

Review Article

Endodontic biofilms: A matter of grave concern in dentistry

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ABSTRACT

Microorganisms irreversibly attach to the solid surfaces and secrete a mucilaginous matrix in which they get embedded and form biofilm. It is one of the basic survival strategies used by microorganisms in all ecosystems in response to stress or nutrient depletion. Clinically, biofilm-mediated persistent endodontic infections are the most frequently encountered lesions in the oral cavity which cause apical periodontitis. Eradicating stubborn endodontic biofilms is a herculean task because of its innate resistance to antimicrobial agents and its formation in anatomical complexities (minute areas) of root canal which are not accessible to mechanical instrumentation; this is giving headaches to dental practitioners because of the increased rate of endodontic treatment failure associated with inadequate removal of biofilms. The aim of this article is to review the role of biofilms in pulpal infections, types of endodontic biofilms, mechanism of antimicrobial resistance, challenges in biofilm elimination and strategies for combating biofilm.

INTRODUCTION

A biofilm is a well - structured framework of surface attached extracellular polymeric matrix which is produced by the bacterial cells enclosed in it. It is basically a layer of condensed microbiota comprising of cells that are irreversibly attached to a substratum or interface or to each other, ingrained in an extracellular polymeric substance (EPS), which consists of polysaccharides, extracellular DNA (eDNA) and extracellular proteins [1, 2]. The human mouth has enormous and diverse microflora, of over 700 species of bacteria and numerous other microorganisms including

fungi, viruses and protozoa, due to the unique environmental conditions for microbial adhesion and growth [3, 4]. Most of the oral microflora capable of forming biofilms are of pathogenic nature [5] and cause oral infections like endodontic disease which is a biofilm-mediated infection [6].

Through many years of observational findings different authors have proposed six criteria for classifying a given infectious disease to be a biofilm-mediated disease [7, 8, 9]. They are as follows:

- 1) The infection causing bacteria are associated or cohered to a surface.
- 2) Infected tissue examination reveals clustered bacteria or microcolonies circumscribed by an extracellular matrix.
- 3) Although dissemination of infection may occur in a secondary phase but generally the infection is confined to a particular site.
- 4) The infection is invincible even after the usage of antibiotics.
- 5) Ineffective host clearance may be exhibited by the location of microbial colonies in areas usually surrounded by host defense cells. The evidence of accumulation of PMNs and macrophages near bacterial aggregates/coaggregates in situ affirms the point for biofilm involvement with disease causation.
- 6) Remission of the disease process occurs by the elimination or drastic disruption of the biofilm structure and ecology.

TYPES OF ENDODONTIC BIOFILMS

1. **Intracanal biofilm:** It is formed on the internal surface of root canal dentin of the infected teeth. Majority of microflora in this biofilm survived as free collections of cocci, rods, filaments, and spirochetes arranged in palisade pattern.
2. **Extraradicular (cementum) biofilm:** It is formed on the surface of root close to root apex of endodontically infected teeth. Such biofilms are common in teeth with asymptomatic apical periodontitis and also in teeth with

chronic apical abscesses and a sinus tract.

3. **Periapical biofilm:** It is formed in the periapical region of the teeth, and is independent of root canal infection.
4. **Foreign body centered biofilm:** In this type, the bacteria adhere to an artificial biomaterial surface like implants and other prosthesis and form biofilm.

BIOFILM AND ENDODONTIC INFECTIONS

In morphological structure of teeth, the dental pulp is surrounded by enamel and dentine which acts as barrier for microbial invasion thereby protecting the soft connective tissue, blood vessels and nerves of the pulp in the root canal. However, any breach in the hard tissues of teeth can allow the microorganisms to penetrate through the pulp, resulting in pulp and periapical tissue necrosis (figure 1). Therefore, to preserve the tooth, it is imperative to remove microorganisms from the root canal, which is done by performing endodontic treatment. In this treatment, chemo-mechanical debridement of dentin walls is performed and sealing is done to prevent recontamination. But, sometimes even a correctly performed endodontic treatment can fail due to the microorganisms which are capable of adhering and surviving in the root canal by biofilm formation. [11, 13, 16-18]

Biofilm formation confers bacteria phenotypic characteristics that are unknown in the planktonic state. When the bacteria are in biofilm state they can change their individual,

genetic and physiological characteristics. This allows them to not only achieve a higher survival rate, but also attain virulence characteristics of other microorganisms present in the biofilm. This is clinically significant for persistent infections as the host's defense system and other measures including chemical or mechanical treatments are not sufficient to eradicate these infections. [11-15]. Sometimes the iatrogenic alterations to the pulpal ecology, such as introduction of root canal disinfectants and changes to oxygen tension during the inception of endodontic therapy, may impact the microbial succession (figure 2). This may further add to the selective pressure of specific species that flourish in oppressive environments and have the potential to form persistent infections. The possible mechanism that enables bacteria to persist is their ability to form biofilm which allows them to survive in the nutrient depleted environments and exhibit resistance to antimicrobial agents [25]. So, from clinical point of view, persistence of biofilm forming microbial cells in the radicular pulp directly impacts the prognosis of endodontic treatment.

In case of persistent infection, *E. faecalis* is the most commonly isolated anaerobic facultative species [26-28]. It survives in the root canal system by adhering to dentin and invading the dentinal tubules, [29, 30] where it form communities organized in biofilms, which may lead to bacterial resistance and persistence even after intracanal antimicrobial procedures [31].

BIOFILM AND ANTIMICROBIAL RESISTANCE

A biofilm is a 3 dimensional structure that works as a microbial battlefield. Biofilms directly impart 10 to 1000 fold increase in

antibiotic resistance to the bacteria living in the biofilm as compared to the same bacteria living in planktonic state. Biofilms help perpetuate antimicrobial resistance (AMR) by 3 main mechanisms [32]:

1. **Resistance at the surface of biofilm:** When an antimicrobial agent is trying to gain access in the sticky, slimy membrane, it initiates the first mechanism at surface levels of the biofilm. Exopolysaccharide, DNA and protein, which are the constituents of the biofilm makes it arduous for antibiotics to work their way through the matrix and reach the target bacterial cells within. Moreover, the slow diffusion of the antibiotic at the surface may render the antibiotic inoperative.
2. **Resistance Within Biofilm Microenvironments:** An antibiotic has to deal with a challenging microenvironment of the biofilm matrix once it is able to invade the primary surface of the biofilm. Various metabolic byproducts, waste and nutrients accumulate at this level. Moreover, oxygen may plummet, thus creating an anaerobic environment. The amalgamation of these factors has varying impacts on antibiotics, depending upon structure and action of each antibiotic. For instance, insufficient oxygen levels decrease the bactericidal efficacy of antibiotics tobramycin and ciprofloxacin, while pH changes can have undesirable effect on aminoglycoside action.
3. **Resistance of Bacterial "Persister" Cells:** Within the biofilm, the small subpopulations of bacteria survive the

antibiotic invasion, by transforming into a “spore-like” state which is immune to extreme conditions, like chemical treatment or antibiotic activity. These cells are called persister cells. They do not divide in the presence of antibiotics and remain dormant. This transformation is not due to any genetic mutation and as the organisms are liberated from the biofilm or begin multiplying again, they return to their pre-persister susceptibility profile.

Furthermore, in biofilm environment, bacteria are in close proximity with multiple microorganisms which permits bacterial communication like quorum sensing or cell-cell signaling and also allows transfer of mobile genetic elements. This results in transmission of resistance information more readily [32]. This bolsters proliferation of antimicrobial - tolerant persister cells that can withstand root canal treatment [33, 34]. So, from above it is clear that acquiring antibiotic resistant state by the bacteria in biofilms is a multifaceted response.

CHALLENGES IN ELIMINATING ROOT CANAL BIOFILM

Despite considerable and clearly evident progress of contemporary endodontics with regards to mechanical instrumentation of radicular spaces, the root canal infections and their associated apical periodontitis lesions remain pervasive [35]. In fact, a recent systematic review has revealed a spike in the prevalence of apical periodontitis lesions during the last 8–9 years, reportedly due to unproficient endodontic and restorative treatments [36]. The prime objective of endodontic treatment is to stave off the

development of apical periodontitis, by eliminating the infected and/or inflamed pulpal tissues and by creating the aseptic intraradicular conditions congenial with periradicular healing, if a lesion already exists. The crux of root canal treatment is to wipe out the infection and further prevent microorganisms from re-penetrating the root canal system.

The two pivotal challenges in removing root canal biofilms: microbiological and anatomical. From microbiological aspect, the infected teeth have densely colonizing biofilms that are robustly attached to the dentin, thus pose a challenge in removal. In addition to it, a typical challenge which appears in the form of anatomical complexities in the root canal system are inter and intra-canal communications/isthmi, lateral and accessory canals and apical delta. From clinical perspective, endodontic practicalities are complicated by the “blind” working areas of root canal system and intricate inter-communicating pathways which are never accessible to mechanical instrumentation. Furthermore, the inherent tolerance of biofilms to antimicrobial agents makes their removal an onerous task [37].

STRATEGIES FOR COMBATING ENDODONTIC BIOFILM

Instrumentation and endodontic irrigants

The purpose of mechanical instrumentation using endodontic files and irrigating the root canals with proteolytic disinfecting solutions is to reduce the microbial load and disrupt the biofilm [38]. However, biofilms in the root canal are difficult to access by using round

endodontic files [39]; hence files with ultramodern shapes have been developed that can move in oval or eccentric paths and are even more effective in canals with oval cross-sections than circular files [40, 41]. A recent study backed up the contention that instruments with a greater taper are crucial in maximizing the effectiveness of reducing bacterial load in the root canals [42]. A new strategy of using single-file systems as well as reciprocating instrumentation have also proven to be effective in bringing down the microbial count in the root canal system. These techniques use only one file to perform the root canal therapy, but are still considered effective in reducing the *E. faecalis* biofilm [43]. Despite of all the advancements in endodontic filing system, it needs to be combined with a suitable antimicrobial irrigant solution, so as to accomplish the chemo-mechanical debridement of the root canal system. The viscosity and surface tension of the irrigant solution used with rotary instrumentation system decides how effectively that irrigant touches the biofilm on the walls of the root canal and the sides of the files being used. Moreover, with the help of surfactants, the problem of vapor locks can be subdued and this allows the easy flow of irrigation fluid into fins, lateral canals and other anatomical areas that are difficult to access. As companion to physical debridement, the contemporary clinical protocol involves flushing the root canals with irrigant solution of 2.5 -6% sodium hypochlorite (NaOCl). Sodium chloride, sodium hydroxide (as a pH modifier) and one or more surfactants are also present in the current formulations of NaOCl. Due to these additives, the hypochlorite anion and hydroxyl ions act together to effectively dissolve vital and non-vital endodontic soft

tissues [44]. NaOCl irrigation solutions have microbicidal actions against both bacteria and fungi; some highly resistant organisms, particularly *E. faecalis* in biofilm state, require longer exposure times of up to 5 min for rendering it inactive [45]. In addition, EDTA has been advocated as a chelating irrigant which when paired with NaOCl bolsters the antibiofilm effects against *E. faecalis* biofilm [46].

Amelioration of endodontic diseases depends largely on effective biofilm removal as well as killing of biofilm bacteria. But due to the complex root canal anatomy, about 35% of the instrumented root canal area remains unaffected with conventional rotary and hand instruments [47]. Therefore, eradication of biofilm bacteria from the root canals depends considerably on the efficacy of endodontic irrigants.

The efficacy of NaOCl can be bolstered by warming the solution [6] and by physical activation of the solution with the help of ultrasonic instruments or pulsed middle infrared lasers (such as Er:YAG or Er,Cr:YSGG lasers). Ultrasonic agitation with piezoelectric ultrasonic instruments creates random cavitation events by using a moving tip, while laser agitation creates orchestrated cavitation events by means of a stationary tip. That is why lasers are a lot better to ultrasonic instruments for agitation [48].

The root morphology is complex in the apical third, with numerous lateral canals, apical delta, and apical ramifications. To disinfect these areas, novel laser protocols and optical fiber tips have been developed to amplify the cavitation and the movement of irrigants in the

root canal system. These remove the smear layer (debris as well as organic residues) created by instruments [49, 50]. A recent study which compared the different activation techniques of irrigants revealed that laser activation of 5.25% sodium hypochlorite enhanced the removal of intracanal *Enterococcus Faecalis* followed by ultrasonic, and sonic activation and there was no statistical difference between the groups [51].

Antimicrobial Medicaments

The intracanal medicaments must have a broad spectrum of antimicrobial activity because of the diverse microflora associated with the endodontic biofilms. Most of the dentists prefer to use calcium hydroxide for antibacterial disinfection in teeth with endodontic infections because of its antimicrobial property and stimulation of pulp cells to differentiate into mineralised tissue forming cells [52-55]. *E. faecalis* is inactivated at a pH above 11 but water-based calcium hydroxide pastes cannot achieve this pH because of pH buffering by dentine proteins [56, 57]. This problem can be solved by using a specific non-water polymer vehicle based paste which deploy calcium hydroxide and achieve a higher pH to render the bacteria inactive by enhancing the release of calcium and hydroxyl ions [58].

Triple antibiotic paste (TAP), is a combination of metronidazole, ciprofloxacin, and minocycline, is extensively used in regenerative endodontic procedure (REP). It has potent effect on infected dentin, intracanal biofilms, and most of endodontic pathogens [59-62]. There is also growing concern about

non-antibiotic antimicrobial agents as endodontic medicaments, which can penetrate biofilms. These include plant-derived phenolics, and nanoparticles such as chitosan that can render both fungi and bacteria inoperable [63].

FUTURE PROSPECTS OF ANTIBIOFILM AGENTS

Nanoparticles

Nanoparticles can directly kill the bacteria or can be modified to increase the drug aqueous solubility and transport into bacterial cells [64]. Nanoparticles synthesized from copper oxide, silver and zinc oxide, and other powders have broad antimicrobial applications [65]. Reactive oxygen species (ROS) are generated by the nanoparticles that are cytotoxic for bacteria. With greater surface area and more charge density, there is higher potential for bacterial interactions. Negatively charged bacterial cell membranes attract the positively charged nanoparticles towards it, thereby the nanoparticles gather on the cell membrane which increase permeability to destroy cells [66-68]. Moreover, cationic nanoparticles accumulate on the negatively charged dentin surface to prevent biofilm formation [69]. Studies have indicated that nanoparticles can also mitigate the risk for bacterial resistance and shields the conventional drugs from pH and/or enzymatic degradation in the biofilm microenvironments [70, 71]. Evidence suggests that silver nanoparticles (AgNPs) [72-74] and biomimetic iron oxide nanoparticles can impair biofilm formation and stave off dentinal tubule infection by *E. faecalis* [75]. AgNPs show the same bactericidal potency as 5.25% NaOCl against *E. faecalis* even at low concentration and could be given due consideration as a non-

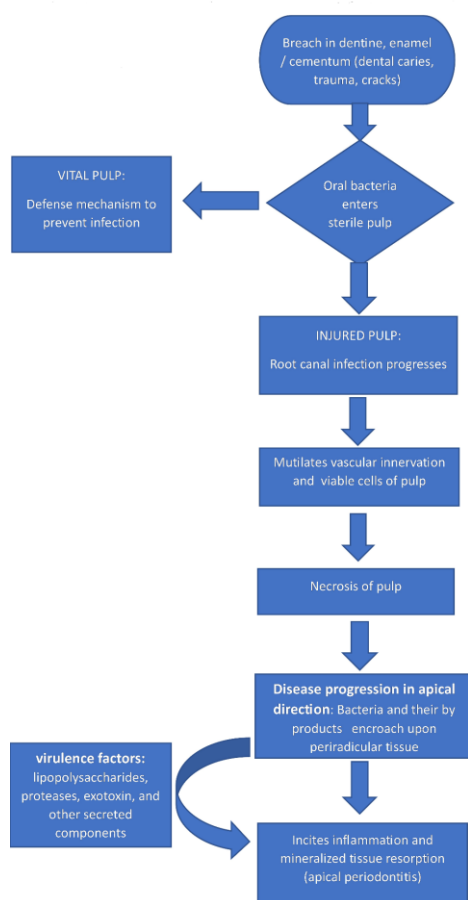


Figure 1: Pathogenesis of apical periodontitis (endodontic infection) [18,19,20,21]

toxic endodontic irrigant [76]. Moreover, chitosan nanoparticles in combination with zinc oxide can completely eradicate biofilms [77]. So, Chitosan nanoparticles can be incorporated into sealers to enhance the microbicidal effect.

Probiotic targeted delivery

Nowadays, probiotic delivery system is being extensively used in eradicating infections. This technique breaks down pathogenic biofilms and

also activates the immune response; consequently this leads to a unique combination of antibacterial-immune treatment regimen [78]. Empirical evidence has revealed that probiotics effectively target and inhibit the pathogenic bacteria and can act as drugs or drug vectors [79, 80, 81]. *Lactobacillus plantarum* (*L. plantarum*) is one such probiotic which inhibits biofilm formation by bacteria namely, *Streptococcus mutans*, *E. faecalis* and *Staphylococcus aureus* [82, 83, 84]. It not only reduced biofilm formation by mono-species but also multi-species biofilm involving *A. naeslundii*, *E. faecalis*, *Lactobacillus salivarius*, and *Streptococcus mutans* [85]. The utilization of *L. plantarum* in future could lead to a new paradigm shift in biofilm-mediated oral diseases prevention.

Phage therapy

Bacteriophages are simply called bacteria eating viruses [86,87]. They have DNA or RNA as their nucleic acid enclosed in a protein shell known as capsid [88]. They inhabit the human oral cavity where the host bacteria are present [89]. They lyse the bacterial cell immediately after their replication and then infects a new host bacteria [90]. They can even disrupt the biofilm by synthesizing polysaccharide depolymerases [91]. Bacteriophages are highly specific for their target bacterial strain, this gives them an edge over the conventional antibiotic therapy; phage therapy even does not promote antibiotic resistance [92]. In previous experimental researches phages for *Actinomyces* species, *Aggregatibacter actinomycetemcomitans*, *Enterococcus faecalis*, *Lactobacillus* species, *Streptococcus* species, and *Veillonella* species have been isolated [93- 98]. Most of the phage

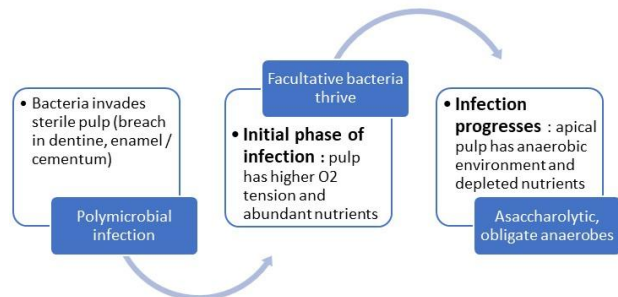


Figure 2: Microbial succession in endodontic infections [22, 23, 24]

therapy related research of oral cavity is in preliminary stages and holds a great potential as a therapeutic agent in biofilm mediated oral infections [93].

CONCLUSION

Endodontic diseases are extensively being associated with biofilm formation. The biofilm environment confers the bacteria with more virulence and antimicrobial tolerance. Inadequate removal of bacteria and biofilm by conventional endodontic treatment techniques are the main culprits for the recurrent cases. The ideal approach for eradication of biofilm will require combination of different techniques discussed in this review; these include state of art instrumentation and irrigation techniques for chemo-mechanical removal of biofilms and obtaining aseptic root canals, which forms the basis for good prognosis of endodontic treatment.

In order to enhance the success rates of root canal disinfection, innovative and effective approaches are being considered which could address the challenge of growing antimicrobial

resistance and removal of stubborn biofilms. Among them, nanoparticles, probiotics and bacteriophages are worth exploring that could open up new prospects for controlling biofilm-mediated endodontic infections.

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