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Revisiting Biofilms and Microbial Interaction in Endodontics Infections: An Updated Review

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Abstract

Biofilms play a critical role in the pathogenesis and persistence of endodontic infections. These structured microbial communities, encased in a protective matrix, exhibit increased resistance to host defenses and conventional treatments. This review explores the formation, composition, and clinical significance of biofilms in endodontics, highlighting recent advances in detection methods and innovative therapeutic strategies. By understanding biofilm dynamics, clinicians can adopt more effective approaches to manage and prevent persistent root canal infections.

Keywords: Biofilms, Endodontic Infections, Oral Microbiology, Pathogenic Bacteria, Quorum Sensing, Polysaccrides.

INTRODUCTION

Biofilm refers to a mode of microbial growth in which dynamic communities of sessile microorganisms irreversibly attach to both solid surfaces and each other. These communities are encased in a self-produced matrix of extracellular polymeric substances (EPS), which provides structural and protective functions [1].

Biofilm research has significantly advanced our understanding of microbial behaviour in endodontics. Biofilms, defined as structured communities of microorganisms enclosed in a self-produced polymeric matrix, play a crucial role in the pathogenesis and persistence of endodontic infections [2]. Unlike planktonic bacteria, biofilm-associated microorganisms exhibit enhanced resistance to antimicrobial agents and host immune responses, presenting challenges in the treatment of endodontic infections [2].

A microbial biofilm is classified as a community based on four fundamental characteristics: [3,4]

1. Self-organization (autopoiesis)-The ability to organize and sustain themselves.

2. Environmental resilience (homeostasis)- Resistance to external disturbances.

3. Collaborative efficiency (synergy)-Enhanced functionality in association compared to individual performance.

4. Unified response (communality)-Collective adaptation to environmental changes.

Oral bacteria, capable of forming biofilms on various surfaces within the mouth, contribute significantly to dental and periodontal diseases. Controlling these biofilms is essential for maintaining oral health and preventing conditions such as dental caries, gingivitis, and periodontitis.

SIGNIFICANCE OF BIOFILMS

Biofilm-embedded bacteria exhibit altered phenotypes, making them highly resistant to antimicrobials, environmental stresses, bacteriophages, and predation by amoebas. This resilience underpins their role in chronic and recurrent infections, as biofilm bacteria evade both treatment and host defenses [5].

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ULTRASTRUCTURE OF BIOFILMS

Mature biofilms consist of heterogeneous microbial clusters, known as microcolonies, that adhere to a solid substrate. These clusters are encased in an EPS-rich glycocalyx matrix, anchoring them to the surface. The biofilm matrix accounts for approximately 85% of its volume, while bacterial cells make up the remaining 15%. Key components of the matrix include polysaccharides, proteins, nucleic acids, and salts [6,7].

Biofilms also feature a network of water-filled channels that function as a rudimentary circulatory system, facilitating nutrient exchange and waste removal between the bacterial cells and their environment. The overall structure, often tower- or mushroom-shaped, is influenced by fluid dynamics, such as the force of flushing liquids [8].

DETACHMENT MECHANISMS

Biofilm detachment plays a crucial role in its development and infection potential. Mechanisms of detachment include: [1,2]

Erosion-Continuous shedding of individual cells or small fragments.

Sloughing-Rapid and extensive loss of biofilm mass.

Active dispersal-The release of resistant cells capable of colonizing new environments, contributing to persistent infections.

BIOFILM FORMATION PROCESS

Biofilm development is a stepwise process occurring in the presence of microorganisms, a solid surface, and a fluid medium. The phases of biofilm formation generally follow the same sequence, though variations may arise based on environmental conditions. The core criteria defining biofilm communities include autopoiesis, homeostasis, synergy, and communality [2].

STEPS IN BIOFILM FORMATION

Biofilm formation is a multi-step process involving the adhesion, growth, and eventual dispersal of microorganisms. The stages are as follows: [3]

Step 1: Adsorption and Conditioning

The initial phase involves the adsorption of organic and inorganic molecules onto a solid surface, forming a conditioning film. This film, primarily composed of proteins and glycoproteins derived from saliva and gingival crevicular fluid, creates a suitable environment for microbial attachment. In dental plaque formation, this process includes conditioning by the salivary pellicle on tooth surfaces.

Step 2: Adhesion and Colonization

Planktonic microorganisms adhere to the surface, and their attachment is reinforced by the production of polymers and the unfolding of cell surface structures. Streptococcus species are the pioneer organisms in this phase, followed by the subsequent attachment of both gram-positive and gram-negative bacteria [2].

Factors influencing bacterial attachment include pH, temperature changes, fluid flow rate, nutrient availability, surface energy of the substrate, bacterial growth stage, cell surface charge, and hydrophobicity. This step consists of three stages:

Phase 1- Transportation of microbes to the substrate and initial attachment. Key adherence factors include fimbriae, pili, flagella, and extracellular polymeric substances (EPS) [3,4].

Phase 2- Microbial and substrate adherence, where weak initial bonds—formed via electrostatic attraction, hydrogen bonds, dipole interactions, and hydrophobic forces—are gradually strengthened by polysaccharide adhesion or ligand formation.

Phase 3- Specific microbial-substrate adherence, involving the binding of bacterial adhesins or ligands to the substrate.

Step 3: Growth and Expansion [5]

During this phase, the initial microbial monolayer attracts secondary colonizers, leading to the formation of microcolonies. These structures grow both laterally and vertically, resembling towers. Two types of microbial interactions occur:

Coadhesion- Recognition between a suspended microbial cell and one already attached to the surface.

Coaggregation- Recognition between genetically distinct cells in suspension, resulting in clump formation. As the biofilm develops, it adopts a corn-cob-like structure due to the arrangement of bacterial cells.

Step 4: Dispersal of Biofilm Microorganisms [3,4]

Biofilm dispersal occurs through two primary mechanisms:

Seeding Dispersal-This is a programmed release of planktonic cells driven by local hydrolysis of the EPS matrix. A subset of cells becomes motile, detaches, and contributes to persistent infections.

Clumping Dispersal-This involves the physical detachment of microcolony fragments, which are transported by bulk fluids to new locations where they establish new biofilms.

Detachment can occur through:

Erosion-Continuous release of single cells.

Sloughing-Rapid detachment of large biofilm segments.

Structural and Phenotypic Features of Biofilms [4,5]

Under electron microscopy, biofilms appear as tower- or mushroom-shaped microcolonies with interspersed fluid channels. These channels facilitate nutrient transport and waste removal.

The phenotype of biofilm bacteria differs significantly from planktonic bacteria due to several factors:

1. The EPS matrix provides protection from environmental stressors.

2. The biofilm structure traps nutrients and metabolites, supporting bacterial survival.

3. Internal compartmentalization within the biofilm enhances organization.

4. Bacteria within biofilms communicate and exchange genetic material effectively, ensuring collective resilience and adaptability.

ORAL BIOFILM AND ITS FORMATION

Oral bacteria have the remarkable ability to form biofilms on various surfaces, including hard and soft tissues. The nature of the biofilm depends on the bacterial species present, the surface composition, and the conditioning layer that coats these surfaces. Oral biofilm development occurs in three sequential steps: pellicle formation, bacterial colonization, and biofilm maturation. These steps are not random but involve complex and coordinated interactions [7,8].

Water constitutes 80% of the oral biofilm, while organic and inorganic components make up the remaining 20%. Microorganisms account for 70–80% of the solid fraction and are more abundant in subgingival biofilms than in supragingival ones. The chemical makeup of the biofilm varies between individuals, different tooth surfaces, and with age. Organic substances, primarily carbohydrates, proteins, and lipids, surround the microorganisms. Inorganic elements such as calcium, phosphorus, magnesium, and fluoride are more concentrated in biofilms than in saliva [9,10].

PROCESS OF BIOFILM FORMATION [11]

1. Pellicle Formation:

Salivary micelle-like globules (SMGs) adhere to the clean tooth surface, forming the acquired enamel pellicle, which serves as the base for a multi-layered biofilm. Calcium ions aid in creating larger globules by neutralizing the negative charges on subunits.

2. Bacterial Attachment and Colonization

Specific oral bacteria attach selectively to the pellicle. These interactions depend on bacterial properties and the pellicle's composition. Gram-positive cocci like *Streptococcus mutans* and *Streptococcus sanguis* are the initial colonizers. Over time, filamentous bacteria such as *Fusobacterium nucleatum* and slender rods adhere, eventually replacing many cocci. As the biofilm matures, vibrios, spirochetes, gram-negative, and anaerobic organisms become more prevalent. Certain bacterial surfaces also serve as attachment sites for smaller coccoid bacteria, forming unique "corn-cob" structures [12].

3. Biofilm Maturation

The biofilm thickens and matures as more microbial species colonize it. Calcification of dental biofilms, known as calculus, occurs due to calcium phosphate precipitation within the biofilm's organic matrix. Factors such as pH, calcium and phosphate levels, fluoride availability, and biological crystallization nucleators influence calculus formation [13].

ENDODONTIC BIOFILMS

Microorganisms in the oral cavity often colonize root canals following pulp tissue breakdown. Biofilm formation in endodontic infections begins after the invasion of planktonic bacteria into the pulp chamber. Inflammatory fluids facilitate bacterial multiplication and attachment to the root canal walls. Necrotic pulp tissue provides a nutrient-rich environment, promoting microbial proliferation. Biofilm-associated bacteria often include gram-negative and facultative or strict anaerobes, with bacilli, filaments, and cocci predominating [2-4].

TYPES OF ENDODONTIC BIOFILMS

1. **Intracanal Biofilms**-These form on root canal dentine and consist of monolayered or multilayered bacterial aggregates embedded in an extracellular matrix [2].

2. **Extraradicular Biofilms-**Found on the root surface near the apex, these biofilms are often associated with chronic apical abscesses and consist of cocci, rods, and filamentous bacteria.

3. **Periapical Biofilms**-Located in the periapical region, these biofilms are rare as most root canal bacteria cannot survive host defenses.

4. **Biomaterial-Centered Biofilms**-These form on artificial surfaces, such as implants, and are resistant to antimicrobial agents [3-5].

ADVANTAGES OF BIOFILM FORMATION FOR BACTERIA

Biofilms provide several benefits for bacterial communities: [1-3]

1. Broader Habitat Range-Early colonizers modify the environment, enabling latecomers to thrive. This can include nutrient availability, reduced oxygen tension, and removal of harmful substances.

2. Enhanced Metabolic Efficiency-Nutritional interdependencies among species create efficient food webs. By-products of one species can serve as nutrients for others.

3.Protection-The biofilm matrix shields bacteria from antimicrobial agents, environmental stress, and host immune responses. Enzymes, metabolites, and bacteriocins produced by certain bacteria within the biofilm can protect neighboring cells.

4.. Genetic Exchange-Close cell associations within biofilms facilitate horizontal gene transfer through conjugation, transformation, and transduction, promoting virulence and antibiotic resistance.

5. Increased Pathogenicity- Biofilms enable collective bacterial behavior, including nutrient acquisition, immune evasion, and tissue invasion, resulting in enhanced disease potential.

QUORUM SENSING IN BIOFILMS

Biofilms employ quorum sensing a cell-density-dependent communication system—to regulate gene expression collectively. This mechanism enables bacteria to behave as a coordinated group, enhancing their survival and adaptability.

BIOFILM FORMATION IN THE ROOT CANAL SYSTEM [3,4,14]

Stages of Biofilm Formation :

1. **Initial Adhesion**-Microorganisms attach to the dentinal walls or organic debris within the root canal.

2. **Microcolony Formation**-Attached microorganisms proliferate and produce extracellular polymeric substances (EPS).

3. **Maturation**- Biofilm develops into a complex three-dimensional structure, with channels allowing nutrient and waste exchange.

4. **Dispersal-** Some cells detach to colonize new surfaces, contributing to infection spread.

CHARACTERISTICS OF ENDODONTIC BIOFILMS [2-4]

Diversity- Biofilms in endodontic infections are polymicrobial, commonly dominated by anaerobic bacteria like Porphyromona and Fusobacterium species.

Resistance- Biofilm bacteria are up to 1,000 times more resistant to antimicrobials compared to planktonic forms.

Role of Biofilms in Endodontic Infections [2,3,15,16]

Primary Endodontic Infections

Primary infections are initiated by bacteria invading the pulp due to caries, trauma, or periodontal disease. Biofilms develop in the root canal system, contributing to pulp necrosis and periapical inflammation.

Secondary and Persistent Infections

Secondary infections occur due to microbial contamination during treatment, while persistent infections are caused by residual biofilms or resistant organisms like Enterococcus faecalis. These infections are major causes of root canal treatment failure.

DIAGNOSTIC TECHNIQUES FOR BIOFILMS [17]

1. Traditional Method

2. Light Microscopy: Basic visualization of biofilm structures.

3. Culture Techniques- Limited utility as biofilm bacteria are often unculturable.

4. Modern Molecular Approach

5. Fluorescence in Situ Hybridization (FISH)-Detects specific bacterial species in biofilms.

6. Confocal Laser Scanning Microscopy (CLSM)-Provides high-resolution imaging of biofilm architecture.

7. Metagenomics-Identifies microbial diversity using 16S rRNA gene sequencing.

MANAGEMENT OF BIOFILMS IN ENDODONTICS [3,4,6,18-20]

Chemical Methods

1. Irrigants - Sodium hypochlorite (NaOCl): Effective in dissolving organic matter and disrupting biofilms.

Chlorhexidine (CHX): Broad-spectrum antimicrobial with substantivity.

2. Interim MedicamentsCalcium hydroxide: Creates an alkaline environment hostile to biofilm survival.

Mechanical Methods

1. **Instrumentation**

- Files and rotary instruments remove infected dentin harboring biofilms.

2. **Irrigation Activation**

- Passive ultrasonic irrigation (PUI) enhances irrigant penetration into biofilms.

- Laser-activated irrigation (LAI) disrupts biofilms through cavitation and shock waves.

Emerging Technologies

1. Nanoparticles- Nanomaterials like silver nanoparticles show promise in biofilm disruption.

2. Antimicrobial Photodynamic Therapy (aPDT)- Combines light and photosensitizers to produce reactive oxygen species that kill biofilm bacteria.

3. Quorum Sensing Inhibitors- Target bacterial communication pathways critical for biofilm formation.

CONCLUSION

Biofilms in the root canal system pose significant challenges to endodontic treatment. A combination of mechanical debridement, chemical disinfection, and advanced technologies is essential for effective biofilm management. Continued research into biofilm behavior and innovative therapeutic approaches holds promise for improving endodontic outcomes. The biofilm mode of bacterial growth confers numerous survival advantages, including antimicrobial resistance, nutrient concentration, genetic exchange, and community communication. These properties make biofilm-associated infections, such as dental plaque and endodontic biofilms, challenging to treat and manage [2-4].

Conflicts of Interest

The author reports no conflicts of interest.

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