Dental Plaque: A Complex Biofilm

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Abstract

Periodontitis is a destructive inflammatory disease of the supporting tissues of the teeth and is caused by specific microorganisms or group of specific microorganisms Inflamed periodontal tissues produce significant amounts of pro-inflammatory cytokines, mainly interleukin1 beta (IL-1â), IL-6, PGE2, and tumor necrosis factor alpha (TNF-á), which may have systemic effects on the host. The main paradigm for periodontal treatment is to remove bacteria and their products from periodontal pockets using non-surgical or surgical periodontal therapy. The biofilm is used to describe the communities of micro-organisms attached to a surface; such microbes are usually spatially organized into three-dimension structure and are enclosed in matrix of extracellular material derived both from the cells themselves and from the environment. Dental plaque biofilm cannot be eliminated. However, the pathogenic nature of the dental plaque biofilm can be reduced by reducing the bio-burden (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 mouth rinse.

Key Words: *Dental Plaque, Biofilm, Quorum Sensing, Oral Hygiene*

Introduction

Mouth acts as a window to lot of systemic diseases and serves as a port of entry of the various infections that can alter and affect the immune status of the person. The oral cavity has the potential to harbor at least 600 different bacterial species, and in any given patient, more than 150 species may be present, surfaces of tooth can have as many as billion bacteria in its attached bacterial plaque and good oral hygiene is the fundamental for oral integrity as it greatly affects the quality of life.[1] Periodontitis is a destructive inflammatory disease of the supporting tissues of the teeth and is caused by specific microorganisms or group of specific microorganisms resulting in progressive destruction of periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession or both. The host responds to the periodontal infections with an array of events involving both innate and adaptive immunity.[2] The link between periodontal disease and systemic diseases has been scientifically proven over last two decades.[3] Association of periodontal infection with organ systems like cardiovascular system, endocrine system, reproductive system, and respiratory system etc. makes periodontal infection a complex multiphase disease. Inflamed periodontal tissues produce significant amounts of pro-inflammatory cytokines, mainly interleukin1 beta (IL-1â), IL-6, PGE2, and tumor necrosis factor alpha (TNF-á), which may have systemic effects on the host. Periodontitis initiates systemic inflammation and can be monitored by inflammatory markers like C-reactive protein or fibrinogen levels.[4]

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Microbiology of Dental Plaque

The microbial etiology of periodontitis has been extensively studied during past few decades, it is now well-known that periodontitis is not associated with a single

microorganism, but is a consortium of bacteria participating in the initiation and progression of periodontitis. Nonspecific accumulation of bacterial plaque was once thought to be the cause of periodontal disease but it is now recognized that periodontitis is an infectious disease associated with a small number of predominantly gram negative microorganisms that exist in subgingival biofilm. Another important issue is co-relation that occurs among bacterial species in the resident biofilm, as well as between their communities and the host tissues. The main paradigm for periodontal treatment is to remove bacteria and their products from periodontal pockets using non-surgical or surgical periodontal therapy. The most significant consequence of biofilm formation on the tooth surface is the continuous release of bacteria cell-surface components into the oral cavity and gingival sulcus. As such, subgingival biofilms constitute a significant continuous bacterial load on the host. These biofilms are self-renewing reservoirs of endotoxin (lipopolysaccharide) and other bacterial toxins that can gain access not only into the surrounding periodontal tissues, but the general circulation as well. Biofilm micro-organisms form distinct three-dimensional structured communities with fluid channels for transport of substrate, waste products and signal molecules. Subgingival biofilms involved in periodontal infection are self-maintaining, self-perpetuating microbial communities and, as such, are a continuous source of bacterial products and toxins into the surrounding gingival tissues and general circulation. The continuous spillover of bacterial toxins into the circulation, or the "dental plaque attack," results in chronic activation of the inflammatory cascade by these bacteria. The matrix that holds the biofilm together is a mixture of polysaccharides, proteins, and DNA secreted by the cells. Micro-organisms undergo a wide range of physiological and morphological adaptations in response to environmental changes. In biofilms, different gradients of chemicals, nutrients, and oxygen create micro-environments to which micro-organisms must adapt to survive. The perception and processing of chemical information from the environment form a central part of the regulatory control of these adaptive responses. Adaptation to a biofilm life style involves regulation of a vast set of genes, and the micro-organisms are thus able to optimize phenotypic properties for the particular environment. Consequently, biofilm micro-organisms differ phenotypically from their planktonic counterparts.

Plaque structure

The structure of dental plaque refers to the manner in which the elements of dental plaque, predominantly bacteria, are organized and interrelated, the studies of dental plaque by light and electron microscopy were crucial in demonstrating that plaque structure is intimately related to microbial composition.[5] Plaque is a living, continuously changing structure with capacity to adapt to ever-changing mechanical, physical, and chemical conditions, it presents exceptionally varied morphological features, and these features may vary with age, extent of maturation, location on tooth surface, diet.[6] A high degree of specificity is found in the interaction between bacteria in dental plaque, as demonstrated by the studies of coaggregation.[7] Environmental conditions on a tooth are not uniform. Differences exist in the degree of protection from oral removal forces and in the gradients of many biological and chemical factors that influence the growth of the resident microflora. These differences will be reflected in variations in the composition of the microbial community, particularly at sites so obviously distinct as the gingival crevice, approximal regions, smooth surfaces, and pits and fissures. For example, fissure plaque will be influenced more by saliva than other sites, whereas gingival crevicular fluid (GCF) has a greater impact on plaque in the gingival crevice. This latter site also has a lower redox potential (Eh) and is colonized by higher numbers of anaerobes, especially proteolytic species which obtain key growth factors from the catabolism of host proteins and glycoprotein in GCF. To clarify the morphological characteristics of dental plaque, some of these studies examined dental plaque on natural teeth; others collected dental plaque on tooth sections or artificial substrates placed in the oral cavity for defined periods of time. Plaque morphology has been examined by light and transmission electron microscopy of sections through the plaque layer. Other studies analyzed plaque by light microscopy of whole or dispersed plaque, or scanning electron microscopy of plaque deposits or plaque deposit replicas, the results of these and other investigations demonstrated that dental plaque is composed of a large variety of bacterial morphotypes with some significant heterogeneity in the appearance of the microbial deposits.[5]

Characteristics of biofilm

A biofilm environment confers certain properties to bacteria that are not seen in the nomadic state, a fact that explains the importance of recognizing dental plaque as a biofilm and not as bacteria in the planktonic state.[8]

Some of the distinctive properties of biofilms are discussed and explained below.

I Structure of Biofilm

Ia) Composition

Biofilms are composed of mirocolonies of bacterial cells (15-20% by volume) that are non-randomly distributed in a shaped matrix or glycocalyx (75-80% volume).[9] The bacteria in the biofilm cluster together to form sessile, mushroom shaped colonies. Each microcolony is an independent community with its own customized living environment. Rapid formation of visible layers of microorganisms due to extensive bacterial growth accompanied by excretion of copious amount of extracellular polymers is typical for biofilms. In the lower levels of most biofilms a dense layer of microbes is bound together in a polysaccharide matrix with other organic and inorganic materials.[10] The successive layer is a loose layer, which is often highly irregular in appearance and may ex-tend into the surrounding medium. The fluid layer bordering the biofilm may have a rather "stationary" sub layer and a fluid layer in motion.[11]

Ib) Voids/Water Channels

There is the presence of voids or water channels between the microcolonies that were present in these biofilms. The water channels permit the passage of nutrients and other agents throughout the biofilm, acting as primitive "circulatory" system.[9] Nutrients make contact with sessile (attached) microcolonies by diffusion from the water channel to the microcolony rather than from the matrix.[10]

Ic) Composition and Shapes of Microcolony

Each micro colony is a tiny, independent community containing thousands of compatible bacteria. Different micro colonies may contain different combinations of bacterial species. Bacteria in the center of a micro colony may live in a strict anaerobic environment, while other bacteria at the edges of the fluid channels may live in an aerobic environment. Thus, the biofilm structure provides a range of customized living environments (with differing pHs, nutrient availability, and oxygen).[12] Micro colonies occur in different shapes in biofilm that are governed by shear forces due to passage of fluid over the biofilm. At low shear forces, the colonies are shaped like towers or mushrooms, while at higher shear forces, the colonies are elongated and capable of rapid oscillation.[9]

Id) Exopolysaccharides- the backbone of the biofilm

Exopolysaccharides are produced by the bacteria in the biofilm, and are the major components of the biofilm making up 50-95% of the dry weight. They play a major role in maintaining the integrity of the biofilm and as well as preventing desiccation and attack by harmful agents.[10] In addition, they also bind essential nutrients such as cations to create a local rich environment favoring specific microorganisms. The EPS matrix could also act as a buffer and assist in the retention of extracellular enzymes (and their substrates) enhancing substrate utilization by bacterial cells. One distinguished feature of the oral biofilms is that many of the microorganisms can both synthesize and degrade the exopolysaccharides.[9]

II Physiological heterogeneity of biofilm

Cells of the same bacterial species can exhibit extremely different physiological states in a biofilm even though they are separated by a distance of as little as 10 microns. The use of microelectrodes has shown that pH can vary quite remarkably over short distances within a biofilm.[9] Measurement of oxygen and other gases has demonstrated that certain microcolonies that are completely anaerobic even though composed of single species and grown in ambient air. Thus, studies to date indicate that the sessile cells growing in mixed biofilm can exist in an almost infinite range of chemical and physical micro habitants within the microbial communities.[10]

III Quorum Sensing

Some of the unique functions of biofilms are dependent on the ability of the bacteria and microcolonies within the biofilm to communicate with one another. Quorum sensing or cell density mediated gene expression in the bacteria involves the regulation of expression of specific genes through the accumulation of signaling compounds that mediate intercellular communication.[9] Quorum-sensing signaling represents a signaling pathway that is activated as a response to cell density.[13] Such systems are found in both Gram-positive and Gram-negative micro-organisms. The stimuli of quorum-sensing systems are signal molecules, called autoinducers. The autoinducers are produced at a basal constant level, and the concentration thus is a function of microbial density. Perception of the signal occurs at a concentration threshold. The term "quorum" is used to describe this kind of signal system, since a certain number of micro-organisms must be present for the signal to be sensed and for the population to respond to the signal.[13] In gram-

positive bacteria the signaling molecules are secreted peptides, whereas in gram-negative bacteria two different systems of quorum sensing, which use different types of autoinducers, have so far been described. The first system was initially described in *Vibrio fischeri* as the mechanism that controls the expression of bioluminescence in this microorganism. Over the past few years, similar systems have also been found in different genera of gram-negative bacteria, and it has become clear that this system monitors the density of cells by producing acylated homoserine lactones (AHLs), whose structure depends on the bacteria that produce them. In *V. harveyi* this first system has been called system 1, and hence the autoinducer that controls it is called AI-1. In this case the hydroxybutyryl homoserine lactone is the autoinducer. A second quorum-sensing system has been described in *V. harveyi*. The structure of the autoinducer for this system, which has been called AI-2, is still unknown, although it has been reported that its synthesis is dependent on the *luxS* gene.[14] This second system seems to be more widespread among the microbial world than the one that uses AHLs as autoinducers, and homologues for *luxS* have been identified in a large number of both gram-positive and gram-negative microorganisms. Autoinducer-2 is an umbrella designation that covers a collection of molecules from the spontaneous rearrangement of 4, 5-dihydroxy-2, 3-pentanedione (DPD), which is the product of LuxS gene. The molecule called autoinducer-2 was a universal signal mediating message among the species in mixed communities. This idea is distinct from the regulation of expression mediated by autoinducer-1, a family of acyl homoserine lactones which regulate gene expression in genetically identical cells. Since bacteria within the biofilms reach a high density, it has been suggested that quorum sensing might play a key role in bacterium-bacterium communication and, therefore, in the formation of biofilms.[15] Quorum sensing may give biofilm their distinct properties. For example, expression of gene for antibiotic resistance at high cell density may provide protection. Quorum sensing also helps the potential to influence community structure by encouraging the growth of beneficial bacteria (to the biofilm) and discouraging the growth of competitors. It is also possible that physiological properties of bacteria in the community may be altered through quorum sensing.[10]

IV Attachment of Bacteria

The key characteristic of a biofilm is that microcolonies within the biofilm attach to a solid surface. Thus, adhesion to a surface is the essential first step in the development of biofilm.[10] Many bacterial species possesses surface structures such as fimbriae and fibrils that aid in their attachment to different surfaces.

Fimbriae: Fimbriae are found in oral bacteria such as *Actinobacillus actinimycetemcomitans* and *Porphyromonas gingivalis*. They are long protein filaments, present singly or in the bundles on the surfaces of the cells.[8] The major component in fimbrillin, a highly antigenic protein encoded by *fimA* in *P.gingivalis* and *flp in A.actinomycetemcomitans.* In both bacteria, fimbriae are thought to be important in colonization because the fimbrial-deficient mutants show reduced ability to bind and invade the epithelial cells and fibroblasts. Fimbriae-mediated epithelial invasion stimulates expression of host cell adhesion molecules such as intercellular adhesion molecule, vascular cell adhesion molecule, P-selectin and E-selectin, thus inducing a massive leukocytic response at the site. *P.gingivalis* fimbriae also stimulate IL-1â, IL-1á, TNF- á and granulocyte-macrophage colony-stimulating factor, leading to bone resorption.[8]

Extracellular proteolytic enzymes: *Tannerella forsythia, P.gingivalis, Treponema denticola* and other oral bacteria produce proteolytic enzymes often displayed on their cell surfaces. Dentilisin (*T.dneticola*), PrtH (*T.forsythia*), and RgpA, RgpB, and kgp (*P.gingivalis*) are the best characterized enzymes in this group. These enzymes have various virulence components, degrade fibrinogen, laminin, fibronectin and several types of collagens.[8]

V Antibiotic Resistance

It has been recognized for considerable period of time that the organisms growing in the biofilms are more resistant to antibiotics than the same species growing in a planktonic (unattached) state.[10] Estimate of 1000 to 1500[16] times greater resistance for biofilm-grown cells than the planktonic cells have been suggested, although these estimates have been considered too high by some investigators. Conventionally, the sensitivity of bacteria to antimicrobial agents is determined on cells grown in liquid culture by the measure-ment of the minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC). Given the decreased sensitivity of an organism on a surface to antimicrobial agents, it has been argued that it would be more appropriate to determine the 'biofilm inhibitory concentration (BIC) and 'biofilm killing concentration (BKC). Thus, it is important to understand the factors leading to antimicrobial resistance in biofilms such as dental plaque.

The important mechanism of resistant appears to be the slower growth rate of bacteria in the biofilm, which makes them less susceptible to many but not all antibiotics. It has been shown in many studies that resistance of bacteria to antibiotics, biocides, or preservatives are affected by their nutritional status, growth rate, temperature, pH and prior exposure to subeffective concentrations of antimicrobials.[9] Variations in any of these parameters can lead to a varied response to antibiotics with in a biofilm. The slower growing bacteria often overexpress non-specific defense mechanisms including heat shock proteins, multidrug efflux pumps and demonstrated increased exopolymer synthesis.[10] When eukaryotic and bacterial cells are exposed to environmental stress (e.g. temperature, pH, redox potential), they synthesize stress proteins such as heat shock proteins. These proteins protect the cells from damaging effects of the environment. Heat shock proteins such as GroEL, GroES, DnaK and HtpG have been studied in oral bacteria; heat shock proteins from *A.actinomycetemcomitans* stimulate the osteoclast activation and epithelial proliferation at low concentrations and are cytotoxic at high doses.[8] The exopolymer matrix of biofilm, although not a significant barrier in itself to the diffusion of antibiotics, does have certain properties that can retard diffusion. For example, strongly charged or chemically highly reactive agents can fail to reach the deeper zones of the biofilm because the biofilm acts as an ion exchange resin removing such molecules from the solutions. In addition, extracellular enzymes such as â lactamase, formaldehyde lyase and formaldehyde dehydrogenase may become trapped and concentrated in the extracellular matrix, thus inactivating the susceptible, typically positively charged, hydrophilic antibiotics. Hydrodynamics and turnover rate of the microcolonies will also impact on antibiotic effectiveness. Alteration of genotype or phenotype of the cells growing within a biofilm matrix is receiving increased attention. Cells growing within the biofilm express genes that are not observed in the same cells growing in planktonic state and they can retain this resistance for some time after being released from the biofilm.

VI Gene Transfer

The high density of bacterial cells in a biofilm also facilitates the exchange of genetic information among the cells of the same species and across species and even genera. Conjugation, transformation and transduction have been shown to occur more easily in a biofilm. Biofilm associated bacteria communicate with each other by way of horizontal gene transfer.[8] Horizontal gene transfer among bacteria is recognized as a major contributor in the molecular evolution of many bacterial genomes.[17] In addition, horizontal gene transfer is responsible for the seemingly uncontrollable spread of antibiotic resistance gene among bacteria in the natural and nosocomial environments. The oral cavity is believed to be an excellent environment in which horizontal gene transfer can occur, as a result of the close and stable proximity of the majority of the bacteria present in dental plaque and the availability of exogenous DNA passing through the oral cavity.

Transformation:- Transformation is defined as the uptake and maintenance of DNA. Competence is the physiological state in which the cells can take up DNA. Some oral bacteria, including members of genus *Streptococcus*, *Neisseria* and *Actinobacillus* are naturally competent and have specialized systems for DNA uptake. Transformation has no requirement for live donor cells because the DNA released upon cell death is the principal source of transforming DNA.[17]

Transduction:- Transduction is a process where bacterial DNA is packaged into phage heads. When the phage infects a suitable host it injects this bacterial DNA, instead of phage DNA, into the new host. One of the main barriers to the activity of bacteriophage in the oral biofilm is the access to the cells within the extracellular polymeric substances secreted by cells themselves when growing as a biofilm.[17]

Conjugation:- Conjugation is the polar transfer of genetic material through direct cell-to-cell contact and is mediated by a variety of specialized genetic elements, such as conjugative transposons and conjugative plasmids. It requires intimate cell-to-cell contact between the donor and recipient cells; this is mediated by variety of specialized intracellular and surface structures.[17]

Conclusion

Dental plaque biofilm cannot be eliminated. However, the pathogenic nature of the dental plaque biofilm can be reduced by reducing the bio-burden (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 mouthrinse. This can result in the prevention or management of the associated squeal, including the development of periodontal diseases and possibly the impact of periodontal diseases on specific systemic disorders.[18] Research on microbial biofilm is proceedings on many dimensions, with specific focus

on elucidation of the genes specifically expressed by biofilm-associated organisms, assessment of different control approaches for either preventing or remediating biofilm colonization of medical devices, and development of new methods for evaluating the efficacy of these treatments.[19]

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