Control of oral biofilms

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Oral biofilms play a major role in the etiology of oral diseases, and have broad implications for quality of life, systemic health and economic costs. Hypotheses regarding the mechanisms by which oral biofilms exert their pathogenic potential have evolved over time. Early theories in which oral diseases were associated solely with the quantity of colonizing biofilms have been replaced by the understanding that emergence of specific pathogenic microorganisms may lead to diseases. New insights into the structure and composition of oral microbial communities have implicated shifts in the composition of the resident microbiota in the development of oral diseases. Improved knowledge about the factors involved in the etiology of oral diseases has resulted in a refinement of traditional approaches for the control of oral biofilms and spurred the development of new preventive and therapeutic strategies.

This volume of Periodontology 2000 provides an in-depth analysis of current beliefs regarding the structure and composition of oral microbial communities, microbial shifts associated with oral health and disease, transmission of oral pathogens, and technologies for the assessment of complex microbial communities. Trends in the prevalence of oral biofilm-associated diseases and their implications are discussed, and approaches to the control of oral biofilms are critically appraised.

Dental plaque biofilms: communities, conflict and control

Dental plaque is a surface-associated, structurally and functionally organized multi-species microbial biofilm. Living in a biofilm community provides many advantages for oral microorganisms (5). It broadens their habitat range, improves metabolism, increases tolerance to antimicrobial agents and host defense mechanisms, and enhances bacterial virulence. The resident microbiota also benefit the host by preventing colonization by potentially pathogenic exogenous microorganisms and contributing to host physiology.

The properties of the oral habitat determine which organisms colonize, grow and predominate, and result in biofilms with distinct species composition in various habitats of the oral cavity, e.g. mucosal surfaces and supra- and subgingival dental surfaces. As a result of the dynamic balance imposed by numerous microbial interactions, component species at a colonization site can remain relatively stable over time. Environmental changes may lead to rearrangement in community structure and composition, and thereby predispose the host site to disease. The dynamic relationship between the resident microbiota and the host in health and disease has led to an ‘ecological plaque hypothesis’, which suggests that successful treatment of oral biofilm-associated diseases requires that the underlying changes in the environment that drive microbial shifts are addressed. Furthermore, rather than trying to eliminate oral biofilms, regimens aimed at treating or preventing oral biofilm-associated diseases should control both the level and activity of the oral microbiota.

Microbial shifts and periodontitis

Due to considerable variation in the composition of the subgingival microbiota between individuals with similar periodontal health or disease status, the etiology of periodontitis cannot be readily explained by a single-pathogen model (2). Current theories favor a shift in the microbial composition with a decrease in beneficial symbionts and an increase in pathogens in the etiology of periodontitis, and thereby consider entire microbial communities as pathogenic.

Scaling and root planing, the mainstay of periodontal therapy, has been shown to reverse the microbial shifts associated with periodontitis and re-establish subgingival microbiota similar to those found in periodontal health. Well-established adjunctive treatments for periodontitis include...
locally delivered and systemically administered antibiotics, antiseptics and host-modulating agents. There is evidence that these adjunctive treatments improve the clinical outcome of periodontal therapy at severely diseased sites compared with scaling and root planing alone. Use of probiotics as an adjunct to scaling and root planing has been shown to delay re-colonization with putative periodontal pathogens in an animal model and to reduce gingival inflammation. The safety and efficacy of this novel approach for the treatment of periodontitis remain to be evaluated in humans.

**Bacterial and viral pathogens in saliva: disease relationship and infection risk**

Bacteria reside in the oral cavity either suspended in saliva or embedded in surface-associated biofilms (11). There is a close inter-relationship between the planktonic and biofilm phases, as bacteria in saliva adhere to oral surfaces to form biofilms and biofilms release bacteria into saliva by shedding. A host of microorganisms, including putative pathogens for oral biofilm-associated diseases such as *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans* and *Streptococcus mutans*, medically relevant bacteria including *Streptococcus pyogenes* and *Helicobacter pylori*, and viruses such as herpesviruses, human immunodeficiency viruses, hepatitis viruses and human papillomaviruses, have been found at elevated levels in saliva of diseased individuals. There is evidence to suggest that saliva serves as a vector for transmission of these microorganisms between individuals. Vertical and horizontal transmission have been demonstrated for *A. actinomycetemcomitans*, *P. gingivalis*, *S. mutans* and several viruses.

The available evidence regarding the diverse microbial communities in saliva indicates that a whole-mouth approach that targets not only surface-associated biofilms but also salivary microorganisms is required for prevention of pathogen transmission and therapy of oral diseases.

**High-throughput methods for analysis of the human microbiome**

Understanding of oral microbial communities, biofilm formation and host-microbial interactions is inherently linked to the availability and capability of technologies that can be used to investigate these complex biological systems (9). As half of the oral microorganisms are non-cultivable, culture-independent approaches are required to comprehensively assess the oral microbiome. Methods that do not require culturing for the identification of microorganisms include quantitative RT-PCR, phylogenetic 16S rRNA gene clone library analysis, checkerboard hybridization, analysis of terminal restriction fragment length polymorphisms, microarray analysis and pyrosequencing. A phylogenetic approach using 16S rRNA gene clone library analysis has been used to investigate the diversity of cultivable and non-cultivable oral bacterial species. This method is laborious, not quantitative and fraught with cloning bias. Fingerprinting of amplified PCR products as performed in terminal restriction fragment length polymorphism analysis allows assessment of complex bacterial communities. As different taxa can have the similarly sized terminal restriction fragments, parallel clone libraries are necessary for identification of bacterial species. The checkerboard hybridization technique using whole genome- or 16S rRNA-based probes has been used to comprehensively examine the microbial composition of oral biofilms. Non-specific target binding is a major shortcoming of this otherwise rapid and sensitive technique.

Although the techniques described above have helped to understand the role of certain oral microorganisms in oral health and disease, these techniques are of limited use when trying to analyze the complete oral microbiome and transcriptome. Next-generation sequencing has the potential to elucidate not only which microorganisms are present in the oral cavity, but also how metabolically active they are and how entire oral communities respond to environmental changes and treatments. Furthermore, it may assist in our understanding of the host response to shifts in oral microbial communities.

**Oral biofilm-associated diseases: trends and implications for quality of life, systemic health and expenditure**

Oral biofilms play a major role in the etiology of periodontal diseases, dental caries, pulpal diseases, apical periodontitis, peri-implant diseases and oral candidosis (1). Caries and periodontal diseases...
remain the most prevalent oral diseases, and are responsible for more than 75% of all tooth extractions. Caries experience in children varies considerably in different parts of the world. It is relatively high in the Americas and in Europe, and lower in most of Africa and Asia. Preventive efforts over the past decades have markedly decreased the caries experience in children, adolescents and young adults in most, but not all, countries of the world. Caries experience in adults remains high, with a prevalence approaching 100% in developed countries. The prevalence of periodontitis has shown heterogenous trends throughout the world. Although periodontal health has improved in some parts of Africa and Asia, the prevalence of periodontitis has remained unchanged or even increased in other parts of Asia, the Americas and Europe. The most dramatic improvement in periodontal health over the past 50 years has been reported in the USA.

Oral biofilm-associated diseases have broad implications that extend beyond the ailments they cause in the oral cavity. They may have an impact on an individual’s ability to function, as well as affecting the perception of well-being in physical, mental and social domains of life. Systemic health is also affected by (i) spreading infections to adjacent tissues and spaces, (ii) hematogenous dissemination of oral biofilm bacteria, or (iii) inflammatory mechanisms. Oral biofilm-associated diseases have a significant cost burden in societies. It has been estimated that more than 85% of all dental services are related to the diagnosis, prevention or treatment of oral biofilm-associated diseases and their sequelae, e.g. lost teeth. At an estimated $81 billion in the USA in 2006, the national expenditure for oral biofilm-associated diseases was greater than for any one of the five most expensive medical conditions, including heart conditions, trauma-related disorders, cancer, mental disorders and pulmonary conditions.

Oral hygiene in the prevention of periodontal disease: the evidence

Self-performed mechanical plaque control is the mainstay in prevention of periodontal diseases (13). A single, self-performed toothbrushing exercise using a manual toothbrush results in a mean reduction of plaque scores of 43%, indicating that most people are not very effective in removing dental plaque from the surfaces of their teeth. Electric toothbrushes, in particular those with a rotating/ oscillating motion, have been shown to increase plaque removal efficacy by 7–17% compared to manual brushes. Plaque control at interproximal tooth surfaces is best achieved using interdental brushes, provided the interdental space is not filled with gingival tissues. Although dental flossing may be the method of choice at sites with intact interdental gingival tissues, the available evidence indicates that self-performed interdental flossing is not very efficacious in removing interproximal dental plaque or improving gingival health. This may be partly due to low compliance with interdental flossing. Oral irrigators have been shown to significantly reduce gingival inflammation, despite minimal efficacy in the removal of dental plaque.

Although toothpastes add little to the plaque removal efficacy of toothbrushing, they are an important vehicle for the delivery of fluorides, and therefore play an important role in the prevention of caries. Further additives in toothpastes, most notably triclosan with polyvinyl methyl ether and maleic acid co-polymer and stannous fluoride, have been shown to enhance the efficacy of toothbrushing in gingival health.

Subgingival air-polishing in the treatment of periodontal biofilm infections

Air-polishing with sodium bicarbonate at a particle size of up to 250 μm is very efficient in removing oral biofilms and stains (8). However, safety concerns regarding damage of exposed root surfaces, gingival tissues and restorative materials have greatly limited its use. Use of fine-grain glycine with a maximum particle size of 63 μm in air-polishing has an abrasive effect on human root surfaces that is approximately 80% less than that of sodium bicarbonate, and it has been shown to be safe when applied directly to gingival tissues and restorative materials. Due to its low abrasiveness, fine-grain glycine expands the potential use of air-polishing to removal of subgingival biofilms. In patients receiving periodontal maintenance care, air-polishing with glycine powder has been shown to be safe and efficacious for removal of subgingival biofilms to a pocket probing depth of up to approximately 4 mm, to achieve significantly greater reduction in subgingival microbial counts, and is perceived as more pleasant than subgingival debridement using curettes. Clinical outcomes were not different for sites treated by air-polishing with glycine powder compared to sites treated with curettes at a 15-month follow-up. When used in conjunction with glycine powder air-polishing,
conventional debridement techniques are needed for the removal of calculus.

Photodynamic therapy in the control of oral biofilms

Photodynamic therapy is based on the fact that light-absorbing photosensitizers can be taken up by bacteria, and, when activated by light, generate cytotoxic singlet oxygen and free radicals (12). Photodynamic therapy has recently been investigated for targeted treatment of oral infections. Antimicrobial photosensitizers such as toluidine blue O and methylene blue, which carry a positive charge, can be activated by a diode laser and directly target both gram-negative and gram-positive bacteria. Several putative periodontal pathogens have been shown to be susceptible to photodynamic therapy using various photosensitizers. Bacteria embedded in biofilms show reduced susceptibility to photodynamic therapy compared to bacteria in planktonic phase. Despite promising in vitro results, the evidence supporting the clinical efficacy of photodynamic therapy in the treatment of periodontitis is controversial at best. Most clinical trials reported so far found no incremental benefit of photodynamic therapy when used as an adjunct to scaling and root planing in the treatment of periodontitis. However, case reports indicated that photodynamic therapy may have potential in the treatment of caries, peri-implantitis, endodontic infections and oral candidosis.

In order to enhance the efficacy of photodynamic therapy, photomechanical waves have been used for the delivery into oral biofilms of photosensitizers, monoclonal antibodies against putative pathogens conjugated with photosensitizers, and photosensitizers encapsulated in polymer nanoparticles. Although photodynamic therapy holds promise in the treatment of several oral biofilm-associated diseases, further clinical trials are required to assess its safety and efficacy in the management of oral biofilm-associated diseases.

Calculus removal and prevention of its formation

Biofilms formed on non-shedding oral surfaces such as teeth or dental restorations may absorb calcium and phosphate ions from saliva or gingival crevicular fluid to form calculus (4). Initially, hydroxyapatite crystals form in the biofilm matrix, and then expand into bacterial cells. Hydroxyapatite, whitlockite, octacalcium phosphate and brushite are the predominant crystal formations in calculus. Calculus in itself appears to be non-pathogenic, but, due to its rough surface, harbors oral biofilms associated with oral diseases. Thus, prevention and removal of supra- and subgingival calculus are critical in the management of periodontal diseases.

Complete removal of subgingival calculus in patients with periodontitis remains an elusive goal. The amount of residual calculus following scaling and root planing depends on a number of factors, including the depth of the periodontal pocket, access to the subgingival root surfaces (closed or surgical approach), tooth type (single- versus multi-rooted), tooth surface (buccal/lingual versus interproximal and flat surface versus furcation), instrument design, and, most importantly, the competency of the person performing the procedure. Flap surgery, in particular in deep periodontal pockets, enhances access to the subgingival root surfaces and results in greater calculus removal compared to scaling and root planing using a closed approach.

Hand instruments, sonic scalers, ultrasonic scalers and Er:YAG lasers have been used for removal of supra- and subgingival calculus. In terms of clinical outcomes, there is no strong evidence to support the superiority of any of these techniques in the treatment of periodontitis. However, an autofluorescence-controlled Er:YAG laser, which selectively removes calculus, has been shown to result in less damage of the root cementum than conventional instrumentation techniques. Whether or not this histological finding is clinically relevant and whether it affects patient-centered outcomes such as root hypersensitivity remain to be demonstrated.

Antimicrobial agents, including triclosan with polyvinyl methyl ether and maleic acid co-polymer, and agents that inhibit crystal growth, such as pyrophosphates, zinc chloride and zinc citrate, have been shown to reduce the formation of calculus. As these substances do not penetrate into the periodontal pocket when incorporated in toothpastes or mouthrinses, their efficacy is limited to prevention of supragingival calculus formation.

Calculus detection technologies and their clinical application

Detecting subgingival calculus and assessing the therapeutic end-point during periodontal therapy
is generally achieved by tactile sensation (6). As tactile calculus detection is generally unreliable, and shows considerable inter-examiner variability, sub-gingival debridement results in varying degrees of residual calculus and removal of root cementum. Calculus detection technologies are intended to assist the clinician during periodontal therapy in selectively removing calculus and biofilm from subgingival root surfaces while preserving the underlying cementum. Reliable calculus detection promises to improve clinical outcomes and reduce adverse events, such as root sensitivity due to over-instrumentation. Currently available devices either only detect calculus or combine the features of calculus detection with the ability to debride tooth surfaces.

Detection-only systems are based on fiber-optic endoscopy or spectro-optical or autofluorescence technologies. Although high sensitivities and specificities for the detection of calculus have been demonstrated for several of these technologies, the effect of improved detection on clinical outcomes following periodontal therapy has not been thoroughly investigated. A major drawback of detection-only systems lies in the need to alternate between detection and debridement. In transition from a detection device to a debridement instrument, information regarding where the detected calculus is located may be lost. Thus, although detection-only devices may assist in determining when calculus has been removed, they do not seem to prevent over-instrumentation.

Combined detection/debridement devices are based on ultrasonic or laser technology. Autofluorescence-controlled activation of an Er:YAG laser has been shown to enable selective removal of calculus while mitigating root damage. Despite these advantages, no differences in clinical outcomes were found following debridement using an autofluorescence-controlled Er:YAG laser compared with conventional mechanical debridement. A novel ultrasonic-based technology that combines calculus detection and debridement has shown good calculus detection capabilities. Trials are currently being performed to assess the clinical value of this combined calculus detection/debridement systems.

Cost-effectiveness of adjunctive antimicrobials in the treatment of periodontitis

Economic evaluations in health care have become increasingly important for health policymakers, third-party payers, health providers and patients in order to decide how limited resources are allocated to reap the greatest health benefit (3). Cost-effectiveness analysis, cost-utility analysis, cost–benefit analysis and cost-minimization analysis have been performed to assess economic outcomes. Incremental cost-effectiveness analysis evaluates a change in costs relative to the change in outcomes. Such an analysis, using quality-adjusted tooth years as the outcome, has shown that use of adjunctive antimicrobials is cost-effective in the treatment of moderate to severe periodontitis. Flap surgery, with or without osseous re-contouring, was found to be dominated by non-surgical therapies and thus not cost-effective. Another cost-effectiveness analysis in the UK indicated that systemic administration of antibiotics as an adjunct to scaling and root planing is twice as cost-effective as the use of locally delivered antimicrobials. As healthcare costs vary between countries, the results of cost-effectiveness analyses cannot be readily extrapolated from one
healthcare system to another. Actual costs must be used when assessing the cost-effectiveness of treatments in a given healthcare system.

**Prevention of crown and root caries in adults**

Prevention of crown and root caries involves three major approaches: (i) controlling the oral biofilm and its composition, (ii) altering the balance between de-mineralization and re-mineralization to avoid net loss of tooth mineral, and (iii) reducing dietary carbohydrate intake (10). Mechanical plaque control using toothbrushes, interdental brushes, dental floss and tooth picks in conjunction with fluoridated toothpaste remains the mainstay of caries control. Use of antimicrobial agents or sugar alcohols as an adjunct to mechanical plaque control in combination with a fluoride toothpaste has shown conflicting results in the prevention of caries. Individuals that benefit most from the use of sugar alcohols are those presenting with a high caries risk.

Although most of the clinical evidence for the efficacy of fluoride in the prevention of coronal caries stems from trials that enrolled children and adolescents, there is some evidence that fluorides are effective in the prevention of coronal caries in adults. Higher concentrations of fluoride are required for the prevention of root caries compared to prevention of coronal caries. Amorphous calcium phosphates appear to have an added effect on tooth remineralization when used in conjunction with fluoride.

Promising novel approaches that have not yet been subjected to rigorous clinical testing include use of antimicrobial peptides targeted at eradication of *S. mutans*, replacement of the indigenous oral *S. mutans* population with lactate dehydrogenase-deficient strains, introduction of probiotic strains, e.g. *Streptococcus rattus* JH145, infiltration of incipient coronal caries with a low-viscosity resin, and sealing of root caries lesions using glutaraldehyde-containing adhesives.

**Candida biofilms and oral candidosis: treatment and prevention**

There are over 350 heterogeneous species of *Candida*, of which *Candida albicans* is the most prevalent isolate from human candidosis (14). Oral candidosis is mostly found in individuals who are receiving immunosuppressive therapy or broad-spectrum antibiotics, or have undergone invasive surgical procedures such as solid-organ or hematopoetic stem-cell transplantations. *Candida* biofilms in human infections occur on the mucosa itself and on biomaterials. Pseudomembranous candidosis and hyperplastic candidosis are associated with a multilayered growth of *Candida* adhering to the mucosal surface. *Candida* biofilms on dental prostheses are mostly associated with chronic erythematous candidosis. Wearing dentures also predisposes edentulous patients to other forms of oral candidosis, such as hyperplastic candidosis and angular cheilitis.

Management of oral candidosis includes correction of the underlying predisposing factors and use of antifungal agents, including polyene, azole and echinocandin antifungals. Fluconazole is the agent of first choice for all forms of oral candidosis other than chronic erythematous candidosis. Nystatin and amphotericin have limited efficacy as they only act topically, but may be used in conjunction with chlorhexidine rinses, 30 min apart, for treatment of chronic erythematous candidosis. Mechanical removal of the biofilm from denture surfaces is an important component of the candidosis treatment, and this may be enhanced by the use of cleansers, including alkaline and neutral peroxide-type, hypochlorite, tetrasodium EDTA or acidic solutions.

**Concluding remarks**

Self-performed and professionally administered control of oral biofilms remains the mainstay in prevention of oral biofilm-associated diseases. In combination with the use of antimicrobials, anti-inflammatory agents, inhibitors of tooth de-mineralization and/or management of disease-specific risk factors, adequate control of oral biofilms can prevent and successfully treat most oral biofilm-associated diseases. However, conventional approaches to controlling oral biofilms are very reliant upon compliance, which varies considerably among individuals. This may explain why the prevalence of oral biofilm-associated diseases remains high in so many populations.

Despite substantial advances in our understanding of the etiology and pathogenesis of oral biofilm-associated diseases, improvements in clinical care have lagged behind. In order to further reduce the prevalence of oral biofilm-associated diseases, it may not be sufficient to provide further oral hygiene
instructions and modify risk behavior. Approaches for the control of oral biofilms that are less reliant upon compliance and regular access to professional dental care and/or disruptive technologies are needed. Treatments that aim to prevent the transmission of putative oral pathogens, inhibit the attachment of oral microorganisms on oral surfaces, or create long-lasting shifts in the oral microbiota hold much promise. Future research exploring these and other avenues will provide guidance on how to better prevent and manage pervasive oral biofilm-associated diseases.

References
