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The oral microbiome in health and disease and the potential impact on personalized dental medicine

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Every human body contains a personalized microbiome that is essential to maintaining health but capable of eliciting disease. The oral microbiome is particularly imperative to health because it can cause both oral and systemic disease. The oral microbiome rests within biofilms throughout the oral cavity, forming an ecosystem that maintains health when in equilibrium. However, certain ecological shifts in the microbiome allow pathogens to manifest and cause disease. Severe forms of oral disease may result in systemic disease at different body sites. Microbiomics and metagenomics are two fields of research that have emerged to identify the presence of specific microbes in the body and understand the nature of the microbiome activity during both health and disease. The analysis of the microbiome and its genomes will pave the way for more effective therapeutic and diagnostic techniques and, ultimately, contribute to the development of personalized medicine and personalized dental medicine.


Keywords: microbiome; genomics; personalized dental medicine; diagnostics; metagenomics; medicine

Introduction

Every human body contains a personalized set of foreign inhabitants essential to maintaining health, yet also capable of eliciting disease. The totality of these microorganisms, their genomes and ecosystems encompasses the microbiome1 (Parahitiyawa et al, 2010). The human and its microbiome together make up a ‘supraorganism’ (Ling et al, 2010; Rajendhran and Gunasekaran, 2010). The number of microbial cells within a human body exceeds the total number of human cells in the body by nearly 10 times (Turnbaugh et al, 2007; Ling et al, 2010). These microorganisms contribute their genome, known as the metagenome, to the human body, multiplying human genes by approximately 100 times (Turnbaugh et al, 2007; Ling et al, 2010; Rajendhran and Gunasekaran, 2010). The activity of the microbiome and, specifically, the expression of its metagenome provide the human with resources and traits that did not originally evolve with the body (Rajendhran and Gunasekaran, 2010). For example, the microbiome contains genes that allow humans to digest certain plant polysaccharides (Rajendhran and Gunasekaran, 2010).

There are various microhabitats throughout the body that contribute to the overall microbiome. The mouth, skin, gut, etc. each contains its exclusive microbiome and metagenome (Badger et al, 2011; Sonnenburg and Fischbach, 2011). Each microhabitat maintains a unique ecosystem with distinct atmospheric and nutritional compositions that provide a setting for symbiotic interactions among the various microbes within that ecosystem and the host. Of note, microbiomes from the same location on the body are more similar among different individuals than microbiomes from different locations on the same individual (Sonnenburg and Fischbach, 2011).

The human microbiome can be classified into a ‘core’ microbiome and a ‘variable’ microbiome (Turnbaugh et al, 2007). The core microbiome is shared among all individuals and is comprised of the predominant species that exist under healthy conditions at different sites of the body (Turnbaugh et al, 2007; Zaura et al, 2009; Sonnenburg and Fischbach, 2011). The variable microbiome is exclusive to the individual and has evolved in response to unique lifestyle, and phenotypic and genotypic determinants (Figure 1). Although individuals share microbiota at similar sites of the body, there are varying differences at the species and strain level of the microbiome that can be as inimitable to the individual as is the fingerprint (Dethlefsen et al, 2007).

As the correlation between the human microbiome and health becomes more evident, microbiome research is becoming central to the advancement of disease diagnos-
tics and therapeutics as well as the development of personalized medicine (Sonnenburg and Fischbach, 2011). Because each individual harbors a unique microbiome that plays a key role in the etiology of disease within the body, disease may manifest and progress differently among different individuals, making personalized medicine imperative for optimal health care.

However, microbiome research must develop a deeper understanding of the fundamentals and specifics of microbial activity within the body during health and disease before it can contribute to personalized care. First, there should be a clear picture of which microorganisms exist in the body and how they affect the host’s physiology and health condition. Then, microbial characteristics of specific diseases should be studied to recognize microbiome patterns that distinguish one disease from another. Finally, proper diagnostic methods and technologies should be developed to enable professionals to identify individual microbial profiles and treat specific microbes responsible for disease.

The emerging field of research that targets the microbiome for therapeutic purposes is known as microbiomics. Microbiomics aims to understand how microorganisms interplay with its host’s physiology and health by analyzing their distinct functions and interrelationships (Rajendhran and Gunasekaran, 2010). Bacteria as a determining factor of disease was a concept that emerged in 1882 when physicist Robert Koch wrote an article discussing bacteria as the etiological agents of tuberculosis (Socransky and Haffajee, 1992). Koch’s article triggered years of microbiology and disease etiology research that eventually led to microbiomics. Figure 2 summarizes several groundbreaking discoveries that were steps to the study of the microbiome today (Socransky and Haffajee, 1992). Today, the Human Microbiome Project (HMP) takes a leading role in human microbiomics. It explores the role of the human microbiome in physiology, health, and disease through metagenomic research, which analyzes the genomes of specific microorganisms (Rajendhran and Gunasekaran, 2009).

Specifically, studies have shown the oral cavity’s microbiome to be a key source in the etiology of many oral and systemic diseases (Scannapieco, 1998; Garcia et al., 2001). Because the oral microbiome is vital to a body’s overall health, it has become an essential focus of microbiomics. It is crucial to unravel the complexities of the oral microbiome to learn the mechanisms by which it maintains health or causes disease.

**Microbiomics and metagenomics**

To truly understand the activity of the microbiome for therapeutic purposes, microbiomics must use metagenomics to sequence and analyze bacterial genomes. The HMP aims to use metagenomic techniques to sequence 3000 microbial reference genomes isolated from various sites around the human body. In doing so, it hopes to contribute to our understanding of how the microbiome correlates with human health by identifying the differences between bacterial genomes that encode for metabolic functions vs disease processes (Rajendhran and Gunasekaran, 2009).

Genome sequencing has greatly enhanced the analysis of pathogenic microorganisms, most of which are uncultivable (Horz and Conrads, 2007). Most metagenomic methods are feasible because they are able to analyze extremely small sample sizes of bacterial genomes. The technique has led to the discovery of new
genes, enzymes, and natural products, which may also be used for the development of novel chemicals and pharmaceuticals in the future (Wong, 2010).

There are several ways to sequence a metagenome or segments of a metagenome, each technique serving a unique purpose. The most common techniques include 16S ribosomal RNA sequencing, pyrosequencing, and shotgun sequencing. Table 1 summarizes some methods used to identify microbes, how they are performed, their purpose, and their positive and negative aspects. As certain metagenomes have already been synthesized and the species identified, researchers can also use more conventional techniques to determine the presence of a microorganism. These techniques include culture-based identification, microscopy, enzyme analysis, and immunological assays. Although less advanced, these procedures complement the sequencing processes by providing information on the physical characteristics and additional behavioral and metabolic properties of microbes (Horz and Conrads, 2007; Filoche et al., 2010). It may be beneficial or even necessary for the future dentist to be familiar with sequencing and the other identification techniques in case these laboratory procedures arrive in the clinical setting for an on-the-spot microbial identification and personalized care.

Microbiomics requires collaboration of metagenomics and clinical research to gather accurate and in depth information about the correlation between the microbiome and human health. Unfortunately, there are many existing factors that inhibit rapid progress of microbiomics, beginning with complications in experimental design. First of all, it is challenging to gather population sizes that are large enough for unbiased test results (Badger et al., 2011). Second, there are factors in sequencing that can contribute to bias, like defining a operational taxonomic unit that may over or underestimate biodiversity in a sample. Researchers must decide whether to sequence full-length genomes or genome segments to determine diversity, or to undergo more expensive procedures such as pyrosequencing. Also, to fully measure the microbial diversity in the oral cavity, there must be a more concrete definition of 'species', which includes not only genotypic characteristics, but phenotypic ones as well (Avila et al., 2009). In

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terms of the oral microbiome, time and composition face significant challenges attributable to the highly dynamic characteristic of the oral cavity and its anaerobic inhabitants (Badger et al., 2011).

Furthermore, Socransky and Haffajee, (1992) presents the complications researchers face when forming conclusions about oral pathogens. It is tricky to define the predominant etiological agent of a polymicrobial disease caused by the opportunistic behavior of pathogenic bacteria. Even in the case of a monomicrobial disease, there is a potential to define the wrong predominant species because a disease caused by a pathogen within a niche may change the environment within that niche, leading other microorganisms to proliferate in numbers that can skew test results. In addition, there is a constantly growing number of newly identified periodontal species, most probably because of the unique composition of every individual oral microbiome (Horz and Conrads, 2007). Advanced technologies and scientific methods are needed to accurately identify specific bacterial compositions characteristic to disease (Horz and Conrads, 2007).

However, the most complicated issue that arises in microbiomics and metagenomics is the significance of studying microbes in isolation and outside the context of their natural habitat within the human body (Keller and Ramos, 2008). To safely mimic and manipulate the microbiome for therapeutic purposes, tests must ensure that microbes operate similarly in vitro and in silico as they do in vivo.

The oral cavity and its microbiome
To understand the role of the oral microbiome within the oral cavity, it is important to analyze its fundamental characteristics and dynamics. The oral cavity harbors over 700 species of bacteria that contribute to the health and physiological status of the oral cavity. Within the oral cavity, there are two types of surfaces for which bacteria can colonize: the hard surfaces of teeth and the soft tissue of the oral mucosa (Zaura et al., 2009). The teeth, gingival sulcus, tongue, cheeks, hard and soft palates, and tonsils (Dewhirst et al., 2010) each provide enriching environments in which microbial communities can flourish. Different types of microorganisms prefer distinct niches according to varying surface structures and functions (Aas et al., 2005). Each niche provides the optimal conditions and nutrients for its populating microbes (Avila et al., 2009). In fact, research has shown the maxilla, hard palate, soft palate, and even the tongue’s lateral sides and dorsal side each to have a different bacterial profile (Aas et al., 2005).

Furthermore, the oral microbiome is extremely dynamic because of the oral cavity’s ‘continuum with the external environment’ (Parahitiyawa et al., 2010). Thus, the oral microbiota has evolved skills to face challenges that are not experienced by other microbiotas (Avila et al., 2009). The oral cavity has multiple essential functions that affect bacterial growth and activity: eating, communicating, and defending against infection. Also, the microbial ecosystem is disturbed by oral hygiene practices. Even oral microbial colonies that are less susceptible to agitation experience changes attributed to diet, age, and health (Parahitiyawa et al., 2010), as well as constant changes in pH, redox potential, atmospheric conditions, salinity, and water activity from saliva (Badger et al., 2011).

Biofilms and saliva
Oral microorganisms adapt to changing environments within protective biofilms (Avila et al., 2009). Biofilms are the complex colonies of microorganisms that predominate both hard and mucosal surfaces in the oral cavity (Flemmig and Beikler, 2011). While the microbial colonies play vital roles in maintaining oral homeostasis, they are also significant players in oral diseases (Flemmig and Beikler, 2011).

Dental plaque is a commonly known multispecies biofilm that packs as layers onto tooth surfaces (Listgarten, 1976; Flemmig and Beikler, 2011). Dental plaque can either entrap and prevent an existing oral pathogen from flourishing, or provide a refuge for a pathogen to hide from salivary flow and the host’s immune system (Avila et al., 2009). Under healthy conditions, an ecological balance between microbe composition and activity keeps biofilms healthy and stable (Flemmig and Beikler, 2011). Yet, detachment of biofilms is necessary because pathogens can manifest within and cause disease (Filoche et al., 2010). Both oral hygiene practices and salivary flow are responsible for this required detachment.

Saliva is crucial to the oral cavity because it plays a key role in maintaining homeostasis and defending from disease (Nieuw Amerongen and Veeman, 2002). While it helps maintain a climate that allows biofilms to flourish, saliva also detaches layers of plaque and contains numerous proteins, minerals, and antimicrobial enzymes that control biofilm build up and activity (Nieuw Amerongen and Veeman, 2002). Saliva also provides nutrients that protect tooth enamel and antibodies that defend the oral cavity and the rest of the body from infection (Nieuw Amerongen and Veeman, 2002; Filoche et al., 2010).

Studies have shown both salivary flow and microbiome composition to be unique to an individual’s oral cavity, also suggesting that dental plaque composition and arrangement are specific to the individual (Filoche et al., 2010). Varying plaque biomass, pH, and microbial response may result from or explain current health and disease conditions, and also may explain why some individuals are more prone to oral diseases than others, despite oral hygiene habits (Filoche et al., 2010).

Oral microbiome of health
The bacterial flora in a healthy oral cavity vs a diseased one is distinctly different, suggesting there may be a profile for a core oral microbiome of health (Aas et al., 2005). According to various studies, identical bacterial sequences have been discovered in the oral cavities of unrelated healthy individuals (Zaura et al., 2009; Bik et al., 2010). Bik et al. (2010) performed a study based on the largest set of near full-length sequences per
healthy individual to date. The analysis identified 10 variables shared between 11 bacterial species. However, the same study also showed that significant interindividual differences exist, supporting the concept of both a core and variable microbiome within the oral cavity. Figure 3 shows the overlapping of specific bacterial genera that were found among the 10 samples. This study and others offer a glimpse into the possible microorganisms that predominate healthy oral cavities. The major genera with the largest representation in healthy oral cavities include the following: Streptococcus, Veillonella, Granulicatella, Gamella, Actinomyces, Corynebacterium, Rothia, Fusobacterium, Porphyromonas, Prevotella, Capnocytophaga, Nisseria, Haemophilis, Treponema, Lactobacterium, Eikenella, Lepotrichia, Peptostreptococcus, Staphylococcus, Eubacteria, and Propionibacterium (Aas et al., 2005; Jenkinson and Lamont, 2005; Zaura et al., 2009; Bik et al., 2010). Developing an in depth definition of health, and understanding molecular differences between health and disease, may give clinicians the ability to recognize and diagnose diseases at an earlier and reversible stage (Zaura et al., 2009).

Oral symbiosis as the determining factor of health and disease

The key to oral health is an ecologically balanced and diverse microbiome that practices commensalism within itself and mutualism with its host (Ruby and Goldner, 2007; Zaura et al., 2009; Filoche et al., 2010). Commensal relationships among microbes allow them to flourish at no expense to their co-habitants and, in turn, maintain biodiversity within the oral cavity. Research has demonstrated such biodiversity to be crucial to health. Analysis of plaque and saliva in healthy adults demonstrated much more diversity than originally hypothesized (Filoche et al., 2010). Oral microbiomes of children suffering from severe dental caries are much less diverse than those of children with oral health (Kanasi et al., 2010). Asymptomatic lesions of infected root canals displayed a higher level of biodiversity than did the symptomatic ones. The need for biodiversity in health may suggest that every species carries out a specific function that is required to maintain equilibrium and homeostasis within the oral cavity.

Furthermore, the relationship between the microbiome and its host during health is mutually beneficial because the host is providing its microbial communities with an environment in which they can flourish and, in turn, keep their host healthy. In health, microorganisms prevent disease progression in several ways: they can prevent the adherence of pathogens onto specific surfaces by occupying the niche preferred by a pathogen, they can actively prevent a pathogen from occupying a site, they can hinder a pathogen’s abilities to multiply, and they can degrade a pathogen’s virulence factors (Socransky and Haffajee, 1992).

However, certain pathological changes within the microbial ecosystem may occur and cause a once-beneficial microorganism to initiate disease within the oral cavity. Ecological shifts that cause pathological changes are: (1) a change in the relationships between the microbes and with the host; (2) an increase in relative abundance; and (3) acquisition of virulence factors (Parahitiyawa et al., 2010). In disease, microbes alter their relationship with their host from mutualistic to parasitic and with other microbes from commensal to opportunistic (Avila et al., 2009; Parahitiyawa et al., 2010). As the pathogenic bacteria flourish, the host becomes infected or prone to infection (Ruby and Goldner, 2007). Pathogens will grow with disregard of its co-habiting bacteria, and any beneficial bacteria will not be able to inhibit the diseases manifestation (Nieuw Amerongen and Veeman, 2002).

Because shifts in relationships, proportion, and virulence properties of microbes seem to affect one another, it is not always certain which ecological shift occurred first. It is also unclear what exactly triggers the initial ecological shift and, in turn, catalyzes the entire cycle (Avila et al., 2009). The major factors that may be responsible for initiating an ecological shift are poor oral hygiene, compromised immune system, and genetics. Figure 4 illustrates the cycle of the ecological shifts the oral microbiome may experience and the contributing factors to these shifts, which eventually cause disease.

Poor oral hygiene is greatly responsible for the accumulation of bacteria within biofilms. Failure to detach accumulating plaque will lead to overgrowth of bacteria that may become pathogenic, reduce biodiversity of the oral cavity, and ultimately cause

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**Figure 3** The inner circle presents the bacterial genera found in all 10 individuals; the second circle, bacteria present in 6–9 individuals; the third circle, bacteria present in 3–5 individuals; and the outer circle, bacteria present in 1–2 individuals. Adapted by permission from Macmillan Publishers Ltd: The ISME Journal (Bik et al., 2010)
diseases such as dental caries or periodontal disease (Zaura et al., 2009). Anaerobic microflora in the crypts of the tongue can also grow out of proportion and develop halitosis, or consistent bad breath (Zaura et al., 2009). Proper oral hygiene practice is crucial because it is the only voluntary way to prevent oral disease.

The presence of an immune system disorder can also cause an ecological shift in the microbiome. As the immune system regulates interactions between the microbiome and the host, a compromised immune system usually disrupts mutual or commensal relationships (Badger et al., 2011). Although microbial relationships during diseased states are parasitic, some pathogens can also facilitate the growth of other pathogen species. In dental caries, Streptococcus mutans is responsible, in part, for creating the lactic acid rich environment in which Veillonella species thrive (Kanasi et al., 2010). In biofilm research, Veillonella species have also been found to enhance the growth of S. mutans (Klutymans et al., 1997). Moreover, compromised immune systems may inhibit the flow of saliva or decrease the amount of nutrients present in saliva, allowing a buildup of dental plaque. For instance, Sjögren’s syndrome is an autoimmune deficiency that attacks the exocrine glands and inhibits the flow of any saliva through the oral cavity, leading to dry mouth and further dental complications (Taubert et al., 2007).

Although usually not obvious, genetic factors can be responsible for ecological shifts that lead to disease. First, genetic factors could contribute to oral disease in an indirect manner. An individual may have a specific genetic makeup that encodes for a permanent immune system disorder, which may then affect the microbiome. For example, a person with Crohn’s disease, an autoimmune disease of the gastrointestinal tract, has a decreased abundance of Bacteroides in the intestines (Badger et al., 2011). A similar situation in the oral cavity could result in a reduction of biodiversity and potentially lead to disease.

Also, because an individual’s genotype contributes to the makeup of its unique microbiome (Turnbaugh et al., 2007), one’s genetic makeup could directly either prevent the existence of certain beneficial bacteria in the body or produce a bodily environment in which certain pathogenic species can reside. For example, 20% of people are long-term carriers of Staphylococcus aureus. These people are more prone to staph infections, especially if the bacteria are not controlled. In addition, certain individuals may lack genes that encode for specific protective proteins and antibodies in saliva and, thus, be more prone to plaque accumulation or cavities.

Once a pathogen possesses virulence factors, exists in abnormal proportions, and demonstrates parasitism, all of the following conditions which are required for disease have been satisfied: (1) The local environment is one in which the species can express its virulence properties; (2) the pathogen is in numbers that exceed the threshold for that host; (3) other bacterial species can foster, or at least not inhibit, the diseases manifestation; and (4) the host is susceptible to this pathogen, i.e., currently compromised immune system or specific genetic composition (Socransky and Haffajee, 1992). Overall, it is crucial that there be an ecological balance among microorganisms to prevent pathological changes and disease from occurring. A healthy microbiome can only be maintained with good oral hygiene and a well-functioning immune system (Nieuw Amerongen and Veeman, 2002).

The oral microbiome and the etiology of major oral diseases

Oral diseases such as dental caries and periodontal disease are among the most prevalent diseases worldwide (Horz and Conrads, 2007; Selwitz et al., 2007), affecting nearly all ages and geographic populations. Hence, discovering the etiological factors responsible for disease activation and progression will make way for advanced methods of treatment and prevention.

Dental caries

Dental caries, also recognized as tooth decay and the primary cause of oral pain and tooth loss, is a disease that can begin as minor surface changes and persist until there are lesions in the dentin (Selwitz et al., 2007). As supragingival biofilm matures on teeth, acid-producing microbial colonies accumulate in dental plaque and lower the pH of the oral cavity, creating an environment in which they can thrive and produce more plaque (Selwitz et al., 2007; Ling et al., 2010). These opportunistic pathogens cause dietary carbohydrates to ferment, producing acidic byproducts that destroy either the enamel of the crown or the root of the tooth. The low-pH environment facilitates the diffusion of calcium,
phosphate, and carbonate out of teeth, which usually protect the enamel from these pathogens. Although a specific microbiome that signals dental caries is yet to be found (Ling et al., 2010), the most common bacteria responsible for dental caries are S. mutans, Streptococcus sobrinus, and Lactobacillus acidophilus (Streckfus and Bigler, 2002; Selwitz et al., 2007). Dental caries is the most preventable and reversible childhood disease, which can be avoided with proper oral hygiene, diet, and fluoride exposure, which enables mineral resorption back into the teeth (Streckfus and Bigler, 2002; Selwitz et al., 2007).

**Periodontal disease**

Periodontal disease also results from subgingival plaque accumulation that causes shifts in the microflora from a healthy state to a diseased state (Horz and Conrads, 2007; Filoche et al., 2010). Periodontal disease is a polymicrobial inflammatory disorder of the periodontium (Pihlstrom et al., 2005). Gingivitis is the mildest form of periodontal disease (Horz and Conrads, 2007). Microorganisms within biofilms begin to form pathogenic characteristics that aggravate and inflame the gingiva when disturbed by actions such as flossing (Pihlstrom et al., 2005). Fortunately, gingivitis is easily reversible with good oral hygiene (Horz and Conrads, 2007).

Periodontitis, on the other hand, is a severe, irreversible infection that attacks all soft tissue and bone that support the periodontium and teeth structures (Horz and Conrads, 2007). Like dental caries, multiple opportunistic pathogens overgrow in dental plaque and these abnormal proportions become pathogenic (Horz and Conrads, 2007; Van Essche et al., 2011). Microbes release proteolytic enzymes that break down host tissue and may result in gingival inflammation, loss of gingival attachment, periodontal pocket formation, and alveolar bone and teeth destruction. The predominant pathogens involved in periodontitis are Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Tannerella forsythia, and Eikenella corrodens, and Treponema denticola (Filoche et al., 2010; Dashiff and Kadouri, 2011).

Periodontitis is extremely difficult to treat because of the nature of the disease, the complications of antimicrobial therapy, and the lack of information on the microbial interactions occurring during the disease. Once pockets form in the periodontium, periodontitis officially becomes irreversible (Pihlstrom et al., 2005). For one, the periodontium is unable to reattach to bone once separated. Also, the causative pathogens deep within the pockets become nearly impossible to target with antimicrobial solutions (Horz and Conrads, 2007). Furthermore, periodontal pathogens develop virulent factors, like encapsulation, that make them resistant to antibiotics (Horz and Conrads, 2007; Van Essche et al., 2011). Pathogens hiding within plaque are one thousand times more resistant to antimicrobials than those which are more exposed (Van Essche et al., 2011). Even if pathogens are successfully targeted, there are high chances of recolonization at treated sites because of bacterial reserves in the mucous membranes that line the oral cavity (Horz and Conrads, 2007). Today, the most adequate treatment for periodontitis is simply reducing the number of pathogens present with antibiotics to maintain control of the disease. As antibiotics destroy an array of communities, they cannot be distributed loosely. The oral cavity should maintain certain Gram-positive bacteria that shield pathogens from damaging hard and soft tissues (Van Essche et al., 2011).

**Oral cancer**

A third oral disease that deserves substantial attention is oral cancer. Oral cancer is the sixth most prevalent cancer, affecting over 300 000 people each year around the world (Gill, 2011). There may be a correlation between the structure and function of the oral microbiome and oral cancer (Meurman, 2010; Gill, 2011). The Gill Lab at the University of Rochester Medical Center has shown that bacterial cells of oral tumors can impact signal pathways that initiate and advance oral cancer (Gill, 2011); however, the sources of activation of cancerous and precancerous oral lesions have yet to be identified (Mehrotra and Yadav, 2006). Researchers should take full advantage of the ability to easily access the oral cavity and examine its microbiome and other precancerous characteristics for the purpose of early diagnosis, effective treatments and better chances of survival (Mehrotra and Yadav, 2006).

Most patients infected with oral cancer practice poor oral hygiene (Meurman, 2010). In general, numerous studies conducted around the world have shown poor oral health and tooth loss to increase the risk of gastric, pancreatic, and other cancers. Inflammation is usually the first symptom of compromised oral health and it gets worse as health regresses. Approximately 15–20% of human tumors contain pathogenic agents derived from inflammatory infections. Proper oral hygiene will maintain control of such inflammatory agents that may contribute to oral cancer (Meurman, 2010).

Conversely, cancer can lead to poor oral health. Carcinogens can introduce toxic agents into salivary fluid that damage DNA, cause mutations, and damage the integrity of oral cavity (Meurman, 2010). The oral cavity reacts to the toxins with an inflammatory response, which then produces the pathogenic agents that contribute to tumor development, thus maintaining a vicious carcinogenic cycle.

Despite the limited research available about the relationship between oral cancer and the oral microbiome, it is well known that the two most important risk factors for oral cancer are tobacco and alcohol (Johnson, 2001). Certain pathogenic strains of oral microorganisms tend to increase carcinogenic acetaldehyde concentrations in saliva when metabolizing ethanol and tobacco smoke (Meurman, 2010; Yang et al., 2011). However, not all who drink alcohol or smoke are subject to oral cancer. Those individuals are at higher risk. Also, each microbiome differs in the rate at which it metabolizes the ethanol and tobacco compounds (Meurman, 2010).
Relationships among oral disease, the oral microbiome, and systemic diseases

The oral cavity is the primary gateway to the human body; therefore, microorganisms that inhabit that area are very capable of spreading to different body sites (Dewhirst et al, 2010). Pathogens that originate in the oral cavity can be frequently detected in blood cultures as they destroy and pass through oral mucous membranes and periodontal pockets (Horz and Conrads, 2007). This suggests a mechanism by which pathogens derived from a periodontal inflammatory response make their way to tumors in the gut or pancreas. Pathogens may enter the blood stream, alter proper immune responses, or produce excessive and deregulated amounts of inflammatory mediators, and in turn, cause disease at different body sites (Williams et al, 2008). Figure 5 illustrates a summary of the pathways that periodontal pathogens may take to a systemic target organ. Persistent inflammation and frequent bacterial attacks not only lead to bacteremia, but also organ abscesses and severe systemic diseases, such as diabetes and cardiovascular disease (Horz and Conrads, 2007; Williams et al, 2008; Meurman, 2010). This correlation further supports the importance of the oral microbiome to overall health.

Diabetes

Diabetes and periodontal disease hold a strong bidirectional relationship (Pihlstrom et al, 2005; Kuo et al, 2008; Williams et al, 2008). In one direction, the bacteria involved in periodontal disease jeopardize the body’s control of glycemic levels (Kuo et al, 2008). Porphyromonas gingivalis, a chief agent in periodontal disease, produces a lipopolysaccharide (LPS) that is toxic to certain cytokine proteins that regulate insulin activity under normal conditions. Other bacterial infections can also decrease the ability of skeletal muscles to uptake insulin-mediated glucose. This can produce whole body insulin resistance (Kuo et al, 2008). Fortunately, periodontal treatments can benefit patients with diabetes by inhibiting pathogen secretions of LPS and improving the body’s glycemic control (Pihlstrom et al, 2005).

Poorly controlled diabetes increases the risk of periodontal disease activation and severity, and the rate of periodontal bone loss (Pihlstrom et al, 2005; Preshaw, 2009; Filoche et al, 2010). A hyperglycemic condition can lead to a chronic inflammatory-immune response that produces an excessive and deregulated amount of inflammatory mediators, such as cytokines and other enzymes (Pihlstrom et al, 2005). The excess inflammatory mediators make their way into the periodontium, causing periodontal detachment, pocket formation, and even alveolar bone destruction (Preshaw, 2009). Diabetics can also result in other oral complications such as burning mouth syndrome, fungal infections, dental caries, and salivary functional disorders (Kuo et al, 2008).

Cardiovascular disease

Periodontal pathogens signal excessive amounts of antigens, endotoxins, cytokines, and C-reactive proteins that also contribute to cardiovascular complications such as lipid deposition, smooth muscle proliferation, and platelet aggregation (Kuo et al, 2008). Pathogens like P. gingivalis and Streptococcus sanguis have abilities to induce platelet aggregation and accumulate as arterial plaque (Williams et al, 2008). Aggregatibacter actinomyctematitans in the periodontal pockets has also been discovered in atherosclerotic plaque (Bahekar et al, 2007). The organism accesses the circulatory system through oral tissue and makes its way to the arteries where it secretes LPS and inflammatory-response mediators, resulting in atherothrombogenesis. As in diabetes, periodontal treatments may also alleviate cardiovascular diseases (Tonetti et al, 2007; Kuo et al, 2008). The exact pathway from cardiovascular disease to periodontal disease has yet to be established.

Disease manifestations in the oral cavity

Analysis of the oral cavity and its microbiome may become a means to diagnose systemic diseases that tend to manifest in the periodontium (Pihlstrom et al, 2005). Herpetic infections, leukemia, tuberculosis, and even dermatological diseases are some examples of diseases that present major gingival swelling, oral lesions, and gingival discoloration because of cellular infiltration (Pihlstrom et al, 2005). These diseases may also ‘invisibly’ manifest in the oral cavity before any symptoms become apparent in the body. For instance, respiratory pathogens may colonize the mouths of individuals with a high risk of pneumonia even when respiratory symptoms are absent (Scannapieco, 1998). The oral biofilms serve as a reservoir for these pathogens and contribute to the disease’s progression. In fact, there is also an altered oral microflora in individuals with HIV, as well as individuals who are pregnant, lactating, or taking antibiotics. Although not all of these conditions are diseases, this evidence suggests that homeostatic alterations in the body manifest in the oral microbiome. Detecting bacteria related to oral or systemic disease at early or asymptomatic stages may increase the chances

Figure 5 Periodonto pathogen pathways from the oral cavity to systemic organs. Adapted from (Scannapieco, 2004)
of rapid disease reversal and alert the patient to practice preventative measures.

**Treatment and prevention methods**

To maintain oral and systemic health, it is vital to protect the periodontium from pathogens that cause inflammatory infections. The foundation of periodontal therapy is anti-infective, non-surgical treatments aimed to control biofilms and the proliferation of pathogenic bacteria within the oral cavity (Pihlstrom *et al.*, 2005). First and foremost, practicing good oral hygiene is the principal preventative measure of oral diseases. Dental professionals may perform scaling and root planning to remove plaque on tooth surfaces or infected tissue of the periodontal pockets. These procedures, combined with persistent oral hygiene practices, can reduce tissue inflammation, pocket depths, and improve periodontal attachment (Pihlstrom *et al.*, 2005).

**Antibiotics**

When manual treatments are supplemented with local and systemic antibiotics, the oral cavity experiences a change in composition and abundance of various bacteria. (Pihlstrom *et al.*, 2005). Local antibiotics kill or freeze an array of species at diseased sites in the oral cavity, as well as heal oral lesions and halt plaque accumulation (Horz and Conrads, 2007). Systemic drugs target pathogens at sites around the body in addition to the oral cavity but are limited to the species they target. They can also reduce any bleeding in the periodontium. However, systemic drugs are conventionally used as a last resort in the treatment of periodontal diseases for cost-effective purposes (Flemmig and Böckler, 2011).

There can be variation in the pathogen and host response to different drugs. For example, growth of *A. actinomycetemcomitans* is inhibited by tetracyclines but unaffected by clindamycin (Horz and Conrads, 2007). Moreover, an individual’s intestinal microbiota affects the metabolism of drugs and toxins. As the human microbiome is unique to the individual, oral vaccines may be processed differently in each body, depending on both oral and gut microbial communities (Ferreira *et al.*, 2010).

Effective use of antibiotics in the future requires genomic analysis of the patient’s oral microbiome to recognize the microbes that are present and to determine whether they will respond to specific treatments. Therefore, the oral microbiome will likely play a central role in the development and advancement of personalized medicine.

**Probiotics and prebiotics**

While antibiotics are synthetic drugs that harm the microflora, probiotics are live microbes that are part of the natural microflora. The utilization of antibiotics implies that disease is already in progress. Instead of fighting to cure disease, medicine today should focus on how to maintain health and prevent disease. Probiotic therapy or ‘bacteriotherapy’ (Rajendhran and Gunasekaran, 2010) has the potential to naturally cure and prevent disease at its early stages by incorporating beneficial bacteria that can reestablish an ecological balance or enhance the biodiversity of a microflora. For example, research showed that individuals with high amounts of *Capnocytophaga ochracea* had lower amounts of *P. gingivalis* and displayed no periodontal disease progression. Individuals with low *C. ochracea* did exhibit disease progression. In probiotic treatment, a patient found to have elevated levels of *P. gingivalis* could be given *C. ochracea* probiotics to reestablish a healthy equilibrium before any periodontal disease generates. Prebiotics may also be used for similar purposes. Prebiotics are oligosaccharides, or complex sugars, that aim to stimulate the growth of beneficial bacteria in the host (Badger *et al.*, 2011). Both probiotics and prebiotics would strengthen the beneficial microbiota so the body can naturally fight off disease-causing agents.

However, a clear definition of health, including the composition and interrelationships of the healthy microbiome, is necessary before any probiotics can be developed or used properly. In addition to the ambiguity of ‘health’, the probiotic industry faces challenges that have prevented their market appearance (Klein *et al.*, 2010). First, the effects of probiotic organisms on the host and the mechanism by which they exert these effects are still uncertain (Sonnenburg and Fischbach, 2011). Blind delivery of probiotics is dangerous because there is yet to be any true evidence of how they influence in vivo physiology and functionality. Second, the probiotic industry bears production parameters it must overcome to be able to create safe and reliable probiotic substances (Klein *et al.*, 2010). The production process would have to consider factors such as the technology used, temperature, and fermentations conditions, oxygen content, organic ingredients used, etc. Therapeutic antibiotics and probiotics face limitations because of a lack of knowledge of the oral microbiome and its numerous, complex constituents.

**Pathogen predators**

Aside from mainstream antibiotics and emerging probiotic approaches, an alternative treatment strategy involving biological antimicrobial agents may establish a groundbreaking approach to curing and preventing disease. Research has discovered bacterial lineages of *Bdellovibrio*, *Bacteriovorax*, and *Peredibacter*, called *Bdellovibrio*-and-like organisms or BALOs, that serve as predators to kill anaerobic, Gram-negative bacteria (Dashiff and Kadouri, 2011; Van Essche *et al.*, 2011). Because most periodontal pathogens are anaerobic, Gram-negative bacteria, these BALOs may play a significant role in the treatment of periodontal infections (Horz and Conrads, 2007; Van Essche *et al.*, 2011). These highly motile species are abundant in aquatic environments but cannot be isolated in large amounts (Van Essche *et al.*, 2011). As BALOs do not prey on Gram-positive bacteria, and most beneficial bacteria in the oral cavity are Gram-positive, BALOs may even be preferred over antibiotics when curing oral diseases.
While antibiotics are non-specific and destroy an array of microbial communities, BALOs will only kill the ‘bad’ microbiome and sustain the ‘good’ microbiome (Dashiff and Kadouri, 2011; Van Essche et al, 2011). Furthermore, antibiotics cannot target pathogens deep within layers of biofilm. BALOs, on the other hand, can infiltrate and attack surface-attached bacteria (Dashiff and Kadouri, 2011). They can also assist antibiotics by detaching biofilm surfaces and exposing the present pathogens (Dashiff and Kadouri, 2011). Mixed microbiota environments do not inhibit BALOs’ predation efficiencies (Van Essche et al, 2011) and neither does saliva nor high temperatures (Dashiff and Kadouri, 2011); however, predator-prey interactions are highly BALO strain specific, which underscores the importance of identifying which pathogens are present for effective therapy (Van Essche et al, 2011).

The utilization of BALOs seems to be a groundbreaking approach to oral disease therapy, as these biological antimicrobial agents possess the benefits of both antibiotics and probiotics. Unfortunately, several parameters exist that prevent the use of BALOs in clinical settings. First, the tests that have been performed to analyze BALOs behavior have only taken place in experimental settings. The use of BALOs as therapeutics requires a better understanding of how these predators function in the physiological setting (Van Essche et al, 2011). Second, high inoculum concentrations of BALOs are more efficient in killing a substantial amount of pathogens (Van Essche et al, 2011); yet, BALOs are not extracted from the environment in plentiful amounts. Finally, BALOs are unable to function under anaerobic conditions. This limitation means that BALOs would not be able to prey on the anaerobic bacteria that reside deep within periodontal pockets formed in periodontitis (Dashiff and Kadouri, 2011).

**Personalized dental medicine**

Because the microbiome is the biomarker of disease activity, further research and advancements in microbiomics and metagenomics are essential to understanding the microbiology and etiology of oral diseases. Genomes collected through metagenomic techniques will not only be used for the analysis of microorganisms, but also in the engineering of therapeutic agents needed to manipulate the microbiome according to personal needs (Park and Kim, 2008). Understanding changes in the oral microbiome at the early stages of chronic oral diseases would allow clinicians to diagnose and treat an unhealthy oral cavity before the appearance of any dental lesions or periodontal pockets (Zaura et al, 2009). Additionally, the use of probiotics or other biological antimicrobial agents at early stages of disease could naturally restore microbial equilibrium and, thus, minimize the need for antibiotics.

The diagram in Figure 6 represents the course of chronic oral disease over time, the clinical tools that should be used to track the disease burden, and the suggested use of probiotics and antibiotics to slow or stop the disease process. For example, if specific pathogens are recognized following screening methods, probiotics could be administered locally, according to the amount and type of those pathogens present. However, the role that each microorganism plays in disease progression or regression must be accurate and well understood for safe and effective manipulation of a microbiome (Zaura et al, 2009). In addition, using such clinical detection methods would require the development of novel technologies, especially for performing on-the-spot tests. Personalized dental medicine that focuses on the oral microbiome will have extensive effects in health care, considering the oral microbiome’s importance to both oral and systemic health.

**Conclusion**

Although invisible to the naked eye, the microbiome should not be underestimated as a key determinant of health and disease. The oral microbial ecosystem is particularly vital to maintaining both oral and overall health in the body. Salivary flow and biofilms on the teeth and soft tissue maintain microbial equilibrium within the oral cavity and protect pathogens from manifesting. Disturbing the homeostasis of the oral cavity can stir pathogen activity and lead to oral disease. Because the oral cavity is the primary gateway to the
body, severe cases of oral disease may result in the spread of infection to other body sites, producing systemic diseases such as cardiovascular disease or exacerbating an already compromised immune system, as in diabetes. Practicing good oral hygiene and maintaining stable oral biofilms is essential to keeping a body healthy and also preventing rapid spread of disease to other individuals.

Microbiomics and metagenomics must collaborate to fully elucidate the nature of the microbiome during both health and disease, which will, subsequently, pave the way for more effective therapeutic and diagnostic techniques. Ultimately, the analysis of the human microbiome will significantly contribute to the development of personalized medicine and personalized dental medicine.

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Author contributions

Zarco researched the topic, wrote the initial draft, and edited the final draft. Vess assisted Zarco in the research, and edited early and late drafts. Ginsburg initiated the idea, guided Zarco and Vess in their research and writing, and edited and approved the final manuscript.

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