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Biofilms in Endodontics-A comprehensive review

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Abstract
Microorganisms represent the primary etiology underlying pulpal and periapical pathologies, frequently organizing into structured, three-dimensional communities known as biofilms on oral surfaces. These biofilms consist of single cells and microcolonies embedded within a hydrated extracellular polymeric substance matrix. Because biofilms exhibit enhanced antimicrobial tolerance, environmental protection, and quorum-sensing capabilities traditional mechanical instrumentation is insufficient, leaving a substantial portion of the root canal system untouched. Effective clinical eradication necessitates a multi-modal approach combining mechanical preparation with chemical irrigants, physical activation systems, and targeted intracanal medicaments Emerging options—ranging from smart irrigants and nanoparticles to biofilm-targeting enzymes and artificial intelligence-driven protocols—further enhance precision and predictability in achieving long-term periapical healing.

Keywords: Biofilm, microflora, *E. faecalis*, qurom sensing, molecular methods

Introduction
Microorganisms are the primary etiology driving pulpal and periapical pathologies [1]. Oral bacteria possess the remarkable capacity to form complex biofilms on diverse surfaces ranging from hard enamel and dentin to soft tissues [1]. Biofilms form three-dimensional structured communities featuring specialized fluid channels for the transport of substrates, metabolic waste products, and signaling molecules [1, 4]. As hypothesized by Svensäter and Bergenholtz, biofilm formation in root canals is initiated following the initial invasion of the pulp chamber by planktonic oral microorganisms subsequent to tissue breakdown [11]. Costerton *et al*. established that these biofilms consist of single cells and microcolonies embedded in a highly hydrated, predominantly anionic exopolymer matrix [4].

Microbiology of Root Canals
The bacterial microflora of the root canal evolves dynamically throughout the infection process. Initially, the environment is dominated by aerobes and facultative anaerobes [1]. As oxygen is depleted, the microbial composition shifts dramatically based on the nature of the endodontic infection [4]. Primary endodontic infections are characterized by initial microbial invasion into the non-treated root canal system and periapical tissues, typically featuring a polymicrobial flora predominantly composed of obligate anaerobes such as Bacteroides, Porphyromonas, Prevotella, Fusobacterium, Treponema, Peptostreptococcus, Eubacterium, and Campylobacter [4, 13]. Conversely, secondary or persistent endodontic infections—manifesting as re-infection, persistent inflammation, or recurrence in previously root-canal-treated teeth—are characterized by a restricted spectrum of highly resistant species [4, 12]. These are predominantly comprised of Enterococci (specifically Enterococcus faecalis), Streptococci, Lactobacilli, Actinomyces, and fungal species such as Candida albicans [4, 18].

Definition and Criteria of Biofilms
A biofilm is defined as a microbial community embedded in a self-made matrix of extracellular polymeric substances (EPS) where cells are irreversibly attached to a solid substratum and to each other [4]. Caldwell *et al*. highlighted four fundamental criteria defining a true biofilm: Autopoiesis, possessing the intrinsic ability to self-organize; Homeostasis, effectively resisting environmental perturbations; Synergy, being significantly more effective in association than in isolation; and Communality, responding to environmental changes as a collective unit rather than as single individual cells [1].

Composition and Ultrastructure
A fully developed biofilm represents a heterogeneous arrangement of microbial cells on a solid surface [4]. Quantitatively, the structural volume is composed of approximately 85% extracellular matrix (comprising proteins, polysaccharides, nucleic acids, and salts) and only 15% bacterial cells [1]. Internal water channels function as a primitive circulatory system facilitating nutrient delivery and waste clearance [1]. Microcolonies periodically detach through active dispersive mechanisms (seeding dispersal), transferring cells, polymers, and precipitates into the surrounding fluid [1]. Furthermore, biofilm-mediated mineralization occurs when metal ions such as Ca^{2+}, Mg^{2+}, and Fe^{3+} readily bind and precipitate within the ionic matrix under favorable microenvironments [1]. Mature biofilms frequently exhibit characteristic tower or mushroom-shaped architectures [1].

Mechanisms of Antimicrobial Resistance
Bacteria residing within biofilms exhibit exceptional tolerance to antimicrobial agents and host defense

mechanisms [4, 18]. This enhanced resistance stems from several distinct mechanisms: The dense EPS matrix acts as a physical diffusion barrier that retards antibiotic penetration and contains inactivating enzymes [4];

Nutrient depletion and waste accumulation induce a state of slow growth or metabolic dormancy, rendering cell-wall-active antibiotics ineffective [4];

Quorum sensing—mediated by chemical signaling autoinducers—coordinates gene expression, virulence factors, and stress responses across the microbial population [1, 4].

Endodontic Biofilm Classifications

Endodontic bacterial biofilms are categorized into four principal clinical classifications based on their anatomical location and substrate:

Intracanal Microbial Biofilms, formed directly on root canal dentin walls, initially identified by Nair (1987) as palisade structures resembling dental plaque [6];

Extraradicular Biofilms, developing on the apical root surface of infected teeth, with strong associations to Fusobacterium nucleatum, Porphyromonas gingivalis, and Tannerella forsythia [5],

Periapical Biofilms, microbial aggregations located within periapical lesions associated predominantly with Actinomyces species [6];

Foreign Body-Centered Biofilms, occurring when bacteria colonize artificial biomaterials such as gutta-percha points in the presence of serum [3].

Role of Enterococcus faecalis in Endodontic Biofilms

Enterococcus faecalis is a Gram-positive, facultative anaerobic coccus strongly implicated in persistent endodontic infections and failed root canal treatments [4, 18]. It exhibits remarkable environmental resilience, tolerating starvation, extreme alkaline pH, high salinity, and temperatures ranging from 10°C to 45°C [1, 18]. The colonization of root dentin by E. faecalis proceeds through three distinct stages: initial cell adhesion forming microcolonies, bacterial-mediated dissolution of the dentin mineral matrix releasing calcium and phosphate ions, and subsequent mineralization of the biofilm matrix [1]. Furthermore, E. faecalis successfully withstands calcium hydroxide intracanal medicaments for extended periods by maintaining internal pH homeostasis via membrane-bound proton pumps [4, 18].

Molecular methods of examination

Modern endodontic diagnostics have moved beyond traditional culture methods—which propagate microbes on artificial media but miss dormant cells and the ~50% of oral flora that are uncultivable[1]—to utilize five core analytical approaches.

Microscopic techniques (SEM, ESEM, TEM, CLSM, and epifluorescence) rapidly screen cell structures and morphotypes, though they lack clinical specificity on their own[3].

Immunological methods (immunofluorescence, flow cytometry, and ELISA) overcome this by using specific antibodies to target microbial antigens or host immunoglobulins[4].

Molecular techniques provide culture-independent genetic profiling through PCR/qPCR for sensitive quantification, Fluorescence In Situ Hybridization (FISH) for spatial mapping, and Next-Generation Sequencing (NGS) or DNA-DNA hybridization to characterize complex microbial communities[2].

Advanced biophysical methods analyze structural and chemical dynamics: Atomic Force Microscopy (AFM) and optical tweezers measure nanoscale bacterial-dentin binding forces[6], FTIR spectroscopy profiles the chemical makeup of the EPS matrix, solid-state NMR maps cellular metabolism, and micromanipulators isolate single cells or specific biofilm micro-compartments[7].

Current and Advanced Therapeutic Options

Eradication of endodontic biofilms requires a comprehensive, multi-modal treatment strategy [7, 8]. Mechanical instrumentation serves as the primary physical disruption method, yet anatomical complexities (such as isthmuses and lateral canals) frequently leave up to 35% of the root canal surface untouched [7, 20]. Consequently, chemical irrigation is mandatory.

Sodium hypochlorite (NaOCl) remains the gold standard as the only irrigant capable of dissolving organic tissue and EPS matrices [6, 8]. Ethylenediaminetetraacetic acid (EDTA) is utilized to remove the inorganic smear layer [6, 8], while chlorhexidine gluconate (CHX) offers valuable antimicrobial substantivity `[8].

To overcome the apical vapor lock effect and enhance irrigant penetration into uninstrumented areas, advanced activation technologies—including passive ultrasonic irrigation (PUI), sonic activation, and laser-activated irrigation (LAI)—are clinically indispensable [7, 9, 15]. Additionally, emerging modalities such as photodynamic therapy (PDT), smart irrigants (MTAD, Tetraclean), bioactive nanoparticles, EPS-degrading enzymes, and artificial intelligence-assisted protocol optimization represent the cutting edge of endodontic disinfection [9, 18].

Conclusions

Endodontic infections are fundamentally biofilm-mediated pathologies characterized by high structural resistance and complex anatomical reservoirs [1, 4]. Because mechanical preparation alone is insufficient for complete debridement, predictable disinfection requires a rigorous, multi-modal protocol integrating chemical tissue dissolution, smear layer removal, and physical activation energy [7, 8]. Future advancements driven by smart biomaterials, targeted enzymatic disruption, and artificial intelligence hold profound promise for optimizing clinical outcomes and ensuring long-term periapical health [9, 18].

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