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Review Article: Biofilm

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ABSTRACT

Most microorganisms in nature attach to surfaces and form matrix embedded biofilms. Biofilms are highly structured and spatially organized, and are often composed of consortia of interacting microorganisms, termed microbial communities, the properties of which are more than the sum of the component species.

Keywords: biofilm, consortia, microorganisms.

INTRODUCTION

It is estimated over 95% of bacteria existing in nature are in biofilms.[1] Biofilms have been likened to miniature cities, with channels and voids permeating densely packed microcolonies, like roads and alleys running between tall buildings.[2]

Biofilms are ubiquitous [3], they form on virtually all surfaces immersed in natural aqueous environments. 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 microenvironments 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 lifestyle 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. [4]

Caldwell et al. [5] highlighted four characteristics of biofilm as follows:

• Autopoiesis – Must possess the ability to self‑organize
• Homeostasis – Should resist environmental perturbations
• Synergy – Must be more effective in association than in isolation
Communality – Should respond to environmental changes as a unit rather than as single individuals.

The typical example of a biofilm is dental plaque. [6]

HISTORY OF BIOFILM

A little more than 300 years ago, the Dutch scientist Anthony van Leeuwenhock scraped material from his own teeth and, using his simple microscopes, noticed a vast accumulation of moving objects not visible to the naked eye. He called these microscopic bodies animalcules because he thought they were tiny living animals. In a report to the British Royal Society, he wrote, "The number of these animalcules in the scurf of a man's teeth is so many that I believe they exceed the number of men in a kingdom." This observation made him the first biofilm experimenter. [3]
Heukelekian and Heller [7] observed the “bottle effect” for marine microorganisms, i.e., bacterial growth and activity were substantially enhanced by the incorporation of a surface to which these organisms could attach.

Zobell [8] observed that the number of bacteria on surfaces was dramatically higher than in the surrounding medium. Jones et al. [9] used scanning and transmission electron microscopy to examine biofilms on trickling filters in a wastewater treatment plant and showed them to be composed of a variety of organisms (based on cell morphology). By using a specific polysaccharide-stain called Ruthenium red and coupling this with osmium tetroxide fixative, these researchers were also able to show that the matrix material surrounding and enclosing cells in these biofilms was polysaccharide.

As early as 1973, Characklis [10] studied microbial slimes in industrial water systems and showed that they were not only very tenacious but also highly resistant to disinfectants such as chlorine.

Based on observations of dental plaque and sessile communities in mountain streams, Costerton et al. [11] in 1978 put forth a theory of biofilms that explained the mechanisms whereby microorganisms adhere to living and nonliving materials and the benefits accrued by this ecologic niche. Since that time, the studies of biofilms in industrial and ecologic settings and in environments more relevant for public health have basically paralleled each other.

A study done by Zaura et al., analyzing the microbiomes of several intraoral niches (tooth surface, check, hard palate, tongue, and saliva) from three healthy subjects using pyrosequencing, highlighted the presence of over 3,600 unique nucleotidic sequences per individual. The highest diversity was associated with tooth samples, while the lowest diversity was observed in the cheek samples. [12]

What is a biofilm?

Biofilms have been defined as matrix embedded microbial populations, adherent to each other and/or to surfaces or interfaces (Costerton et al. 1995).[13] It is a mode of microbial growth where dynamic communities of interacting sessile cells are irreversibly attached to a solid substratum, as well as to each other.[6,14]

Biofilm mode of growth is advantageous for microorganisms, as they form three‑dimensional structured communities with fluid channels for transport of substrate, waste products, and signal molecules. [15,6] Biofilm formation in root canals is probably initiated sometime after the first invasion of the pulp chamber by planktonic oral microorganisms after some tissue breakdown, as hypothesized by Svensäter and Bergenholtz.[6]Costerton et al.[16] stated that biofilm consists of single cells and microcolonies, all embedded in a highly hydrated, predominantly anionic exopolymer matrix.

COMPOSITION:

A fully developed biofilm is described as a heterogeneous arrangement of microbial cells on a solid surface. It is composed of matrix material consisting of proteins, polysaccharides, nucleic acids, and salt, which makes up 85% by volume, while 15% is made up of cells. [17]

Biofilm‑mediated mineralization occurs when the metal ions including Ca2+, Mg2+, and Fe3+ readily bind and precipitate within an ionic biofilm under a favourable environment. [6]

MATURATION OF BIOFILM :

As biofilm get matured, its structure and composition are modified according to the growth conditions, nature of fluid movements, physicochemical properties of the substrate, nutritional availability, etc.
The water channels are regarded as a primitive circulatory system in a biofilm. These microcolonies have a tendency to detach from the biofilm community and have the highest impact in chronic bacterial infection. During the process of detachment, the biofilm transfers cells, polymers, and precipitates from the biofilm to the fluid bathing the biofilm, which is important in shaping the morphological characteristics and structure of mature biofilm.[14] It is considered as an active dispersive mechanism (seeding dispersal).

Development of biofilm

Stage 1: The first step consists of formation of a conditioning layer. it is formed by the adsorption of inorganic and organic molecules to the solid surface.

Stage 2: The next step in biofilm formation is the adhesion of microbial cells to this layer. There are many factors that affect bacterial attachment to a solid substrate. These factors include pH, temperature, surface energy of the substrate, flow rate of the fluid passing over the surface, nutrient availability, length of time the bacteria is in contact with the surface, bacterial growth stage, as well as bacterial cell charge, and surface hydrophobicity. Physicochemical properties such as surface energy and charge density determine the nature of initial bacteria. The microbial adherence to a substrate is also mediated by bacterial surface structures such as fimbriae, pili, flagella, and glycocalyx [18]

Stage 3: During this stage, the monolayer of microbes attracts secondary colonizers forming microcolonies and the collection of colonies gives rise to the final structure of biofilm. Microcolony formation and co-adhesion take place before a monolayer is established along lateral and vertical growth of indwellers gives rise to micro colonies similar to towers. [2]19 Two types of microbial interactions occur at the cellular level during the formation of biofilm. One is the process of recognition between a suspended cell and a cell which is already attached to substratum. This type of interaction is termed co-adhesion. In the second type of interaction, genetically distinct cells in suspension recognize each other and clump together. This type of interaction is called coaggregation. These associations are highly specific and occur between coaggregating partners only. [18]

CHARACTERSTICS OF BIOFILM

Bacteria in a biofilm have the ability to survive tough growth and environmental conditions. This unique capacity of bacteria in a biofilm state is due to the

Following features:

• Residing bacteria are protected from environmental threats; trapping of nutrients and metabolic cooperation between resident cells of the same species and/or different species is allowed by the biofilm structure
• It also exhibits organized internal compartmentalization which helps the bacterial species in each compartment with different growth requirements
• By communicating and exchanging genetic materials, these bacterial cells in a biofilm community may acquire new traits. [20]

Bacterial biofilm provides a setting for the residing bacterial cells to communicate with each other. Some of these signals, produced by the cells, may be interpreted not just by members of the same species but also by other microbial species. [6,21]

Biofilms in dentistry

Oral bacteria have the capacity to form biofilms on distinct surfaces ranging from hard to soft tissues. Formation of oral biofilm involves three basic steps: Pellicle formation, bacterial colonization, and biofilm maturation. The organic substance surrounds the microorganisms of the biofilm and contains primarily carbohydrates, proteins, and lipids. [22] Carbohydrates include glucans, fructans, or levans. The proteins found in the supragingival biofilm are derived from saliva, while the proteins in the subgingival biofilm are derived from gingival sulcular fluid. The lipid content may include endotoxins (LPS) from Gram-negative bacteria.[18]

The inorganic elements found in a biofilm are calcium, phosphorus, magnesium, and fluoride. The concentrations of these inorganic elements are higher in biofilm than in saliva. [24]

The characteristics of the biofilm formed depend upon the residing bacterial species, the surface or substratum composition, and the conditioning layer coating the surfaces on which they are formed. The chemical composition of biofilm differs among individuals, between tooth surfaces, in an individual and with age.

Human saliva contains proline-rich proteins that aggregate together to form micelle like globules called salivary micelle-like globules (SMGs). SMGs from saliva get adsorbed to the clean tooth surface to form acquired enamel pellicle, which acts as a “foundation” for the future multi layered biofilm. The globular micelles of acquired enamel pellicles are characterized by a negatively charged (calcium binding) surface and hydrophobic interior. Presence of calcium facilitates the formation of larger globules by bridging the negative charges on the subunits. The initial attachment of bacteria to the pellicle is by selective adherence of specific bacteria from oral environment. Innate characteristics of the bacteria and the pellicle determine the adhesive interactions that cause a specific organism to adhere to the pellicle. [6,18,25]

Dental biofilm consists of a complex mixture of microorganisms that occur primarily as microcolonies. The population density is very high and increases as biofilm ages. The prospect of developing dental caries or gingivitis increases as the number of microorganisms increases. The acquired pellicle attracts Gram-positive cocci such as S. mutans and S. sanguis, which are the pioneer organisms in the plaque formation. Subsequently, filamentous bacterium such as F. nucleatum and slender rods adheres to primary colonizers.[14,18, 26]

Gradually, the filamentous form grows into the cocci layer and replaces many of the cocci. Vibrios and spirochetes appear as the biofilm thickens. More and more gram-negative and anaerobic organisms emerge as the biofilm matures. Interestingly, it is not only the surface of tooth that can be attached by bacterial cells. [26,18]

The surface of some bacteria (bacilli and spirochetes) also can serve as attachment sites for certain smaller coccoids. This coaggregation of F. nucleatum with coccoid bacteria gives rise to "corn cob" structure, which is unique in plaque biofilms. The presence of these bacteria makes it possible for other non-aggregating bacteria to coexist in the biofilm, by acting as coaggregating bridges. Calcified dental biofilm is termed as calculus. It is formed by the precipitation of calcium phosphates within the organic plaque matrix. Factors that regulate the deposition of minerals on dental biofilms are physicochemical factors such as plaque pH, local saturation of Calcium, Phosphate, and availability of fluoride ions and biological factors such as presence of crystallization nucleators/inhibitors from either bacteria or oral fluids. Localized super saturation of calcium and phosphate ions provides the driving force for mineralization. The inorganic or mineral fraction consists of calcium phosphates, magnesium, fluoride, and carbonate, and they make up 70% to 80% weight of dental calculus.[24,25] Various mineral phases namely, HAP, whitlockite, octacalcium phosphate, and brushite have been reported in calculus. [18]

Control biolfim formation :

As eliminating surface‑adherent biofilm bacteria is a challenge, different antimicrobials (ranging from antimicrobial irrigants to advanced and microbial methods such as lasers, photoactivated disinfection, and nanoparticles) are employed in the management of biofilms.The application of nanoparticles to control biofilm formation within the oral cavity, as a function of their biocidal, anti-adhesive, and delivery capabilities, is worthy of serious consideration.[27]
Laser-activated irrigation using the photon-induced photoacoustic streaming technique of 6% sodium hypochlorite significantly improved the cleaning of biofilm-infected dentine compared with passive ultrasonic, sonic or mere needle irrigation.

There is some evidence showing that the use of oral mouthwashes reduces cariogenic plaque .The prescription most indicated for this situation is the use of the mouthwash twice a day, once before going to sleep and once in the morning, with quantities of mouthwash varying between 10 and 20 ml.
According to the data set out, it was observed that the use of mouthwashes based on chlorhexidine, octenidine, essential oil (Listerine), Cetylpyridinium, NaF, and AmF/SnF2 were shown to be effective in the reduction of cariogenic plaque. Oral micro-organisms grown in biofilms are shown to be up to 250 times more resistant to CHX than their planktonic counterparts (Pratten and Wilson, 1999). Even after a 60-minute exposure to 0.2% CHX, substantial numbers of bacteria in the biofilm remained vital (Wilson et al., 1998) [28]

CONCLUSION

The formation of biofilms carries particular clinical significance because not only host defense mechanisms, but also the therapeutic efforts including chemical and mechanical anti-microbial treatment measures, have a most difficult task to deal with organisms that are gathered in a biofilm.

Developing oral prophylactic strategies through interference with two-component systems or quorum-sensing of biofilm micro-organisms represents an interesting future challenge. Unlike strategies that target microbial viability, such approaches may interfere with microbial adaptive pathways without killing the micro-organisms. Therefore, resistance development would probably represent a minor problem.

While the stages of biofilm formation seem to follow basically the same model in various micro-organisms, the biofilm architecture and molecular mechanisms involved in biofilm formation appear to differ. The mechanisms involved inbiofilm formation by P. aeruginosa are some of the best-characterized and have served as a model for new hypotheses on mechanisms used by other micro-organisms. Information on the genetic regulation of oral biofilm formation, however, is still lacking. A better understanding of these processes is necessary to the development of novel strategies for oral disease prevention and control.

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