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ROLE OF ENDODONTIC BIOFILM IN DENTISTRY

Dr. Lovin Valsan Mathew* Post Graduate Student, Department of Conservative Dentistry And Endodontics, Maratha Mandal's Nathajirao G.halgekar Institute of Dental Science And Research Centre, Belgaum, Karnataka. Pin. 590010. *Corresponding Author

Dr. Veerendra M. Uppin Professor, Department of Conservative Dentistry and Endodontics, Maratha Mandal's Nathajirao G.Halgekar institute of Dental Science and Research Centre, Belgaum, Karnataka

Dr. Madhu Pujar Professor and HOD, Department of Conservative Dentistry And Endodontics, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Science And Research Centre, Belgaum, Karnataka

ABSTRACT

Endodontic infection is caused by microorganisms colonizing as surface aggregates in the root canal system. These complex microbial communities are called biofilms that harbours numerous microorganisms with altering ecological requirements and pathological potential. The root canal system's intricacy and unpredictability, along with the multi-species nature of biofilms, make disinfection extremely difficult. The primary goal of endodontic treatment is to eliminate the biofilm from the root canal walls which is responsible for endodontic infection. The most important factor for failure of root canal treatment is the persistence of microorganisms as bioflms in the root canals. Eradication of biofilm is made possible by specific instruments and disinfecting chemicals in the form of irrigants and/or intracanal medicaments. Endodontic research has focused on the characterization of root canal biofilms and the clinical methods to disrupt the biofilms along with microbial killing. The aim of this narrative is to review the mechanisms of biofilms' formation, their roles in pulpal and periapical pathosis, the different types of biofilms, the factors influencing biofilm formation, the mechanisms of their antimicrobial resistance, techniques to identify biofilms and the role of root canal disinfectants on biofilm removal.

INTRODUCTION

A biofilm is a highly organised structure made up of bacterial cells encased in a self-produced extracellular polymeric matrix that is linked to a surface. [1] It can also be thought of as a layer of microbiota condensation or a microbial-derived community composed of cells that are irreversibly attached to a substratum or interface, or to each other, and embedded in a matrix of extracellular polysaccharides as well as extracellular DNA (eDNA) and extracellular proteins. [1,2]

Bacteria in biofilms have distinct physiological features than bacteria in culture media because microorganisms in biofilms are shielded from environmental stimuli by their matrix. [3]

Microbial biofilms in the root canal are extremely resistant to endodontic disinfectants. The intricate and unpredictable character of root canal anatomy, as well as the multi-species biofilm, exacerbates the difficulties in removing microbial biomasses from there. [4, 5] As a result, the purpose of this communication is to provide an overview of the biofilm idea and to examine how it may apply to endodontic diseases.

Endodontic Biofilm

The flora is typically polymicrobial, with obligate anaerobic bacteria as predominant organisms. In 1955, *Mazzarella and colleagues* discovered that the number of bacteria isolated from teeth differed slightly. [7] Streptococci, staphylococci, corynebacteria, yeasts etc are the most common Gram-positive organisms. Spirochetes, neisseria, bacteroides, fusobacterium, pseudomonas, coliform bacteria etc are the Gram-negative bacteria. [7]

Endodontic biofilms are classified as intracanal biofilm, extraradicular biofilm and periapical biofilm. Extraradicular biofilm forms on the root surface of an endodontically infected tooth near the root apex, while intracanal biofilm forms on the dentin wall of an infected root canal. Periapical biofilm can exists in endodontically affected teeth even if infection is not present. When bacteria bind to an artificial biomaterial surface, a foreign body centred biofilm is created. [6]

Characteristics Of Endodontic Biofilm

Biofilm is embedded in a self-made glycocalyx matrix on which aggregates and co-aggregates of various microorganism species are permanently connected. [6] Proteins, polysaccharides, nucleic acids, and salts constitute 85% of matrix material, while salts constitute 15%. [7] Bacteria with varied growth requirements can thrive in their own micro-environments owing to the biofilm's well-organized internal compartmentalization. The interstitial voids or channels provide a mechanism of circulating nutrients and exchanging metabolic products with the bulk fluid layer. [8, 9] Composition and structural changes occur as the biofilm matures and bacteria begin to separate from the surface (seeding dispersal). The polymeric compounds breakdown in the fluid and surrounds the biofilm leading to chronic infections. [7]

The extracellular polysaccharide (EPS) protects all resident bacteria in the biofilm from pH fluctuations, osmotic shock, UV radiation, and desiccation. Antimicrobial tolerance of biofilm is considered to be influenced by long term drug use, nutrient depletion and presence of highly resistant phenotype. [10]

Quorum sensing is a type of bacterial cell-to-cell communication system that allows bacteria to communicate with one another via signalling molecules. [11] It aids bacteria in acquiring new features through the exchange of genetic material, as well as in dealing with environmental challenges. [12]

Formation Of Endodontic Biofilm

Stage 1: Organic and inorganic molecules adsorb on a solid surface, resulting in the formation of a conditioning layer.

Stage 2: Planktonic cells are attached to a polymeric matrix.

Phase 1: Bacteria attach to solid surfaces using surface features such as fimbriae, pilia, and flagella.

Phase 2: Electrostatic interaction allows bacteria to interact with the substrate on a surface-specific level.

Phase 3: The development of a polysaccharide adhesion or ligand that binds to specific receptors on the surface.

Stage 3: Growth of bacteria and expansion of biofilm can be seen in this stage.[6]

Methods Of Studying Biofilms In Endodontics

Biofilms are analyzed by focusing at the consortia of microorganisms as a whole and considering the relationships between different species. *Microtiter Plate-Based Systems* are a closed system with no inward or outward flow in the reactor, making them excellent for efficient biofilm disinfection and screening. The *flow displacement system* is an open system that includes a growth medium containing nutrients that are added at a constant rate.[13]

Modified Robbins Device uses silicone or hydroxyapatite discs as a bacterial growth substrate allowing continuous biofilm production when exposed to fluid flow. Because it facilitates biofilm formation at varying flow velocities and cell-to-fluidic volume ratios, the *Microfluidic Device* could be useful in polymerase chain reaction, protein analysis, and DNA sequencing. *Confocal Laser Scanning Microscopy* allows for 3-Dimensional reconstruction of the biomass whereas *Scanning Electron Microscopy* allows qualitative examination of microbial communities.[13]

Atomic Force Microscopy (AFM) analysed the contact forces between bacterial cells and their substrate, and found that root canal irrigants can alter bacterial adhesion to canal walls. *Optical tweezers* based on lasers can be used to study bacteria-collagen interactions. *Fourier Transform Infrared Spectroscopy* (FTIR) quantify and qualitatively examine the chemical composition of the mature biofilm. [6] *Nuclear magnetic resonance*, *Green fluorescent protein*, *Fluorescent Microscopic Techniques*, etc are some of the other recent methods for studying biofilm models.[13]

Removal Of Endodntic Biofilms

Root canal irrigation has the goals of dissolving necrotic pulp tissues, disrupting endodontic biofilms, neutralising endotoxins, and removing the smear layer. Sodium hypochlorite (NaOCl) is a proteolytic irrigant which is considered as the most potent disinfectant in endodontics. The concentration of NaOCl used in endodontic therapy ranges from 0.5 to 6%, with antibacterial activity not being affected by the concentration, but tissue dissolution and biofilm disruption being unaffected. [14] Warming the solution, using an activation approach, increasing the volume of the irrigant, lowering the pH of the irrigant, etc enhances endodontic biofilm removal.[13]

Because of its lesser toxicity than NaOCl, a 2 % concentration of chlorhexidine (CHX) gluconate is recommended as a root canal irrigant. When combined with cetrimide, CHX aids in the breakdown of the EPS matrix. When compared to CHX alone, CHX Plus, which incorporates surface modifiers, showed higher levels of bacteriocidal activity. [13] Alexidine, Octenidine hydrochloride, Iodine potassium iodide etc have been proven to aid in the disruption of endodontic biofilm.[13]

The chelating chemical *ethylenediaminotetraaceticacid* (EDTA) can eliminate the inorganic portion of the smear layer. It's a demineralizing agent that, when combined with NaOCl, facilitated E. faecalis biofilm removal. Maleic acid and Peracetic acid are two other demineralizing agents that have been demonstrated to be effective against E. Fecalis.[14]

In planktonic and biofilm states, MTAD (a mixture of 3% doxycycline, 4.25 % citric acid, and 0.5 % Tween 80) and QMiX (a mixture of CHX, EDTA, and a detergent) have been demonstrated to be as effective as NaOCl and superior to CHX against Enterococcus fecalis. When 18% etidronic acid is combined with 5% NaOCl, it has remarkable antibiofilm activity against E. fecalis biofilms. Berberine, Morindacitrifolia, and Curcumin, have been tested against root canal biofilms and found to be effective alone or in conjunction with other root canal irrigants.[13]

The permeability of cell walls is altered by chitosan, zinc oxide and silver (10-100nm) nanoparticles, resulting in cell death. The efficiency of rose bengal-functionalized calcium silicate nanoparticles against monospecies and multispecies biofilms has been extensively researched. By forming collagen cross-links with proteins, photodynamic treatment would disinfect the root canals. The adherence of E. Fecalis biofilms in the root canals was also significantly reduced using calcium silicate nanoparticles and bioactive glass powder coated with AgNp.[13]

The eradication of multi-species biofilms is aided by enzymatic irrigation with 1% trypsin and 1% proteinase K. Both aerobic and anaerobic bacteria were successfully killed by trypsin with ultrasonic activation. Intracanal medicaments like as calcium hydroxide, double antibiotic paste, triple antibiotic paste, and others have shown different efficiency against bacteria in endodontic biofilm. Sonic and ultrasonic agitation; photoactivated disinfection; laser activated disinfection; microbubble emulsion etc also assists in the detachment of surface adhering bacteria.[13]

CONCLUSION

The virulence features and survival strategies of biofilm bacteria have been the focus of endodontic biofilm research. The assessment of virulence expression in in-vivo models that mimic the real-life situation in the root canal is a future research challenge. The application of the biofilm paradigm to endodontic microbiology will be critical in assisting us in better understanding not only the pathogenic potential of the root canal microbiota, but also the foundation for innovative infection control and treatment techniques.

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