Antibiofilm protocols in modern endodontic treatment

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Abstract
Endodontic biofilm exist in the form of aggregates and a coaggregate attached by glycocalyx membrane is considered as primary etiology for endodontic infections. The primary goal of endodontic treatment is to eliminate the biofilm from root canal walls which is responsible for endodontic infection. Biofilm formed by endodontic pathogens is resistant to antimicrobial disinfection procedures there fore new approaches for elimination of biofilm have been introduced such as lasers, photo activated disinfection and nanoparticles.

Keywords: Biofilm, Anti biofilm, Root canal irrigation, Intracanal medicaments.

Introduction
The ability of oral microorganisms to adhere to hard surfaces in presence nutrition rich environment leads to formation of slimy layer rich in polysaccharides and protein known as biofilm. Biofilm is a polymicrobial in nature consisting a dynamic community of interacting sessile cells.[1] Infected root canals include aerobic, anaerobic, gram positive and gram negative microorganisms which have a capacity to form biofilm on hard and soft tissues of the oral cavity.[2]

History
Van Leeuwenhoek, discovered microorganisms attach and grow universally on exposed surfaces which exhibits different genetic characteristics and growth rate.[3] Miller observed microbiological changes in infected pulp tissue and found that there are different microorganisms especially surface associated microorganisms show different phenotypic and genetic characteristics as compared to planktonic microorganisms. In 1984 Miller published report on microbiological investigation of infected pulp tissue and found that flora in coronal, middle and apical third is different.[4] Kakeishi et al in his experiments on germ free rats proved that germfree rats did not develop pulpal infection whereas rats exposed to oral microorganisms developed pulpal necrosis and periapical lesion.[5]

Characteristics of endodontic biofilms
Biofilm is embedded in self made matrix of glycocalyx (extracellular polymeric substances) on which aggregates and co aggregates of different species of microorganisms are irreversibly attached to the substratum.[6]

Biofilm is structurally made up polymeric substance upon which surface adherent micro colonies and cells are attached. 85% of matrix material is made up of proteins, polysaccharides, nucleic acids and salts, 15% made up of salts. Water channels are important circulatory system which helps in exchanging the nutrients, metabolites and genetic materials among them. [7]

As the biofilm matures due to environmental changes the composition and structural changes takes place and microorganisms start detaching from the surface (seeding dispersal).The polymeric substances are dissolved in fluid surrounding the biofilm which is responsible for chronic infections. [8]

According to Cardwell et al Biofilm shows four characters

Autopoiesis - Ability to self organize in nutrient rich environment.

Homeostasis - ability to resist environmental disturbances

Synergism- must be more effective in association then isolation
Communality- during environmental changes the microorganisms should act as single unit.[9]

Due to aforementioned characteristics, bacteria in a biofilm have a unique ability to survive challenging environmental conditions. Additionally the microorganisms exhibit organized internal compartmentalization which help the microorganisms with similar growth requirement to reside in one compartment. The bacteria acquire new traits by communicating and exchanging genetic material. Bacterial cells communicate with each other through signaling molecules the process is known as quoram-sensing. [10]

Stages and factors affecting the development of biofilm

For the biofilm to develop three things are required: Bacterial cells, fluid medium and solid substrate.
Stage 1; Due to adsorption of organic and inorganic molecules on solid surface a conditioning layer is formed.
Stage 2: attachment of Planktonic cells
Phase 1.bacteria attach to the surface by their surface structures such as fimbriae, pilia, flagella
Phase 2.bacterial surface specific interaction with the substrate by electrostatic interaction
Phase 3.with the formation of polysaccharide adhesion or ligand which binds to surface specific receptors
Stage 3: bacterial growth and biofilm expansion [11, 12]

Various biofilm models and assessment methods

Biofilm characterization such as type of microorganisms, surface characteristics and viability of microorganisms can be characterized by biofilm assay such as colorimetric techniques, physical methods and molecular methods.[14]Recently interaction forces among bacterial cells and to their substrate and was assessed by Atomic Force Microscopy (AFM) based on this finding root canal irrigants which alter the physicochemical properties of dentin can influence the bacterial adhesion on canal wall.[15] Micromanipulators which assess individual cells or compartments in the biofilm structure.[16] Laser based optical tweezers noninvasive and noncontact tools which can probe the interaction between bacteria and collagen. Chemical composition of the mature biofilm can be assessed qualitatively and quantitatively by Fourier Transform Infrared Spectroscopy (FTIR).[17] Constituents of bacterial biofims can be studied by Solid State nuclear magnetic resonance (NMR). Biofilm identification and characterization is further advanced by green fluorescent protein (GFP) tagging, Confocal laser scanning microscopy (CLSM), Flow cytometry, and fluorescence *in situ* hybridization (FISH).[18,19]

Endodontic biofilms

More than 400 different microbial species were identified from different apical periodontitis cases by advanced pyrosequencing techniques. Endodontic pathogens fall in to 15 major phyla such as Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Spirochaetes, and Synergistes.

The mechanism of biofilm formation involves penetration of microorganisms in pulpal space where it attaches and spreads further. Once the microorganisms form biofilm the infection gets established and subsequent pulp destruction takes place.At some point of time after the infection gets established a steady state is reached where bacterial mass is held up by host defense mechanism. The zone of demarcation may be inside the root canal or at the apex or on the external root surface near the exit of the foramen.[20]

With progression of the disease the endodontic microbiota will undergo transition from aerobic to predominantly anaerobic in nutrition depleted environment. The anatomical and geometrical complexities of root canal shelter microorganisms which are difficult to clean.[21]

Endodontic biofilms are classified as

Intracanal biofilm: formed on the dentin wall of infected root canal, the bacterial structures consist of filaments, rods and cocci which are arranged in palisade pattern.
Extraradicular biofilm: are formed on the root surface of endodontically infected tooth adjacent to root apex

Periapical biofilm: these isolated, periapical biofilms are seen in endodontically infected teeth even in absence of infection.

Foreign body centered biofilm: when bacteria adhere to an artificial biomaterial surface and forms a biofilm. It is usually associated with implants and other prosthesis.[21-23]

Role of *E.FECALIS* in biofilm

E fecalis is the most commonly found anaerobic facultative microorganism in persistant root canal infections. Endodontic microorganisms have unique capacity to form a biofilm on the root canal walls. Microorganisms associated with biofilm formation exchange gene via horizontal gene transfer between clinically relevant species. E fecalis can inactivate calcium hydroxide dressing by ph homeostasis but at ph 11.5 it is not able to survive.[24]

Current therapeutic options endodontic biofilms

Endodontic irrigating solutions possess both antimicrobial and antibiofilm properties to eradicate microorganisms existing in biofilm mode of growth. Although current irrigation regime using sodium hypochlorite exhibits excellent antimicrobial property but it has irritating effect on soft tissues, to overcome this disadvantage herbal alternatives such as tulsi, neem, green tea extract, berberine are found to be effective which possess minimum cytotoxicity and side effects.[25]

There are other irrigating solutions such as cholorhexidine and cetrimide are less effective then sodium hypochlorite against e- fecalis biofilm. Chelating agents such as ethylene diamino tetra acetic acid (EDTA) and Maleic acid (MA) which are effective in smearlayer removal.

Biopure MTAD is a universal irrigating solution which consist of tetracycline and acid detergent which removes the smear layer and is effective against e.fecalis. A new irrigant Tetra clean also contain doxycycline hycalate and detergent at lower concentration then MTAD which removes the smear layer and microorganisms in less than 4 minutes. Combination of different irrigation protocols such as root canal agitation with ultrasonic instruments, apical negative pressure irrigation systems such as ENDOVAC and Rinse Endo systems are found to be beneficial in elimination of biofilm. Affective elimination of biofilm microorganisms from root canal walls can be achieved by newer promising treatment modalities like photoactivated disinfection procedures, lasers and nanoparticles.

Conclusion

Endodontic microorganisms exist in the form surface aggregates on the root canal wall and even in the dentinal tubules which are difficult to eradicate by traditional root canal irrigation regieme. E.fecalis is the most common microorganism associated with persistant endodontic infections which is resistant to antimicrobials by horizontal gene transfer mechanism. Newer irrigation and instrumentation protocols using contemporary irrigation solutions along with lasers, nanoparticles and photo activated disinfection protocols are found to be promising.

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Conflict of Interest

None.

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