Ministry of higher education and scientific research Babylon university faculty of dentistry conservative department

Biofilm in endodontic infections

A project submitted to department of operative, Babylon university /collage of dentistry in partial fulfillment of requirement for the degree of bachelor in dentistry

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2022A.D **1442A.H**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((هوَ الَّذِي بَعَثَ فِي الأُمِّيِّينَ رَسولا مِنْهمْ يَتْلو عَلَيْهِمْ آيَاتِهِ وَيزَكِّيهِمْ وَيعَلِّمهم الْكِتَابَ وَالْحِكْمَةَ وَإِنْ كَانوا مِنْ قَبْل لَفِي ضَلالٍ مبِين))

صدق الله العظيم

(سورة الجمعة، اية ٢)

الاهداء

الى الشموس الطالعة والأقمار المنيرة والانجم الزاهرة...

النبي الأعظم والائمة الاطهار عليهم السلام

الى من لا تورق اغصاننا الا بماء حنانها... وبركات دعائها...

الى ذلك النور البهي المشع بالأمل والسعادة...

الى امي الغالية...

الى من قوم أخلاقي بعد ان خلقني ربي...

الى من أرشدني الى العلم ...والشمعة التي أضاءت دربي..

الى ابي العزيز...

الى الازهار الملتفة حولي بقلوبهم ومن اشد بهم ازري..

اخواني واخواتي

الى كل من وقفوا بجانبي...

الى كل من له مكانة في قلبي...

اهدي عملي هذا مع كل الحب والامتنان

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Abbreviation

E. faecalis: Enterococcus faecalis bacteria.

(BBR): Berberine.

Str.mutans: Streptococcus mutans.

F. nucleatum: Fusobacterium nucleatum.

(NaoCl): sodium hypochlorite.

(EDTA): Ethylenediaminetetraacetic acid.

CHx: Chlorohexidine.

(MA) Maleic acid.

(CTR): Cetrimide.

Introduction

Endodontic disease refers to infection or inflammation the pulp of the tooth. Is biofilm mediated infection, and primary aim in the management of endodontic disease is the elimination of bacterial biofilm from the root canal system. The most common endodontic infection is caused by the surface-associated growth of microorganisms. It is important to apply the biofilm concept to endodontic microbiology to understand the pathogenic potential of the root canal microbiota as well as to form the basis for new approaches for disinfection. Biofilms and microbial aggregates are the common mechanisms for the survival of bacteria in nature.Microbial biofilms play an essential role in several infectious diseases such as pulp and periradicular pathosis [1].

Aim of this study:

The main goal of this study to explain the role of biofilm in primary infection of tooth pulp.

Review

Almost 700 bacterial species can be found in the oral cavity, with any particular individual harboring 100–200 of these species. Infection progress to apical once the root canal is infected coronally until bacterial products or bacteria themselves got the capability to infect the periapical tissues, which leads to apical periodontitis. Endodontic infections have a polymicrobial nature, with obligate anaerobic bacteria conspicuously dominating the microbiota in primary infections as in (figure1) . The most common bacteria found in endodontic biofilms gram negative anaerobic rods are: F. nucleatum, Prevotella spp. and C. rectus. The most common Gram-positive bacteria are: Streptococci (S. mitis, S. gordonii, S. anginosus, S. oralis), Lactobacilli (L. paracasei, L. acidophilus), Staphylococci, E. faecalis, O. uli, P micra, P. alactolyticus, Propionibacterium spp., Actinomyces spp., Bifidobacterium spp. and Eubacterium spp. Sometimes, yeasts, commonly C. albicans, are also found in small amounts.

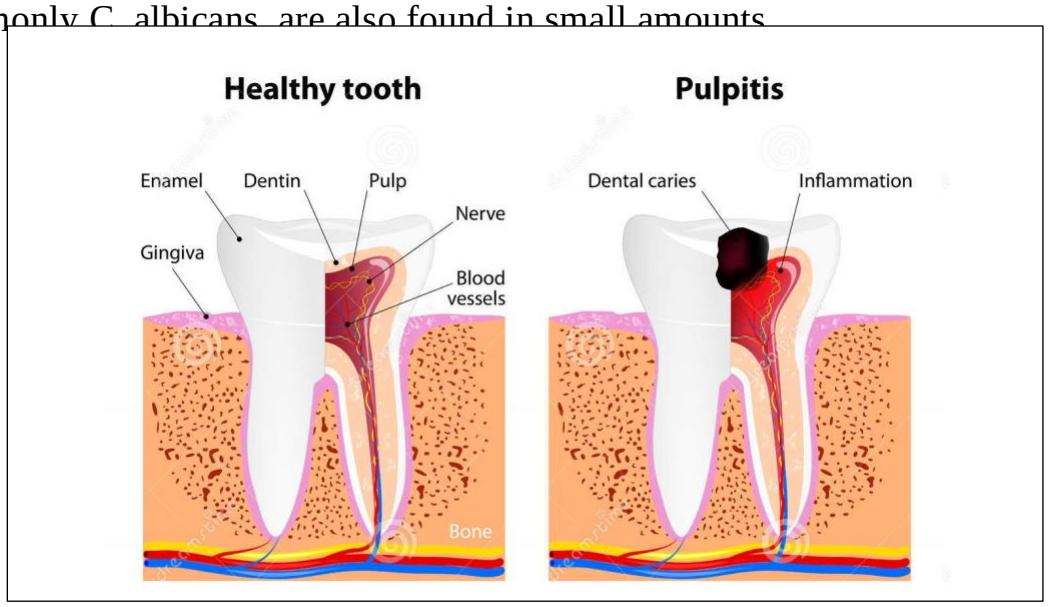


Figure 1. healthy tooth vs a pulpitis tooth after dental caries.

Role of E-faecalis in biofilm

Among different clinical bacterial isolates recovered from endodontic infections, E.faecalis is the only species that has been widely studied for its capacity to form biofilms. [2-3] If bacteria participate in gene exchange within a biofilm via horizontal gene transfer, processes leading to a spread of antibiotic resistance genes between different clinically relevant species can be accelerated. [4] horizontal gene transfer rates are typically higher in biofilm communities, compared with those in planktonic niches. Thus, there is a connection between biofilm formation and horizontal gene transfer. In addition to this, the persistence of endodontic bacteria via biofilm formation underlines the necessity for more effective methods not only to completely eliminate bacteria during endodontic retreatment but also to isolate all the existing microorganisms during the microbiological sampling from infected root canals. It should also be kept in mind that the complex anatomy of the root canal poses further difficulties because biofilms of persistent microorganisms within the root canals may also be located on the walls of ramifications and isthmuses.

E. faecalis is a **gram-positive**, facultative anaerobic coccus that is strongly associated with endodontic infections. Being an opportunistic pathogen, it causes nosocomial infections and is frequently isolated from the failed root canals undergoing retreatment [5-6]. Enterococci are gram-positive cocci that can occur singly, in pairs, or as short chains see (figure 2). They are facultative anaerobes which have the ability to grow in the presence or absence of oxygen. They can grow in extremely alkaline pH, salt concentrated environment, in a temperature range of 10–45°C, and survive a temperature of 60°C for 30 min. E. faecalis is able to

suppress the action of lymphocytes, potentially contributing to endodontic failure.[7] E. faecalis in dentinal tubules can resist intracanal dressings of calcium hydroxide for over 10 days by forming a biofilm that helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non-biofilm producing organisms. Calcium hydroxide, a commonly used intracanal medicament, may be ineffective to kill E. faecalis on its own, if a high pH is not maintained. [7] E. faecalis has the ability to form biofilm that can resist calcium hydroxide dressing by maintaining pH homeostasis, but at a pH of 11.5 or greater, E. faecalis is unable to survive.

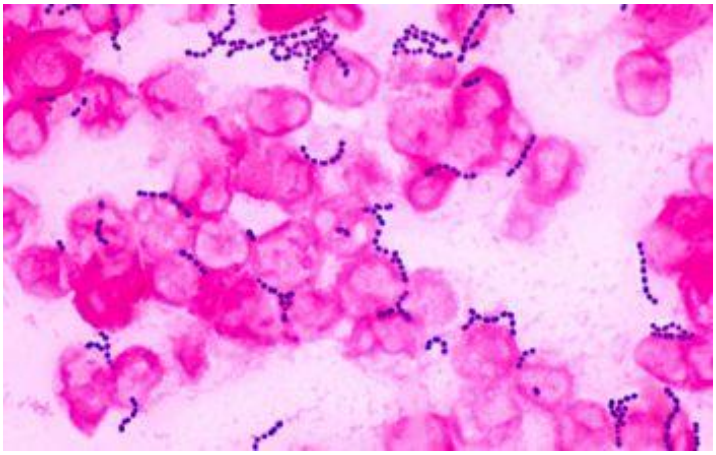


Figure 2. E. faecalis shape.

Development of biofilm

Bacteria can form biofilms on any surface that is bathed in a nutrient-containing fluid. The three major components involved in biofilm formation are bacterial cells, a solid surface and a fluid medium.

Biofilm formation occurs in three stages given below:

Stage 1: Adsorption of inorganic and organic molecules to the solid surface occurs, leading to the formation of conditioning layer like show in (figure 3).

Stage 2: Adhesion of microbial cells to the conditioned layer: There are many factors that affect the bacterial attachment like pH, temperature, surface energy of the substrate, nutritional availability, time of contact of bacteria, bacterial cell surface charge and surface hydrophobicity like show in (figure 3).

The bacteria substrate interaction occurs in three phases:

- **Phase 1:** Transport of microbe to substrate surface which is mediated by fimbriae, pili, flagella and extracellular polysaccharides (glycocalyx).
- **Phase 2:** Initial non-specific microbial–substrate adherence which occurs due to combination of electrostatic attraction, covalent and hydrogen bonding, dipole and hydrophobic interaction.
- **Phase 3:** Specific microbial substrate adherence phase. In this phase, adhesin or ligand on the bacterial cell surface binds to receptors on the substrate.

Stage 3: Development of biofilm and biofilm expansion occurs. In this stage, monolayer of microbes attracts secondary colonizers forming microcolony, and the collection of microcolonies gives rise to the final structure of biofilm like show in (figure 3).[8-9]

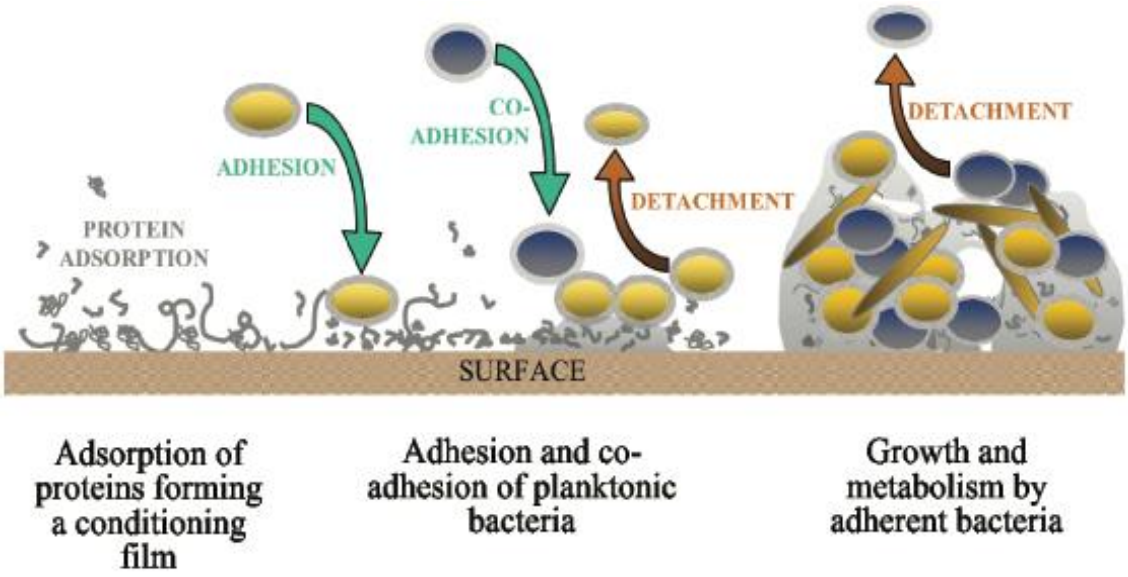


Figure 3. Stages of biofilm formation.

Endodontic bacterial biofilms can be categorized as

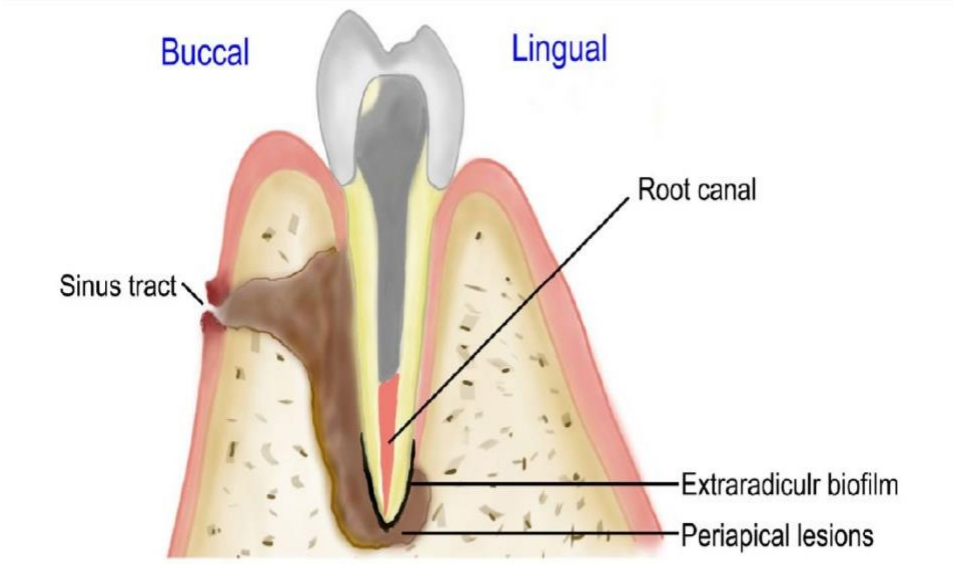
1) Intracanal microbial biofilms

They are microbial biofilms formed on the root canal dentin of an endodontically infected tooth (figure 4).[10]

2) Extraradicular microbial biofilms

They are also termed as root surface biofilms which are formed on the root (cementum) surface adjacent to the root apex of endodontically infected teeth.[11]

Extraradicular biofilms are reported with asymptomatic periapical periodontitis and in chronic apical abscesses with sinus tracts like show in figure 4. Sometimes, the extraradicular biofilm becomes calcified and gets associated with periapical inflammation and delayed periapical healing in spite of adequate orthograde root canal treatment.[12]



3) Periapical microbial biofilms

They are isolated biofilms found in the periapical region of endodontically infected teeth. Periapical biofilms may or may not be dependent on the root canal. These microorganisms have the ability to overcome host defense mechanisms, thrive in the inflamed periapical tissue and subsequently induce a periapical infection .[13]

4) Biomaterial-centered infection

Biomaterial centered infection is caused when bacteria adhere to an artificial biomaterial surface and form biofilm structures.[14] Presence of biomaterials in close proximity to the host immune system can increase the susceptibility to biofilm. In endodontics, biomaterial-centered biofilms form on root canal obturating materials. These biofilms can be intraradicular or extraradicular depending on whether the obturating material is within the root canal or has extruded beyond the root apex like in (figure 5).

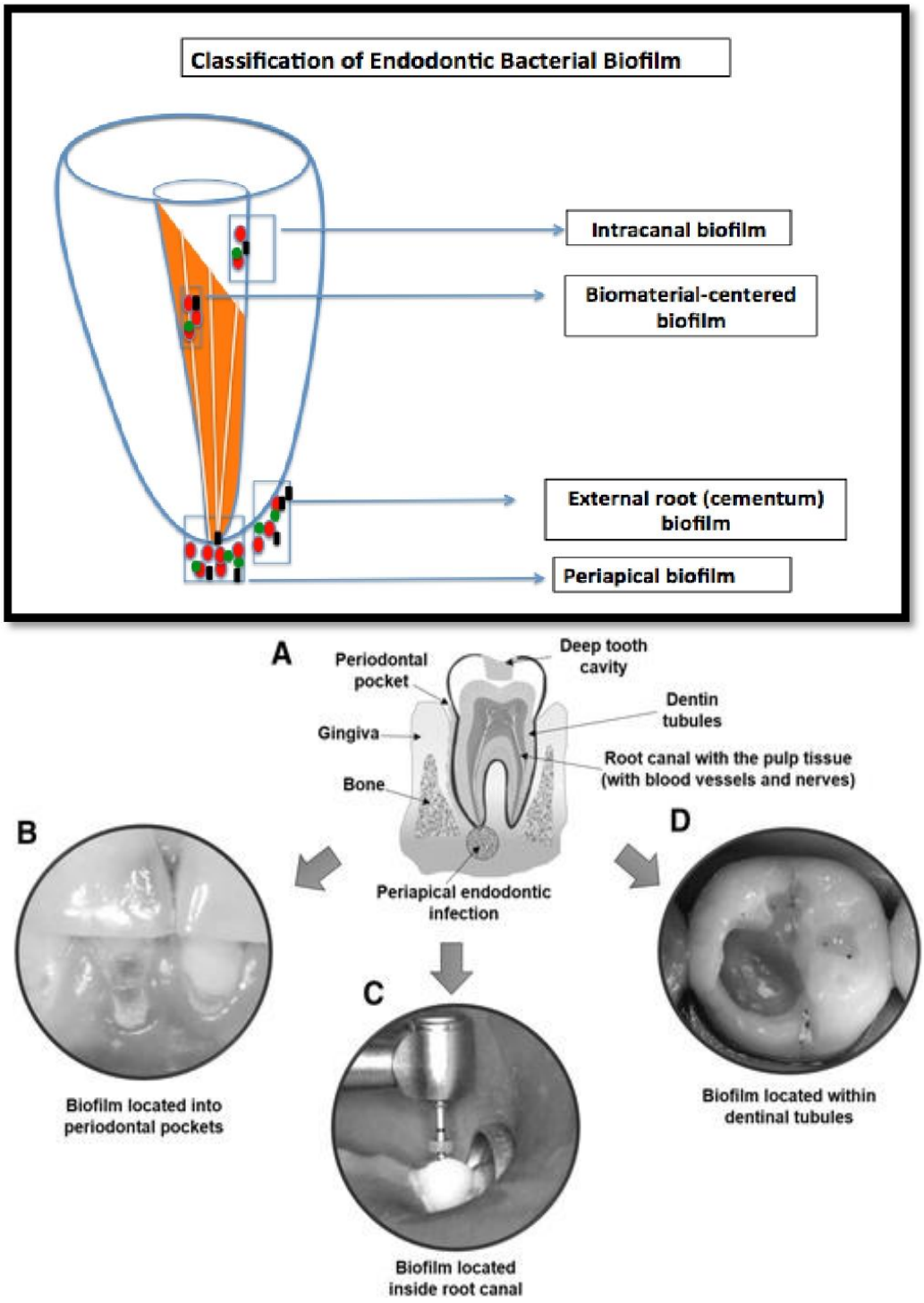


Figure 5. Biofilm in different location within the tooth.

Physico-chemical properties of biofilm

Microorganisms in aggregates such as flocs, film and sludge do not only display mechanical biochemical and biological, but also physical and physicochemical properties. Among these are:

- stability.
- binding of water.
- diffusion.
- Sorption.
- mass transport.
- and optical properties,
- and friction resistance.

These properties are chiefly caused by the extracellular polymeric substances (EPS) which fill the space between the cells and account for a considerable proportion of the organic carbon content of biofilms.

The EPS consist not only of polysaccharides but also of considerable amounts of protein; nucleic acids and lipids are also found in the EPS. Above all, the EPS form the morphology and internal structure of biofilms, including surface pores and channels. The EPS provide a matrix which allows the cells to maintain their position for a much longer period of time compared to the planktonic mode.

This facilitates the formation of synergistic microconsortia of different species which can perform orchestrated degradation processes.

Mechanical stability of biofilms includes aspects such as sloughing of the biomass in biofilm reactors, resulting in possibly adverse effects to the process. On the other hand, when biofilms have to be removed as biofouling layers, it is the cohesive and adhesive forces which have to be overcome.

Three types of weak interactions have to be considered hydrogen bonds, electrostatic interactions and van der Waals interactions.[15]

Current therapeutic options against endodontic biofilm

Effects of various irrigating systems

One role of root canal irrigation is to help in the killing of bacteria and the removal of the bacterial biofilm from uninstrumented surfaces (30–50% of the root canal wall).[16] Antimicrobial irrigating solutions and other locally used disinfecting agents and medicaments play a key role in the eradication of microbes like show in (figure 5). An ideal root canal irrigant should have high efficacy against microorganisms in biofilms while being systemically non-toxic and non-caustic to periodontal tissues.[17-18]

There are many type of irrigation material like show in figure 6

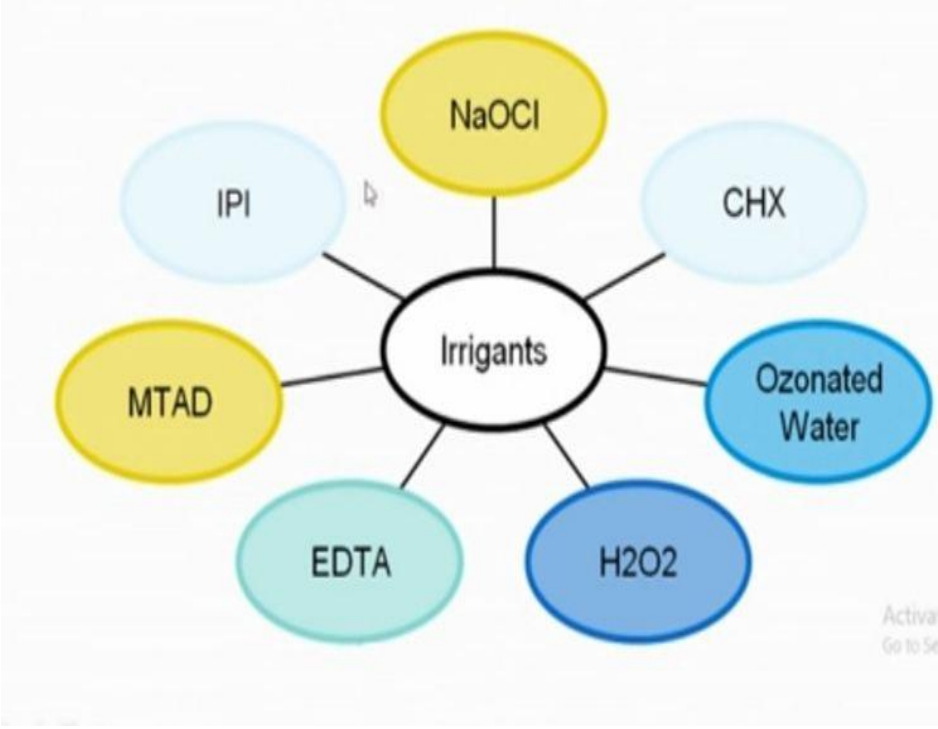


Figure 6: type of irrigation material

Although current irrigation regimens using **sodium hypochlorite (NaOCl)** exhibit excellent antimicrobial activity, caustic and toxic effects to vital tissues are often noted. There is a need for agents that are both antibacterial and exert minimal tissue irrigating effect

(NaOCl) is a frequently used irrigating solution in endodontics because of its ability to dissolve necrotic tissue as well as its potent antimicrobial action.[36] However, it has not been reported to have any residual antimicrobial activity.[37] Other irrigating solutions such as chlorhexidine (CHX) and cetrimide (CTR) are less effective than (NaOCl) in eradicating E. faecalis biofilm, but CHX has substantive properties and is able to inhibit adherence of certain bacteria to dentin.[38] like show in figure 7

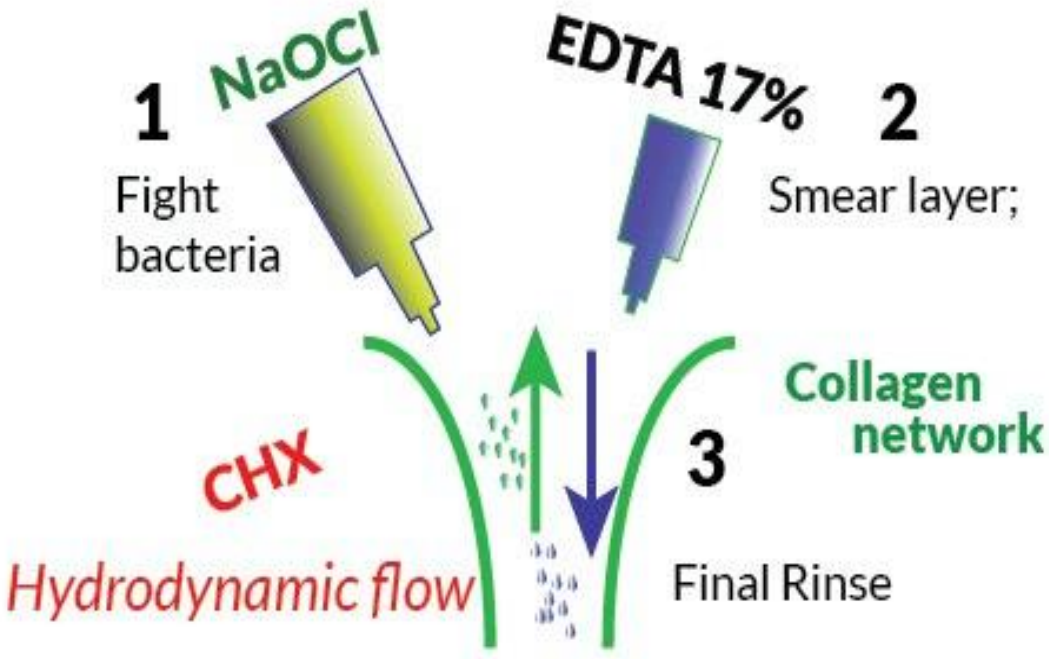


Figure 7 : NaOCl act as irrigation solution

Chelating agents are used to remove the smear layer produced during mechanical instrumentation. Although ethylenediaminetetraacetic acid (EDTA) is one of the most commonly used agents, its antimicrobial activity against biofilms is a matter of some controversy.[39-40] Maleic acid (MA) a mild organic acid, has been more

recently proposed for use as a final irrigating solution, as an alternative to EDTA,[41] because of better smear layer removal from the apical third of the root canal system by MA [42] and its lower toxicity. Furthermore, its antibacterial activity has been shown in vitro against E.faecalis biofilm.[43] Different protocols and/or combinations of irrigating solutions are used in the final irrigation of the root canals, but their residual activity known is not well.

Effects of instrumentation on biofilms

Microorganisms that play an important role in periradicular diseases grow mostly in sessile biofilms, aggregates, and co-aggregates.[44-45-46] By mechanical instrumentation and irrigation with tissue-lytic and microbial solutions and antimicrobial medicaments in the root canal, the microbial load is reduced leading to disruption of biofilm.[47]

Previous studies have shown that instrumentation and antibacterial irrigation with NaOCl would eliminate bacteria in 50–75% of the infected root canals at the end of the first treatment session, whereas the remaining root canals contain recoverable bacteria.[48-49-50] In their study, Nair *et al*. showed that 88% of root canal–treated mandibular molars showed residual infection of mesial roots after instrumentation, irrigation with NaOCl, and obturation in a one-visit treatment. BioPure MTAD has been described as a universal irrigating solution.[51] Torabinejad [52]have shown that MTAD removes the smear layer safely; also, it is effective against E. faecalis and it can eliminate bacteria in human root canals that had been infected by whole saliva.[53] A new irrigant, Tetraclean, which is mixture of doxycycline hyclate present at a lower concentration than MTAD, an acid, and detergents, has the ability to eliminate microorganisms and smear layer in dentinal tubules of infected root canals with a final 4-min rinse.[54] Consequently, recent laboratory studies have

focused on evaluating the effectiveness of root canal irrigants and medicaments against E. faecalis. Many of these studies have grown the bacterial strains as planktonic cultures (bacteria in suspension). However, planktonic bacteria do not usually comply with the in vivo growth conditions found in an infected tooth, in which bacteria grow as a biofilm on the dentinal wall. Therefore, all studies about the clinical action of endodontic irrigants should be conducted with bacteria in "biofilm form." Up to now.

Systimc use of antibiotic in treatment endodontic infections:

In addition to normal endodontic procedures, adjunctive strategies may be needed. Antibiotics are unnecessary in irreversible pulpitis, necrotic pulps, and localized acute apical abscesses [55], but they are necessary if there is abscess, antibiotics are useful adjuncts in specific cases as they assist in the prevention of the spread of infection. Clearly, the clinician must identify these specific cases correctly and caution must be exercised both during the prescription of specific antibiotics and the duration of administration.

Conclusion

The most common endodontic infection is caused by the surface-associated growth of microorganisms(biofilms).It is important to apply the biofilm concept to endodontic microbiology to understand the pathogenic potential of the root canal microbiota as well as to form the basis for new approaches for disinfection. It is foremost to understand that how the biofilm formed by root canal bacteria resists endodontic treatment measure. Bacterial etiology has been confirmed for common oral diseases such as caries and periodontal and endodontic infections. Bacteria causing these diseases are organized in biofilm structures, which are complex microbial communities composed of a great variety of bacteria with different ecological requirements and pathogenic potential. The biofilm community not only gives bacteria effective protection against the host's defense system but also makes them more resistant to a variety of disinfecting agents used as oral hygiene products or in the treatment of infections. Successful treatment of these diseases depends on biofilm removal as well as effective killing of biofilm bacteria. So, the fundamental to maintain oral health and prevent dental caries, gingivitis, and periodontitis is to control the oral biofilms. From these aspects, the formation of biofilms carries particular clinical significance because not only host defense mechanisms but also therapeutic efforts including chemical and mechanical antimicrobial treatment measures have the most difficult task of dealing with organisms that are gathered in a biofilm.

Appendix

Table 1 - Antibiotics prescribed in endodontics

ANTIBIOTIC	LOADING DOSE	MAINTENANCE DOSE
Amoxicillina with or without clavulanic acid	1000mg	500mg q8 h or 875mg q12 h
Clindamycin	600mg	300mg q6 h
Clarithromycin	500mg	250mg q12 h
Azithromycin	500mg	250mg q24 h
Metronidazole	1000mg	500mg q6 h

Pulp/Periapical condition	Clinical and radiographic data	Antibiotics as adjunct
Symptomatic irreversible pulpitis	• Pain • No others symptoms and signs of infection	NO
Pulp necrosis	• Nonvital teeth • Widening of periodontal space	NO
Acute apical periodontitis	• Pain • Pain to percussion and biting • Widening of periodontal space	NO
Chronic apical abscess	• Teeth with sinus tract • Periapical radiolucency	NO
Acute apical abscess with no systemic involvement	• Localized fluctuant swellings	NO
Acute apical abscess in medically compromised patients	• Localized fluctuant swellings • Patient with systemic disease causing impaired immunologic function	YES
Acute apical abscess with systemic involvement	• Localized fluctuant swellings • Elevated body temperature (>38 °C) • Malaise • Lymphadenopathy • Trismus	YES
Progressive infections	• Rapid onset of severe infection (less than 24 h) • Cellulitis or a spreading infection • Osteomyelitis	YES
Persistent infections	• Chronic exudation, which is not resolved by regular intracanal procedures and medications	YES

Table2. the indication of antibiotics in endodontic.

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