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Managing Denture Biofilm Related Diseases

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Review

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ABSTRACT

The oral cavity harbors more than 700 microbial species and is one of the most complex ecosystems ever described. While the majority of these microbes are considered commensal, some of them are responsible for oral infectious diseases such as dental caries, periodontitis, halitosis and stomatitis. The advancement of modern science has greatly furthered our understanding of oral microbes and their roles in host health and disease. It has also led to the development of new tools for early detection, effective treatment, and prevention of oral microbial infections. This perspective provides a general understanding of oral microbiology, and its clinical relationship to oral infectious diseases, with a specific focus on denture-related microbial infections. The perspective also discusses the potential for developing innovative interventions for managing denture-related disease based on recent advances in our understanding of oral microbiology and denture-associated biofilms.

KEYWORDS: Stomatitis; Oral microbiota; *Candida albicans*.

ABBREVIATIONS: SAP: Aspartyl proteinases; PL: Phospholipase; PDT: Photodynamic therapy; STAMPs: Specifically Targeted Antimicrobial Peptides.

INTRODUCTION

The association between microbes and oral diseases had long been suspected. Dr. W. D. Miller is generally recognized as the father of modern dental microbial pathogenesis. His 1890 seminal book titled *Microorganisms of the Human Mouth*[1] makes the first connection between bacteria in dental plaque and tooth decay, and remains a foundation of current understanding of dental disease. For a long time, oral microbes had been indiscriminately regarded as pathogens. In fact, their removal from the oral cavity has become the main objective of dentists. Not until recently, did we realize that like microbes associated with other parts of the human body, most of the oral microorganisms are commensal and might have protective role in preventing the colonization of pathogens.[2,3] More importantly, increasing evidence suggests that oral infectious diseases such as dental caries and periodontitis are often the result of the disturbed host homeostasis, and an imbalanced oral microbial ecology often leads to overgrowth of otherwise low abundant opportunistic pathogens.[4,5]

Recent advances in molecular biological techniques are broadening our understanding of bacterial diversity and the societal community interactions which occur between species in the oral cavity.[6] This has led to tremendous advances in our understanding of oral microbiology and its involvement in health and disease, including tooth decay, gum diseases, as well as the diseases associated with artificial dental apparatus introduced through modern dentistry.[6,7]

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ADVANCING UNDERSTANDING OF ORAL MICROBIOLOGY THROUGH MOLECULAR BIOLOGICAL APPROACHES

Our bodies are home to a multitude of microbial organisms that form distinct microflora inhabiting the gut, skin, vagina and oral cavity. These microbial communities have been of great interest to scientists in recent years due to their impact on host health and disease. Increasing lines of evidence indicate that these commensal microbiotas have important metabolic, trophic, and protective functions and greatly affect the host's physiology and pathology.[8,9] For example, the importance of the gut flora in digesting unutilized substrates, training the immune system and protecting against epithelial cell injury is well appreciated,[10-12] and we are beginning to understand its potential role in systemic diseases, such as inflammatory bowel disease[13,14] and obesity.[15]

Molecular biological tools have been critically important for identifying the diversity of these host-associated microbiotas, including the oral microbial community.[16] Prior to the availability of such tools, determining the diversity of complex microbiological communities, such as those of the oral microbiota had been essentially dependent on the ability to culture and identify individual organisms. However, we then realized that only a small fraction of the organisms comprising these microbial communities has been isolated.[6] In fact, accumulating lines of evidence suggested that there are extensive physical and metabolic interactions between different microbial species within the same community, which are essential for the growth and persistence of certain microbes[17] and make them recalcitrant to cultivation. The power of molecular biological approaches, such as culture-independent 16S rRNA gene sequencing-based methods allows us to identify yet-uncultivable species and provides a more comprehensive and detailed inventory of human oral microbiota.[6]

The studies using culture-independent approaches have revealed the sheer magnitude of the diverse microbes, including yet-uncultivable species residing within the oral cavity.[18,19] The human mouth is estimated to harbor more than 700 different bacterial species, comprising one of the most complex microbial flora.[18] The diversity of microorganisms that inhabit the oral cavity includes bacteria, archaea, protozoa and fungi.[20,21] An interesting perspective regarding diversity of the oral flora is the presence of Archaea as a constituent.[22] Phylogenetically, Archaea is among the oldest known type of prokaryotes; it has previously been isolated from ocean bottom, and yet also appears to be a colonizer of the human oral cavity with yet-to-be-determined role in oral microbial ecology.[23] The diversity of the microbial flora reflects tremendous genetic information and immense bio-physiological potential that may have huge impact on host health and disease. If we consider that an average bacterial species has 2,000-6,000 genes, then an oral bacterial population of some 700 individual species represents a pool of over 1 million genes, 10 times more than human host genes. This provides the oral microbial environment with a huge quantity of information related to unique metabolic pathways, the generation and secretion of various factors that can control and modify their ecological niche, and factors that may impact function of the human host.

THE STRUCTURE OF DENTAL AND DENTURE PLAQUE

Bacteria in the oral cavity often reside within biofilms, such as those that form dental and gingival plaque.[24] For edentulous and partially edentulous individuals who wear dentures, a denture-associated biofilm, or denture plaque, forms on the denture surface and could potentially serve as a reservoir of pathogenic microbes for infections.[7]

Dental and denture plaque are not simple matrices. They consist of a diverse collection of microbial species, and furthermore have a highly organized structure in which different species can occupy specific sites or niches within the biofilms.[25] During dental biofilm formation, bacteria that are early colonizers, such as Streptococci (i.e., *S. gordonii*, *S. oralis*, etc.) with specific adhesins can effectively bind to proteins deposited as a pellicle coat on the tooth surface. This is followed by the subsequent recruitment of intermediate and late colonizing species through cell-cell coadhesion *via* specific adhesin-receptor interactions.[25] These specific bacterial physical associations eventually generate a highly structured microbial community, which we recognize as dental plaque or biofilms.[5,6,26] Furthermore, bacterial species within dental biofilm are often engaged in extensive signaling and metabolic interactions to ensure their survival within the microbial community.[6]

Dental biofilms of healthy subjects harbor a commensal oral microbial community with properties that limit the invasive potential of opportunistic pathogens.[3,27] And likemost ecological communities, once established, dental biofilm generally has a stable and controlled population of different organisms and displays resilience to environmental disturbance.[28,29] However, as will be discussed in the following section, the microbial composition of denture biofilm flora and their pathogenic potentials could differ significantly from that of healthy dental biofilm, thus contributing to the pathogenesis of denture-biofilm related diseases, such as stomatitis.

CONNECTIONS BETWEEN MICROBES AND ORAL DISEASES ASSOCIATED WITH DENTURE WEARING

Denture stomatitis is a common disorder in subjects wearing dentures, which are prostheses that provide important functional and esthetic improvements for edentulous and partially edentulous patients.[30,31] The disorder is characterized as inflammation and erythema of the oral mucosal areas covered by the denture. The current view regarding the etiology of denture stomatitis is that it is a multifactorial infectious disease. It involves a number of associative factors, including denture-induced trauma, continual denture wearing and denture plaque harboring pathogenic microbes, such as Candida.[30] Among those

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factors, the microbial biofilm formed on the denture surface plays a significant role in contributing to the disease pathogenic process. Whereas the normal commensal oral microbial community could prevent infection by interfering with the invasive potential of opportunistic pathogens, this is altered in the denture biofilm. Indeed, the microbial composition of the biofilm which forms on denture surfaces differs significantly from that observed in the oral cavity of healthy individuals.[32,33] This could be due to the fact that denture wearing can alter normal oral physiology by affecting normal salivary flow that plays an important role in shaping the microbial community.[34] Meanwhile, the older segment of the population often has a comparable higher proportion of denture wearer.[35] These individuals are more likely to have systemic health conditions and could have already had imbalanced oral microbial ecosystem due to disturbance in host homeostasis. Furthermore, a denture provides a unique abiotic surface for microbial colonization, which often leads to the development of a denture biofilm with microbial composition and structure different from normal oral biofilm.[7,32]

Compared to the normal oral and dental biofilms, denture biofilmsare associated with a much higher occurrence of *Candida* yeasts, particularly *Candida albicans*.[36] *C. albicans* is a commensal fungal species commonly colonizing human mucosal surfaces. It co-exists with diverse oral microbial species and has long been adapted to the human host.[37] In healthy individuals, *C. albicans* is usually a minor component of their oral microfloras. However, under conditions of immune dysfunction or local predisposing factors such as poor oral hygiene or ill-fitted dentures, colonizing *C. albicans* can become an opportunistic pathogen. In these patients, *C. albicans* becomes more predominant and invasive, causing recurrent mucosal infections such as denture stomatitis.[38] The presence of *C. albicans* on denture and oral mucosal surfaces of denture wearers is positively associated with denture stomatitis.[39] The virulence factors of *C. albicans* have been well documented.[40] Among them, multiple host recognition biomolecules, such as Als1p and Hwp1p,[41,42] as well as the secreted enzymes, including Aspartyl proteinases (SAP)[43] and Phospholipase (PL)[44] have been shown to play important role in determining *C. albicans'* pathogenicity. Meanwhile, its polymorphic growth patterns[40] as well as phenotypic switching[45] have also been implicated in contributing to its virulence. While *C. albicans* infection cannot be claimed as the single causal pathogen for inducing denture stomatitis, it has a strong associative presence when the disorder occurs, and its eradication from denture and mucosal surfaces is associated with reversal of the condition.[46,47] Hence, it is generally accepted that *C. albicans* is a main opportunistic pathogen which is involved in the development and pathogenesis of denture stomatitis. Meanwhile, certain bacterial species, such as *Prevotellasp*., *Veillonellasp*. and *Staphylococcus sp*. have been found to be enriched in denture biofilms,[48,49] although their potential role in denture stomatitis pathogenesis remains to be determined. More importantly, increasing lines of evidence indicate the extensive Candida-bacterial interactions, which could impact their pathogenicity.[37] For example, co-infection of *C. albicans* and *Staphylococcus aureus* has been shown to lead to increased mortality in animal model.[50] A better understanding of the physiology of Candida and bacteria co-existence and the inter-kingdom Candida-bacterial interactions would shed light on the impact of polymicrobial infection on the etiology of denture-related stomatitis.

TREATMENT OF DENTURE-RELATED INFECTIOUS DISEASE

Plaque formed on the denture surface often serves as a reservoir of opportunistic pathogens, including *C. albicans* for infections. In addition to maintaining good general oral hygiene, the most recommended approach to managing and preventing microbial-related disease associated with denture use is for patients to maintain a high level of denture hygiene by appropriate cleaning.[51,52] Common approaches to denture cleaning utilized by patients include brushing with abrasive cleansers, such as toothpastes, and washing or soaking dentures using commercial chemical cleansers with antimicrobial compounds designed for this purpose. The latter is preferred, as brushing with abrasive cleansers has been shown to be less effective for removal of the biofilm, and furthermore, can roughen the denture surfaces and result in more rapid bacterial adherence and biofilm growth.[7,53] However, as observed with other biofilms, a problematic issue associated with denture plaque is that it reduces the effectiveness of antimicrobial, including antifungal treatment.[54] The mechanisms by which biofilm environments enhance antimicrobial resistance are not fully understood. However, putative mechanisms likely include decreased ability of the antimicrobial agents to penetrate and diffuse within the biofilm matrix, protective functions conferred to the putatively susceptible bacteria due to slower growth rates and even changes in phenotype, and perhaps protective factors secreted by other microbes within the biofilm community which can degrade the applied antimicrobial agents.[55] In addition, the materials used in the manufacture of dentures can also affect adherence and colonization by microbes, including *C. albicans*, as well as impact the efficacy of antimicrobial treatment on thebiofilm.[56]

Soaking dentures in an appropriate commercial cleanser has been shown to be effective in removing attached microbes without increasing surface roughness.[57] Overnight denture removal is also important for controlling denture plaque, as it isolates the denture from salivary secretion that provides nutrients for microbial growth of denture biofilm. In addition to maintaining denture hygiene, various antimicrobials, including imidazole (clotrimazole, ketaconazole), triazole (fluconazole, itraconazole) and polyene (nystatin, amphotericin B) antifungals for treating Candida, and antibiotics for treating bacterial pathogens were also recommended for controlling denture-related mucosal infections.[58,59]

More recently, a new antifungal therapeutic approach Photodynamic (PDT) therapy has been used to treat denture stomatitis.[60] PDT uses a photosensitizing agent and light of appropriate wavelength. The interaction between the photosensitizer and light in the presence of oxygen produces reactive species that induce cell damage and death.[61] In a recent clinical trial, PDT was shown to be as effective as topical nystatin in the treatment of denture stomatitis.[62] Since PDT can effectively kill Candida species, including strains resistant to conventional antifungal agents,[63] it has been regarded as a promising method for the treatment of dental stomatitis. Recurrence of stomatitis is frequently observed within short period of time after stopping antifungal treatment.[64] This is likely due to reinfection by residual pathogens that remain within plaque on dentures and are resistant to treatment. Meanwhile, many patients failed to respond to the usual treatment, largely due to the development of drug resistance of candida species. For patients with systemic diseases, such as type 2 diabetes mellitus or being immunocompromised,[65] they often show less responsiveness to the treatment as well. When treating these patients, combined efforts including antifungal treatment and improving patients overall health status are critical in determining the outcome.[66]

The knowledge we are gaining from molecular biological studies of dental and denture biofilms is contributing to the development of novel therapeutic tools.[6,67] One approach is to build on our ability to identify specific pathogenic organisms that inhabit the biofilm, and develop therapeutics that specifically target these organisms. An example of this approach undertaken by our research group is the development of STAMPs (Specifically Targeted Antimicrobial Peptides).[68,69] A typical STAMP consists of two functional moieties conjoined in a linear peptide sequence: a nonspecific antimicrobial peptide serves as the killing moiety, whereas a species-specific binding peptide provides specific binding to a selected pathogen and facilitates the targeted delivery of the attached antimicrobial peptide. The feasibility of this approach has been demonstrated by the development of C16G2, a STAMP specifically targeting *S.mutans*, the bacterium known to cause dental caries. C16G2 has been shown to remove *S. mutans* within *in vitro* multi-species biofilms with high efficacy and specificity,[68,70] and is under further animal and human evaluations.[71] The successful demonstration of this targeted approach could serve as proof-of-concept for applying this technology to the treatment of denture-related Candida infections.

CONCLUSIONS

The past decade has witnessed significant advances in our understanding of oral microbiota. We now better understand the structural and functional complexity of dental and denture plaque, and a strong connection between oral microbial ecology and host health and disease has been established. It is well known that the control of microbial pathogens, such *C. albicans* on dentures and in the oral cavity is critical for the oral health of denture wearers. Continued efforts using modern scientific methods will help us develop more diagnostic tools and therapeutic interventions for the identification, treatment and prevention of denture infections. New and improved approaches will be able to treat and control denture infections with less physical damage to denture surfaces by providing improved mechanisms for killing and removing microorganisms in the denture biofilm. We can envisage products that will have targeted killing of selective pathogens without affecting other commensal species within the same denture biofilm. Finally, we can also expect to see new products that will be able to enhance natural oral immunity, and provide cavity protection or control gingival disease in dentate individuals, and other inflammatory disorders in denture wearers.

CONFLICTS OF INTEREST

The authors declare that Wenyuan Shi is an employee of C3 Jian, Inc. which has licensed technologies from UC Regents that could be indirectly related to this research project.

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