Role of Endodontic Biofilms

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Abstract
Biological communities consisting of sessile cells embedded in a self-produced extracellular polymeric matrix (containing polysaccharides, DNA and other components). The formation of biofilms might facilitate certain survival and virulence characteristics under some situations. Several mechanisms have been postulated in the biofilm antimicrobial resistance, which includes: slow penetration of the antimicrobial agent into the biofilm, changes in the chemical micro-environment within the biofilm leading to zones of slow or no growth, adaptive stress responses and presence of a small population of extremely resistant “persister” cells. Biofilm biology has become an expanding field of research and the knowledge accumulated suggests that organisms growing in biofilms develop properties different to those dwelling in the planktonic stage. This review article covers the concept of biofilms and its role in endodontic infections.

Keywords: Biofilm; Endodontic biofilm; Bacterial adaptation; Microbial ecology; Pathogens

Introduction
Microorganisms are essential in the development of periradicular diseases and are the major causative factors associated with endodontic treatment failures. "Bacteria-associated endodontic failures together with pulp-periapical infections refractory to conventional treatment represent the unresolved bacteriological problems in endodontics" [1]. It is evident that an infected root canal system is a unique niche for a range of species of microorganisms. The composition of root canal microflora has been the focus of considerable research and interest over the years. Results of studies have clearly defined the microbial differences between primary endodontic treatment and also retreatment [2]. Apical periodontitis persisting after root canal treatment presents a more complex etiological and therapeutic solution [3]. Another important factor is that the microbes in the root canals grow not only as planktonic cells, but also form biofilms consisting of a complex network of different microorganisms.

The term 'biofilm' was introduced to designate the thin layered condensation of microbes (e.g. bacteria, fungi, protozoa) that may occur on various surface structures in nature. Free-flowing bacteria existing in an aqueous environment, so-called planktonic microorganisms are a prerequisite for biofilm formation. Biofilms are highly organised structures consisting of mushroom-shaped clumps of bacteria bound together by a carbohydrate matrix that contains water channels to deliver nutrients and remove wastes. Bacteria sequestered in biofilms are shielded and are often harder to kill than their planktonic counterparts. Biofilm bacteria are 1000 times more resistant to phagocytosis, antibodies and antibiotics [4].

The dominant mechanisms of biofilm resistance are due to:

a. Delayed penetration of antimicrobial agents through the exo-polysaccharide complex.
b. Modified nutrient environments and suppression of growth rate within the biofilm, thus affording protection from antimicrobial killing.

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c. A subpopulation of microorganisms in a biofilm can develop into a spore state that is highly protected: a phenotypic state known as a “persister”.

Most antimicrobial agents may be effective on the superficial layer of microorganisms in a biofilm, as the matrix layer may prevent direct contact of the agents with the microorganisms [5]. In the endodontic field, biofilms did not receive wide attention until it was reported by Sen et al. [6]. The genera most frequently implicated as persistent are streptococci, enterococci, staphylococci, fusobacteria, peptostreptococci, and lactobacilli.

Biofilms -An Over-View

Structure

The basic structural units of a biofilm are the colonies or cell clusters formed by the surface adherent bacterial cells. The bacterial cells are distributed in a spatial manner within a biofilm. A glyocalyx matrix made up of extra-cellular polymeric substances surrounds the microcolonies and anchors the bacterial cell to the substrate. The biofilm structure by volume is made up by 85% with matrix material and the rest with cells. The structure and composition of a biofilm modifies according to the environmental conditions. The structural feature of a biofilm that has the highest impact in chronic bacterial infection is the tendency of microcolonies to detach from the biofilm community; and during this process of detachment, there is transfer of particulate matter from the biofilm to the fluid bathing the biofilm. Detachment occurs by two types; erosion where there is continual detachment of single cells and small portions of the biofilm and by sloughing, which is rapid and massive loss of biofilm. Detachment plays an important role in shaping the morphological characteristics of and also the structure of a mature biofilm.

Composition

Biofilms are composed of carbohydrates, proteins and lipids which make up the organic portion; and calcium, phosphorus, magnesium ad fluoride which make up the inorganic portion [7].

Characteristics [8,9]

Bacteria in a biofilm show distinct capacity to survive tough growth and environmental conditions; this is due to the following features:

a. Biofilm structure protects the residing bacteria from environmental threats
b. Biofilm structure permits trapping of nutrients and metabolic cooperativity between resident cells of same species and/or different species
c. Biofilm structure allows bacterial species with different growth requirements to survive
d. Bacteria in biofilms may communicate and exchange genetic materials to acquire new traits.

Endodontic Pathogens

According to the results of studies, primary root canal infection is a dynamic process and bacterial species differ during various stages. Steeg and van der Hoeven [10] showed that the most important factors are availability of nutrition, oxygen level (redox potential) and the local pH within the root canal. Facultative anaerobic bacteria grow well in anaerobic conditions, their primary source of energy being carbohydrates. Obviously they flourish when there is decreased availability of carbohydrates in the root canals. Endogenous proteins and glycoproteins are the main nutrients in the root canal system of primary endodontic cases. The main source of proteins in the root canal is a process of degradation of the small volume of pulpal tissue and influx of exudates from periapical tissues into the canal due to inflammatory process. Bacterial metabolism of the serum-like fluid also causes reduction of the redox potential and a rise in the pH within the root canal [11].

Currently, there is no substantial evidence indicating that certain microorganisms are more virulent than others. Sundqvist and Figdor [2] stated that a proper definition for endodontic pathogens should include every organism capable of inducing the tissue destruction in apical periodontitis. In reality, however, the majority of endodontic-microbiology studies refer to the endodontic pathogen as the bacteria isolated from a symptom-associated root canal that grows in the laboratory in a specific media. By this approach, the most frequently recovered species will assume the role of major endodontic pathogen.

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With the exception of Actinomyces, other species commonly associated with persistent intraradicular infection such as candida and enterococci are opportunistic pathogens. For microbes to maintain apical periodontitis and continue to cause disease, they must not only survive in the root-filled canal, but also possess the pathogenic properties necessary to perpetuate inflammation external to the root canal system. In general, microorganisms involved in persistent infections implement one of the three strategies to evade immune response-sequestration, cellular or humoral evasion [12]. Sequestration involves a physical barrier between the microbe and the host; cellular evasion means that microorganisms avoid leukocyte dependant antibacterial mechanisms and humoral evasion means that extracellular bacteria avoid the host’s antibodies and complement.

The frequent occurrence of E. faecalis in the potential colonization and overgrowth in endodontic infections as the dominant organism in post-treated apical periodontitis has often been isolated from root canals; and its pathogenicity is well documented [2]. E. faecalis is an opportunistic pathogen and one of the leading causes of nosocomial infections. The ability of E. faecalis to form biofilms may confer an ecological advantage in certain situations. In endodontic infections, E. faecalis first adheres to the tissue surfaces by a physical association; in a second step there is permanent bonding by specific bacterial adhesins to complementary receptors on the host surfaces. Once the bacterial cell is bound, it is able to use available nutrients and a biofilm structure is necessary to contend with host defense mechanisms and for resistance to antibacterial treatments. For these reasons, experimental data suggest that viable E. faecalis cells can be recovered from root canals after an effective chemo-mechanical instrumentation treatment [13,14]. When compared to detection of E. faecalis by culturing (24-70%), E. faecalis has been found at higher percentages (67-77%) when a PCR detection method is used [15].

E. faecalis possesses certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones and lipoteichoic acid [15]. It has been shown to adhere to host cells, express proteins and alter host responses [15,16]. E. faecalis suppresses the action of lymphocytes, potentially contributing to endodontic failure [17]. The potential survival and virulence factors of E. faecalis can be summarised as:

a. It endures prolonged periods of nutritional deprivation
b. Binds to dentin, proficiently invading the dentin tubules
c. Alters the host responses
d. Suppresses the action of lymphocytes
e. Utilises serum as nutritional source
f. Forms a biofilm

Prevotella species such as P. intermedia and P. nigrescens were more often found in infected root canals; these two species have been cultured from 26-40% of root canals of teeth with apical periodontitis [18]. Further, P. nigrescens was more common than P. intermedia [19]. Some species of microorganisms are strongly associated with primary endodontic cases. These are Fusobacterium nucleatum, Veillonella parvula, Eubacterium and other species. In root canals, some of them are associated with other species; and numerous studies have shown the importance of food chain in which the metabolism of one species supplies nutrients for the growth of others. One example of synergistic association between microbial species could be the strong association of F. nucleatum with P. micros, P. endodontalis and Campylobacter rectus. Strong associations were also detected between Pr. intermedia and P. micros and also between P. anaerobius and the Eubacteria and Peptostreptococcus anaerobius [20].

Conditions for Persistent Infection

Persistent endodontic treatment disease involves multiple microbial and location factors. Microorganisms must possess an ability to survive the antimicrobial treatment and require ‘persistence’ characteristics such as a capacity for starvation survival and an ability to utilize serum-like periapical transudate as a source of nutrition. The location of the microbes within the root canals is crucial for access to nutrients; they must be situated near the apical foramen and also have an open communication for the free exchange of fluid, molecules and to inflame the periapical tissue. Together, these microbial characteristics and the opportunities of location determine whether microorganisms that survive treatment are able to maintain apical periodontitis following such treatment.

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Endodontic Biofilms

Though surface-associated microbial communities are the main form of colonization and retention by oral bacteria, biofilms also form in the root canals having the same properties as the parent communities colonising the enamel and cementum. Biofilms form when planktonic bacteria in a natural liquid phase are deposited on a surface containing an organic conditioning polymeric matrix or “conditioning film”. According to Svensater and Bergenholtz [8], the biofilms in root canals are initiated at some time after the first invasion of the pulp chamber by planktonic oral organisms. There is inflammation which moves towards the apex providing the fluid vehicle for the invading planktonic organisms so that they multiply and attach to the root canals. The bacteria in the infected root canals, is a restricted group compared to oral flora and largely comprised of facultative bacteria and strict anaerobes.

The progression of infection alters the nutritional and environment of the root canals; the initial polymicrobial environment of the infected root canal becomes more anaerobic thus depleting the nutritional level. These changes will offer a tough ecological niche for the surviving microorganisms. The endodontic bacterial biofilms can be categorised as:

- Intracanal biofilms
- Extraradicular biofilms
- Periapical biofilms
- Biomaterial centered infections

During the various stages of biofilm development, cells are in different physiological states. The cells that are at the base of the biofilm, may be dead whereas those at the top may be actively growing. The majority of the time cells even with extremes of diversity, are in a state equivalent to cells in the stationery phase of growth [21,22]. From the perspective of the persisting root canal flora, the “stationery-phase” cells might maintain a low but sufficient metabolic activity to provoke periapical inflammation.

The characteristic features in cell-cell and microbe-substrate interactions were explained based on the phenomena of microbial adherence [23,24]. Many studies have shown the ability of *E. faecalis* to resist starvation and also develop biofilms under different environmental and nutrient conditions; but they modified according to the prevailing conditions. *E. faecalis* produced typical biofilm structures with bacterial cells and water channels under nutrient-rich environment.

*In vitro* experiments have revealed three distinct stages in the development of *E. faecalis* biofilm:

**Stage 1:** Formation of microcolonies on the root canal

**Stage 2:** There is bacterial-mediated dissolution of the mineral fraction from the dentin substrate thus leading to increase in calcium and phosphate ions; finally promoting the mineralization of the biofilm

**Stage 3:** The mature biofilm structure formed after 6 weeks carbonated-apatite structure as compared to natural dentin which had carbonated fluorapatite structure

Anti-Microbial Agents and Biofilms

Anti-microbial agents have been developed and optimised for their activity against fast growing, dispersed populations containing a single organism. Antibiofilm substances can inhibit biofilm formation (preventive effect) or alternatively act on biofilms already formed (therapeutic effect). The mechanism of action against established biofilms may be through disruption of biofilm biomass and/or direct killing of the biofilm bacteria. It is very important for an endodontic irrigant or medicament to act primarily on established biofilms attached to the root canal walls so as to promote their elimination.

Some of the newer Antibiofilm agents like Farnesol, Xylitol, Lactoferrin and also Salicylic acid have removed the biofilm. Farnesol has a unique property of both inhibition of biofilm formation and also disrupts the already formed biofilms [25-27]. Farnesol, when applied topically reduces the biofilm matrix content [26] and it also kills biofilm bacteria [28]. Xylitol only minimally reduces bacterial viability in biofilms [29]; but can synergistically act with Farnesol inhibiting the growth of *Staphylococcus aureus* [30,31]. Lactoferrin has great potential to act synergistically with xylitol to disrupt biofilm structure and reduce bacterial viability [29,32]. Specifically, xylitol disrupts biofilm integrity whereas Lactoferrin permeabilizes bacterial membranes [29].

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Salicylic acid, prevents bacterial attachment to medical devices [33] and inhibits biofilm formation [34,35]; Salicylic acid preferentially affects certain species.

Conclusion

It is evident that in primary endodontic cases, root canal environment provides nutritional supply rich with peptides and amino acids for bacterial inhabitants of root canal system favouring the growth of anaerobic proteolytic species. The formation of biofilms carries particular clinical significance for defense mechanisms, and therapeutic benefits including chemical and mechanical antimicrobial treatment measures. As far as endodontic infections are concerned, the biofilm concept has gained very little attention. Further research is required to explore the conditions that may affect the efficacy of antimicrobials so that their clinical effects can be better predicted.

Bibliography


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