

## EFFECT OF BIOFILM IN ENDODONTICS: A REVIEW

Swetha HB, \* Shubhashini N, \*\* Vinay Chandra R, \*\*\* Thokala Dhamodaran, †  
Anoop P ††

\* Reader, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental College, Bengaluru, Karnataka, India

\*\* Reader, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental College, Bengaluru, Karnataka, India

\*\*\* Professor, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental College, Bengaluru, Karnataka, India

† Senior Lecturer, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental College, Bengaluru, Karnataka, India

†† Senior Lecturer, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental College, Bengaluru, Karnataka, India

### ABSTRACT

Biofilm biology has become an expanding field of research in human, industrial and environmental ecosystems. The knowledge accumulated suggests that organisms growing in biofilms develop properties different to those dwelling in the planktonic state. It is obvious that this realization and the fact that biofilms afford the resistant microorganisms resistance against harmful exogenous influences including antimicrobial agents, is rather new to endodontology. Thus, one future challenge for research in endodontology is to assess virulence expressions in vivo and in situ models with microenvironment resembling the real life condition in root canal. Furthermore, the understanding of biofilm concept will play a crucial role in helping us to assess and understand not only the pathogenic potential of root canal microbiota, but also the basis for new approaches to infection control.

**KEYWORDS:** Biofilms; periapical infections; pulpal infections; microbiota

### INTRODUCTION

Biofilms is a mode of microbial growth where dynamic communities of interacting sessile cells are irreversibly attached to a solid substratum, as well as each other, and are embedded in a self made matrix of extra cellular polymeric substances. Microbial biofilm is considered a community and the microorganisms living in the community must possess the ability to self organize (Autopoiesis), resist environmental perturbations (Homeostasis), must be more effective in association than in isolation (Synergy) and respond to environmental changes as a unit rather than single individuals (Communality).<sup>[1]</sup>

### SIGNIFICANCE OF BIOFILMS

It is one of the basic survival strategy employed by microorganisms in all natural and industrial ecosystems in response to starvation. The sessile microbial cells in a biofilm state differ greatly from their planktonic counterparts.<sup>[2]</sup> Inside a biofilm, the bacterial cells exhibit altered phenotypic properties and are protected from antimicrobial environmental stresses, bacteriophage and amoeba. Biofilms are responsible for most of the chronic infections and almost all recalcitrant infections in human beings, as bacteria in biofilm are resistant to both antimicrobials and host defence mechanisms. Three factors essential for biofilm are microorganisms, solid substrate and fluid channels (Fig. 1).<sup>[3]</sup>

### COMPOSITION OF BIOFILM

Biofilm consists of matrix material 85% vol. and 15% cells. A fresh biofilm is made up of biopolymers such as polysaccharides, proteins, nucleic acids and salts. A glycocalyx matrix is made up of EPS which surrounds the microcolonies and anchors the bacterial cells to the substrate.<sup>[4]</sup> Typically, a viable fully hydrated biofilm appears as tower or "mushroom shaped structure". The overall shape of biofilm structure is determined by force generated by flushing of fluid media. The water filled channels which are regarded as a primitive circulatory system in a biofilm, intersect the structure of biofilm to establish connections between the microbial colonies. Presence of water channels facilitates efficient exchange of materials between bacterial cells and bulk fluid, which in turn helps to coordinate functions in a biofilm community.<sup>[1]</sup> The structural feature of biofilm that has the highest impact in chronic bacterial infection is the tendency of microcolonies to detach from the

biofilm community. Detachment is through Erosion are sloughing. Detachment has been understood, to play an important role in shaping the morphological characteristics. It is also an “active dispersal mechanism” or “Seeding dispersal” where a detached cell forms resistance traits which are the source of persistent infections.<sup>[4]</sup>

#### **DEVELOPMENT OF BIOFILM**

Microorganisms can form biofilm on any surface that is bathed in nutrient containing fluid. First step is the adsorption of inorganic and organic molecules to solid surface creating the conditioning layer.<sup>[11]</sup> Once the conditioned layer is formed, the next step is the adhesion of microbial cells to this layer. There are many factors that affect bacterial attachment to a solid substrate such as pH, temperature, surface energy of substrate, nutrient availability, and length of the time the bacteria is in contact with the surface and bacterial cell surface charge.<sup>[4]</sup> In addition, the microbial adherence to substrate is also mediated by bacterial surface structure such as fimbriae, pili, flagella and extrapolymeric substances. The bacterial cell surface structures form bridges between the bacteria and conditioning layer.<sup>[5]</sup> These bridges are combination of electrostatic attractions, covalent and hydrogen bonding and dipole interaction. Initially, the bond between the bacteria and the substrate may not be strong.<sup>[7]</sup> However with time, these bonds gains in strength, making the bacteria substrate attachment irreversible. Finally, a specific bacterial adhesion with a substrate is produced via polysaccharide adhesion.<sup>[8]</sup> Next step is the Biofilm growth and expansion. During this step, the monolayer of microbes attracts secondary colonizers forming microcolonies forming the final structure of biofilm. A mature biofilm will be metabolically active community of microorganisms where individuals share duties and benefits. The bacterial cells in a biofilm will exhibit considerable variation in its genetic and biochemical constitutions compared to its planktonic counterparts (Fig. 3).<sup>[9]</sup>

#### **MICROBIAL INTERACTIONS IN A BIOFILM**

It's by coadhesion or coaggregation, coadhesion is a process of recognition between a suspended cell and a cell already attached to substrate while Coaggregation is a process where genetic distinct

cells in suspension recognize each other and clump together.<sup>[11]</sup>

#### **CHARACTERISTICS OF BIOFILM**

Bacteria in a biofilm show distinct capacity to survive tough growth and environmental conditions. This unique capacity of bacteria in biofilm is due to it protects the residing bacteria from environmental thrats. Structures of biofilm permit trapping of nutrients and metabolic cooperatively between resident cell of same species and different species. Biofilm structure display organized internal compartmentalization and Bacterial cells in biofilm may communicate and exchange genetic materials to acquire new traits (Fig. 4).<sup>[11]</sup> The mechanisms responsible for resistance may include the resistance associated with extracellular polymeric substance, growth rate and nutrient availability and adoption of resistant phenotype (metabolic alterations and genetic alterations).<sup>[10]</sup>

#### **ENDODONTIC BIOFILMS**

Endodontic microbiota is established to be less diverse compared to oral microbiota. This transition in the microbial population is more conspicuous with the progression of infections<sup>10</sup>. Furthermore, clinical investigations have shown that the complete disinfection of the root canal system is very difficult to achieve because microorganisms are found to persist in the root canal system complexities such as apical portions, deltas, isthmuses and lateral canals etc. These anatomical complexities in the root canal systems shelter the adhering bacteria in a biofilm from cleaning and shaping procedures.<sup>[1]</sup> Additionally, biofilm mode of microbial growth offer advantages like resistance to antimicrobials, increase local concentration of nutrients, provides opportunities for genetic exchange and ability to communicate between different bacterial populations of same and different species.<sup>[2]</sup>

Endodontic biofilms are of four types:

1. Intracanal biofilm
2. Periapical biofilm
3. Extraradicular biofilm
4. Biomaterial centered biofilms

*Intracanal biofilms* - microbial biofilms that are formed on root canal dentine of endodontically infected teeth. Intracanal microbiotas in an endodontically infected tooth exist as both loose microbial cells and biofilm structures, made up of cocci, rods, and filamentous bacteria.<sup>[11]</sup> It is

monolayered or multilayered in structure (Fig. 5). Studies have revealed the ability of *E. faecalis* to resist starvation and develop biofilms under different environmental and nutrient conditions (aerobic, anaerobic, nutrient rich and nutrient deprived conditions).<sup>[2]</sup>

*Stages in the development of E. faecalis:*

Stage I: *E. faecalis* adhered and formed microcolonies on dentin surface.

Stage II: *E. faecalis* induces bacterial mediated dissolution of mineral fraction from dentin and increases calcium and phosphates ions and promotes calcifications of *E. faecalis* biofilms.<sup>[1]</sup>

A recent investigation has highlighted the ability of *E. faecalis* to coaggregate with *F. nucleatum*. The coaggregation of *E. faecalis* and *F. nucleatum* provides the ability of these microorganisms to coexist in microbial community and contribute to endodontic infection.<sup>[4]</sup> The inherent capacity of *E. faecalis* to resist the bactericidal action of many antimicrobials along with its ability to form biofilm under tough environmental conditions and nutritional conditions contribute to its persistence in recalcitrant endodontic infections.<sup>[11]</sup>

#### **EXTRARADICULAR BIOFILMS**

These biofilms are also known as “root surface biofilms”. These biofilms are microbial films formed on the root surface adjacent to root apex of endodontically infected teeth.<sup>[1]</sup> This type of biofilms is reported in teeth with asymptomatic periapical periodontitis and teeth with chronic apical abscess associated with sinus tracts. These biofilms consists of cocci and rods and filamentous species, with cocci attached to tooth substrate. Clinical evidence of calcified biofilms on extraradicular regions were also reported. Ricucci et al 1995 - reported the presence of calculus like deposit on root apex of teeth extracted due to post-treatment periapical periodontitis. Harn *et al.*, 1998 - calculus like deposit on apical root surface of tooth with lesion refractory to root canal therapy.<sup>[3]</sup>

#### **PERIAPICAL MICROBIAL BIOFILMS**

These are isolated biofilms found in periapical region of an endodontically infected tooth. The microbiota in majority of teeth associated with apical periodontitis is restricted to root canal as most of the microbial species that infect the root canal are opportunistic pathogens that dont have the ability to survive the host defence mechanisms in periapical tissues. Rarely,

microbial species or even strains within species may possess strategies to survive and thus infect periapical tissues.<sup>[3]</sup> The members of genus *Actinomyces* and *P. propionicum* have been demonstrated in asymptomatic periapical lesions refractory to endodontic treatment. These microorganisms have the ability to overcome host defence mechanisms, thrived in inflamed periapical tissue and subsequently induce a periapical infection.<sup>[12]</sup>

#### **BIOMATERIAL CENTERED INFECTION (BCI):**

It is caused when microorganisms<sup>[1]</sup> adheres to an artificial biomaterial surface and forms biofilms structures. Presence of biomaterials in close proximity to host immune systems can increase the susceptibility to Biomaterial centred biofilms. Biomaterial centered infection is one of the major complications associated with prosthesis and or implant related infections.<sup>[1]</sup> Furthermore, biofilms are extremely resistant to host defence mechanisms and antibiotic treatment, BCI are rarely resolved and often the only solution to an infected biomaterial is the surgical removal. In BCI, various microorganisms are involved and species like *Staphylococcus aureus*, *Enterococcus faecalis*, streptococci and *Pseudomonas aeruginosa* and fungi (*Candida albicans*) are most commonly isolated species. In endodontics Biomaterial centered biofilms would form on root canal obturating materials and can be intraradicular or extraradicular.<sup>[2]</sup>

#### **CONCLUSION**

Biofilm biology has become an expanding field of research in human, industrial and environmental ecosystems. The knowledge accumulated suggests that organisms growing in biofilms develop properties different to those dwelling in the planktonic state. It is obvious that this realization and the fact that biofilms afford the resistant microorganisms resistance against harmful exogenous influences including antimicrobial agents, is rather new to endodontology. Hence, the conditions under which biofilms occur in endodontic infections and the measures that ought to be taken for their eradication, are not well understood. Thus, one future challenge for research in endodontolgy is to assess virulence expressions in in-vivo and in - situ models with microenvironment resembling the real life condition in root canal. Furthermore,

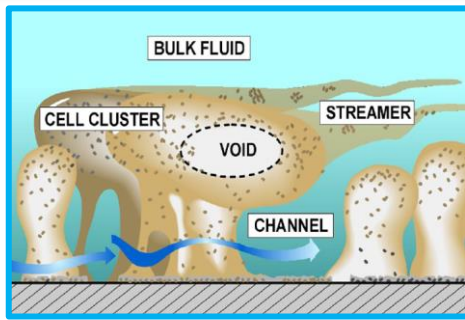


Fig. 1: Factors essential for Biofilm

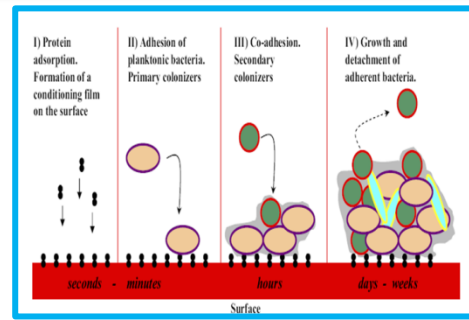


Fig. 2: Development of biofilm

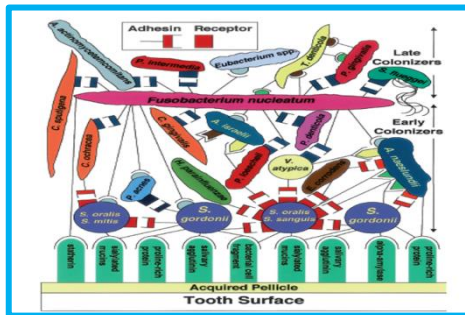


Fig. 3: Mature biofilm

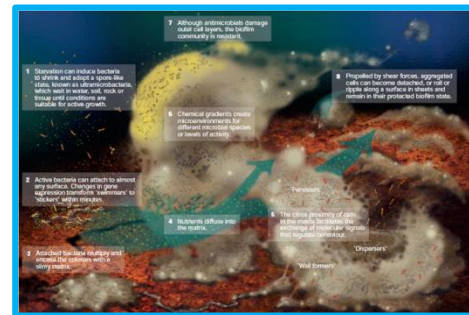


Fig. 4: Characteristic of biofilm

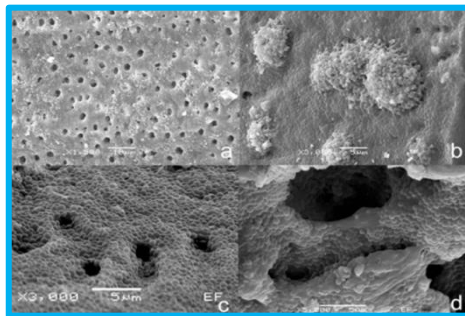


Fig. 5: E. faecalis biofilm on root dentinal surface

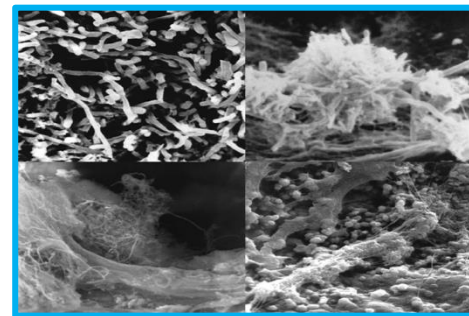


Fig. 6: Apical biofilm on external root surface

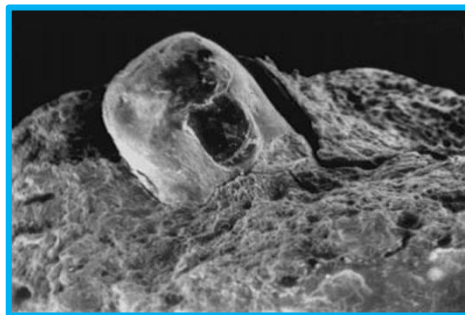


Fig. 7: Biofilm on extruded gutta percha

the understanding of biofilm concept will play a crucial role in helping us to assess and understand, not only the pathogenic potential of root canal microbiota, but also the basis for new approaches to infection control. How bacteria adapt their properties under different conditions as well as how biofilms are organized in root canals are important issues to be addressed on the road to clearer understanding of how the root

canal bacteria resist endodontic treatment procedures.

**CONFLICT OF INTEREST & SOURCE OF FUNDING**

The author declares that there is no source of funding and there is no conflict of interest among all authors.

**BIBLIOGRAPHY**

1. Kapil Jhaharia. Biofilm in endodontics: A review. J Int Soc Prev Community Dent 2015;5(1);1-12.
2. Leonardo MR. EM evaluation of bacterial biofilm and microorganisms on the apical external root surface of human teeth. J Endod 2002;28;815-8.
3. Chavaz de Paz. Redefining the Persistent Infection in Root Canals: Possible Role of Bio film Communities. J Endod 2007;33;652-62.
4. Toledo. Enterococcal Surface Protein, Esp, Is Involved in E.faecalis biofilm formation.

- Appl Environ Microbio 2001;99;231-52.
5. Munson MA, Pitt-Ford T, Chong B, Weightman A, Wade WG. Molecular and cultural analysis of the micro flora associated with endodontic infections. *J Dent Res* 2002;81:761-6.
  6. Moliz. *Enterococcus faecalis* Biofilms Eradication by Root Canal Irrigants. *J Endod* 2009;34;1-10.
  7. Giardino. Comparative Evaluation of Antimicrobial Efficacy of Sodium Hypochlorite, MTAD, and Tetraclean against *Enterococcus faecalis* Biofilm. *J Endod* 2007;1;44-51.
  8. Grivet JP, Delort AM, Portais JC. NMR and microbiology: From physiology to metabolomics. *Biochimie* 2003;85:823
  9. Noiri Y, Ehara A, Kawahara T, Takemura N, Ebisu S. Participation of bacterial biofilms in refractory and chronic periapical periodontitis. *J Endod* 2002;28:679-83
  10. Takemura. Single species biofilm forming ability of root canal isolates on gutta percha points. *Eu J Or Sci* 2004;29;400-3
  11. Distel. Biofilm Formation in Medicated Root Canals. *J Endod* 2002;31;380-6.
  12. Walker. Microbial biofilm formation in DUWS and their control using disinfectants. *Jour Dent* 2007;36;500-8.