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Review Article

Endodontic Biofilm: A Review

Dhanu Thomas*, B. S. Keshava Prasad

D A Pandu Memorial RV Dental College, Department of Conservative Dentistry and Endodontics.

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ABSTRACT

Endodontic biofilms are structured microbial communities encased in a self-produced extracellular matrix, adhering to the root canal system's intricate surfaces. These biofilms play a pivotal role in the pathogenesis of pulpal and periapical diseases, contributing significantly to both primary and persistent endodontic infections. Their complex architecture and resistance to antimicrobial agents and host defenses pose a major challenge to effective root canal disinfection. The root canal system's anatomical complexities—such as isthmuses, lateral canals, and dentinal tubules—further facilitate biofilm survival and re-establishment post-treatment. Advances in microbiological techniques have revealed the polymicrobial nature of these biofilms, predominantly composed of facultative and obligate anaerobes, including Enterococcus faecalis, Fusobacterium nucleatum, and Prevotella species. This abstract explores the formation, structure, and resistance mechanisms of endodontic biofilms, along with current and emerging strategies for their disruption, including the use of irrigants, intracanal medicaments, ultrasonic activation, and novel approaches like photodynamic therapy and nanoparticles. Understanding the biology of endodontic biofilms is critical for improving clinical outcomes in endodontic therapy.

INTRODUCTION

A biofilm (Costerton et al.1995) is a polymicrobial community, with a biofilm growth pattern, living embedded in a self-produced matrix of highly hydrated extracellular polymeric substances (EPS)[1]. Biofilms are sessile multicellular microbial communities where microbes are enmeshed in a self-made extracellular polymeric substance (EPS, usually a polysaccharide), and firmly attached to surfaces[2]. These surfaces include root canal walls that provide a niche for bacteria. It is a community of microorganisms, such as bacteria, that are capable of living and reproducing as a collective entity known as a colony. It is a complex covering on the bacterial cells that provides survival support under unfavorable conditions such as antibiotic actions,

***Corresponding Author:** Dhanu Thomas

Address: *D A Pandu Memorial RV Dental College , Department Of Conservative Dentistry And Endodontics.*

Email : dhanuthomas8@gmail.com

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host immune actions, nutritional limitations, and environmental limitations. It is made up of various biological molecules such as carbohydrates, lipids, and proteins, which are synthesized and regulated by the bacterial cell itself. Challenges and difficulties in the eradication of root canal microorganisms could be classified into the anatomical, microbiological, environmental, and ecological factors[3]. From 1683 to 1708, Anthony van Leeuwenhoek (1632–1723) of Delft, the Netherlands, used his crude microscope to analyse and characterise biofilms on material from his own mouth, where he discovered aggregated microorganisms in the "scurf of the teeth" and from "particles scraped off his tongue"[4]. In marine microbiology, the word "film," which describes bacterial adhesion, aggregation, and proliferation on surfaces, was used to differentiate adherent (sessile) bacteria from free-swimming bacteria ‘planktonic’ organisms from 1933 to 1935[5].

Biofilm Formation

The development of biofilm involves four main stages, starting with cellular attachment, to formation of microcolonies, then biofilm maturation, and finally dispersion[6]. Microorganisms initially attach to a surface, often through reversible interactions like flagella and pili-mediated motility. After initial contact, cells undergo irreversible attachment, facilitated by surface proteins and extracellular polymeric substances (EPS). Following irreversible attachment, bacterial cells initiate the formation of microcolonies through the assembly of previously attached cells and cell division[7].

Biofilm Species

Endodontic biofilms are complex communities of various bacterial species, primarily gram-positive and gram-negative bacteria, that are crucial in the pathogenesis of endodontic infections. Key species involved include Enterococcus faecalis, Actinomyces, Streptococcus, Fusobacterium nucleatum, and Propionibacterium.

Factors Influencing Attachment:

Hydrodynamic forces, Brownian movements, electrostatic interactions, and Van der Waals forces all play a role in bacterial deposition and attachment influencing the initial stages of bacterial colonization and biofilm formation[8,9]. Once attached, bacteria begin to divide and produce EPS, forming a matrix that encapsulates the cells. The EPS matrix contributes to the formation of a three-dimensional biofilm structure. During this phase, microbial cells engage in cross-talk/communication via “quorum sensing”, producing autoinducer signals, which in turn lead to the activation of biofilm-specific genes. In the final stage, some cells detach from the biofilm and disperse, potentially colonizing new surfaces[6]. Species interact within the polymicrobial community through cooperative metabolism. The interaction occurs through cross feeding[10]. Endodontic biofilm resistance refers to the increased tolerance and survival of bacteria within a biofilm community in the root canal compared to planktonic cells. This resistance is attributed to various mechanisms involving altered physiology, functional redundancy and via adaptive extremophiles[11]

Biofilm Assessment Methods

Biofilms are studied in two ways: in one, the consortia of microorganisms as a single unit and, in the second, by studying the effects and relationships between one species and others[12]. Biofilm assessment models aids in understanding interaction of microorganism within the biofilm colony they replicate in vivo like condition[13]. Model development and studies are needed to explore the conditions that may affect the efficacy of endodontic anti-microbials in vivo so that their clinical effects can be better predicted. Biofilm models are categorized into four types: in vitro, ex vivo, nonmammalian in vivo, and mammalian in vivo[14]. These models serve the purpose of cultivating different microorganisms. However, in endodontics, the focus is primarily on the development and use of in vitro biofilm models. The properties of biofilms can be evaluated by analyzing the quantity and variety of microorganisms, their viability, age, thickness, structural features, and surface texture. Different techniques are used for biofilm assays, including colorimetric methods, microscopy, microbiological cultures, physical methods, biochemical approaches, and molecular techniques[13].

Endodontic Treatment And Disinfection

The methods of removal of endodontic biofilm are categorized into - Traditional methods ii. Contemporary approaches The challenge in endodontic treatments is for the irrigants to reach the minute areas and facilitate the removal of the inflamed or necrotic tissues within the biofilm. Current approaches to endodontic treatment are based on instrumentation, combined with irrigation and medication of the root canal system[15]. Besides, root canal ramifications, dentinal tubules, and other anatomical irregularities inaccessible to endodontic instrumentation and intracanal substances represent an additional difficulty for the treatment.[16]

Standard Chemomechanical Preparation:

Endodontic treatment uses mechanical tools and chemical solutions to clean the root canal. Mechanical tools, like stainless steel or NiTi rotary instruments, break apart and remove biofilm from canal walls. - Chemical solutions flush out debris and kill germs.Sodium hypochlorite (NaOCl) is the main chemical used, effective against bacteria and capable of dissolving tissue, but it works best with direct contact. Ethylenediaminetetraacetic acid (EDTA) is used with NaOCl to remove inorganic material and clear the smear layer, allowing better access to biofilm. Chlorhexidine (CHX) offers a lasting antimicrobial effect but doesn't dissolve tissue.Combining these solutions with strong irrigation techniques enhances fluid flow and improves biofilm removal.

Improved instrumentation of root canal

Eccentric or oval path-shaped files are more effective in removing the root canal debris than traditional round files. The viscosity and surface tension of the antimicrobial irrigant used with endodontic files influence how well that irrigant touches the biofilm on the walls of the root canal and the sides of the files being used[17]. Methods to enhance the action of NaOCl against root canal biofilms include adding various detergents such as cetrimide or benzalkonium chloride to lower the surface tension and enhance penetration of the solution into dentinal tubules. enhance the action of NaOCl include adding calcium hydroxide, which enhances its antimicrobial actions and reduces the levels of bacterial endotoxins, and physical activation of the solution, by using ultrasonic instruments or pulsed mid-infrared lasers (such as Er:YAG or Er,Cr:YSGG lasers)[18].

Nanoparticles

Nanoparticles are increasingly incorporated into various endodontic applications, including irrigants, intracanal medicaments, and sealers. Notably , NP-enhanced irrigants, such as silver nanoparticles combined with sodium hypochlorite (NaOCl) and light-activated curcumin-loaded nanoparticles, demonstrate superior capabilities in biofilm removal compared to traditional treatments. Additionally, nanoparticles serve as efficient carriers for delivering antibiotics and photosensitizers directly into biofilms.

A. Characteristics of NPs

1. Size range (1–100 nm)

2. Ability to penetrate biofilm matrices and bacterial cells

B. Mechanisms of action [19]

1. Disruption of bacterial cell walls and membranes

2. Generation of reactive oxygen species (ROS)

3. Release of metal ions impacting microbial enzymes and DNA replication

Antimicrobial photodynamic therapy (APDT)

A two-step procedure involving: Injection of a photosensitizer into the tissue and Illumination of the treated tissue. The activated dye produces reactive oxygen species (singlet oxygen and free radicals) that can destroy bacteria, fungi, and disrupt the biofilm matrix Common Photosensitizers: Methylene blue, Toluidine Blue O, cationic porphyrins, phthalocyanines, and chlorins are effective photosensitizers[20]. APDT uses both laser and non-laser light sources. Lasers provide focused and consistent light, making them ideal for complex areas like root canals. High-intensity dental lasers have been employed to enhance disinfection of the root canal. Laser light (e.g., from Nd:YAG, diode, or Er:YAG lasers) delivered via a fine fiber can penetrate deep into dentin (up to 1000 µm) and kill bacteria through heat and photothermal effects[21]. The thermal energy causes vaporization of water in biofilms, disrupts cell walls, and can even burn organic matter. Studies show lasers can significantly reduce intracanal bacterial counts, and they are especially useful in sterilizing dentinal tubules beyond what liquid irrigants reach.

Phage therapy

Phage therapy is gaining renewed interest as a method to target antibiotic-resistant bacteria associated with biofilms. A bacteriophage is a virus that specifically attacks and kills harmful bacteria by entering their cells, disrupting their metabolic functions, and inducing cell lysis. Additionally, phages can carry specialized enzymes that break down bacterial capsules, aiding in the elimination of bacterial defenses[22].

Role of plasma

Atmospheric pressure cold plasma is an effective therapy in endodontics for its strong sterilization effect on fully matured biofilm within a few minutes. It has been accepted to be mechanically safe for its low temperature and does not alter the microhardness and roughness of root canal dentin significantly

Biofilm modifiers [23]

These agents modify the biofilm without hampering or killing all the useful microorganisms in the biofilm. The various biofilm modifiers acting against different bacterial strains include Furanone C-30 , Quercetin , Antimicrobial peptide Cec4 , etc. They act by downregulating the quorum sensing genes and affect the motility of the microorganisms.

Targeted Antimicrobial Peptides (STAMPs):These synthetic proteins are designed to bind to harmful bacteria such as S. mutans or E. faecalis and deliver a lethal effect, while preserving beneficial bacteria.

Disrupting Communication: Certain compounds can block bacterial communication, preventing them from coordinating biofilm formation and causing disease.

Using Enzymes: Enzymes like DNase and dispersin B are being investigated to effectively break down biofilms in cleaning solutions[24].

Ozone and Electric Currents: The use of ozone therapy has been tested both as an alternative agent to NaOCl and as a complementary disinfection source in chemo-mechanical canal preparation[25]. Electric currents improve the efficiency of biocides and antibiotics in destroying bacterial biofilms, and it has been suggested that electric currents could be considered as a safe and inexpensive method to increase the entrance of different therapeutic agents into the cells. These therapies are being studied for their potential to enhance biofilm removal[26].

Future Research will have to be directed towards the following:

- Complete Biofilm Eradication
- Biofilm-Targeted Therapeutics
- Enhanced Diagnostics and Monitoring
- Understanding Microbial Interactions
- Biomaterials and Host Response Modulation

CONCLUSION

Summary: Endodontic biofilms cause persistent root canal infections, posing significant challenges to successful therapy due to their robust defense mechanisms. Factors like the protective biofilm matrix and complex canal anatomy make elimination difficult.

Current Efforts: Research has advanced our understanding of biofilm formation and resistance, leading to improved treatment strategies that include mechanical, chemical, and nanotechnological approaches. Clinicians now adopt anti-biofilm strategies, such as irrigant activation and combination disinfectants, to enhance outcomes.

Challenges: Predictable biofilm eradication is still elusive, with some cases failing due to residual infections. The diversity of microbial species means there is no one-size-fits-all solution, and management often focuses on reducing bacterial load rather than achieving complete sterilization.

Future Outlook: Future endodontic treatments aim to integrate new technologies and tailored therapies to combat biofilm resilience. Research is addressing diagnostic gaps and developing therapeutics like targeted peptides and smart delivery systems. Combinations of preventive strategies and advanced treatments are expected to improve root canal success rates by effectively managing endodontic biofilms.

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