projects focusing on specific diseases [34,35]. The HMP1 produced abundant community resources including nucleotide sequences of microorganisms [36,37], protocols for reproducible microbiome sampling and data curation [38,39], bioinformatics methodology, and epidemiology [40,41]. One of the important findings of HMP1 was that the taxonomic composition of microorganisms alone was not strongly associated with the phenotype of the host. This tended to be better predicted by a wide range of microbial molecular functions or personalized strain-specific compositions. Based on this, the Integrative HMP (iHMP), the second phase of HMP (HMP2), was promoted to understand host–microbiome communications, including molecular mechanisms and immunity [42,43]. Unlike HMP1, which focused on the microbiome, HMP2 expanded the scope to include both the microbiome and the host in three longitudinal cohort studies on preterm birth based on vaginal microbiomes, inflammatory bowel diseases based on gut microbiomes, and prediabetes based on gut and nasal microbiomes [43]. The studies of HMP2 included both microbial and host-specific multi-omics data such as genome, epigenome, transcriptome, metabolome, and proteome. In addition to HMP, Metagenomics of the Human Intestinal Tract (MetaHIT) has produced the resources and specialty needed to understand human microbiomes.

6. Oral Microbiome

The composition of the oral microbiome differs according to its habitat [2]; the microbial population forms a unique identity and plays an important role in nutritional, defensive, and physiological activities [44]. The oral environment is a heterogeneous ecological system and it is suitable for the growth of many microorganisms due to appropriate temperature and moisture, and provides host-derived nutrients such as gingival crevicular fluid and salivary proteins [45]. The hard structures in the mouth, including tooth and dental restorations such as restoration material, dental implants, and prosthesis, provide unique, non-shedding surfaces that affect biofilm formation and calculus deposition [46,47]. In the formation of the oral microbiome, the transmission of microbes from mother to baby at birth is the initial stage, and delivery types, such as vaginally born or caesarean section, affect the composition of the microbiome [48,49]. Subsequently, feeding type also influences the composition of the oral microbiome [50], and the eruption of a tooth makes a new environment for microbial colonization [51]. After tooth eruption, the composition of the oral microbiome becomes increasingly complex with age, and transition from deciduous to permanent teeth significantly alters the dynamics of the oral microbiome [52].

Human Oral Microbiome Database (HOMD)

The main purpose of HMP was to produce 1000 microbial reference genomes, including about 300 among the oral microbes [53]. As a national resource project, the data of HMP were released rapidly, and a Data Analysis and Coordinating Center was established. Additionally, comprehensive analyses of body site-specific data from HMP1, including oral, gut, skin, and vagina data, and taxonomic classification of newly identified microbes, were needed. The HOMD is the first oral-specific public database providing the scientific community with comprehensive information on oral microbiome species [54]. The HOMD database and web-based interface were set up under the Foundation for the Oral Microbiome and Metagenome from the National Institute of Dental and Craniofacial Research, and were organized based on taxonomic classifications, which were identified based on 16S rRNA sequencing data [2,54]. The named oral species and taxa identified in 16S rRNA sequencing were placed dependent on their 16S rRNA types and their unique human oral taxon (HOT) number. The HOT interconnects phylotypes, phenotypes, and the genomic, bibliographic, and clinical information of each taxon.

7. Host–Oral Microbiome Interactions in Health

The oral cavity has evolved to improve oral health and fosters highly personalized microbiomes that exist dynamically in balance with the host. The symbiotic relationship
between host and microbiome maintains microbial homeostasis; however, dysbiosis, a breakdown of the microbial homeostasis, induces oral disease and increases the risk for systemic diseases. The inseparable relationship between the host and microbiome is formed over a long time by facing various changes that force the adaptation of the oral microbiome to the new environment [55]. A bidirectional relation is characterized by the microbe providing the host with abilities it lacks alone, while the host provides an appropriate environment for microbial growth [56]. The host factors can positively affect the microbiome, making balance and diversity between the species, thus inducing symbiosis and an absence of pathology. On the contrary, the host can also create a negative influence [56,57]. This co-evolution between the host and microbiome succeeded in achieving a complex biological process in which the existence of independent entities would be impossible. The mutual benefits from the maintenance of a balanced host–oral microbiome ecology can be distorted to induce a shift from a healthy and symbiotic relation to a pathologic and dysbiotic one [58,59]. This distortion can result from changes in the oral microbiome as well as in the host [60]. Even though the host and the microbiome are equivalent factors, early studies have focused on finding the pathological oral microbiome, and the role of the host in maintaining a healthy oral microbiome was overlooked. Based on this, recent research trends have moved to focus on the host factors and combined the role of host–oral microbiome in the development of a healthy and balanced oral ecology, and extended to systemic disease and oral disease [61,62]. The host factors are largely classified into two, intrinsic and extrinsic, factors (Table 1). The oral microbiome is associated with a variety of oral diseases. Recently, there has been growing evidence that the oral microbiome is closely related to physical conditions, with many intermediate host factors [63].

| Factor          | Reference                                                                 |
|-----------------|---------------------------------------------------------------------------|
| Genetics        | Genetic polymorphism in miRNA202 is involved in hBD1 salivary level as well as caries experience [64] |
|                 | Genes expressed in dental enamel development are associated with molar-incisor hypomineralization [65] |
|                 | GLUT2 and TAS1R2 genotypes individually and in combination are associated with caries risk [66] |
|                 | Host genetic control of the oral microbiome in health and disease [67] |
|                 | Microbial abundance and some aspects of the microbial population structure are influenced by heritable traits in saliva [68] |
| Immunity        | Immune cell network mediating immune surveillance at oral mucosa and gingiva [69,70] |
|                 | The innate host response in caries and periodontitis [71] |
|                 | Secretory immunity with special reference to the oral cavity [72] |
| Attachment surface | Surface properties influence oral biofilm formation [73] |
|                 | Differences in relation to the microbial diversity of modified resins during the initial phase of biofilm maturatiion [74] |
|                 | Biomaterial-associated infection of implants and devices [46] |
| Diet            | Vegan diet influences on the human salivary microbiota [75] |
|                 | Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms [76] |
| Cigarette smoking | Smoking decreases structural and functional resilience in the subgingival ecosystem [77] |
|                 | Firmicutes were statistically elevated in smokers at the expense of Proteobacteria and Fusobacteria in non-smokers [78] |
|                 | Tobacco smoking affects the salivary gram-positive bacterial population [79] |
| Alcohol         | Alcohol affects to the oral microbiome composition [80–82] |
| Oral hygiene    | Toothbrushing frequency is related to the incidence and increment of dental caries [83] |
| Socioeconomic status | Socioeconomic factors, such as education and income, are associated with disparities in the prevalence and severity of periodontal disease [84] |
|                 | A strong association between cariogenic bacteria and socioeconomic status was found [85] |
|                 | Differences in socioeconomic status were reflected in the bacterial profile of saliva [86] |
8. Potential Clinical Application of Oral Microbiomes

With various “omics” studies, information on the composition of oral microbiomes is available. This vast amount of oral microbiome data, which were procured via HMP, could be the fundamental basis of clinical applications including early diagnosis, predictive treatment, and prevention [87,88]. The general microbial screening for diagnosis is performed using saliva and site-specific screening with gingival crevicular fluid and dental biofilm [89]. Saliva is a useful diagnostic fluid, providing the overall microbiome and proteome or metabolomic data from bacterial metabolic or host inflammatory products for personalized monitoring. This combined information from saliva can be used to predict susceptibility to oral diseases, including dental caries or periodontitis, with higher specificity [89,90]. Microbial screening of the mouth can be applied not only with oral diseases, but also with systemic diseases due to their reciprocal association.

9. Oral Disease and Systemic Disease

The commensal microbiome plays an important role in maintaining oral and systemic health. The breakdown of the microbial balance induces pathologic conditions such as periodontal disease, dental caries, and endodontic disease, which are associated with systemic diseases including diabetes [91], cardiovascular disease (CVD) [92], respiratory disease [93], and cancer [94] (Figure 3). The links between oral diseases and systemic health are complicated and bidirectional in many ways [95]. Among many oral diseases, periodontitis has a close relationship with non-communicable diseases (NCDs); particularly, diabetes and CVD. When periodontitis is left untreated, it could lead to the loss of periodontal supporting tissue due to microbial infection. Oral pathologic microbiomes could release virulence factors, inducing an inflammatory response, and invade the body through pathogenic lesions, which increases the risk of exacerbating NCDs [96].

Dental caries
Periodontal diseases

Diabetes
Cardiovascular disease
Chronic respiratory disease
Cancer

Figure 3. The oral microbiome affects both oral and systemic diseases. Adapted from Cho et al. (2021).

9.1. Non-Communicable Diseases

NCDs are diseases that are not transmissible between people, and are mainly chronic conditions with slow progression [97]. Prevalent NCDs include diabetes mellitus (DM), CVD, chronic respiratory diseases, and cancer [98]. The prevalence of NCDs is globally increasing with an aging population, which makes it a significant burden to the healthcare sector [99].
9.1.1. Cardiovascular Disease

CVD is a general term for atherosclerotic diseases, and atherosclerotic CVD is one of the leading causes of death in the world [100]. Generally, circulating leukocytes in the blood vessels do not adhere to the endothelium under normal conditions. However, when an inflammatory response occurs, various adhesion molecules, such as vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and P-selectin, are expressed in the endothelial cells, inducing the adhesion of leukocytes [101]. When leukocytes adhere to the endothelium, they migrate into the intima, and diapedesis occurs via the expression of matrix metalloproteinases [102]. Several studies regarding the link between periodontitis and CVD have reported their positive association [100,105,106]. Several risk factors including smoking, diabetes, hypertension, and hypercholesterolemia have been focused on as the contributory factors in atherosclerosis; however, recent studies have suggested that immune and inflammatory mechanisms caused by oral bacteria could be an important factor in atherosclerosis [103,104]. Oral bacteria, such as periodontal and cariogenic pathogens, are known as etiological factors in the development of atherosclerosis, which is the first step of CVD. To investigate the association between oral microbiomes and atherosclerosis, several studies have been performed examining atheromatous lesions with various molecular biologic techniques. Fundamentally, periodontal pathogen were cultured in atheromatous plaque [105], and fluorescence in situ hybridization [106], DNA–DNA hybridization [107], and real-time PCR [108] results showed the presence of oral pathogens in atheromatous lesions. Based on these results, oral microbiome studies have been widely conducted on oral bacteria-induced atherosclerotic CVD (Table 2).

Table 2. Oral Microbiome Related to CVD.

| Mechanism                          | Organism | Result                                                                 | Reference |
|------------------------------------|----------|------------------------------------------------------------------------|-----------|
| Endothelial cell invasion          | P. gingivalis (P.g.) strain 381 | Infection of human aortic endothelial cells with invasive P. g. strain 381 resulted in the upregulation of 68 genes that code for the pro-inflammatory cytokines, adhesion molecules, and chemokines. In addition, P. g. induces procoagulant effects including enhanced tissue factor expression and activity, and suppression of tissue factor pathway inhibitors | [109]     |
|                                    | F.nucleatum (F.n.) | Co-infection with F. n. resulted in a 2–20-fold increase in the invasion of endothelial cells by P. g. strains | [110]     |
| Endothelial cell activation        | A. actinomycenecomitans (A.a.) | A.a. infection in apoliipoprotein E-deficient mice increased expressions of ICAM-1, E-selectin, P-selectin, MCP-1, chemokine (C-C motif) ligand 19 (CCL19), CCL21, and CCR7 in the aorta | [111]     |
|                                    | P.g.     | Coculture of endothelial cells with P.g. increased ICAM-1, VCAM-1 and P-and E-selectins | [112,113] |
| Oxidative stress-mediated mechanism| P.g.     | P.g. cleaves apoB-100 and increases the expression of apoM in LDL in whole blood | [114]     |
| Metalloproteinase-mediated mechanism| A.a.     | A.a. induces MMP-9 expression and proatherogenic lipoprotein profile in apoE-deficient mice | [115]     |
9.1.2. Diabetes Mellitus

Periodontitis and DM are representative chronic and high-prevalence diseases in the dental and medical fields, respectively [121]. DM is a metabolic disorder characterized by prolonged high blood glucose level, which could lead to systemic complications such as CVD and circulatory problems, including peripheral vascular disease. In 2017, the International Diabetic Federation listed periodontitis as a risk factor of DM [122]. The majority of studies on DM and periodontitis have focused on type 2 DM [123,124], and the relationship of type 1 DM with periodontal disease in young patients [125]. The association between these two diseases has been studied for many years, and it is believed that the two have bidirectional links, implying that DM is a risk factor of periodontitis and periodontitis adversely affects glycemic control [126–128]. It has been known that the oral microbiota plays an important role in the relationship between DM and periodontitis because it affects blood glycemic control [129]. Disturbances in the oral microbiome are considered to be factors of periodontal disease initiation and progression and DM [130], and several studies have been conducted to understand this cause-and-effect relationship (Table 3). Matsha et al. showed the alteration in the composition of oral microbiomes across glycemic status as well as different stages of periodontal disease using 16S rDNA sequencing in dental plaque samples from South Africa [91]. Additionally, other studies with high-throughput metagenomic sequencing (16S rDNA or rRNA) of oral microbiomes also demonstrated the role of the oral microbiome in the development of DM [131–133]. Recently, Preshaw et al. reported that the treatment of periodontitis could reduce inflammation in DM patients, indicating that diabetes and periodontitis together increase systemic inflammation [134]. Similarly, a systematic review has proven that periodontal treatment, such as scaling and root planing, improves the glycemic control in DM [135]. In contrast, some reports raised the question about the effect periodontal treatment has on the glycemic control of DM patients [136,137], and showed there is no difference in oral microbiota between those with and those without DM [138,139]. These contrasting results might be due to the different types of detection methods, sampling conditions, and analysis techniques used; therefore, a comprehensive understanding with more controlled procedures and analyses would be necessary to explain the link between oral microbiome and DM.

### Table 3. Oral Microbiome’s Relationship with DM.

| Organism | Result | Reference |
|----------|--------|-----------|
| P.g. | P.g. triggers periodontal tissue destruction and increases insulin resistance | [140,141] |
| P.g. | P.g. with type II fimbriae is a critical infectious factor in the deterioration of periodontitis with DM | [142] |
| P.g. | P.g. is involved with insulin resistance in DM | [143–145] |
Table 3. Cont.

| Organism           | Result                                                                 | Reference |
|--------------------|------------------------------------------------------------------------|-----------|
| Actinobacteria     | Higher abundances of taxa in the phylum Actinobacteria were associated with lower diabetes risk | [131]     |
| A.a.               | A.a. was associated with periodontitis in DM patients                   | [146]     |
| P.g., T. forsythia, T. denticola | Poor glycemic control is associated with increased proportions of red-complex microbes | [144]     |

10. Conclusions and Future Perspective

In this review, we explored the close relationship between oral microbiomes and host health. The novel technological advances in sequencing have greatly accelerated our ability to identify the microbiomes present in clinical samples taken from the oral cavity at the species level. The accumulation of knowledge about oral microbiomes is driving this research in new directions, and extended analysis of transcript (transcriptome), protein (proteome), and metabolic products (metabolome) provides insight into host–microbial interaction in oral and systemic diseases. The current state of this oral microbiome research, which has been reported so far, shows that oral diseases are complex diseases reflecting changes in microbial components and host immune responses, and are interrelated with systemic health. The combined study with multi-omics data from a host and their microbiome will facilitate advances in personalized medicine (Figure 4).

![Figure 4](image_url)

**Figure 4.** Personalized medicine with host–microbiome combined analysis.

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References

1. Priya Nimish Deo, R.D. Oral microbiome: Unveiling the fundamentals. J. Oral Maxillofac. Pathol. 2019, 23, 122–128.
2. Dewhurst, F.E.; Chen, T.; Izard, J.; Paster, B.J.; Tanner, A.C.R.; Yu, W.H.; Lakshmanan, A.; Wade, W.G. The Human Oral Microbiome. J. Bacteriol. 2010, 192, 5002–5017. [CrossRef] [PubMed]
3. Lu, M.Y.; Xuan, S.Y.; Wang, Z. Oral microbiota: A new view of body health. Food Sci. Hum. Well. 2019, 8, 8–15. [CrossRef]
4. Aas, J.A.; Paster, B.J.; Stokes, L.N.; Olsen, I.; Dewhirst, F.E. Defining the normal bacterial flora of the oral cavity. J. Clin. Microbiol. 2005, 43, 5721–5732. [CrossRef] [PubMed]
5. Lim, Y.; Totsika, M.; Morrison, M.; Punyadeera, C. Oral Microbiome: A New Biomarker Reservoir for Oral and Oropharyngeal Cancers. THERANOSTICS 2017, 7, 4313–4321. [CrossRef] [PubMed]
6. Zhao, H.S.; Chu, M.; Huang, Z.W.; Yang, X.; Ran, S.J.; Hu, B.; Zhang, C.P.; Liang, J.P. Variations in oral microbiota associated with oral cancer. Sci. Rep. 2017, 7. [CrossRef] [PubMed]
7. Liu, S.L.; Bauer, M.E.; Marsh, P. Advancements toward a systems level understanding of the human oral microbiome. Front. Cell. Infect. Microbiol. 2014, 4. [CrossRef]
8. Ogunrinola, G.A.; Oyewale, J.O.; Oshamika, O.O.; Olasehinde, G.I. The Human Microbiome and Its Impacts on Health. Int. J. Microbiol. 2020, 2020, 8045646. [CrossRef]
9. Kilian, M.; Chapple, I.L.; Hannig, M.; Marsh, P.D.; Meuric, V.; Pedersen, A.M.; Tonetti, M.S.; Wade, W.G.; Zaura, E. The oral microbiome—An update for oral healthcare professionals. Br. Dent. J. 2016, 221, 657–666. [CrossRef] [PubMed]
10. Aguiar-Pulido, V.; Huang, W.; Suarez-Ulloa, V.; Cickovski, T.; Mathee, K.; Narasimhan, G. Metagenomics, Metatranscriptomics, and Metabolomics Approaches for Microbiome Analysis. Evol. Bioinform. Online 2016, 12, 5–16. [CrossRef] [PubMed]
11. Mall, M.A.; Dubey, A.; Kumar, A.; Yadav, S.; Hashem, A.; Abd Allah, E.F. Exploring the Human Microbiome: The Potential Future Role of Next-Generation Sequencing in Disease Diagnosis and Treatment. Front. Immunol. 2018, 9, 2868. [CrossRef] [PubMed]
12. Lane, N. The unseen world: Reflections on Leeuwenhoek (1677) ‘Concerning little animals’. Philos. Trans. R. Soc. Lond. B Biol. Sci. 2015, 370. [CrossRef] [PubMed]
13. Rajfer, J. Antonie van Leeuwenhoek (1632–1723). Invest. Urol. 1976, 14, 83. [PubMed]
14. Hugenholtz, P.; Goebel, B.M.; Pace, N.R. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J. Bacteriol. 1998, 180, 4756–4774. [CrossRef] [PubMed]
15. Hébert, P.D.; Cywinska, A.; Ball, S.L.; deWaard, J.R. Biological identifications through DNA barcodes. Philos. Trans. R. Soc. Lond. B Biol. Sci. 2003, 270, 313–321. [CrossRef] [PubMed]
16. Lander, E.S.; Linton, L.M.; Birren, B.; Nusbaum, C.; Zody, M.C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.; et al. Initial sequencing and analysis of the human genome. Nature 2001, 409, 860–921. [CrossRef] [PubMed]
17. Carrascos-Ramíro, F.; Peiro-Pastor, R.; Aguado, B. Human genomics projects and precision medicine. Gene Ther. 2017, 24, 551–561. [CrossRef] [PubMed]
18. Hebert, P.D.; Cywinska, A.; Ball, S.L.; deWaard, J.R. Biological identifications through DNA barcodes. Philos. Trans. R. Soc. Lond. B Biol. Sci. 2003, 270, 313–321. [CrossRef] [PubMed]
19. Zaura, E. Next-generation sequencing approaches to understanding the oral microbiome. Adv. Dent. Res. 2012, 24, 81–85. [CrossRef] [PubMed]
20. Wepner, J.J.; Zhou, D.; Caporaso, J.G.; Knight, R.; Angenent, L.T. Comparison of Illumina paired-end and single-direction sequencing for microbial 16S rRNA gene amplicon surveys. ISME J. 2012, 6, 1273–1276. [CrossRef] [PubMed]
21. Hugenholtz, P.; Goebel, B.M.; Pace, N.R. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J. Bacteriol. 1998, 180, 4756–4774. [CrossRef] [PubMed]
22. Hebert, P.D.; Cywinska, A.; Ball, S.L.; deWaard, J.R. Biological identifications through DNA barcodes. Proc. Biol. Sci. 2003, 270, 313–321. [CrossRef] [PubMed]
23. Ruppert, K.M.; Kline, R.J.; Rahman, M.S. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Glob. Ecol. Conserv. 2019, 17. [CrossRef]
24. Simon, C.; Daniel, R. Metagenomic analyses: Past and future trends. Appl. Environ. Microbiol. 2011, 77, 1153–1161. [CrossRef]
25. Hettich, R.L.; Fan, C.; Chourey, K.; Giannone, R.J. Metaproteomics: Harnessing the power of high performance mass spectrometry to identify the suite of proteins that control metabolic activities in microbial communities. AnaI. Chem. 2013, 85, 4203–4214. [CrossRef]
26. Bostanci, N.; Bao, K. Contribution of proteomics to our understanding of periodontal inflammation. Proteomics 2017, 17. [CrossRef]
107. Elkaim, R.; Dahan, M.; Kocgozlu, L.; Werner, S.; Kanter, D.; Kretz, J.G.; Tenenbaum, H. Prevalence of periodontal pathogens in subgingival lesions, atherosclerotic plaques and healthy blood vessels: A preliminary study. J. Periodontal Res. 2008, 43, 224–231. [CrossRef]

108. Gaetti-Jardim, E.; Marcelino, S.L.; Feitosa, A.C.R.; Romito, G.A.; Avila-Campos, M.J. Quantitative detection of periodontopathic bacteria in atherosclerotic plaques from coronary arteries. J. Med. Microbiol. 2009, 58, 1568–1575. [CrossRef] [PubMed]

109. Chou, H.H.; Yumoto, H.; Davey, M.; Takahashi, Y.; Miyamoto, T.; Gibson, F.C., 3rd; Genco, C.A. Porphyromonas gingivalis fimbria-dependent activation of inflammatory genes in human aortic endothelial cells. Infect. Immun. 2005, 73, 5367–5378. [CrossRef] [PubMed]

110. Saito, Y.; Fujii, R.; Nakagawa, K.L.; Kuramitsu, H.K.; Okuda, K.; Ishihara, K. Stimulation of Fusobacterium nucleatum biofilm formation by Porphyromonas gingivalis. Oral Microbiol. Immunol. 2008, 23, 1–6. [CrossRef] [PubMed]

111. Zhang, T.; Kurita-Ochiai, T.; Hashizume, T.; Du, Y.; Oguchi, S.; Yamamoto, M. Aggregatibacter actinomycetemcomitans accelerates atherosclerosis with an increase in atherogenic factors in spontaneously hyperlipidemic mice. FEMS Immunol. Med. Microbiol. 2010, 59, 143–151. [CrossRef] [PubMed]

112. Khlgatian, M.; Nassar, H.; Chou, H.H.; Gibson, F.C., 3rd; Genco, C.A. Fimbria-dependent activation of cell adhesion molecule expression in Porphyromonas gingivalis-infected endothelial cells. Infect. Immun. 2002, 70, 257–267. [CrossRef] [PubMed]

113. Takahashi, Y.; Davey, M.; Yamoto, H.; Gibson, F.C., 3rd; Genco, C.A. Fimbria-dependent activation of pro-inflammatory molecules in Porphyromonas gingivalis infected human endothelial cells. Cell. Microbiol. 2006, 8, 738–757. [CrossRef] [PubMed]

114. Bengtsson, T.; Karlsson, H.; Gunnarsson, P.; Skoglund, C.; Elison, C.; Leanderson, P.; Lindahl, M. The periodontal pathogen Porphyromonas gingivalis cleaves apoB-100 and increases the expression of apoM in LDL in whole blood leading to cell proliferation. J. Intern. Med. 2008, 263, 558–571. [CrossRef] [PubMed]

115. Tuomainen, A.M.; Jauhiainen, M.; Kovanen, P.T.; Metso, J.; Paju, S.; Pussinen, P.J. Aggregatibacter actinomycetemcomitans induces MMP-9 expression and proatherogenic lipoprotein profile in apoE-deficient mice. Microb. Pathog. 2008, 44, 111–117. [CrossRef] [PubMed]

116. Yumoto, H.; Chou, H.H.; Takahashi, Y.; Davey, M.; Gibson, F.C., 3rd; Genco, C.A. Sensitization of human aortic endothelial cells to lipopolysaccharide via regulation of Toll-like receptor 4 by bacterial fimbria-dependent invasion. Infect. Immun. 2005, 73, 8050–8059. [CrossRef]

117. Nakamura, N.; Yoshida, M.; Umeda, M.; Huang, Y.; Kitajima, S.; Inoue, Y.; Ishikawa, I.; Iwai, T. Extended exposure of lipopolysaccharide fraction from Porphyromonas gingivalis facilitates mononuclear cell adhesion to vascular endothelium via Toll-like receptor-2 dependent mechanism. Atherosclerosis 2008, 196, 59–67. [CrossRef] [PubMed]

118. Gibson, F.C., 3rd; Hong, C.; Chou, H.H.; Yamoto, H.; Chen, J.; Lien, E.; Wong, J.; Genco, C.A. Innate immune recognition of invasive bacteria accelerates atherosclerosis in apolipoprotein E-deficient mice. Circulation 2004, 109, 2801–2806. [CrossRef] [PubMed]

119. Brodala, N.; Merricks, E.P.; Bellinger, D.A.; Damrongsi, D.; Offenbacher, S.; Beck, J.; Madianos, P.; Sotres, D.; Chang, Y.L.; Koch, G.; et al. Porphyromonas gingivalis bacteria induces coronary and aortic atherosclerosis in normocholesterolemic and hypercholesterolemic pigs. Arterioscler. Thromb. Vasc. Biol. 2005, 25, 1446–1451. [CrossRef]

120. Lalla, E.; Lamster, I.B.; Hofmann, M.A.; Bucciarelli, L.; Jerud, A.P.; Tucker, S.; Lu, Y.; Papapanou, P.N.; Schmidt, A.M. Oral infection with a periodontal pathogen accelerates early atherosclerosis in apolipoprotein E-null mice. Arterioscler. Thromb. Vasc. Biol. 2003, 23, 1405–1411. [CrossRef]

121. Pizzo, G.; Guigla, R.; Lo Russo, L.; Campisi, G. Dentistry and internal medicine: From the focal infection theory to the periodontal medicine concept. Eur. J. Intern. Med. 2008, 19, 496–502. [CrossRef] [PubMed]

122. Sanz, M.; Ceriello, A.; Buysschaert, M.; Chapple, I.; Demmer, R.T.; Graziani, F.; Herrera, D.; Jepsen, S.; Lione, L.; Madianos, P.; et al. Scientific evidence on the links between periodontal diseases and diabetes: Consensus report and guidelines of the joint workshop on periodontal diseases and diabetes by the International Diabetes Federation and the European Federation of Periodontology. J. Clin. Periodontol. 2018, 45, 138–149. [CrossRef] [PubMed]

123. Padhi, S.; Nayak, A.K.; Behera, A. Type II diabetes mellitus: A review on recent drug based therapeutics. Biomed. Pharmacother. 2020, 131, 110708. [CrossRef]

124. Chatterjee, S.; Khunti, K.; Davies, M.J. Type 2 diabetes. Lancet 2017, 389, 2239–2251. [CrossRef]

125. Lalla, E.; Cheng, B.; Lar, S.; Kaplan, S.; Softness, B.; Greenberg, E.; Goland, R.S.; Lamster, I.B. Diabetes mellitus promotes periodontal destruction in children. J. Clin. Periodontol. 2007, 34, 294–298. [CrossRef] [PubMed]

126. Taylor, G.W. Bidirectional interrelationships between diabetes and periodontal diseases: An epidemiologic perspective. Ann. Periodontol. 2001, 6, 99–112. [CrossRef] [PubMed]

127. Negrato, C.A.; Tarzia, O.; Chelli, L.M. Periodontal disease and diabetes mellitus. J. Oral Microbiol. 2013, 21, 1–12. [CrossRef] [PubMed]

128. Taylor, J.I.; F reshaw, P.M.; Lalla, E. A review of the evidence for pathogenic mechanisms that may link periodontitis and diabetes. J. Clin. Periodontol. 2013, 40 (Suppl. S14), S113–S134. [CrossRef]

129. Mealey, B.L.; Oates, T.W.; American Academy of. P. Diabetes mellitus and periodontal diseases. J. Periodontol. 2006, 77, 1289–1303. [CrossRef] [PubMed]

130. Signorini, L.; Inchingolo, A.D.; Santacroce, L.; Xhajanka, E.; Altini, V.; Bordea, I.R.; Dipalma, G.; Cantore, S.; Inchingolo, F. Efficacy of combined sea salt based oral rinse with xylitol in improving healing process and oral hygiene among diabetic population after oral surgery. J. Biol. Regul. Homeost. Agents 2020, 34, 1617–1622. [CrossRef] [PubMed]
131. Long, J.; Cai, Q.; Steinwandel, M.; Hargreaves, M.K.; Bordenstein, S.R.; Blot, W.J.; Zheng, W.; Shu, X.O. Association of oral microbiome with type 2 diabetes risk. *J. Periodontal Res.* 2017, 52, 636–643. [CrossRef] [PubMed]

132. Ogawa, T.; Honda-Ogawa, M.; Ikebe, K.; Notomi, Y.; Iwamoto, Y.; Shiroyabashi, I.; Hata, S.; Kibi, M.; Masayasu, S.; Sasaki, S.; et al. Characterizations of oral microbiota in elderly nursing home residents with diabetes. *J. Oral Sci.* 2017, 59, 549–555. [CrossRef] [PubMed]

133. Saeb, A.T.M.; Al-Rubeaan, K.A.; Aldosary, K.; Raja, G.K.U.; Mani, B.; Abouelhoda, M.; Tayeb, H.T. Relative reduction of biological and phylogenetic diversity of the oral microbiota of diabetes and pre-diabetes patients. *Microb. Pathog.* 2019, 128, 215–229. [CrossRef] [PubMed]

134. Preshaw, P.M.; Taylor, J.J.; Jaedicke, K.M.; De Jager, M.; Bikker, J.W.; Selten, W.; Bissett, S.M.; Whall, K.M.; van de Merwe, R.; Areibi, A.; et al. Treatment of periodontitis reduces systemic inflammation in type 2 diabetes. *J. Clin. Periodontol.* 2020. [CrossRef] [PubMed]

135. Baeza, M.; Morales, A.; Cisterna, C.; Cavalla, F.; Jara, G.; Isamitt, Y.; Pino, P.; Gamonal, J. Effect of periodontal treatment in patients with periodontitis and diabetes: Systematic review and meta-analysis. *J. Appl. Oral Sci. Revista FOB* 2020, 28, e20190248. [CrossRef] [PubMed]

136. Phillips, N.M. Does Treatment of Periodontal Disease Improve Glycemic Control in Diabetes? *Am. J. Nurs.* 2012, 112, 22. [CrossRef]

137. Janket, S.J.; Wightman, A.; Baird, A.E.; Van Dyke, T.E.; Jones, J.A. Does periodontal treatment improve glycemic control in diabetic patients? A meta-analysis of intervention studies. *J. Dent. Res.* 2005, 84, 1154–1159. [CrossRef]

138. Sastrowijoto, S.H.; Hillemans, P.; van Steenbergen, T.J.; Abraham-Inpijn, L.; de Graaff, J. Periodontal condition and microbiology of healthy and diseased periodontal pockets in type 1 diabetes mellitus patients. *J. Clin. Periodontol.* 1989, 16, 316–322. [CrossRef] [PubMed]

139. Tervonen, T.; Oliver, R.C.; Wolff, L.F.; Bereuter, J.; Anderson, L.; Aeppli, D.M. Prevalence of periodontal pathogens with varying metabolic control of diabetes mellitus. *J. Clin. Periodontol.* 1994, 21, 375–379. [CrossRef] [PubMed]

140. Kuo, L.C.; Polson, A.M.; Kang, T. Associations between periodontal diseases and systemic diseases: A review of the interrelationships and interactions with diabetes, respiratory diseases, cardiovascular diseases and osteoporosis. *Public Health* 2008, 122, 417–433. [CrossRef]

141. Makuri, N.; Ojima, M.; Kou, Y.; Furuta, N.; Okahashi, N.; Shizukuishi, S.; Amano, A. Relationship of Porphyromonas gingivalis with glycemic level in patients with type 2 diabetes following periodontal treatment. *Oral Microbiol. Immunol.* 2008, 23, 348–351. [CrossRef] [PubMed]

142. Ojima, M.; Takeda, M.; Yoshioka, H.; Nomura, M.; Tanaka, N.; Kato, T.; Shizukuishi, S.; Amano, A. Relationship of periodontal bacterium genotypic variations with periodontitis in type 2 diabetic patients. *Diabetes Care* 2005, 28, 433–434. [CrossRef] [PubMed]

143. Tian, J.; Liu, C.; Zheng, X.; Jia, X.; Peng, X.; Yang, R.; Zhou, X.; Xu, X. Porphyromonas gingivalis Induces Insulin Resistance by Increasing BCAA Levels in Mice. *J. Dent. Res.* 2020, 99, 839–846. [CrossRef] [PubMed]

144. Aemaimanan, P.; Amimanpan, P.; Taweechaisupapong, S. Quantification of key periodontal pathogens in insulin-dependent type 2 diabetic and non-diabetic patients with generalized chronic periodontitis. *Anaerobe* 2013, 22, 64–68. [CrossRef] [PubMed]

145. Arimatsu, K.; Yamada, H.; Miyazawa, H.; Minagawa, T.; Nakajima, M.; Ryder, M.I.; Gotoh, K.; Motooka, D.; Nakamura, S.; Iida, T.; et al. Oral pathobiont induces systemic inflammation and metabolic changes associated with alteration of gut microbiota. *Sci. Rep.* 2014, 4, 4828. [CrossRef]

146. Castrillon, C.A.; Hincapie, J.P.; Yepes, F.L.; Roldan, N.; Moreno, S.M.; Contreras, A.; Botero, J.E. Occurrence of red complex microorganisms and Aggregatibacter actinomycetemcomitans in patients with diabetes. *J. Investig. Clin. Dent.* 2015, 6, 25–31. [CrossRef] [PubMed]