REVIEWS

Oral Microbiology: Past, Present and Future

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Abstract

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Since the initial observations of oral bacteria within dental plaque by van Leeuwenhoek using his primitive microscopes in 1680, an event that is generally recognized as the advent of oral microbiological investigation, oral microbiology has gone through phases of "reductionism" and "holism". From the small beginnings of the Miller and Black period, in which microbiologists followed Koch's postulates, took the reductionist approach to try to study the complex oral microbial community by analyzing individual species; to the modern era when oral researchers embrace "holism" or "system thinking", adopt new concepts such as interspecies interaction, microbial community, biofilms, poly-microbial diseases, oral microbiological knowledge has burgeoned and our ability to identify the resident organisms in dental plaque and decipher the interactions between key components has rapidly increased, such knowledge has greatly changed our view of the oral microbial flora, provided invaluable insight into the etiology of dental and periodontal diseases, opened the door to new approaches and techniques for developing new therapeutic and preventive tools for combating oral poly-microbial diseases.

Keywords oral microbiology, biofilms, dental caries, oral pathogenesis

Introduction

Like many other biological sciences, the study of microbiology has gone through phases of "reductionism" and "holism". For a long time, microbiologists took the reductionist approach to study complex microbial communities by analyzing individual bacterial species. The strategy was to understand the whole by examining smaller components, and has been the hallmark of much of the industrial and scientific revolutions for the past 150 years. While reductionism has greatly advanced microbiology, it was recognized that assembly of smaller pieces cannot explain the whole! Modern microbiologists are learning "system thinking" and "holism." From global gene regulation to "meta-genomics" to "biofilms", microbiology is entering an exciting new era with emphasis on revealing and decoding the interactions of different elements within a microbial community. The knowledge obtained from "system thinking" is changing our understanding of microbial physiology and our ability to diagnose/treat microbial infections, and will have great impact on oral microbiology as well.

Commonly known as "dental plaque", oral microbial communities is one of the most complex bacterial floras associated with human body. So far, more than 700 different bacterial species have been identified from human oral cavity, and the majority of them are associated with dental plaque (Paster *et al.*, 2001; Aas *et al.*, 2005; Paster *et al.*, 2006). Extensive animal and clinical studies have indicated that the oral microbial flora is responsible for two major human oral diseases: dental caries (tooth decay) and periodontitis (gum disease) (Loe

et al., 1965; Socransky, 1979; Dahlen, 1993; Marsh, 1994; Nishihara and Koseki, 2004). For a long time, oral microbiologists followed guideline of Koch's postulates, and used reductionism approach to try to identify the key pathogens responsible for oral microbial pathogenesis (Slots and Listgarten, 1988; Dzink *et al.*, 1988; Haffajee and Socransky, 1994). However the limitation of reductionism forced microbiologists to embrace new concepts such as interspecies interaction, microbial community, biofilms, poly-microbial diseases, etc. These new research directions have revealed many new physiological functions critical for the pathogenesis, which result from interactions between different components within oral microbial flora, and might not be observed with individual organisms. The new "system thinking", empowered with the modern techniques for analyzing complex microbial community serves as a new foundation for studying oral microbial community, providing invaluable insight into the etiology of dental and periodontal diseases, developing new therapeutic and preventive tools for combating oral poly-microbial diseases.

Oral microbiology at early stage

Although minute and primordial, bacteria are incredibly versatile and diversified. Judging by their numbers and biomass, they are arguably the most successful living organisms on earth. They can tolerate environmental extremes and colonize almost every habitat on earth, including human oral cavity. However, due to their minuteness, their existence was not revealed until fairly recent times, around 1680, when van Leeuwenhoek (1632–1723) (Gest, 2004), a Dutch dry goods merchant, observed and described first microorganisms in the tartar from his teeth with his primitive microscope. In his notebook, he recorded "I didn't clean my teeth for three days and then took the material that has lodged in small amounts on the gums above my front teeth...... I found a few living animalcules." The microbes sketched in his notebook are now known as some of the most abundant bacteria resided within oral cavity, including cocci, spirochetes, and fusiform bacteria. These fascinating observations at the birth of microbiology had already signaled the complexity of the oral micro-

bial community.

Revealing the infectious nature of dental caries and periodontitis

Like many other sub-disciplines of microbiology, the early stage of development in oral microbiology was largely driven by medical needs. As two of the most prevalent human oral diseases, dental caries and periodontitis have always been the focus of oral microbiology research.

Dental caries is a human oral disease with a long history. Archaeological evidence shows that tooth decay is an ancient disease dating far back into prehistory (Suddick and Harris, 1990). Interestingly, studies by Moore and Corbett of the dentition of Britons in various periods demonstrated that, until about 1850, caries occurred relatively infrequently; after 1850, coinciding with the increasing availability of cane sugar and refined flour, there has been an explosive increase in dental caries lesions (Nikiforuk, 1985). Even from ancient time, long before the visual observation of microorganism, people had suspected the possible causative link between certain form of living organism—described as "tooth worm" in a Sumerian text about 5000 BC, and dental caries (Suddick and Harris, 1990). However, the revelation of the true identity of "tooth worms" had to wait for another 6000 years.

Miller was arguably one of the most important individuals who greatly advanced oral microbiology. As a practicing dentist with a thorough training in natural sciences, he spent his evenings and weekends at Koch's laboratory for the purpose of identifying the "germs" that were responsible for tooth decay. In his 1890 book titled "*Microorganisms of the Human Mouth*" (Miller, 1890), he proposed a "chemoparasitic" theory which suggested that, in susceptible hosts who frequently consumed fermentable carbohydrates, oral microorganisms would convert these carbohydrates into acid, which would result in the demineralization of teeth. Miller's chemoparasitic theory, together with the description of "gelatinous microbic plaques"—now commonly known as "dental plaque", by Black and Williams (Black, 1898; Williams, 1898), provided the key elements for our modern concept of the etiology of dental caries.

Due to the limited bacterial isolation and cultivation technique available in 19th century, Miller was unable to identify the causative agent(s) of dental caries. In 1924, Clarke first isolated a bacterial species from human dental caries site, it was named *Streptococcus mutans* (*S. mutans*), and was shown to be capable of fermenting several sugars and producing a pH of 4.2 in glucose broth (Clarke, 1924). But unfortunately, Clarke was unable to demonstrate that this organism actually caused caries.

Since 1950, there has been an increasing interest in using experimental animal to better understand the nature and etiology of oral disease, including dental caries. Many relatively sophisticated animal models had been developed which has greatly facilitated oral microbiology studies (Jordan, 1971; Navia, 1977). In 1960, two important paper were published which were considered the cornerstone of dental caries research. In the first paper, using hamster as animal model, Keyes revealed the infectious and transmissible nature of dental caries (Keyes, 1960). Then in another elegant paper, Fitzgerald and Keyes successfully demonstrated caries induction in an animal harboring a "conventional" microflora, by a single type of streptococcus—the same bacterial species that had been isolated by Clarke more than 30 years ago (Fitzgerald and Keyes, 1960).

Other than dental caries, another major human oral disease is periodontitis, it is widely regarded as the second most common disease worldwide. The involvement of bacteria in the development of periodontal disease was suggested by the fact that administration of penicillin inhibited periodontitis in hamster (Mitchell and Johnson, 1956); while the infectious nature of this disease was substantiated by demonstration of its transmissibility (Keyes and Jordan, 1964). Early experimental animal study also identified several oral isolates, such as *Actinomyces* species, which could potentially play an important role in eliciting the disease (Howell *et al.*, 1965).

Establishing dental plaque as the cause of dental caries and periodontitis

One of the early achievements in oral microbiology is to link dental plaques to dental and periodontal diseases. As mentioned earlier, dental plaque was one of the first substances van Leeuwenhoek examined under his microscope (Gest, 2004). His recording of the living microorganisms within the plaque gave the first hint to the complex nature of dental plaque in term of its diversified microbial inhabitants. Later on, both Erdi and Ficinus described the presence of microorganisms within the "membrane" on teeth (Suddick and Harris, 1990). However, the full implications of dental plaque were not realized until the publication of Black's 1898 paper, in which he referred dental plaque as "gelatinous microbic plaques", a gelatin-like substance that carried microorganisms (Black, 1898). Based on his clinical experience and experimentation, he believed that the cause of dental caries was due to the attack from acids generated by bacteria within these plaques. Black's work, together with Miller's chemoparasitic theory established the important role of dental plaque in the etiology of dental caries and has become one of the essential paradigms of oral biology. And the progress in dental plaque study has become a major indicator of the development of oral microbiology.

Oral microbiology—Present

Genetic studies of bacterial isolates from dental plaque

Since the establishment of microbes within dental plaque as the cause of dental and periodontal diseases, oral microbiologists followed the guideline of Koch's postulates and tried to isolate specific microorganisms that could be the causative agents and responsible for these disease. However, like in other microbiology fields, one of the challenges faced by early researchers in oral microbiology was the isolation and cultivation of "pure" bacterial culture. By the 1960s, anaerobic bacteria had been recognized as the predominant microbial component of the gastro-intestinal tract (including oral cavity) associated flora of human and mammals (Rosebury, 1962). During the process of bacterial cultivation, researchers realized that the majority of these anaerobic bacteria cannot be cultivated aerobically, or with the conventional anaerobic cultivation technique, such as, anaerobic culture

tube introduced by Laidlaw (Laidlaw, 1915), the vacuum jar designed by Noguchi (Noguchi, 1911), and the anaerobic container—the "McIntosh bomb" developed by McIntosh and Fildes (McIntosh and Fildes, 1916). The introduction of anaerobic glove boxes—a primitive version of now widely used anaerobic chamber, by Socransky (Socransky *et al.*, 1959) and Rosebury *et al.* (Rosebury and Reynolds, 1964) in the 1960s greatly facilitated the isolation and cultivation of anaerobes, particularly those obligate (strict) anaerobic microorganisms, from human oral cavity.

With improved anaerobic cultivation technique and availability of newly developed complex media for bacterial cultivation, pure cultures of more than 300 different oral bacteria species have been isolated from oral cavity in the past 40 years, including bacterial species harbored in both supra-gingival and sub-gingival dental plaque samples taken from healthy and diseased sites (Kolenbrander, 2000). The isolation and cultivation allowed detailed phenotypic and genetic study on those clinically important bacteria, such as *Streptococcus, Actinobacillus, Actinomyces, Porphyromonas* and *Treponema* species (Kolenbrander, 2000). The combination of classic genetic approaches, such as isolation and characterization of mutants, with molecular genetic techniques, including recombinant DNA methodology developed in the late 1970s and genetic mapping (Cohen *et al.*, 1973), has been used to genetically manipulate and characterize those microorganisms important in dental caries and periodontal diseases. The genetic studies, together with the additional bio-informatic information obtained from whole genomic sequences of many oral bacteria (Zhulin, 2009) allow microbiologists to dissect the development and function of oral microbial community at the molecular level. Progress has been made in determining the molecular genetic basis of biofilm formation of oral isolates using data from *in vitro* studies on either single, dual species or multi-species systems. Studies have shown that in certain virulent organisms many different types of genes encoding a variety of functions are required for biofilm development and successful establishment within the dental plaque, including genes encoding general stress response pathways, quorum-sensing systems, two-component systems sensing environmental cues,

and those encoding surface adhesions involved in cell-cell or cell-to-genes encoding surface interactions (Kolenbrander, 1988; Kolenbrander, 2000; Davey and Costerton, 2006; Kuramitsu *et al.*, 2007).

Getting a closer look at dental plaque

The dental plaque has long been linked to the etiology of dental and periodontal diseases. The concept proposed by Black more than 100 years ago that caries was due wholly to attack from acids produced by bacteria within dental plaque has stimulated considerable scientific investigation on dental plaque. In the past few decades, there has been a tremendous amount of work and numerous exciting discoveries made during these investigations, ranging from dental plaque's microbial composition, structure to its formation process, from its physiology properties to different kinds and levels of bacterial interactions within these densely populated microbial community. The virtual explosion of our knowledge regarding dental plaque has had a profound impact on our understanding of the etiology of dental and periodontal diseases (Kolenbrander, 1988; Kolenbrander *et al.*, 1999; Kolenbrander, 2000; Kolenbrander *et al.*, 2002; Kolenbrander *et al.*, 2005; Davey and Costerton, 2006; Kuramitsu *et al.*, 2007).

Before the 1960s, although microorganisms within dental plaque were held responsible for the etiology of dental and periodontal disease, it was generally believed that diseases were the result of an increased mass of bacteria rather than qualitative differences in the composition of the microbiota. This was largely due to the lack of suitable cultivation techniques and the gaps in the knowledge of the taxonomy of the oral microbial flora. This conventional wisdom was challenged by Listgaten (Listgarten, 1976). By analyzing the dental plaque associated with periodontally healthy and diseased teeth using electron microscopy, he observed that distinct qualitative differences exist between supra- and sub-gingival, healthy and disease associated plaque (Listgarten, 1976). These original microscopic observations were later confirmed by improved culture-dependent and non-culture dependent analysis.

In 1978, Costerton invented the word "biofilm", referring to the matrix-enclosed bacterial com-

munity (Costerton *et al.*, 1978). Studies have shown that bacteria within biofilms usually display different phenotypes compared with their counterparts. They are more resilient and more resistant to external insults. Within biofilm, there are extensive metabolites exchanges, signaling trafficking and different levels of interactions among different species (Costerton *et al.*, 1987; Costerton *et al.*, 1995; Costerton *et al.*, 1999). The introduction of biofilm theory into oral microbiology field provided impetus for researchers to take a closer and thorough look at the dental plaque, the first biofilm described by van Leeuwenhoek.

Detailed analysis of a microbial community requires the full knowledge of its endogenous inhabitants. However, for a long time, our understanding of the microbial world has been hampered by the intrinsic limitation of the conventional culture-dependent methods. According to Staley and Konopka, fewer than 1% of the organisms are able to grow under laboratory conditions, suggesting that our views of the complexity and genetic diversity of microbial communities based on cultivation strategies are severely biased (Staley and Konopka, 1985). Fortunately, Woese and coworkers discovered three decades ago that the conservation as well as the variation within the sequences of 16S rRNA-encoding genes allows for the construction of evolutionary trees for bacteria to the species level (Saiki *et al.*, 1985; Woese, 1987). This finding in combination with the development of PCR methods in 1985 (Saiki *et al.*, 1985) opened the door for culture-independent analyses and classification of previously unknown members of many different microbial communities, including the resident flora of oral biofilms. The past two decades has witnessed a virtual explosion in the development of high-throughput tools for microbial community analysis. Some of these methods directly examine nucleic acids isolated from samples of microbial communities, such as microarrays (Small *et al.*, 2001) and checkerboard DNA-DNA hybridization method (Socransky *et al.*, 1994), while others are PCR based, such as denaturing gradient gel electrophoresis (DGGE) (Nakatsu, 2007) or temperature gradient gel electrophoresis (Yoshino *et al.*, 1991), terminal restriction fragment length polymorphism (T-RFLP) (Liu *et al.*, 1997; Thies, 2007), automated ribosomal intergenic spacer analysis (Cardinale *et al.*, 2004), and denaturing high-performance liquid chromatography (DHPLC) (Domann *et al.*, 2003). Most of these approaches were originally developed for analyzing environmental microbial communities and have been used to study human microbial ecology, including oral microbial analysis. These culture-independent techniques have revealed a whole new microbial world within dental plaque with great genetic diversity. Over 700 bacterial species (gram-positive, gram negative bacteria and archaea) have been identified form the human oral cavity, majority of them are associated with dental plaque, making the oral microbial community one of the most complex microbial floras in the human body (Aas *et al.*, 2005; Paster *et al.*, 2001; Aas *et al.*, 2005; Paster *et al.*, 2006). Based on our current knowledge, supragingival plaque is dominated by gram-positive bacteria, including *Streptococcus sanguinis, S. mutans, Streptococcus mitis, Streptococcus salivarius*, and lactobacilli, while the subgingival plaque is made up primarily by gram-negative anaerobic bacteria, such as *Aggregatibacter (Actinobacillus) actinomycetemcomitans, Tannerella forsythia, Campylobacter* spp., *Capnocytophoga* spp., *Eikenella corrodens, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia*, and oral spirochetes such as *Treponema denticola*. In both cases, the microbial communities on teeth and gingival tissues can accumulate high concentrations of bacterial metabolites (*e.g.*, fatty acid end products, ammonia, hydrogen peroxide, oxidants, and carbon dioxide) in their local environments, which influence the bacterial species within the microbial community, as well as the host (Carlsson, 1997).

To study the formation and architecture of dental plaque, several experimental designs have been developed. These include *in vitro* systems ranging from simple batch culture system (*e.g.*, the Zürich Biofilm Model) (Guggenheim *et al.*, 2004), constant depth film fermentor (CDFF) (Kinniment *et al.*, 1996), saliva-conditioned flow cell (Foster and Kolenbrander, 2004), to artificial mouths (Pigman and Elliott, 1952; Russell and Coulter, 1975), as well as *in situ* device for the *in vivo* generation of intact dental plaque biofilms on natural tooth surface in human subjects (Wood *et al.*, 2000). Using imaging tools such as the scanning electron microscope (Listgarten, 1976; Theilade *et*

al., 1976) and, more recently, the confocal laser scanning microscope (Netuschil *et al.*, 1998; Wood *et al.*, 2000), and coupled with variety of staining techniques, such as fluorescence *in situ* hybridization (FISH) (Foster and Kolenbrander, 2004; Wagner *et al.*, 1994), oral microbiologist now realized that dental plaque are not unstructured, homogeneous deposits of cells, but complex, well organized communities of surface-associated cells enclosed in a polymer matrix containing open water channels and fluid-filled voids. *In vitro* and *in vivo* study on the formation and development of dental plaque revealed that during the formation process, some oral bacteria are early colonizers that express biochemical components allowing them to effectively adhere to targeted tissues (teeth or periodontal tissue). The later colonizers often contain components that enable them to adhere to the early colonizers, often bringing metabolic or other competitive advantages. Within an established dental plaque, specific bacterial species are often found located adjacent to each other or mixed together to form unique structures that often confer adherence or growth advantages. Previous comprehensive reviews by Kolenbrander *et al.* should be consulted for assessment of these important properties (Kolenbrander, 1988; Kolenbrander *et al.*, 1999; Kolenbrander, 2000; Kolenbrander *et al.*, 2005; Kolenbrander *et al.*, 2006).

Community based microbial pathogenesis

The improved cultivation technique allows successful isolation and characterization of microbial species from dental plaque, which naturally prompted oral microbiologists to connect specific bacterial species to certain diseases, according to Koch's postulates. For example, as a result of such strategies, the acid-producing oral bacterium *S. mutans* present in supragingival plaque was selected for its positive correlation with dental caries, animal study also demonstrated its ability to induce dental caries. And this has become the base for "Specific plaque hypothesis" proposed by Loesche, which proposed that only a few specific species, such as *S. mutans* and *Streptococcus sobrinus*, are actively involved in the disease (Loe *et al.*, 1965). However, additional scientific data have suggested that such a simple correlation may be an oversimplification.

Unlike many known medical pathogens that are "foreign invaders with specific virulence factors", the oral "pathogens" such as *S. mutans* are part of the normal flora (Aas *et al.*, 2005). While they express certain pathogenic factors (such as acid production in this case), a dynamic balance of both synergistic and antagonistic interactions with its neighboring bacteria plays an essential role in determining whether these pathogenic factors cause damage or not (Kleinberg, 2002; Marsh, 2005). As proposed by Marsh in his "Ecological plaque hypothesis", in the case of complex biofilms, it is not merely the presence of a single organism in a complex community which determines the properties of a biofilm, but it is the interactions between the biofilm residents which is crucial (Marsh, 2005). As an example, in the presence of nearby base-producing bacteria, *S. mutans* in dental plaque may not be as pathogenic to the host. Thus, for dental caries, it is now generally recognized that this disease results not solely because of the presence of *S. mutans* or any single organism in dental plaque. Rather, it is the result of the interaction of multiple acid-producing organisms such as *S. mutans* with other biofilm residents (Kleinberg, 2002; Marsh, 2005). Such a community- and microbial ecology-based pathogenic theory serves as a new concept for understanding the relationship between dental plaque and the host in health or disease, as well as suggesting new strategies for disease treatment and prevention.

Dental plaque is a microbial community with great structural complexity and genetic diversity. Its bacterial composition remains relatively stable despite regular exposure to minor environmental perturbations. This stability (microbial homeostasis) is due in part to a dynamic balance of both synergistic and antagonistic microbial interactions (Marsh, 1994; Kleinberg, 2002). This suggests that the residents in this community should display extensive interactions while forming biofilm structures, carrying out physiological functions, and inducing microbial pathogenesis. Extensive *in vitro* and animal studies have revealed a multitude of bacterial interspecies interaction as described in resent reviews. These interactions include (i) competition between bacteria for nutrients, (ii) synergistic interactions which may stimulate the growth or survival of one or more residents, (iii) produc-

tion of an antagonist by one resident which inhibits the growth of another, (iv) neutralization of a virulence factor produced by one organism by another resident, and (v) interference in the growth-dependent signaling mechanisms of one organism by another (Kolenbrander, 2000; Kolenbrander *et al*., 2005; Kolenbrander *et al*., 2006; Kuramitsu *et al*., 2007; Marsh, 1994; Marsh, 2005). In a micro-Gaia community, these interactions could be envisaged as forms of "war and peace" among the bacterial residents of a biofilm.

Multispecies communities like dental plaque can produce polymicrobial infections in which microorganisms interact in a synergistic fashion, leading to pathogenesis. Periodontal diseases represent one of the best-documented polymicrobial infections. *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella forsythia* are strongly implicated clinically as a pathogenic consortium in the etiology of adult periodontitis (Socransky *et al*., 1998; Feng and Weinberg, 2006). It is anticipated that a better understanding of the mechanisms involved in periodontitis may provide opportunities to more critically evaluate the role of different virulence factors involved in these mixed infections. This may provide useful general information for investigators in the development of novel diagnostic, preventive, and treatment strategies against polymicrobial infections

Oral microbiology—Future

The future direction of oral dental plaque study—Metagenomics

The aforementioned high-throughput tools for microbial community analysis are largely based on PCR amplification of 16S rRNA sequences from microbial communities, which are relatively short, often conserved but varied enough to differentiate bacteria at species level. Although these approaches can provide us with the microbial composition within the community, unless we have genomic or other research data on those identified species, it reveals very limited information regarding what functions they might carry out within the flora. The introduction and application of "metagenomics" approach has greatly enhanced and will continue

to increase our ability to study microbial community, including dental plaque, in greater detail.

The term "metagenomics" was first invented by Handelsman (Handelsman *et al*., 1998), and it is defined as "the application of modern genomics techniques to the study of communities of microbial organisms directly in their natural environments, bypassing the need for isolation and lab cultivation of individual species". The advances in refinements of DNA amplification, bioinformatics, and enhanced computational power for analyzing DNA sequences have enabled the adaptation of shotgun sequencing, such as chip-based pyrosequencing, to metagenomic samples (Breitbart *et al*., 2002; Edwards *et al*., 2006). The approach randomly shears DNA, sequences many short sequences, and reconstructs them into a consensus sequence (Breitbart *et al*., 2002).

By performing metabolic function analyses on genes identified *via* metagenomic approach, researchers are able to retrieve information both on which organisms are present and more importantly, what functions or metabolic processes are possible in that particular community (Gill *et al*., 2006). Using comparative genetic studies coupled with expression experiments such as microarray and proteomics, microbiologist will be able to piece together a metabolic network that goes beyond species boundaries, and gain valuable insight into the metabolism within the community. Recently, comparative metagenomic study has been initiated to try to compare the microbial community within dental plaque associated with healthy and diseased sites. The results should become available in the next few years. It is anticipated that such comparison will assist in identifying potential pathogenic organisms which may not have been detected using currently available technologies (Kumar *et al*., 2003).

Evidence based dental caries diagnosis

Although the infectious nature of dental caries has been proved for more than 100 years, instead of treating it as infectious disease, traditional dentistry still holds sway and focuses on treating the symptom (repair the damaged tooth) *via* surgical approaches. The recent advanced knowledge we have gained about caries pathogenesis allows us to

understand that a comprehensive analysis of dental caries should be more than detecting tooth demineralization sites and repairing damaged teeth with surgical approaches. Instead, it should include the detection of cariogenic bacteria and plaque acidogenicity, followed by a comprehensive treatment of dental caries that includes the elimination of cariogenic bacteria, the reduction of plaque acidogenicity and the enhancement of tooth reminerlization (Tsang *et al.*, 2006). The combination of accurate detection of oral bacteria and *in situ* monitoring of plaque pH—such as the poly-aniline-based planer pH sensor developed by scientists at Jet Propulsion Laboratory, and combined NMR confocal microscopy from Pacific Northwest Laboratory which can monitor pH gradient within dental plaque with high sensitivity and in real time (Majors *et al.*, 2005), is opening a new chapter for cariology. Early detection and quantification of cariogenic bacteria in plaque or saliva samples can help clinicians take preventive measures to stop caries development, much like early detection of cancer markers can be detected before overt/detectable cancerous lesions develop. New antibody or nucleotide based bacterial detection techniques have been developed for the detection of cariogenic bacteria in chairside or laboratory settings (Shi *et al.*, 1998). In conjunction with nanotechnology development, there tests can be further developed into different forms of nano-chips for detection of multiple pathogens in the clinical settings (Li *et al.*, 2005).

New approaches for controlling dental and periodontal diseases

Current preventive dental therapy is primarily focused on removing dental plaque. Since it is now known that dental plaque is made of large numbers of commensal bacteria together with a limited number of pathogens (Aas *et al.*, 2005; Paster *et al.*, 2006), such an approach may not be effective since the "remove all or kill all" approach creates open, non-competitive surfaces for pathogens to repopulate the oral cavity. With our new understanding of the oral microbial community interactions, and the wide recognition of microbial ecology based theory on dental and periodontal diseases, there is now interest in approaches that selectively inhibit oral pathogens or modulate the microbial composition of dental plaque to control community based microbial pathogenesis. Among them, the probiotic approach has been a popular methods used to affect microbial communities.

The term "probiotics" refers to "live microorganisms, which when administered in adequate amount, confer a health benefit on the host" (Guarner *et al.*, 2005). Recently, more evidence suggested that probiotic therapy might be applied to the maintenance of oral health (Caglar *et al.*, 2006; Meurman and Stamatova, 2007). Using randomized controlled trials, Meurman *et al.* demonstrated that long-term consumption of milk containing the probiotic *Lactobacillus rhamuosus* GG strain reduced initial caries in kindergarten children (Nase *et al.*, 2001). While Hillman and colleagues introduced a non-acid poducing *S. mutans* strain that produce a bacteriocin active against other *S. mutans* strains into the oral cavity to replace the naturally occurring cariogenic strains. Both *in vitro* and animal model asscessment suggested its potential in reducing *S. mutans* colonization. This approach is awaiting evaluation for its efficacy in humans (Hillman, 2002).

Since the beneficial effects of probiotic therapy are mainly achieved through modulating existing microbial flora associated with the host, thus obtaining a balanced and healthy microbe-host relationship, instead of using live organisms, microbiologists are now developing novel techniques and products that do not involve live bacteria, yet generate targeted effects against pathogenic factors or organisms, thus, achieving similar probiotic effects (He *et al.*, 2009). One good example is the targeted antimicrobial therapy *via* a novel specifically targeted-anti-microbial peptides (STAMPs) technology (Eckert *et al.*, 2006). A "STAMP" is a fusion peptide with two moieties: a killing moiet made of a non-specific anti-microbial peptide and a targeting moiety containing a species-specific binding peptide. The targeting moiety provides specific binding to a selected pathogen and facilitates the targeted delivery of an attached anti-microbial peptide. In one of their published papers, Eckert *et al.* explored a pheromone produced by *S. mutans*, namely competence-stimulating peptide (CSP), as a STAMP targeting domain to mediate *S. mutans*-specific delivery of and killing domain.

They discovered that such STAMPs were potent against *S. mutans* grown in liquid as well as in biofilm states (Eckert *et al*., 2006). The STAMPs were capable of eliminating *S. mutans* from multispecies biofilms without affecting closely related non-cariogenic oral streptococci, indicating the potential of these molecules to be developed into "probiotic" antimicrobials that may selectively eliminate pathogens while preserving the protective benefits of the normal flora. This proof-of-principle demonstration using *S. mutans* suggests that it may be possible to develop other STAMPs which are specifically targeted to other pathogens, including periodontal pathogens within oral cavity.

Conclusions

Since the initial observations of bacteria within dental plaque by van Leeuwenhoek using his primitive microscopes in 1680, an event that is generally recognized as the advent of oral microbiological investigation, our ability to identify the resident organisms in dental plaque and decipher the interactions between key components has rapidly increased, particularly during the past decade. Continued expansion of such information in the future will have a great impact on oral microbiology and dentistry as well. We envision that in the future, empowered with the detailed knowledge of the microbial community ecology within dental plaque, the invention and application of new diagnostic tools, dentistry will be an evidence-based dental practice emphasizing the triple-pronged approach of early detection, effective and sustainable treatment, and prevention. Specifically, pathogen-based early detection will become routine, which will allow instantaneous chairside quantification of cariogenic bacteria in plaque or saliva samples. This clinical adjunct will help the clinician reinforce the concept of dental caries as an infectious process and will facilitate immediate, evidence-based treatment decisions.

The progress in oral microbiology will continue to provide in-depth understanding of the ecological tug-of-war between the indigenous microbial flora and the cariogenic, periodontal pathogens. And it will become a gateway leading to more specific and practive therapeutic approaches in combating dental and periodontal diseases.

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