

## Review Article



# Endodontic biofilms: contemporary and future treatment options

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Received: Nov 20, 2018

Accepted: Dec 26, 2018

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### Funding

This work was supported by the Korea Health  
Technology R&D Project through the Korea  
Health Industry Development Institute (KHIDI),  
funded by the Ministry of Health & Welfare  
(HI17C1377), Republic of Korea.

### Conflict of Interest

No potential conflict of interest relevant to this  
article was reported.

## ABSTRACT

Apical periodontitis is a biofilm-mediated infection. The biofilm protects bacteria from host defenses and increase their resistance to intracanal disinfecting protocols. Understanding the virulence of these endodontic microbiota within biofilm is essential for the development of novel therapeutic procedures for intracanal disinfection. Both the disruption of biofilms and the killing of their bacteria are necessary to effectively treat apical periodontitis. Accordingly, a review of endodontic biofilm types, antimicrobial resistance mechanisms, and current and future therapeutic procedures for endodontic biofilm is provided.

**Keywords:** Antimicrobial resistance; Endodontic biofilm; Intracanal disinfection; *Lactobacillus*; Lipoteichoic acid

## ENDODONTIC BIOFILMS

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 [1]. These surfaces include root canal walls that provide a niche for bacteria [2,3]. Despite intracanal disinfection and a drastically changed environment, bacteria can be detected in post-treatment samples.







Biofilm formation is dependent on a surface conditioning layer, the properties of which determine microbial attachment and composition, as microbes within a planktonic phase are delivered [3]. Bacterial attachment to the substrate is dependent on surface energy, temperature, pH, fluid flow rate, duration of contact, surface hydrophobicity, and nutrient availability [4]. Bacterial structures such as pili, flagella, EPS and polysaccharide-specific adhesins/ligands are important for adherence [5]. Proliferation and metabolism of attached microorganism creates structurally organized mixed microbial communities [3], and this monolayer then attracts secondary colonizers that form microcolonies and the final biofilm structure [6,7].

In endodontics, 4 types of biofilms, including intracanal, extraradicular, periapical, and biomaterial-centered biofilms were reported [4,8]. Nair reported that despite

**Author Contributions**

Conceptualization: Yoo YJ, Han SH, Kum KY; Data curation: Yoo YJ, Kim AR, Perinpanayagam H; Formal analysis: Yoo YJ, Kim AR; Funding acquisition: Kum KY; Investigation: Yoo YJ, Kim AR; Methodology: Perinpanayagam H, Kum KY; Project administration: Kum KY; Resources: Perinpanayagam H, Kim AR; Software: Kim AR, Oh S; Supervision: Han SH, Kum KY; Validation: Han SH, Kum KY; Visualization: Yoo YJ, Kim AR, Kum KY; Writing - original draft: Yoo YJ, Kim AR, Kum KY; Writing - review & editing: Yoo YJ, Perinpanayagam H, Han SH, Kum KY.

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instrumentation, irrigation, and obturation for single-visit treatment of mandibular first molars with primary apical periodontitis, microorganisms persisted within biofilms in untouched areas of canals and isthmuses [9], which is called as an intracanal biofilms.

Extradicular biofilms were reported in teeth with asymptomatic apical periodontitis, as well as those with chronic apical abscesses and sinus tract [4,8]. Ricucci *et al.* [10] discovered calculus-like deposits on root tips of teeth with secondary (post-treatment) apical periodontitis. Noiri *et al.* [11] found glycocalyx-like structures had almost completely covered gutta-percha cones recovered from beyond the apex, and bacteria on the external root surfaces in the extracted teeth in cases of 'refractory periapical pathosis'.

Certain strains can survive and infect periapical tissues as periapical biofilms [2]. *Propionibacterium propionicum* and various Actinomyces have been demonstrated in asymptomatic periapical lesions refractory to endodontic treatment [12]. Some Actinomyces have fimbriae for coaggregation, adherence to canal walls and dentinal debris forced through the apical foramen during treatment [13]. Bacteria may evade host defenses by building cohesive colonies including many branching and filamentous bacteria enmeshed in protein-polysaccharide matrix [13].

Bacteria can also adhere to artificial biomaterial surfaces and form biofilm structures [14] that cause biomaterial-centered infections. Gram-positive facultative anaerobes with serum colonize and form EPS on gutta-percha [14]. These biofilms on obturating materials can be both intra- and extra-radicular, when the material has extruded beyond the apex.

## MECHANISMS OF ANTIMICROBIAL RESISTANCE

The polysaccharide matrix in biofilms retards diffusion of antibiotics and inactivating extracellular enzymes such as  $\beta$ -lactamase may become concentrated [15]. Microbial cells communicate by quorum sensing to encourage the growth of species beneficial to biofilm structure [1,16]. Subpopulations within a biofilm can alter gene expression to remain protected [17]. Cells remain interiorly where they are protected from medicaments that act only on the microorganisms in the biofilms periphery. Bacterial cells grow more slowly with less metabolism in biofilms than when planktonic, and thereby elude antimicrobial agents [15]. They halt growth with nutrient depletion or waste product accumulation, further protecting them from antibiotics [17]. The altered pH and oxygen level within biofilms may further impair antibiotics [18].

## CURRENT AND FUTURE THERAPEUTIC STRATEGIES AGAINST ENDODONTIC BIOFILM

### Irrigants for biofilm eradication

Microorganisms grown within biofilms are 1,000–1,500 times more resistant to antimicrobials than planktonic bacteria [6,19]. Sodium hypochlorite (NaOCl) has been widely used as an endodontic irrigant due to its potent antimicrobial action and necrotic tissue dissolving property. Regarding the recalcitrant bacteria, mostly *Enterococcus faecalis* (*E. faecalis*) biofilm, it was reported that treatment of *E. faecalis* lipoteichoic acid (LTA) with NaOCl resulted in the impairment of immunostimulating activity by the delipidation of

glycolipid moiety structure [20]. NaOCl could impair toll like receptor 2 activation of *E. faecalis* and induce inflammatory mediators, and damage the LTA structure, potentially through deacylation [20]. Furthermore, NaOCl is the most effective antimicrobial irrigant against multi-species biofilm [21]. Given that the dual-species biofilms or the aged biofilms were more resistant to NaOCl than monospecies biofilms or the young biofilms [22], many researches found that high concentration NaOCl was the only irrigant effective in disrupting multi-species biofilm and eradicating bacterial cells [23-26].

Chlorhexidine (CHX) digluconate is a broad spectrum antimicrobial disinfectant that has antimicrobial substantive activity [27-29], and thus has been widely used as an auxiliary canal irrigant or a canal soaking agent against *E. faecalis* biofilms [30,31]. A recent study demonstrated that CHX attenuates the activity of *E. faecalis* LTA [32]. Kim *et al.* [33] compared the antimicrobial activity of alexidine (1%) and CHX (2%) on *E. faecalis* by using dentin block model according to soaking time (5 and 10 minutes). And they found that there was no significant difference in the number of bacteria adhering after the first minute of exposure and the most effective irrigant at disrupting biofilms was NaOCl [25]. Despite these antimicrobial activities, CHX cannot be used as main root canal irrigant because it does not have tissue solvent activity [30].

In addition to smear layer removal, EDTA irrigation can be beneficial in disruption of biofilm. Ozdemir *et al.* [34] demonstrated that combination of EDTA and NaOCl significantly reduced the amount of intracanal biofilm in both young and old aged biofilms. Soares *et al.* [35] reported that the NaOCl-EDTA alternating irrigation was a promising regimen for elimination of intracanal *E. faecalis* biofilms.

### **Intracanal medicament for biofilm eradication**

#### *1. Calcium hydroxide*

Calcium hydroxide (CH) is a widely used intracanal medicament that has broad antimicrobial activity, which is dependent on the release of aqueous hydroxyl ions to raise pH so that microbes cannot survive [36]. Elevated pH alters membrane integrity, and the hydroxyl ions are highly reactive with biomolecules [37].

Yet, intracanal CH was reported to be ineffective in preventing *E. faecalis* biofilm formation in root canals [19], while still being effective in eliminating their biofilm [38]. Brändle *et al.* [39] evaluated the effects of growth condition (planktonic, mono- and multi-species biofilms) on the susceptibility of *E. faecalis*, *Streptococcus sobrinus* (*S. sobrinus*), *Candida albicans* (*C. albicans*), *Actinomyces naeslundii* (*A. naeslundii*), and *Fusobacterium nucleatum* to alkaline stress. The findings showed that planktonic microorganisms were most susceptible; only *E. faecalis* and *C. albicans* survived in saturated solution for 10 minutes, and the latter also survived for 100 minutes [39]. Dentin adhesion was the major factor in improving the resistance of *E. faecalis* and *A. naeslundii* to CH, whereas the multispecies context in a biofilm was the major factor in promoting resistance of *S. sobrinus* to the disinfectant. In contrast, the *C. albicans* response to CH was not influenced by growth conditions [39].

In addition to the effect of hydroxyl ion, damage in the lipid moieties of bacterial virulence factors composed of glycolipids might be a unique detoxification mechanism of CH. *E. faecalis* is known to be resistant to CH, owing to their proton pump for internal pH maintenance and inhibitory dentin buffering effect. However, it was recently found that CH could attenuate

the abilities of not only *E. faecalis* but also its LTA to stimulate murine macrophages, and could reduce TNF- $\alpha$  or NO production [40]. As an underlying mechanism, Baik *et al.* [40] reported that CH could deacylate the LTA from *E. faecalis*, resulting in the impairment of LTA immunostimulating activity. CH can also inactivate lipopolysaccharide (LPS) in gram-negative bacteria, via hydrolysis of fatty acid in the lipid A moiety [41-44].

### 2. Chlorhexidine

Positively charged CHX molecules interact with negatively charged membrane phospholipids to enter and permeabilize microbial cells [45]. It was reported that CHX could alter cell walls and nucleoprotein coagulation, even in *C. albicans* [46,47]. In dentin block model, CHX showed superior antifungal activity compared to CH, up to 400  $\mu\text{m}$  depth dentinal tubules [46]. Additionally, CHX binds to hydroxyapatite and reduces microbial colonization on dentin surfaces, which provides substantive antimicrobial activity [27].

Subsequent analyses of biofilm spatial arrangements showed differences between the single- and dual-species biofilms in microstructural alterations in response to CHX exposure. Dual-species biofilms, but not single-species biofilms, had formed distinct clusters that were considered to account for the increased resistance to CHX [48].

### 3. Human beta defensins

Human beta defensins (HBDs) are cationic antimicrobial peptides that are critical host defense against microbes [49]. They bind to the negatively charged molecules on bacterial surface and disrupt bacterial membranes [50]. HBDs differ in amino acid sequences, structure, cysteine residues with disulfide bridges, charge, and affinity for bacterial membrane targets such as LPS in gram-negatives and LTA in gram-positives [51]. The antimicrobial effects of HBDs differ with bacterial strains due to variations in their LPS and LTA structure [52]. HBD-3 is strongly inhibitory, whereas HBD-1, -2, and -4 have weak antimicrobial effects on *E. faecalis* [53].

HBD-1, -2, -3, and -4 are produced in normal and inflamed dental pulp [54,55]. They may protect the pulp from inflammation induced by LTA of gram-positive bacteria and LPS of gram-negative bacteria [56]. Synthetic HBD-3 consisting of the C terminal 15 amino acids (HBD3-C15) was reported to be effective for disinfecting endodontic biofilm including *C. albicans* [46,57,58].

### 4. Triple antibiotic paste

Triple antibiotic paste (TAP), a mixture of metronidazole, ciprofloxacin, and minocycline, is widely used in regenerative endodontic procedure (REP). It is effective on infected dentin, intracanal biofilms, and the majority of endodontic pathogens [59-62]. But its toxicity to residual undifferentiated cells and periapical tissues limits its application in REP.

## Laser-assisted eradication of biofilms

A low power laser directed at the access cavity combined with a photosensitizing agent was bactericidal on *S. intermedius* biofilms in root canals, but less effective than NaOCl (3%) irrigation [63]. Er:YAG laser was effective on apical root apex biofilms *in vitro* [64]. However, endodontic pathogens in biofilms were difficult to eradicate despite direct laser exposure *ex vivo* [65]. Er:YAG laser was effective against biofilms of *A. naeslundii*, *E. faecalis*, *Lactobacillus casei* (*L. casei*), *Propionibacterium acnes*, *F. nucleatum*, *Porphyromonas gingivalis*, or *Prevotella nigrescens*, *in vitro*, but not against biofilms of *L. casei* [66].

### Effect of *Lactobacillus plantarum* LTA

*Lactobacillus plantarum* (*L. plantarum*) is a probiotic [67] that is known to have anti-inflammatory and anti-biofilm effect [68]. Bacterial cell wall components especially LTA inhibit *Streptococcus mutans* (*S. mutans*), *E. faecalis*, and *Staphylococcus aureus* (*S. aureus*) biofilm formation by controlling gene expression, quorum sensing, and inhibiting exopolysaccharides production [69-71]. Furthermore, *L. plantarum* LTA also disrupted preformed biofilm of *E. faecalis* and *S. aureus* [69,71]. Interestingly, *L. plantarum* LTA reduced not only mono-species biofilm, but also multi-species biofilm consisting of *A. naeslundii*, *E. faecalis*, *Lactobacillus salivarius*, and *S. mutans* [72], and it also cooperatively enhanced disruption of oral multispecies biofilm when combined with CH and CHX intracanal medicaments (unpublished data).

### Effect of nanoparticles, photodynamic therapy, ozone, and enzymes

Nanoparticles synthesized from powders of silver, copper oxide, and zinc oxide, and other powders have broad antimicrobial applications [73]. These nanoparticles generate reactive oxygen species (ROS) that are cytotoxic for bacteria. Higher surface area and more charge density mean greater potential for bacterial interactions. Numerous positively charged nanoparticles accumulate on negatively charged bacterial cell membranes, which increase permeability to destroy cells [74-76]. Additionally, cationic nanoparticles adhere to negatively charged dentin surface to prevent biofilm formation [77].

In photodynamic therapy (PDT), a photosensitizer is preferentially localized in tissue and subsequently activated by appropriate wavelength light to generate reactive oxygen that kill bacteria [78]. There have been numerous *in vitro* studies on PDT in root canal disinfection [79-83]. However, penetration of the activating light and the photosensitizer may be limited within root canal structures. When microorganisms were sensitized with methylene blue (25 µg/mL, 5 minutes), all bacterial species except *E. faecalis* (53% killing) were destroyed. When this was followed by the addition of red light CH (222 J/cm<sup>2</sup>) with an optical fiber, almost all (97%) *E. faecalis* biofilm bacteria in root canals were eliminated [83].

Ozone gas (HealOzone, KaVo, Biberach, Germany) has yielded inconsistent result in destroying endodontic pathogens. These inconsistencies may have been due to variation in concentration and duration of application [84-86]. There is conflicting evidence on its antimicrobial efficacy and reduced effects on sessile versus planktonic bacteria [87].

Natural plant extracts such as polyphenols, *Morinda citrifolia*, and turmeric, as well as enzymes, such as dispersin B and proteinase K, have been proposed for treating biofilm medicated infections. But studies are needed to demonstrate their efficacy.

## CONCLUSIONS

Endodontic infection is caused by the surface-associated growth of microorganisms. Applying the biofilm concept to endodontic microbiology helps to understand the pathogenic potential of the root canal microbiota and to form the basis of new approaches in root canal disinfection. Recent developments in biocompatible intracanal medicaments including synthetic HBDs and *L. plantarum* LTA could open up new avenues as an ideal therapeutic agent to eradicate endodontic biofilm.

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