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Oral Biofilm- A Review

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Abstract

Microbial biofilms are complex communities of bacteria and are common in the human oral cavity and in the environment. Dental plaque is structured microbiology, and pathophysiology of dental biofilms. Biofilm increases bacteria's resistance to both the host's defense system and antimicrobial action. If it's not removed regularly, the biofilm undergoes maturation and can lead to dental caries, gingivitis and periodontitis. In addition, subgingival biofilm in patients with periodontitis, has been associated with various systemic diseases like cardiovascular disease, diabetes mellitus, respiratory disease, and adverse pregnancy outcomes. So it's important to understanding of the nature and pathophysiology of the dental biofilm to implement proper management strategies. Although dental biofilm cannot be eliminated by daily oral care but it can be reduced and controlled. Biofilm can be controlled by daily regimen of thorough mechanical oral hygiene procedures, including tooth brushing and interdental cleaning. The root canal anatomy provides excellent conditions for a biofilm to develop which is one of the main causes for caries and later progress to pulpal diseases. The following review article explores the biofilm formation which commences from adhesion of planktonic microorganisms to a surface followed by colonization, coadhesion, growth and maturation and finally detachment of some microorganisms on the tooth surface and inside the root canal.

Keywords: Biofilm, Bacteria, Quorum sensing, Root canal.

Introduction

Oral biofilms are structured dental plaque are attached to a solid surface like the enamel, cementum or dental implants[(1)] and are embedded in polysaccharide matrix.[(2)] Oral biofilms help bacteria adhere to the to the surface[(3,4)] and responsible for antibiotic resistance.[(5)] Biofilms are also found in dental unit water lines, dental prosthetic appliances and on oral mucosa. Biofilm in the form of supragingival plaque causes caries and subgingival plaque causes gingivitis and periodontitis.[(6-9)] Therefore, it's important to control the dental biofilm is a major objective of dental professionals to maintain optimal oral health. Bacterial biofilms are also found in a variety of sites within the human body.

Dental plaque exhibits an open architecture which consists of channels and voids, which helps to achieve the flow of nutrients, waste products, metabolites, enzymes, and oxygen.[(3)] Due to this structure, a variety of microbial organisms forms biofilms, including both aerobic and anaerobic bacteria.

Quorum sensing is the means of communication between these bacteria to regulate a wide range of behavior patterns among them. Bassler in 2002 reveal the basic machinery of cell—cell signaling in microbial communities. The signal machinery bacteria use to coordinate a variety of their activities is identified by various studies.[(10-12)]

Quorum sensing is widely employed by a variety of gram-positive and gram-negative bacterial species to coordinate various activities in biofilms. Quorum-sensing-interfering compounds have either a positive or a negative effect on the expression of bacterial phenotypes regulated by quorum sensing.[(13-15)] These studies of bacterial quorum sensing have also suggested several ideal targets for drug design which can be promising in preventive and therapeutic aspects of periodontal diseases and dental caries.[(16)]

Bacteria in biofilms encounter much higher local cell densities than free-floating, planktonic cell populations. An obvious consequence of this is the elevated levels of metabolic by-products, secondary metabolites and other secreted or excreted microbial factors that biofilm cells encounter.[(12,15)] Of particular interest are intercellular signaling molecules called the 'quorum-sensing molecules.' This ability, termed quorum sensing, functions through the secretion and detection of autoinducer molecules which accumulate in a cell density dependent manner. When the concentration of autoinducers reaches a threshold level, activation of the receptor leads to a signal transduction cascade to switch on specific genes in the bacterial cells, leading to a coordinated population response. As a group, bacteria behave in one way when there are few bacteria around them and in a different way when there are many bacteria present (Table 1).

Table 1: Key players in quorum sensing

Autoinducers
Acyl homoserine lactones
Autoinducer 2
Cyclic dipeptides
Bradyoxetin
Other types of autoinducers
Autoinducer Synthases
AHL synthases

AI-2 synthase
Synthases for other types of autoinducers
Quorum Sensing Regulators
LuxR-type regulators
LuxP/Q-type regulators

1. **Pathways of entry of microorganisms into the pulp:** The portals of entry of microorganisms into the pulp chamber are Fig. 1.[17]

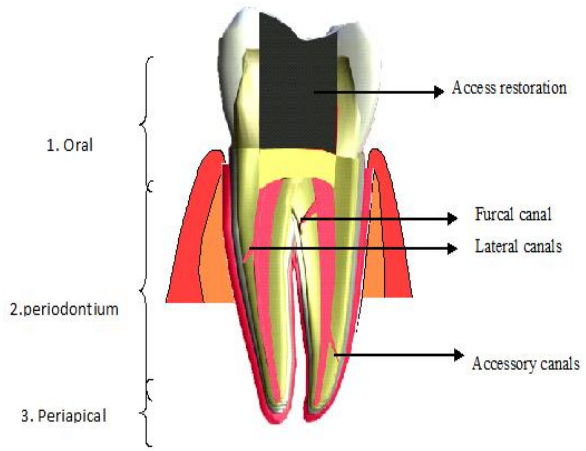

Fig. 1: Schematic diagram representing the portals of entry of infection into the root canal

2. Formation of biofilm

Formation of biofilm on the tooth surface: Biofilm formation occurs in the presence of microorganisms, fluid and solid surface. The phases for microbial community to develop a biofilm and colonize the environment may sometimes be unusual, but basically occur with the same sequence of developmental steps (Fig. 2).

a. **Deposition of conditioning film**: Inorganic and organic molecules like proteins and glycoproteins from saliva and gingival crevicular fluid adsorbed to the solid surface which leads to the formation of conditioning film.[18]

b. **Adhesion and colonization of planktonic microorganisms:** Adhesion and colonization of conditioning film is strengthened by polymer production and unfolding of cell surface structures. Main organism which is involved in the formation of biofilm is Streptococcus followed by subsequent attachment of gram positive and gram negative bacteria.[19] Factors affecting bacterial adhesion are pH, temperature, flow rate of fluid, nutrient supply, surface energy of the substrate, bacterial content, bacterial growth stage, bacterial cell surface charge, and surface hydrophobicity.[20] Changes in pH can frequently exploited in the production of detergents and disinfectants and used to kill bacteria. The optimum temperature for a microorganism is associated with an increase in nutrient intake resulting in a rapid formation of biofilm. Higher surface energy of the surface will help in faster growth of the bacteria.

c. **Bacterial growth and biofilm expansion:** single layer of microorganisms attracts secondary colonizers to form microcolonies. These microcolonies form by co-adhesion and coaggregation. Coadhesion is a process of recognition between a suspended cell and cell already attached to substratum. Coaggregation is a process where genetically distinct cells in suspension recognize each other and results in a clump formation. At the end of this stage biofilm is seen as corncob structure.[21]

d. **Detachment of biofilm microorganisms into their surroundings**: which are of two types – seeding disposal and clumping dispersal. Seeding dispersal it is detachment of Planktonic bacterial cells by local hydrolysis of the extracellular polysaccharide matrix, and conversion of a subpopulation of cells into motile planktonic cells.[22] Clumping Dispersal: A physical detachment pathway in which a fragment of a microcolony detaches from the biofilm.

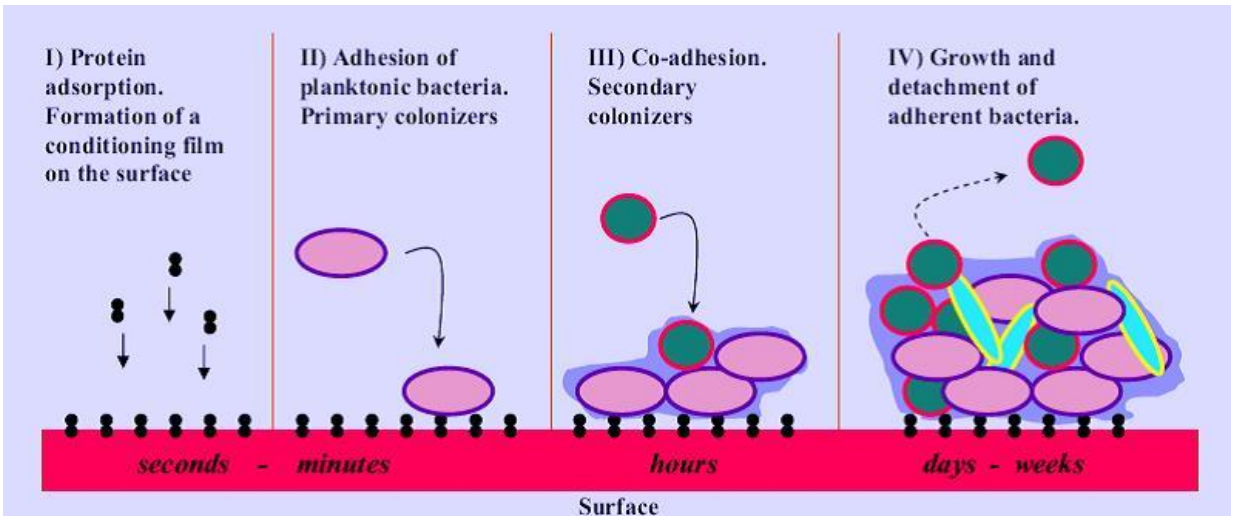

Fig. 2: Stage of biofilm formation

The cells within biofilms produce either matrix made up of extracellular polymeric substances (EPS)[23] or cell surface structures (capsule). EPS protects the biofilm bacteria UV radiation, pH shifts, osmotic shock and dessication.[24] EPS can also engulf metals, cations and toxins.[25] The activity of positively charged antimicrobial agents is neutralized by negatively charged EPS.[26]

Water channels in the biofilm facilitates efficient exchange of materials between outer and inner fluid medium to provide sufficient nutrition to nutrient deprived ecosystem.[27] Bacteria's in a biofilm can communicate, exchange genetic materials and also acquire new traits. Communications in biofilm are of two types which includes intraspecies communication[28] and inter species communication.[29]

Intraspecies communication include Quorum sensing (Fig. 3) which is mediated by low molecular weight molecules, which can affect the metabolic activity of neighboring cells and coordinate the functions of resident bacterials cells inside the biofilm.[28] Interspecies communication includes release of peptide molecules by streptococci to the bacteria within the biofilm.

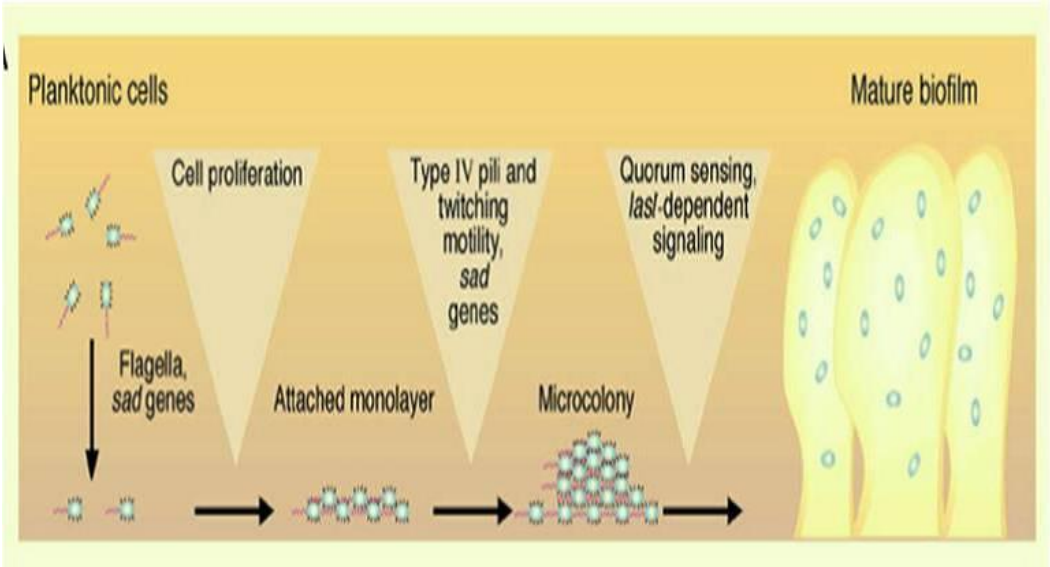

Fig. 3: Interspecies communication (Quorum sensing)

Biofilm in root canal

Bacteria present in the oral cavity serves as primary source of biofilm formation inside the root canal. The anatomical complexities in the root canal system provide shelter to bacteria.[30]

Biofilm formation in root canals is initiated at after the first invasion of the pulp chamber by planktonic oral organisms. At this point, the inflammatory lesion moves successively toward the apex and provide the fluid vehicle for the invading planktonic organisms so these can multiply and continue attaching to the root

canal walls. The necrotic pulp tissue provide favorable environment for microbial proliferation due to the presence of organic residue or nutrients. Gram-negative bacteria are more dominant than Gram- positive bacteria. Facultative or strict anaerobic microorganisms are more frequent than aerobic microorganisms.

Endodontic biofilm can be of various categories

1. Intracanal biofilm
2. Extra radicular biofilm
3. Periapical biofilm
4. Foreign body centered biofilm

Intracanal biofilm are microbial biofilm seen on the root canal dentine of infected tooth. Nair in 1987 First identified the biofilm under transmission electron microscopy.[31] intra canal biofilm contains loose collections of cocci, rods, filaments and spirochetes apart from this bacterial condensations were seen as palisade structure similar to dental plaque seen on tooth surface.[32]

Extra radicular biofilm are root surface biofilms formed on cementum adjacent to the root apex of endodontically infected teeth.[33] Main bacteria detected in extra radicular biofilm were Fusobacterium nucleatum, Porphyromonas gingivalis and Tannellera forsythensis by using PCR based 16s rRNA gene assay.[34]

Periapical microbial biofilms are isolated biofilms in the periapical region of endodontically infected teeth. Bacteria in such biofilm should have the capacity to overcome host defense mechanisms and result in periapical lesions.[35] Microorganisms seen in this biofilm are Actinomyces species and P. Propionicum.

Foreign body –centered biofilm is seen when microorganisms adheres to an artificial biomaterial surface and forms biofilm.[36] it is also called as biomaterial centered infection. Bacteria like S. aureus, Streptococci, Enterococci, P. Aeruginosa and fungi are commonly isolated from infected biomaterial surfaces.

3. **Changing Views of Dental Plaque:** Understanding and characterization of dental plaque have undergone significant evolution. Loesche[37] proposed non-specific and a specific plaque hypothesis for periodontal disease initiation and progression. The non-specific plaque hypothesis proposed that the entire microbial community of plaque which seen on tooth surfaces and in the gingival sulcus contributed to the development of periodontal disease. Under this hypothesis, development of periodontal disease was considered on the basis of quantity of plaque.

In specific plaque hypothesis theory specific pathogenic microorganisms (quality) found in the plaque, which are primarily responsible for inducing disease and disease progression.[38,39]

4. **Biofilm and Oral Disease:** Biofilms can exist on oral mucosa, the tongue, biomaterials used for restorations and dental appliances and tooth surfaces above and below the gingival margin. The oral health professionals should communicate to their patients that both dental caries and periodontal disease are infectious diseases resulting from dental plaque biofilm accumulation. These diseases require specific strategies for prevention and treatment. These strategies account for the ongoing challenge of successful management periodontal disease and it's progression.[40]

Gurenlian, in 2007[41] stated that Teaching patients to use daily brushing, interdental cleaning, and antimicrobial mouthrinses will definitely help in preventing biofilm formation in oral cavity and improvement in oral health. According to Seneviratne et al. In the healthy state, both plaque biofilm and adjacent tissues maintain a delicate balance, establishing a harmonious relationship between the two.[42]

As the biofilm matures and proliferates, pathogenic bacteria produces soluble compounds which penetrate the sulcular epithelium. These compounds stimulate host cells to produce chemical mediators associated with the inflammatory process.[43]

Interleukin-1 beta (IL-1β), prostaglandins, tumor necrosis factor alpha (TNF-α), and matrix metalloproteinases are mediators that recruit neutrophils to the area by chemotaxis and increases permeability of gingival blood vessels and permitting plasma proteins to migrate from within the blood vessels into the tissue.[44] Additional mediators are produced as the gingival inflammatory process continues, and more inflammatory cell types such as neutrophils, T cells, and monocytes are recruited to the area.

Cytokines are produced in the tissues as a response to the chronic inflammatory process, and these cytokines may further increase the local inflammatory response and affect the initiation and progression of systemic inflammation and disease.

Breakdown of gingival collagen and accumulation of an inflammatory infiltrate as a result of this chronic inflammation, leading to the clinical signs of gingivitis. In some individuals, this gingivitis will progress into periodontitis by breakdown of collagen in the periodontal ligament and resorption of the supporting alveolar bone. By controlling dental plaque biofilm we can prevent gingivitis as well as periodontitis.

Periodontal Biofilm Infection and Systemic Health

Many studies have demonstrated an association between periodontitis and systemic diseases and conditions, like cardiovascular disease, diabetes mellitus, respiratory disease, adverse pregnancy outcomes, obesity, pancreatic cancer, and Alzheimer's disease. Meenawat et al,[45] concluded that Periodontal disease was more evident in type 1 diabetes mellitus patients and periodontal inflammation is greatly

increased in subjects with longer disease course, poor metabolic control and diabetic complications. Al-Zahrani concluded in his study that In a younger population, overall and abdominal obesity are associated with increased prevalence of periodontal disease, while underweight (BMI < 18.5) is associated with decreased prevalence.[46] In contradict to the above statements, Miller showed till date no one has evaluated the effects of periodontal therapy on the metabolic state of the poorly-controlled diabetic patient.[47]

The association between periodontal disease and systemic diseases may relate to the ability of subgingival plaque bacteria and/or their products which gain access to the systemic circulation through the ulcerated sulcular epithelium of the periodontal pocket. In this way, both oral and systemic inflammation caused by a dental biofilm infection.[38]

Strategies for Managing Dental Biofilm to Promote Health

Though dental biofilm cannot be completely removed, its pathogenicity can be decreased through effective oral hygiene measures like Daily tooth brushing, interdental cleaning, and antimicrobial mouthrinses to reduce the bacterial biofilm and to help prevent periodontal diseases. Effect of antimicrobial mouthrinses is accepted by American Dental Association.[48] Oral mucosa can serve as a reservoir for pathogenic bacteria that can be transferred to the tooth surface and sulcus through saliva. Supplementing mechanical cleaning methods with topical antimicrobials may also play an important role in reducing reservoirs of bacteria that are unaffected by brushing and flossing directed at the tooth surface.

Eradication of biofilm from the root canal

Biofilm are found to be resistant to many antimicrobials like amoxicillin, doxycycline and metronidazole.[49] Sodium hypochlorite is an effective root canal irrigant to destroy all forms of Enterococcus faecalis.[50-52] 2% Chlorhexidine gel or liquid form is effective to eliminate Enterococcus faecalis from dentinal tubules up to 100micrometer.[53]

The new techniques include use of ultrasonic activation of the irrigation, ozone, plasma dental probe, photoactivated disinfection (PAD) with low-energy laser for removal of the biofilm. However, NaOCl was the main method to eliminated biofilm. Root canal after plasma treatment for 5 min the Scanning electron microscopy showed complete destruction of endodontic biofilms for a depth of 1 mm.

The Er: YAG laser have produced excellent results due to its capacity for ablating hard tissue with very less thermal effects. They are considered to be most effective tool for the removal of apical biofilm. Photodynamic therapy is the latest method used to destruct endodontic biofilm. It involves the killing of microorganisms when a photo sensitizer selectively accumulated in the target is activated by a visible light of appropriate wavelength.

PAD is a unique combination of a photosensitizer solution and low-power laser light. The photosensitizer, which is mostly colored, adheres to or gets absorbed by microbial cells. The low-power laser will destruct the target area and inactivate the microbial invaders. The photosensitizer then binds to microbial cell walls or even enters the cells. Further, the Laser light activates the photosensitizer and creates a cascade of energy transfer and variable chemical reactions in which singlet oxygen and free radicals play an important role (Fig. 4).

Usually sodium hypochlorite, antibiotics and other methods against microbial threats need a lot of time to inactivate the microbes. PAD needs a maximum of 150 seconds. PAD is effective against most of the microorganisms.[54]

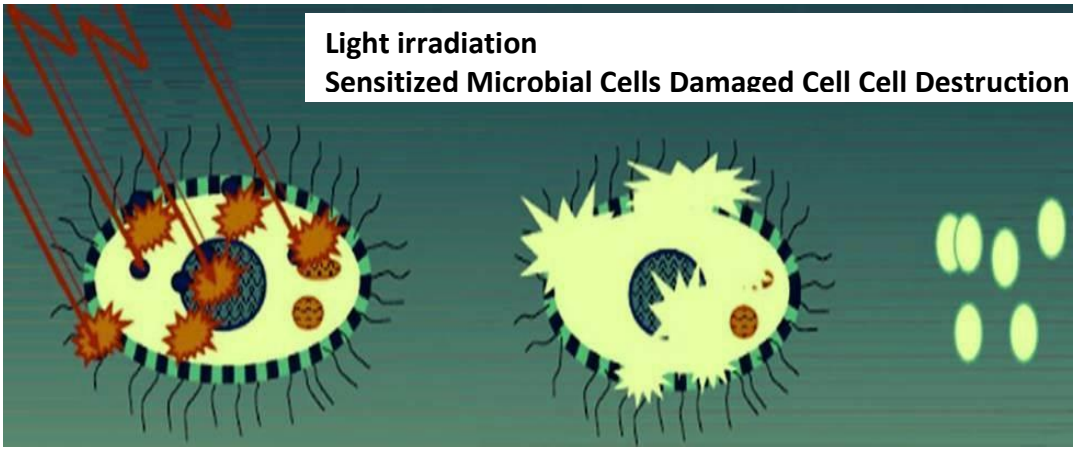

Fig. 4: Action of Photoactivated laser

Conclusion

Dental biofilm is a complex, organized bacterial community that is the primary etiologic factor for dental caries and periodontal diseases. Although the dental biofilm cannot be removed it can be decreased with mechanical and chemotherapeutic oral hygiene methods. The concept of biofilm to endodontic microbiology will play an important role in helping us to understand the root canal microbiota and also the basis for new approaches for disinfection.

Although additional research is required to eliminate biofilm from the oral cavity and root canals which should cost effective and potent in action without effecting normal tissues.

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