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Dental Plaque Biofilm: Development, Pathogenicity and Analysis

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ABSTRACT

Biofilms are communities of microorganisms which are found attached to a surface. They develop on both biotic and abiotic surfaces and could act as a source of infection. The development of biofilms is a complex process and it involves several steps such as initial adhesion, reversible binding of bacteria to the solid surface, production of exopolysaccharide matrix, irreversible binding to the surface, maturation of biofilm structure, disintegration and dispersion of organized structure and the formation of new habitats. The biofilm exhibits unique properties of protecting host defences and desiccation, persistence in the flowing system, heterogeneity, spatial organization and resistance to antimicrobial agents through its ability to influence gene expression and phenotype. Quorum sensing, a means of the cell to cell communication is closely interconnected to the development of biofilm formation and inhibition. The dental plaque is the most common and well known oral biofilm. The preponderance of biofilm-associated diseases and its resistance in eradication has potentiated the need for further research in this field. Hence, the purpose of the review is to enlighten the importance of dental plaque as a biofilm, its properties, pathogenicity and analysis of biofilm.

Keywords: Biofilms, Dental Plaque, Microbiome, Quorum sensing.

INTRODUCTION

The evolution of the planktonic state of microbes leads to their association as small communities that form a complex matrix-like structure called as biofilms.1 Biofilms are aggregates of micro-organisms in which the associated cells are frequently embedded in a self-produced matrix of extracellular polymeric substances (EPS) that are adherent to each other and/or a surface. In general, microbes are free-floating organisms, considered as planktonic and are characterized based on their growth characteristics in different nutrient-rich media. This term "Microbiome" was proposed by Joshua Lederberg and embraced by the Human Microbiome Project, refers to the microorganisms found in the human oral cavity and signifies the ecological community of commensals, both symbiotic and pathogenic which share our body space acting as major determinants of health and disease.2 Currently, Microbiome defines as of genomes living in the same habitat. Any change in the surrounding environment affects these planktonic organisms, this, in turn, leads to multiple regulatory signals being influenced, resulting in the reorganization of their spatial and temporal forms. All this reprogramming causes the biofilm to become highly complex and dynamic colonization needed for their survival.

Within oral biofilms, dental plaque is the most common and well known oral biofilm. In the oral cavity, the biofilm is built on hard surfaces like a tooth, that is being continuously irrigated by saliva. Also, the biofilm gets established on soft tissues such as tongue and gingival.[3,] [4]The preponderance of biofilm-associated diseases and its resistance in eradication has potentiated the need for further research in this field. A literature search was carried out on biofilms about oral health using search engines like PubMed, PubMed Central, Medline and Google scholar has been taken into account in reviewing the pathogenesis and significance of biofilms in this article. The review brings an understanding of the intricate formation, heterogeneity and adaptation of the microbiome within biofilms and their implications concerning oral health.

Composition

The formed biofilm is composed of bacterial cells, and non-cellular materials cocooned in an extracellular matrix composed primarily of polysaccharide material produced by the bacteria themselves. This contributes to ~90% of its biomass. Carbohydrate–binding proteins, pili, flagella, adhesive fibres and extracellular DNA (e DNA) are some of the other components found within the biofilm.[5]

Stages of Biofilm formation

Biofilm formation is a very complicated process and occurs at several stages. Several relevant factors and favourable conditions must exist in the oral cavity for the favourable formation of biofilm. However, the formation of biofilm is characterized into five phases.

The Adherence or attachment phase

In this phase, the microorganism attaches to the solid surfaces like enamel, tooth root or any dental prosthesis. The attachment occurs by two mechanisms: sucrose dependent and sucrose independent.[6,7,8] It is influenced by many factors such as pH, oxygen concentration, temperature, nutrients, nutrient concentration, osmolality and iron.

Irreversible binding or connection of bacteria with the surface, by exopolysaccharide matrix formation that forms the protection from host defence system.[9] Adherence is followed by proliferation and intercommunication among the bacterial cells through chemical signals. These signals, on crossing the threshold activate the genetic mechanisms responsible for exopolysaccharide production, a major component of the matrix. This matrix then traps nutrients and planktonic bacteria as cell aggregates with lowered motility.[10]

In the maturation phase, the matrix is still being developed and there are other bacterial species join to form the biofilm. In this stage, the glucans, fructans are synthesized by bacteria and form the plaque matrix. This matrix is biologically active where it retains water, nutrients and enzymes inside the biofilm structure.[11] Following maturation, there is a succession of a bacterial accumulation from initial dominance to predominance with gram-positive cocci. The newly forming bacterial species get adhere to the already attached species thereby reaching a thickness of 100mm or above. Rickard et al, in 2003 referred this phenomenon as co-aggregation, where the presence of one microorganism facilitates the survival of new organisms.[12] Thus, the mature biofilm is a result of adhesion, growth and removal of several species of microorganisms. The growth and development of mature biofilm last till it reaches a critical size, where shear forces cannot disturb the biofilm. When the biofilm reaches stage IV, it was believed that it is possible to further inhibit its formation. This is as a result of interaction between specific species present in the biofilm. In the final phase, cell dispersion occurs where certain bacteria tend to leave the Biofilm after developing the planktonic phenotype.[13]

Thus, the structure and evolution of biofilm communities depends on the factors such as nutrient resources, attachment efficiency, genotypic factors, substratum, cyclic stage, anti effective hostile forces, physiochemical environment, mechanical factors and shear forces and types of surfaces.[14-18] All this is mostly enhanced and made possible through several regulatory mechanisms like two-component regulatory system, quorum sensing, cyclic di-GMP signalling and stigmergic factors(different spp. after differentiation into distinct cell types are kept together through intermolecular signals "stimulation affecting performance").[19] This has been validated in studies done on pseudomonas biofilm formation.[20]

Quorum sensing is a means of the cell to cell communication where gene expression is synchronized in response to the density of the cell population. It is closely interconnected to the development of biofilm formation and its inhibition through the action of stress response genes and cell signalling.[21] The extracellular molecules, pheromones (chemical substances which when liberated triggers a social response in members of the same spp), acylated homoserine lactone (acyl-HSL)[1] liberated via quorum sensing enable communication between the bacteria. These signals are then translated to concerted gene expression which causes cellular reprogramming by altering the expression of surface molecules, nutrient utilization and virulence. This makes the bacteria more equipped to survive in unfavourable conditions. This is responsible for the viability of the biofilm community and is utilized by both the pathogenic and non-pathogenic bacteria.

Pathogenicity

The biofilm protects its component microbes from host defences like desiccation through its ability to influence its environment and by altering the gene expression and phenotype of the resident organisms. This results in the bacteria exhibiting features of persistence in the flowing system, heterogeneity, attachment to a solid surface, spatial re-organization and resistance to antimicrobial agents. Biofilms provide a constant supply of nutrients and are well hydrated keeping the organisms viable.[5,22] Inter bacterial communications help in spreading drug resistance and other factors that enable increased virulence.[5] The pathogenicity of a particular organism is expressed as a variation of damages caused by the microorganism itself as a response to pathogens. The pathogenicity has been classified into six types of pathogens and areas follows:[23]

Class I: In this type, the microorganism is referred to as either opportunistic or commensal. The organism attacks the host with poor immune response, but these organisms cannot damage the host with a good immune response.

Class II: These categories the organisms that can cause damage to people with normal and impaired immune response. The damages are more frequent and severe in a patient with poor immune response.

Class III: This class is similar to that of class II where the microorganism affects to hosts with normal and impaired immune response. The difference is that class III is associated with a high mortality rate due to the proliferation of cells to different organisms.

Class IV: This class of microorganism damages host of extremely impaired immunity or hyperimmune activity. This is seen in relatively small groups of pathogens causing symptomatic infections with impaired immunity or with prolonged immune response.

Class V: This class of microorganism causes damage in any condition of the immune system, but mostly in the acute phase of immune system overactivity.

Class VI: This class of microorganisms causes damages only in the conditions of a strong immune response of the host.

Formation and behaviour

Biofilms could be formed on a variety of both biotic and abiotic surfaces, [22,24] ranging from living tissues, dead tissue like sequestra of dead bone, to indwelling medical devices. Aquatic systems, both natural and industrial piping systems shows biofilm formation.[1] Biofilm formation could be the result of single species colonization or a mixture of species involvement.[22] Though the antigens liberated by sessile bacterial cells stimulates antibody production, the antibodies are not effective in killing the bacteria within the biofilm and end up causing damage to the surrounding tissues.[25]Recently organisms within the biofilm, have shown varied behaviour, having found to exhibit a new character of producing more than one biofilm. Candida albicans, common oral pathogen, for instance, produces two biofilms that are outwardly similar but functionally different, one being pathogenic and resistant to any challenge, while the other being sexually-oriented and non-resistant.

Methods to detect biofilms

Recalcitrant infections, known for their resistance to antibiotics owe their properties of restricted penetration to an expression of genes that cause resistance to biofilm-producing bacteria. This validates the need to detect and assess the biofilm and its associated microbiome. The different modes of detection modalities are

1) Tissue Culture Plate Method (TCP), also called a microwell plate assay: The culture of the microorganisms is followed by staining and detecting biofilm formation through the reading of the optical density using Elisa reader. This method uses 96-well microtiter plates. This is the most commonly used method and is reproducible, cost-effective and efficient.[26]

2) Tube Method: A qualitative assessment involving the formation of visible film lines on the walls and floor of the tube after culturing of the organisms in soyositive broth followed by washing and staining procedures.

3) The Congo Red Agar Method (CRA): Microorganisms show black colony formation indicative of positive film formation.

4) Bioluminescent Assay: Attenuated Total Reflecting Spectroscopy (ATR) -This method tries to monitor the conditioning films that are precursors to actual biofilm formation.

5) Piezoelectric Sensors: monitors accumulating mass of the film through its sensors.

6) Calgary device: This device calculates the minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC) and minimum biofilm eradication concentration (MBEC) of cells. This method used modified 96 well microtitre plate with pegs that can be removable from the lid.[27]

7) Bioflux device: This method is used to assess the biofilms in the dynamic state, unlike static biofilms. It is used to assess the variability of biofilms. The Bioflux system uses micro fluids to assess the in vivo growth conditions for live cells. An inverted microscope is used to quantify and visualize the growth of each channel with phase, fluorescence and confocal microscopy.[28]

8) Confocal Laser scanning microscopy: Structure and viability of biofilm can be analysed using this tool. The advantage of this method is that prior treatment of biofilm can be eliminated. SYTO 59 which is a cell-permeable fluorescent stain is used to stain all cells red and STYOX which is a cell impermeable fluorescent stain labels cell wall in green.[29]

9) Atomic Force Microscopy (AFM): This method uses a sharp probe, which is attached to the cantilever. Non-contact AFM and contact AFM are the two methods used to measure the surface of the biofilm. This method is used to assess the biofilm adhesion and bonding strength to various substrates.[30] Studies have shown the TCP method to have better detection ability when compared to the tube method and the Congo red agar method.[31-33]

Dental plaque

The general properties of biofilm comprises of open architecture with protection from desiccation and other host defences and shows enhanced resistance to antimicrobials through inhibitor neutralization. Novel expression of genes includes a broad range of habitat and exhibits heterogeneity both spatially and environmentally. This leads to a more efficient metabolism.[34] Dental plaque biofilm also exhibits these features. The Dental plaque biofilm shows the presence of channels and voids and produces extracellular polymers to form a functional matrix. Various mechanisms like ß-Lactamase production, synthesis and up-regulation of novel proteins, cell to cell signalling, change in oxygen gradient and pH level are enhanced. This leads to the growth of obligate anaerobes in an aerobic environment showing increased resistance to chlorhexidine and antibiotics.[20]

Dental plaque is the most commonly reported type of biofilm in the human body.[35] Dental plaque-host associated biofilms are made of soft deposits and have to be differentiated from the material. Mineralization of these plaques leads to calculus formation which could be either supragingival or subgingival. Marginal plaque leads to gingivitis and supragingival plaque to caries, while subgingival plaque causes periodontitis and soft tissue destruction. A Study by Tanner et al demonstrates dental plaque composition to be of varied diversity on anaerobic cultivation and isolation using enriched blood and acid agars.[36]

More than 1000 distinct microbial species of bacteria that can form plaque has been identified so far. Majority of organisms failed to grow in vivo.[37-41] Through molecular methods, five dominant phyla of bacteria have been identified in the dental plaque: Actinobacteria, Fusobacteria, Proteobacteria, Bacteroidetes and Firmicutes. These phyla constitute a majority of microflora.[42,43] Studies have shown that streptococcus species constitute a majority of the bacteria in dental plaque biofilm.[40,42-45] It is followed by genes Veilonella, Granulicatella, Fusobacterium, Neisseria.

Based on the formation of plaque-forming microorganisms, they are divided into two types: early colonizers and late colonizers. Early colonizers include Streptococci, Veilonella, Haemophilus, Capnocytophaga, Ekenella, Prevotella and Actinomyces, whereas late colonizers include Treponema, Porphyromonas gingivalis, Eubacterium, Aggregatibacter actinomycetemcomintans.[46] The plaque formation in the oral cavity is purely based on ecological plaque hypothesis.[47] This theory demonstrates that changes in the oral environment affect changes in the bacterial species in dental plaque. Carbohydrates observed from the dietary products are considered primarily responsible for many physiological and biochemical changes occurring in the biofilm. Excess intake of carbohydrate results in the increased number of streptococcus and lactobacillus species thereby decreasing the pH of the oral cavity leading to organic acid production. Besides, frequent consumption of sugars promotes decalcification of tooth by the acid formation and by the production of exopolysaccharides leading to the development of dental caries.[48]

Moreover, taking into note the complexity of interactions between different microorganisms and the number of organisms is essential in the formation of biofilm and this further helps in caries development.

CONCLUSION

From various studies, it was concluded that less than 50% of oral biofilm bacteria are cultured in vitro. An oral microbiome analysis, based on the metabolic phenotypic characters as well as genotypic characters of the host will allow us to understand the factors responsible for maintenance of host microbial homeostasis. However, further studies should aim at assessing the genetic maps which help us to

develop new diagnostic methods and thus individualized therapy.

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