Microbiomics of Oral Biofilms: Driving The Future of Dental Research

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ABSTRACT

Oral infectious diseases such as dental caries, periodontal disease, endodontic infections, oral candidiasis and peri-implantitis cause major health problems worldwide. All of these infectious diseases are associated with the biofilm growth mode of the oral pathogens. In the past, researchers often attempted to examine the association of single pathogens with particular dental diseases such as in the case of *Streptococcus mutans* acting as an aetiological agent for dental caries and the so-called "red-complex" bacteria for periodontal disease. However, with the recent advent of OMICS biology techniques such as genomics, transcriptomics, proteomics, it is possible to gain new insights into the host-microbial interaction, microbial community structure and composition in the oral cavity. The new studies on oral microbiomics can unravel the facets of the aetiopathology of oral diseases as never seen before. This mini-review will provide an history and overview of some of the existing DNA sequencing platforms employed to study the microbiomics of oral biofilms and the exciting future ahead for dental research.

Introduction

Oral diseases represent a major health burden worldwide. A wide spectrum of oral conditions is often encountered among the human population. The World Health Organization reported that dental cavities are prevalent in about 60–90% of schoolchildren and nearly 100% of adults. Severe forms of periodontal disease, one of the most common oral diseases in humans affects at least 15-20% of adult population.[1] Globally, about 30% of people aged 65–74 have no natural teeth.[2] Oral cancer is one of the most prevalent forms of cancer in the South-East Asia (SEA) region. In addition, oral mucosal diseases, infections of the salivary glands and oral infections with systemic effects are common in various patient populations. Hence, dental professionals have a significant role to play not only in enhancing the esthetics aspects but also in improving the oral and systemic health of people.

A significant number of oral diseases are infectious in nature, including dental caries, periodontal diseases, oral candidiasis, endodontic infections and peri-implantitis. The role of pathogenic microorganisms in the occurrence and

spread of these infections has been long established. In some anecdotal reports from ancient Mediterranean societies and China, dental decay was attributed to the presence "tooth worm".[3] In order to cure these diseases, "tooth doctors" aimed to eliminate the so-called "oral pathogen". A clearer picture of this "pathogen" emerged with the work of Antonie Leeuwenhoek in 1663.[4] Leeuwenhoek examined the "white little matter' in between his teeth using his single-lens microscope and termed them "animalcules". He reported his observations to the Royal Society of London, as "a living of animalcules swimming nimbly than any I have ever seen…the big short bending their body into curves in going forward". From another one of his experiments, Leeuwenhoek described that, "there are more animals living on the teeth than men in a whole kingdom, and mainly in those people that do not clean their mouth". These experiments showed that "animalcules" or bacteria have some association with the oral health status of the individual. Subsequently, Clarke in 1924, proposed *Streptococcus mutans* to be the etiological agent for dental caries.[5] The pioneering work by Socransky *et al.* (1988) proposed that "red-complex" bacteria *Porphyromonas gingivalis*, *Treponema denticola*, and *Tanerella forsythia* were pathogens responsible for chronic periodontitis.[6] Bacterial pathogen *Aggregatibacter actinomycetem-comitans* (previously known as *Actinobacillus actinomycetemcomitans)* was found to play a key role in aggressive periodontitis (or localized juvenile periodontitis).[7] *Enterococcus faecalis* has been implicated in endodontic infections.[8]

Until 1970s, pathogenicity models used to describe infectious diseases were based on studies of bacterial cultures in suspension or in the "planktonic mode". At that time, it was believed that bacteria prefer to live in the planktonic lifestyle. This concept was later changed due to the pioneering work of William "Bill" Costerton and colleagues, who introduced the concept of microbial biofilms.[9,10] Biofilms are surface attached microbial communities. Biofilms exhibit phenotypic traits that are different from their "planktonic' counterparts. Studies have shown that at least 65-80% of all infectious diseases are linked to the biofilm mode of microorganisms.[11] The most important feature of biofilms is their higher

resistance to antimicrobials and tolerance against immune response.[12] Because of this property, biofilms formed on body surfaces and medical devices are very difficult to eradicate, which may result in dire consequences to the affected patients.

Properties of Microbial Biofilms

Biofilm formation of microbial species generally follow a similar pattern, although there may be some variations among different species and strains.[13-14] Microbes in free-floating or "planktonic" mode, when in close proximity to biotic or abiotic surfaces, encounter attractive as well as repulsive forces between the organism and the surface. These include electrostatic and hydrophobic interactions, steric hindrance, van der Waals forces, temperature, and hydrodynamic forces. Apart from the properties of the microbial cell wall, surface properties such as surface charge, roughness, hydrophobicity, configuration topo-graphy, and surface free energy also determine the attachment of microbes to a particular surface. Following surface attachment, the adhered cells multiply and form micro-colonies. In parallel, the attached cells also secrete extracellular polymeric substances (EPS) which serve to form a protective matrix around the cells. With time, the biofilm matures resulting in an organized three-dimensional structure.[14] In the final step of this sequence of events, some of the attached cells may disperse from the biofilms to colonize new surfaces (Figure 1). These dispersed cells are known to be different from the "original planktonic" organisms. For instance, dispersed cells are more resistant to antimicrobials than planktonic cells.

The establishment of biofilm lifestyle makes the microorganisms highly resistant to anti-microbials. For example, biofilm infections of Gram-positive or Gram-negative microorganisms may not be eradicated by commonly used antibiotics such as penicillin or metronidazole, which work well for their planktonic counterparts.[15] Similarly, commonly used mouthwashes containing 0.2 % chlorhexidine are not active against biofilms of oral pathogens.[16] Higher drug resistance of the

biofilm mode of growth has been attributed to the following factors: i) altered physiological status of the biofilm cells, ii) protective effect of EPS, iii) presence of a highly drug tolerant subpopulation called persisters, iv) higher anti-oxidative capacities of biofilm cells, and v) differential gene expression profiles of biofilm cells.[12]
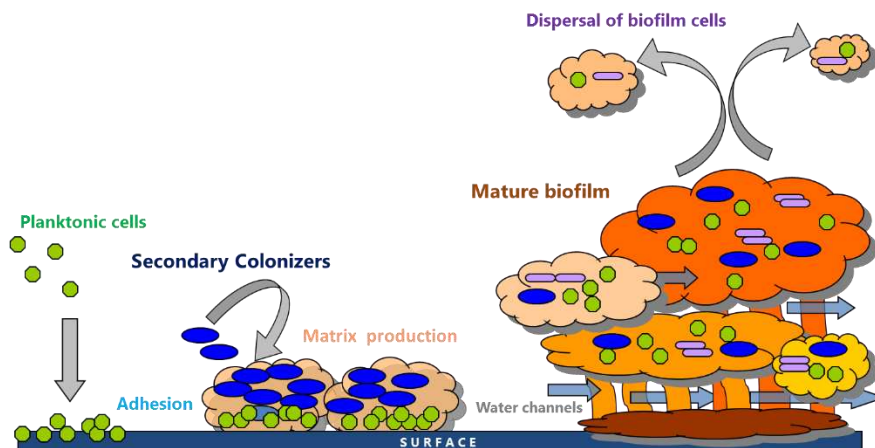

Figure 1. Sequential events taken place during microbial biofilm formation (Reproduced with permission from Seneviratne *et al.*, 2008).

Oral Biofilms

Human body surfaces such as oral cavity, respiratory tract, gastrointestinal tract, genital organs as well as conjunctiva are exposed to external environments and are invariably colonized by various microbial populations, creating unique niches.[17] An interesting feature of the oral cavity is that it provides both hard and soft surfaces for microbial colonization. Therefore, the microbial communities formed on tooth surfaces or dental plaque are different from the ones formed on oral mucosal surfaces. Saliva contains bacteria from various oral niches as well external transient microorganisms.

Dental plaque is an archetypical biofilm.[18] Assessment of the population dynamics of dental plaque or other oral biofilms may be useful in the diagnosis and evaluation of various oral conditions. However, a complete analysis of all the micro-organisms in biofilms is not possible using culture-dependent techniques. Before the advent of DNA-based sequencing techniques, the composition of dental plaque was estimated to be around 200 species. In order to obtain a comprehensive picture of the dental plaque composition, National Institute of Health (NIH) initiated the Human Microbiome Project (HMP) using high-throughput techno-logies such as 16S rDNA sequencing.[19]

Oral Microbiomics

The use of high-throughput sequencing techniques has made it feasible to study the whole microbial community in both health and disease.[20] There are many aspects of diseases that researchers would like to query, such as the etiology, diagnostic and prognostic biomarkers as well as the therapeutic modalities. In the past, most of the oral diseases related studies were hypothesis-driven. Though, these studies have contributed to findings of etiological agents as well as therapeutic modalities, the realization of the complexity of biological systems makes it imperative to look at the big picture. This necessity of understanding how a system works propelled efforts in the development of omics biology. The term omics encompasses various

fields such as genomics, proteomics, transcriptomics, metabolomics, and epigenomics, etc. The initial phase of omics studies is generally hypothesis-free. Hence, the idea behind any omics study is to obtain a complete view of the feature under examination. The holistic evaluation of microbial communities and composition has initiated the field of microbiomics.

One of the commonly used techniques in oral microbiomics is to study the microbial composition and community structure through DNA sequencing of dental plaque or saliva samples of the study population. DNA sequencing involves the precise determination of the order of the four nucleotides, namely, adenine, guanine, cytosine, and thymine—— occurring in a strand of DNA. Gilbert and Sanger pioneered the development of the first DNA sequencing methods.[21,22] Consequently, the first whole genome sequence of bacteriophage φX174 was obtained using Sanger sequencing in 1977[23], followed by the sequencing of Epestein-Barr virus in 1984, and *Saccharomyces cerevisiae* in 1996.[24] In more recent times, shotgun sequencing methods are preferred over the traditional sequencing methods due to their improved speed and accuracy. The first draft of the human genome was released in 2001 by Human Genome Organization (HUGO) using shotgun sequencing methods.[25,26] Next generation sequencing (NGS) is the latest in a series of advanced DNA sequencing technologies that provides a much higher throughput and sequencing depth than that of the conventional methods. Pyrosequencing is one of the NGS techniques that eliminates the need for cloning and sequencing by amplifying a single DNA molecule.[27,28] Roche 454 pyrosequencer can generate up to one million copies in a run with read lengths of 500 to 600 bases.[29] The resultant sampling depth allows for the detection of even rare and low abundance bacterial taxa.[30]

Pyrosequencing methods are able to provide a holistic view of the diversity and composition of oral biofilms revealing the remarkable diversity of oral microbiome.[31,32]. A study of saliva and supragingival plaque samples employing pyrosequencing methods estimated the presence of approximately 19,000 phylotypes in human oral microflora, a considerably higher number than in previous reports.[31] However, in pyrosequencing platforms such as Roche 454, the high

cost of reagents remains a drawback.[33] A combination of 454 and Illumina sequencing platforms has also been used in a recent study to obtain the first gene catalog of dental plaque microbiome.[34] Other sequencing platforms such as Illumina GAIIx and HiSeq 2000 instruments have helped in identifying more than 175 bacterial species at a greater than 90% accuracy rate in human saliva.[35] The SOLiD system (Small Oligonucleotide Ligation and Detection System) based on sequencing by ligation of dye-labeled oligonucleotidesgenerates up to 4 gigabytes of sequence, but with short read lengths of only 35 nucleotides.[27]

In contrast, the Pacific Biosystem system allows for very long read lengths of greater than1,000 nucleotides, but with the setback of the highest error rates (ca. 17%) among all the NGS platforms.[36] The choice of an appropriate sequencing system may vary from sample to sample. Therefore, for different sample types, an optimal balance of factors such as cost, efficiency, and accuracy may help in deciding upon a suitable platform. However, it is also to be kept in mind that 16S rDNA sequencing can provide only the taxonomic details of the sample under investigation, without providing functional characterization.[37]

The high throughput studies of dental plaque and other oral biofilms have revealed that there is a remarkable diversity observed in the oral microbiome.[38] In addition, oral microbiota may also be linked with the systemic diseases such as respiratory tract infections, gastrointestinal diseases, cardiovascular disease, and adverse gestational outcomes makes.[39] However, defining a health or disease-associated core microbiome for oral diseases is still a very difficult task. Human oral microbiome studies have suggested that due to subtle variations, there is a unique microbiome fingerprint for every individual.[40] In addition, there may be variations in the formation of dental plaque biofilms. A recent study demonstrated differences can exist in the ultra-structure and morphology of the dental plaque biofilms of "slow" and "fast" plaque formers.[41] Future oral microbiomics studies should therefore be directed

towards addressing these differences. The detection limit and accuracy of NGS sequencing supersedes the power of traditional culture-dependent techniques. Therefore, future oral microbiomics studies will certainly expand our knowledge on the etiopoathology of dental infectious diseases.

Conclusion

The advent of microbiomics has opened up a new avenue for unravelling the etiopathology of oral biofilm-associated diseases. Although these novel omics techniques have not yet been adequately employed for the above-mentioned purpose at present, growing interest in the field is expected to drive dental research in the future. As Martha Somerman, director of the National Institute of Dental and Craniofacial Research (NIDCR) stated in 2013, omics biology will be instrumental in devising oral health informatics profiles of the individual in future.[42]

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