ISSN 1807-5274
Rev. Clín. Pesq. Odontol., Curitiba, v. 5, n. 1, p. 53-57, jan./abr. 2009

THE BIOFILM CONCEPT AND ITS ROLE IN PREVENTION OF PERIODONTAL DISEASE

O conceito de biofilme e seu papel na prevenção da doença periodontal

Nitin Khuller

MDS, Department of Periodontics, ITC Centre for Dental Studies and Research, Ghaziabad, Índia, e-mail: nkhuller@yahoo.com

Abstract

OBJECTIVE: To provide an overview of the history of oral microbiology, a discussion of dental plaque as both a microbial community and a biofilm, and a review of the measures available to control the oral microflora. **METHODOLOGY**: The author reviewed the literature related to oral microbiology and associated infectious diseases. He also examined articles that detailed the structure and physiology of biofilms, including dental plaque biofilms. **RESULTS and DISCUSSION**: Biofilms cannot be eliminated. The pathogenic nature of the dental plaque biofilm can be diminished in the oral cavity by reducing the bioburden and effectively maintaining a normal oral flora via oral hygiene procedures that include daily toothbrushing, flossing and rinsing with an antimicrobial mouthrinse. An oral hygiene regimen that includes rinsing with an antimicrobial mouthrinse is a practical approach to the prevention and management of periodontal diseases. This strategy may have wider benefits when the link between periodontal disease and certain systemic diseases is considered. **CONCLUSION**: An effective oral hygiene regimen can help control dental plaque biofilm and associated periodontal diseases.

Keywords: Oral Pathogens. Koch's postulates. Dental plaque biofilm. Microbial community. Antimicrobial mouthrinses.

Resumo

***OBJETIVOS**: Apresentar uma visão panorâmica da história da microbiologia bucal, uma discussão sobre a placa dental como uma comunidade microbiana e como biofilme, e uma revisão das medidas disponíveis para controle da microflora bucal. **METODOLOGIA**: O autor revisou a literatura relacionada com microbiologia bucal e doenças infecciosas associadas. Igualmente examinou artigos que detalharam a estrutura e a fisiologia dos biofilmes, incluindo biofilme da placa dental. **RESULTADOS E DISCUSSÃO**: Biofilmes não podem ser eliminados. A natureza patogênica do biofilme placa dental pode ser diminuída pela redução da carga biolótica e*

mantendo efetivamente a flora normal por meio de procedimentos de higiene bucal, que incluem escovação diária, uso de fio dental e colutórios com antimicrobianos. Essa estratégia pode ter benefícios amplos quando a ligação entre doença periodontal e certas doenças sistêmicas é considerada. ***CONCLUSÃO****: O regime efetivo de higiene bucal pode ajudar no controle do biofilme placa dental e doenças periodontais associadas.*

Palavras-chave*: Patógenos bucais. Postulados de Koch. Biofilme placa dental. Comunidade microbiana. Colutórios antimicrobianos.*

INTRODUCTION

Microbial biofilms are common in nature. Virtually any fluid environment in which microorganisms are subject to stress or flow can create conditions for biofilm growth. The mouth is an ideal environment for biofilm development. The importance of dental plaque biofilms for oral and dental disease was proposed recently (1). This article provides an overview of microbiology and a discussion of dental plaque as both a complex microbial community and a biofilm. Although dental plaque cannot be eradicated, it can be controlled with oral hygiene measures that include a daily regimen of brushing, flossing and rinsing with an antimicrobial mouthrinse.

OVERVIEW OF THE MICROBIOLOGY

Discoveries in the field of clinical microbiology occurred from the late 1800s through the early 1900s. For the first time, scientists identified microbial pathogens as the cause of many systemic diseases of medical importance (2). Among the great contributors to this era were Louis Pasteur– who proved that spontaneous generation of organisms did not exist, established that disease can be caused by a single organism and developed the "germ theory" –and Joseph Lister, who integrated the germ theory into surgical practice. Building on the theory that specific pathogens cause disease, German physician Robert Koch developed four criteria that had to be met to establish a causal relationship between a pathogen and a disease. The implication was that removal or reduction of the pathogen might halt or reverse the disease process. Koch's postulates became an integral part of microbiology, though Koch later recognized the limitations of his theory, namely that some people can be asymptomatic carriers of disease.

PERIODONTAL MICROBIOLOGY

From the mid-1960s through the 1970s, the nature of dental plaque became a significant focus for dental scientists and the dental research community. Emphasis was placed on factors contributing to the diversity of microbial ecosystems, including pH, oxidation-reduction potential and nutritional requirements. In 1976, Loesche (3) recognized the importance of the plaque ecosystem and proposed both a nonspecific and a specific plaque hypothesis for oral disease progression. The nonspecific plaque hypothesis maintained that periodontal disease resulted from an "elaboration of noxious products by the entire plaque flora." Large accumulations of plaque would produce large amounts of noxious products, which would essentially overwhelm the host defense and cause periodontal disease. Thus, all the microorganisms within plaque were viewed as contributing to the development of periodontal disease, and identifying a single microorganism was not important. Oral hygiene measures that seek to remove as much of the total plaque mass as possible became paramount for the maintenance of oral health.

In contrast, the "specific plaque hypothesis" stated that only certain organisms within the plaque complex were pathogenic, and pathogenicity depended on the overgrowth or selection of more virulent microorganisms. This hypothesis postulated that specific pathogens result in periodontal disease because these organisms are associated with cellular challenges that result from the host's inflammatory

and immune responses. After recognizing that early plaque colonizers are predominantly gram-positive and later organisms are predominantly gram-negative, Socransky and colleagues (4, 5) defined the organisms within the subgingival microbiota, placing them in five "complexes."

This concept emphasized that microorganisms create their own habitat, interact with each other and are implicated in disease severity (4, 5). The organisms in the plaque reflected the environmental conditions. The most virulent combinations were strict anaerobes, and the less virulent microorganisms thrived in a relatively low-oxygen (microaerophilic) environment. In a detailed analysis using a checkerboard DNA-DNA hybridization approach of more than 13,000 subgingival samples from nearly 200 adults, Socransky and colleagues (5, 6) demonstrated that certain bacterial complexes were associated with either health or disease. The presence of certain complexes such as the "red complex" were associated more commonly with clinical indicators of periodontal diseases and were detected rarely in the absence of bacteria from other complexes (5, 6).

BIOFILM

In recent years, dental plaque has been evaluated and discussed as a biofilm. In 2002, Donlan and Costerton (7) offered the most salient description of a biofilm. They stated that a biofilm is "a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription."

In fact, a biofilm is an accumulation of microbial cells within a matrix, optimizing the use of the available nutri tional resources. The preferred method of growth of any microbial species is in the attached or sessile phenotype. Nevertheless, every microorganism identified as a human pathogen has the potential to exist in either a planktonic phenotype or a biofilm (8). However, microorganisms have a propensity to exist as an attached multispecies biofilm. Biofilms are found throughout the body and in the environment and can be found lining dental unit waterlines, catheters and prosthetic heart valves. A biofilm is organized to maximize energy, spatial arrangements and movement of nutrients and byproducts. Physical composition, degree of organization and multispecies organization characterize organism concentration can approach 1011 or 1012 colony-forming units per milliliter. At this phase, new antigens may be expressed, genetic exchange enhanced and membrane transport maximized. Stage IV (apoptosis or death) signals detachment, eroding or sloughing from the biofilm.

Microbial communities

Most natural biofilms contain multiple species and are termed "microbial communities." Evidence is accumulating that the aggregated organisms are not merely passive neighbors, but rather are involved in a wide range of physical, metabolic and molecular interactions (1). The cooperative communal nature of a microbial community provides advantages to the participating microorganisms. These advantages include a broader habitat range for growth, an enhanced resistance to antimicrobial agents and host defense, and an enhanced ability to cause disease (as certain microorganisms act more pathogenic as coaggregates than as single agents) (1). Evidence suggests that gene expression may be altered within a biofilm, which may be in the four stages of biofilm growth. Whether the organism is planktonic or exists as part of a biofilm, there are four similar phases in the lifecycle. Stage I is the quiescent or least metabolically active state. Conversion or transformation from Stage I to Stage II requiressignificant genetic up-regulation. Stage III involves maturity of the biomass, and total response to the specific surface on which the bacterium has settled (9). Boles and colleagues (10) proposed that the environmental heterogeneity that develops in biofilms can accelerate diversity in bacterial populations as a form of "biological insurance" in which cells are better prepared to cope with adverse conditions.

Communication among bacteria within the biofilm usually is carried out by bacterial products that are able to diffuse away from one cell and enter another (9). Gram-positive bacteria generally communicate via small diffusible peptides, while many gramnegative bacteria secrete acyl homoserine lactones to communicate.

DENTAL PLAQUE BIOFILM

Dental plaque incorporates all of the features of biofilm architecture and microbial community interaction, but it is different in that it has more than 700 contributing oral microbial species in the oral cavity and a distinct method of condition the tooth surface (11). Only 20 to 25 percent of the oral environment is tooth surface, (12) and mucosal surfaces are important contributors to periodontal microbial biofilms. For tooth surfaces, pellicle formation is the preconditioning stage that defines the reversible irreversible attachment of the colonizing bacteria. Attachment is defined as a slime layer forming around the colonizing pioneer bacteria, which consist mainly of gram-positive cocci and rods that divide and form microcolonies. If this early supragingival plaque is unregulated owing to the absence of effective oral hygiene, the bacterial composition can mature into a more complex flora in a three-stage scenario.

The first stage is predominantly Gram-positive cocci and is represented by the streptococcal species, the second stage is cross-linking via fusobacterium species, and the third stage is predominantly gram negative organisms. Mature oral biofilms are robust and resilient, acting as reservoirs of antibiotic resistance and virulence in deep periodontal pockets. Their uncontrolled growth eventually may lead to periodontal disease. A defining characteristic of the multispecies dental plaque biofilm, as well as other microbial biofilms, is communication either from cell to cell or from microcommunity to macrocommunity. This dynamic communication, called "quorum sensing," and regulation provide a mechanism for bacteria to monitor each other's presence and to modulate gene expression in response to changes in population density (13).

Determining the pathogenicity of dental plaque biofilm

Dental biofilm pathogenicity in the mouth is magnified by two biofilm characteristics: increased antibiotic resistance and the inability of the community to be phagocytized by host inflammatory cells. Three mechanisms can account for increased antibiotic resistance. One is the failure of the antibiotic agent to penetrate the extracellular matrix into the full depth of the biofilm (12). The second mechanism suggests that at least some of the cells in a biofilm experience nutrient limitation and, therefore, exist in a slow-growing or starved state. Slow-growing or non-growing cells are not highly susceptible to antimicrobial agents (14). Finally, individual antibiotic resistance increases as a result of genetic changes such as mutations or gene transfer and can result in a loss of susceptibility among the multispecies microcommunities.

MANAGEMENT OF DENTAL PLAQUE BIOFILM

The treatment of infectious diseases has been driven by clinicians' recognition of Koch's postulates. Dentistry's understanding of the predominant phenotypes associated with the dental biofilm has necessitated a shift in the treatment paradigm. The shift in the treatment paradigm incorporates the ecological plaque hypothesis, which states that disease prevention should not only focus on the inhibition of putative pathogens, but also on interference with environmental factors that drive selection and enrichment for these bacteria as reported by Marsh (1). Prevention via maintenance of a normal health associated ecosystem is key. The key characteristics of biofilm that could be targets for pathogen management include its behavior as an adhesive mass with viscoelastic properties, its activity as a coordinated multispecies community in which cells communicate via small molecules, and its inflammatory disease potential. In the pathogen management process, first "focused" or "targeted" energy is delivered to the biofilm via regular meticulous tooth brushing and flossing or via professional sonication and scaling and root planing to overcome viscoelasticity and reduce the pathogenic burden. Second, antimicrobial therapies, including using mouthrinses, can interfere with the shift from Stage I biofilm to Stage II biofilm by application at key intervals to impede the attachment and maturation of the biofilm. Third, use of inflammatory modulators such as low-dose doxycycline (not acting as an antibiotic) may need to be considered to address local tissue inflammation. The underlying risk factors for the periodontal disease also should be identified and addressed.

CONCLUSION

Dental plaque biofilm cannot be eliminated. However, the pathogenic nature of the dental plaque biofilm can be reduced by reducing the bioburden (total microbial load and different pathogenic isolates within that dental plaque biofilm) and maintaining a normal flora with appropriate oral hygiene methods that include daily brushing, flossing and rinsing with antimicrobial mouthrinses. This can result in the prevention or managementof the associated sequelae, including the development of periodontal diseases and possibly the impact of periodontal diseases on specific systemic disorders.

REFERENCES

1. Marsh PD. Dental plaque: biological significance of a biofilm and community life-style. J Clin Periodontol. 2005;32(supp 6):7-15.
2. Overman PR. Biofilm: a new view of plaque. J Contemp Dent Pract. 2000;1(3):18-29.
3. Loesche WJ. Chemotherapy of dental plaque infections. Oral Sci Rev. 1976;9(1):65-107.
4. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol 2000. 2005;38(2):135-87.
5. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134-44.
6. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. Periodontol 2000. 2002;28(1):12-55.
7. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15(2):167-93.
8. Palmer RJ, Gordon S, Cisar JO, Kolenbrander PE. Coaggregration-mediated interactions of streptococci and actinomyces detected in initial human dental plaque. J Bacteriol. 2003;185(11):3400-9.
9. Watnick P, Kolter R. Biofilm, city of microbes. J Bacteriol. 2000;182(10):2675-9.
10. Boles BR, Thoendel M, Singh PK. Self-generated diversity produced "insurance effects" in biofilm communities. Proc Natl Acad Sci USA. 2004;101(47):16630-5.
11. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE . Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 2005;43(11):5721-32.
12. Kerr WJ, Kelly J, Geddes DA. The areas of various surfaces in the human mouth from nine years to adulthood. J Dent Res. 1991;70(12):1528-30.
13. Camilli A, Bassler BL. Bacterial small-molecule signaling pathways. Science. 2006;311(5764):1113-6.
14. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284 (5418):1318-22.
15. Dietrich T, Garcia RI. Associations between periodontal disease and systemic disease: evaluating the strength of the evidence. J Periodontol. 2005;76(11 Suppl)2175-844.

Received: 01/15/2009
Recebido: 15/01/2009

Accepted: 02/28/2009
Aceito: 28/02/2009