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Biofilms in Endodontics

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Abstract:

Biofilm is one of the basic survival strategies employed by microorganisms in all natural and industrial ecosystems in response to starvation. They are responsible for most of the chronic infections and almost all recalcitrant infections in human beings, as bacteria in biofilm are resistant to both antimicrobials and host defense mechanisms. Biofilms may be associated with each branch in dentistry. However, as an endodontist who are more viable regarding these biofilms during an endodontic procedure should have in-depth knowledge to achieve the treatment goals during the procedure.

Key Words: Biofilms, endodontics, infections

Introduction

Microorganisms can form a biofilm on any surface that is bathed in nutrient containing fluid. Development of biofilm is influenced by physiochemical property of components involved in the biofilm. pH, temperature, surface energy of substrate, nutrient availability, and length of the time the bacteria is in contact with the surface and bacterial cell surface charge may play a key role in biofilms. Studies have shown that biofilm is made up of single cells and microcolonies, all embedded in a highly hydrated, predominantly anionic exopolymer matrix.[1]

Stages in the Development of Biofilm

1. First step is the interaction and adsorption of inorganic and organic molecules to solid surface creating the conditioning layer
2. Once the conditioning layer is formed; finally, it undergoes adhesion of microbial cells to this layer.

In addition, the microbial adherence to substrate is also mediated by bacterial surface structure such as fimbriae, pili, flagella, and extra polymeric substances (EPS). The bacterial cell surface structures form bridges between the bacteria and conditioning layer. Initially, the bond between the bacteria and the substrate may not be strong. However with time, these bonds gains in strength, making the bacteria substrate attachment irreversible.

Significance of biofilms

Inside a biofilm, the bacterial cells exhibit altered phenotypic properties and are protected from antimicrobial environmental stresses, bacteriophage, and amoeba. Biofilms are responsible for most of the chronic infections and almost all recalcitrant infections in human beings, as bacteria in the biofilm are resistant to both antimicrobials and host defense mechanisms.

Ultrastructure of Biofilm

A fully developed biofilm is described as a heterogeneous arrangement of microbial cells on a solid surface. Basic structural unit microcolonies or cell clusters formed be surface adherent bacterial cells. Microcolonies comprises a discrete unit of densely packed bacterial cells aggregates.

Three factors essential for biofilm are:

1. Microorganisms
2. Solid substrate
3. Fluid channels.

Composition of biofilm

Biofilm consists of matrix material 85% volume and 15% cells. A fresh biofilm is made up of biopolymers such as polysaccharides, proteins, nucleic acids, and salts. A glycocalyx matrix is made up of EPS, which surrounds the microcolonies and anchors the bacterial cells to the substrate. Tower or mushroom shaped structure is typically characteristic feature of a viable fully hydrated biofilm. The overall shape of biofilm structure is determined by the force generated by flushing of fluid media. There is an important aspect when we see the composition of biofilm these are water-filled channels. These water channels act as a primitive circulatory system in a biofilm, intersect the structure of biofilm to establish connections between the microbial colonies. Biofilm community comprises efficient exchange between bacterial cells and fluid.[2] Detachment has been understood to play an important role in shaping the morphological characteristics. It is also an "active dispersal mechanism" or "seeding dispersal" where detached cells forms resistance traits which is the source of persistent infections. Erosion as the term suggests that is

the continual detachment of single cell and small portions of biofilm that plays a very important role in detachment. Sloughing a process of rapid, massive loss of biofilm is also another method of detachment.

Endodontic Biofilms

This transition in the microbial population is more conspicuous with the progression of infections. Furthermore, clinical investigations have shown that the complete disinfection of the root canal system is very difficult to achieve because microorganisms are found to persist in the root canal system complexities such as apical portions, deltas, isthmuses, and lateral canals. Various anatomical complexities in the root canal systems result in the adhering bacteria in a biofilm from cleaning and shaping procedures. During biomechanical preparation, apical biofilm plays very key rule clinically because they are inherently resistant to antimicrobial agents and cannot be removed by biomechanical preparation alone. This may cause failure of endodontic treatment as a consequence of persistent infection.[3]

It has been suggesting that persisting infections subsequent to endodontic therapy during root canal procedures are caused by one or two bacterial species that are too robust to be eliminated by conventional treatment measures.[4] Kakehashi *et al*. showed that apical periodontitis will develop if the canal system is contaminated with bacteria.[5] Multiple *in vitro* studies have shown that even the best root canal treatment can allow leakage of bacteria and their byproducts through an apparently well-filled canal system. Yet, other studies have shown that even in the presence of obvious contamination, periapical disease does not necessarily develop in all patients.[6,7] The work of Ray and Trope provided important insight into what may be conceived as a paradigm shift in endodontic treatment philosophy in their quest to determine which aspect of treatment had a greater impact on the outcome of root canal treatment.[8] As an endodontist, we have to know the treatment procedure and also have the ability to give the best root canal treatment.

Endodontic biofilms are of four types:

a. Intracanal biofilm
b. Periapical biofilm
c. Extraradicular biofilm
d. Biomaterial centred biofilms.

Intracanal biofilms

Microbial biofilms that are formed on root canal dentin of endodontically infected teeth. Intracanal microbiota in endodontically infected teeth exists as both loose microbial cells and biofilm structures, made up of cocci, rods, and filamentous bacteria. It is monolayered or multilayered in structure.

Extraradicular biofilms

These biofilms are also known as "root surface biofilms." These biofilms are microbial films formed on the root surface adjacent to root apex of endodontically infected teeth.

Extra radicular biofilm

This type of biofilms is reported in teeth with asymptomatic periapical periodontitis and teeth with chronic apical abscess associated with sinus tracts. These biofilms consist of cocci and rods and filamentous species, with cocci attached to tooth substrate. Ricucci and Siqueira reported the presence of calculus like deposit on root apex of teeth extracted due to post-treatment periapical periodontitis.[9] Tseng *et al*. suggested that calculus like deposit on apical root surface of tooth with lesion refractory to root canal therapy.[10]

Periapical microbial biofilms

These are isolated biofilms found in the periapical region of an endodontically infected tooth. The microbiota in the majority of teeth associated with apical periodontitis is restricted to root canal as most of the microbial species that infect the root canal are opportunistic pathogens that do not have the ability to survive the host defense mechanisms in periapical tissues. Rarely, microbial species or even strains within species may possess strategies to survive and thus infect periapical tissues.

Biomaterial centered infection (BCI)

It is caused when microorganisms adhere to an artificial biomaterial surface and form biofilms structures. Biomaterials play an important role, and hence presence of biomaterials in close range to host immune systems can increase the susceptibility to biofilms. BCI is one of the major complications associated with prosthesis and or implant related infections. Studies have shown that NaOCl was the most effective agent, capable of eradicating the biofilms after 1 min at a concentration of 0.00625%. Chlorhexidine eradicated biofilm after 5 min at 2%. It has been documented that ethylenediaminetetraacetic acid and citric and phosphoric acid solutions were not effective against the biofilms at any concentration or time-tested.[11]

Conclusion

Biofilm is an expanding field of research which involves around human, industrial, and environmental ecosystems. Thus, one future challenge for research in endodontology is to assess virulence expressions in *in vivo* and *in situ* models with microenvironment resembling the real life condition in the root canal. Furthermore, the understanding of biofilm concept will play a key role in helping us to assess and understand not only the pathogenic potential of root canal microbiota but also the basis for new approaches to infection control.

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