Review Article

# Biofilm in endodontics: A review

**Kapil Jhajharia, Abhishek Parolia[1], Vikram Shetty K, Lata Kiran Mehta[2]**

Department of Conservative Dentistry and Endodontics, Faculty of Dentistry, Melaka Manipal Medical College, Melaka, [1]Department of Restorative Dentistry, Faculty of Dentistry, International Medical University, Kuala Lumpur, Malaysia, [2]Department of Pedodontics and Preventive Dentistry, P. D. M. Dental College and Research Institute, Jhajjar, Haryana, India

**Corresponding author** (email: <kapilbds@gmail.com>)
Dr. Kapil Jhajharia, Assistant Professor, Department of Conservative Dentistry and Endodontics, Faculty of Dentistry, Melaka Manipal Medical College, Melaka, Malaysia.

## Abstract

Endodontic disease is a 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. It is foremost to understand how the biofilm formed by root canal bacteria resists endodontic treatment measures. 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. The aim of this article was to review the mechanisms of biofilms' formation, their roles in pulpal and periapical pathosis, the different types of biofilms, the factors influencing biofilm formation, the mechanisms of their antimicrobial resistance, techniques to identify biofilms.

**Key words:** *Biofilms, intracanal medicaments, periapical infections, pulpal infections, root canal irrigation*

## INTRODUCTION

The most common etiology for the pulpal and periradicular pathologies is the microorganisms or the microflora. Infection in the oral cavity is caused by a number of organisms from different species found in the human mouth.[1] These oral bacteria have the capacity to form biofilms on distinct surfaces ranging from hard to soft tissues. So, the fundamental to maintain oral health and prevent dental caries, gingivitis, and periodontitis is to control the oral biofilms.[2-4]

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Biofilm mode of growth is advantageous for microorganisms, as they form three-dimensional structured communities with fluid channels for transport of substrate, waste products, and signal molecules.[5] Biofilm formation in root canals is probably initiated sometime after the first invasion of the pulp chamber by planktonic oral microorganisms after some tissue breakdown, as hypothesized by Svensäter and Bergenholtz.[6]

Costerton *et al*.[7] stated that biofilm consists of single cells and microcolonies, all embedded in a highly hydrated, predominantly anionic exopolymer matrix.

Bacteria can form biofilms on any surface that has nutrient-containing fluid. biofilm formation mainly involves the three major components: Bacterial cells, a solid surface, and a fluid medium.[7]

## HISTORY OF BIOFILM

Rediscovery of a microbiological phenomenon, first described by van Leeuwenhoek, that microorganisms attach to and grow universally on exposed surfaces led to studies which revealed that surface-associated microorganisms (biofilms) exhibited a distinct phenotype with respect to gene transcription and growth rate. These microorganisms involved in biofilm elicit specific mechanisms for initial attachment to a surface, development of a community structure and ecosystem, and detachment.[8]

In 1894, Miller published his findings on the bacteriological investigation of pulps.[9] He observed many different microorganisms in the infected pulp space and realized that some were uncultivable when compared with the full range observed by microscopy, and that the flora was different in the coronal, middle, and apical parts of the canal system.[10]

Kakehashi *et al*. exposed the dental pulps of conventional and germ-free rats to the oral cavity and reported that only conventional rats with an oral microbiota showed pulp necrosis and periradicular lesions.[11]

Bacteria may sometimes be unaffected by endodontic disinfection procedures in areas such as isthmuses, ramifications, deltas, irregularities, and dentinal tubules.[12]

## DEFINITION OF BIOFILM

Biofilm is embedded in a self-made matrix of extracellular polymeric substances (EPS) and is a mode of microbial growth where dynamic communities of interacting sessile cells are irreversibly attached to a solid substratum, as well as to each other.[13]

## BASIC CRITERIA FOR A BIOFILM

Caldwell *et al*.[14] highlighted four characteristics of biofilm as follows:

- Autopoiesis – Must possess the ability to self-organize
- Homeostasis – Should resist environmental perturbations
- Synergy – Must be more effective in association than in isolation
- Communality – Should respond to environmental changes as a unit rather than as single individuals.

The typical example of a biofilm is dental plaque.[14]

## COMPOSITION OF BIOFILM

A fully developed biofilm is described as a heterogeneous arrangement of microbial cells on a solid surface. The basic structural unit, microcolonies or cell clusters, is formed by the surface-adherent bacterial cells.[15] It is composed of matrix material consisting of proteins, polysaccharides, nucleic acids, and salt, which makes up 85% by volume, while 15% is made up of cells.[16,17]

As biofilm get matured, its structure and composition are modified according to the environmental conditions (growth conditions, nature of fluid movements, physicochemical properties of the substrate, nutritional availability, etc.)[18] The water channels are regarded as a primitive circulatory system in a biofilm.

These microcolonies have a tendency to detach from the biofilm community and have the highest impact in chronic bacterial infection.

During the process of detachment, the biofilm transfers cells, polymers, and precipitates from the biofilm to the fluid bathing the biofilm, which is important in shaping the morphological characteristics and structure of mature biofilm.[19] It is also considered as an active dispersive mechanism (seeding dispersal).[20]

Biofilm-mediated mineralization occurs when the metal ions including Ca2+, Mg2+, and Fe3+ readily bind and precipitate within an ionic biofilm under a favorable environment.[21]

## CHARACTERSTICS OF BIOFILM

The organic substances surround the microorganisms of a biofilm and contain primarily carbohydrates, proteins, and lipids. Among the inorganic elements in biofilms are calcium, phosphorous, magnesium, and fluoride.[22]

Bacteria in a biofilm have the ability to survive tough growth and environmental conditions. This unique capacity of bacteria in a biofilm state is due to the following features:

- Residing bacteria are protected from environmental threats; trapping of nutrients and metabolic cooperation between resident cells of the same species and/or different species is allowed by the biofilm structure

- It also exhibits organized internal compartmentalization which helps the bacterial species in each compartment with different growth requirements
- By communicating and exchanging genetic materials, these bacterial cells in a biofilm community may acquire new traits.[23]

Bacterial biofilm provides a setting for the residing bacterial cells to communicate with each other. Some of these signals, produced by the cells, may be interpreted not just by members of the same species but also by other microbial species.

Quorum sensing is process by which communications between these bacterial cells is established through signaling molecules in a biofilm.[24,25]

## FACTORS AFFECTING FORMATION OF BIOFILM

### Development of biofilm

The three components involved in biofilm formation are: Bacterial cells, a fluid medium, and a solid surface.

### Stages

Stage 1 (formation of conditioning layer): Adsorption of inorganic and organic molecules to the solid surface, creating what is termed a conditioning layer

During dental plaque formation, the tooth surface is conditioned by the saliva pellicle.

Stage 2 (planktonic bacterial cell attachment): Adhesion of microbial cells to this layer.[26]

#### *Stages of biofilm formation*

*Phase 1 (transport of microbe to the substrate surface):* The nature of initial bacteria–substrate interaction is determined by physicochemical properties such as surface energy and charge density. The bacteria adhere to a substrate by bacterial surface structures such as fimbriae, pili, flagella, and EPS (g1ycocalyx). Bridges are formed between the bacteria and the conditioning film by these bacterial structures.[26]

*Phase 2 (initial non-specific microbial–substrate adherence phase):* Molecular-specific interactions between bacterial surface structures and substrate become active. These bridges are a combination of electrostatic attraction, covalent and hydrogen bonding, dipole interaction, and hydrophobic interaction.

*Porphyromonas gingivalis, Streptococcus mitis, Streptococcus salivarius, Prevotella intermedia, Prevotella nigrescens, Streptococcus mutans, and Actinomyces naeslundii* are some of the oral bacteria possessing surface structures.[27,28]

*Phase 3 (specific microbial–substrate adherence phase):* With the help of polysaccharide adhesin or ligand formation which binds to receptors on the substrate, specific bacterial adhesion with a substrate is produced.[29,30]

Stage 3 (bacterial growth and biofilm expansion).

Microcolony is formed by the monolayer of microbes which attracts secondary colonizers, and gives rise to the final structure of biofilm.[31] This metabolically active community of microorganisms is a mature biofilm where individuals share duties and benefits.[32]

Two types of microbial interactions occur at the cellular level during the formation of biofilm:

- Co-adhesion
- Co-aggregation.[33,34]

## BIOFILM MODELS AND BIOFILM ASSESSMENT METHODS

The number and type of microorganisms, vitality (dead/living cells) of the resident microbial population, age, thickness (monolayered or multilayered),) structure (homogeneous, irregular, dense, porous), and surface topography (peaks and valleys) of biofilms can be characterized by biofilm assay which involves different techniques such as colorimetric techniques, microscopic techniques, microbiological culture techniques, physical methods, biochemical methods, and molecular methods.[35]

## MISCELLANEOUS ADVANCED TECHNIQUES

Recently, the forces of interaction among bacterial cells and between bacterial cells and substrates has been studied by atomic force microscopy (AFM).[36,37] The technique is also used to measure the interaction forces between bacteria and substrates.[38]

Using this concept, the effects of endodontic irrigants on the adherence of *Enterococcus faecalis* to dentin have been studied and it was found that chemicals which altered the physicochemical properties of dentin might influence the nature of bacterial adherence and adhesion forces to dentin that are the factors in biofilm formation. Recently, micromanipulators have been used to sample individual cells or biofilm compartments. Laser-based optical tweezers are

noninvasive and non-contact tools that can probe the interaction between microscopic objects such as bacteria and collagen. They give more information about the forces of interaction between bacteria and substrate quantitatively.[39]

Fourier transform infrared (FTIR) spectroscopy is used to characterize the chemical composition of mature biofilm structures qualitatively and quantitatively.[40] Similarly, solid-state nuclear magnetic resonance (NMR) is a powerful analytical tool to study the constituents of bacterial biofilm, as well as to obtain metabolic information in planktonic cells, adherent bacterial cells, and *in situ* biofilm bacteria. These are noninvasive biophysical techniques.[41,42] Recent advances in micromanipulator-assisted analysis, green fluorescent protein (GFP) tagging, confocal laser scanning microscopy (CLSM), flow cytometry, and fluorescence *in situ* hybridization (FISH) have made biofilm characterization very comprehensive.

## BIOFILMS IN DENTISTRY

Formation of oral biofilm involves three basic steps: Pellicle formation, bacterial colonization, and biofilm maturation. The organic substance surrounds the microorganisms of the biofilm and contains primarily carbohydrates, proteins, and lipids.[43,44]

The inorganic elements found in a biofilm are calcium, phosphorus, magnesium, and fluoride. The concentrations of these inorganic elements are higher in biofilm than in saliva.[45]

Salivary micelle-like globules (SMGs) from saliva get adsorbed to the clean tooth surface to form acquired enamel pellicle, which acts as a "foundation" for the future multilayered biofilm.[46]

Presence of calcium facilitates the formation of larger globules by bridging the negative charges on the subunits.[47]

The initial attachment of bacteria to the pellicle is by selective adherence of specific bacteria from the oral environment. Innate characteristics of the bacteria and the pellicle determine the adhesive interactions that cause a specific organism to adhere to the pellicle. Dental biofilm consists of a complex mixture of microorganisms that occur primarily as microcolonies. The population density is very high and increases as biofilm ages. The acquired pellicle attracts gram-positive cocci such as *Str. mutans* and *Streptococcus sanguis*, which are the pioneer organisms in plaque formation. Subsequently, filamentous bacterium such as *Fusobacterium nucleatum* and slender rods adhere to primary colonizers. Gradually, the filamentous form grows into the cocci layer and replaces many of the cocci. Vibrios and spirochetes appear as the biofilm thickens. More and more gram-negative and anaerobic organisms emerge as the biofilm matures. Interestingly, it is not only the surface of tooth that can be attached by bacterial cells. The surface of some bacteria (bacilli and spirochetes) also can serve as attachment sites for certain smaller coccoids. This co-aggregation of *F. nucleatum* with coccoid bacteria gives rise to "corn-cob" structure, which is unique in plaque biofilms.[48]

The presence of these bacteria makes it possible for other non-aggregating bacteria to coexist in the biofilm, by acting as co-aggregating bridges.[49]

Calcified dental biofilm is termed as calculus. It is formed by the precipitation of calcium phosphates within the organic plaque matrix, which depends on plaque pH, local saturation of calcium and phosphate, and availability of fluoride ions and biological factors such as crystallization nucleators/inhibitors from either bacteria or oral fluids.[50-52]

## ENDODONTIC BIOFILMS

### Biofilm classification

Endodontic bacterial biofilms are classified as:

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

The characteristic features in cell–cell and microbe–substrate interactions were explained based on the phenomena of microbial adherence.[53-55]

Studies have established 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). However, the physicochemical properties of *E. faecalis* biofilms were found to modify according to the prevailing environmental and nutrient conditions. *E. faecalis* under nutrient rich environment produces typical biofilm structures with characteristic surface aggregates of bacterial cells and water channels. Viable bacterial cells were present on the surface of the biofilm. Under nutrient-deprived environment (aerobic and anaerobic), irregular growth of adherent cell clumps were observed. Laser scanning confocal microscopy displayed many dead bacterial cells and pockets of viable bacterial cells in this biofilm structure.

The development of *E. faecalis* biofilm on the root canal dentin involves three stages as follows:

Stage 1: Microcolonies are formed as *E. faecalis* cells adhere on the root canal dentin surface

Stage 2: Bacterial-mediated dissolution of the mineral fraction from the dentin substrate leads to localized increase in the calcium and phosphate ions causing mineralization (or calcification) of the *E. faecalis* biofilm

Stage 3: Due to this interaction of bacteria and their metabolic products on dentin, *E. faecalis* biofilm is mineralized.[56-58]

Recent investigations have shown that *E. faecalis* has the ability to co-aggregate with *F. nucleatum*. The co-aggregation interactions between *E. faecalis* and *F. nucleatum* suggested the ability of these microorganisms to coexist in a microbial community and contribute to endodontic infection. These apical biofilms cannot be removed by biomechanical preparation alone as they are inherently resistant to antimicrobial agents. Numerous studies have shown the presence of rods, cocci, bacilli, and spirochetes on the root surfaces in cases of refractory periodontitis.[59,60]

## MICROBIAL DIVERSITY IN ENDODONTIC BIOFILM

More than 1000 different bacterial species have been identified in the oral cavity by culture and independent molecular microbiology, but with advanced massively parallel DNA pyrosequencing techniques, the number may be higher.[61,62]

Specifically, the diversity of the endodontic microbiota has also been unraveled by numerous culture and molecular studies. Collectively, different forms of apical periodontitis and more than 400 different microbial species have been identified in endodontic samples from teeth. These taxa are usually found in combinations involving many species in primary infections and fewer ones in secondary/persistent infections.[63]

At high phylogenetic levels, endodontic bacteria fall into 15 phyla, with the most common representative species belonging to the phyla Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Spirochaetes, and Synergistes.[64-66]

In addition to bacteria, other microorganisms can be found in endodontic infections. Archaea and fungi have been only occasionally found in intraradicular infections,[67-69] though the latter can be more prevalent in treated teeth with post-treatment disease.[70]

## ENDODONTIC BIOFILM FORMATION MECHANISM

First, there is penetration of the organism in the pulp where it attaches and spreads further along the root canal. Possibly, it is after biofilm formation that the infectious process gains sufficient power to cause subsequent destruction of the pulpal tissue. At some point in the breakdown process, however, a steady state is reached where the bacterial mass is held up by host defense mechanisms. The demarcation zone may be inside the root canal near the root canal exit,[71] at the foramen, or, as demonstrated by scanning electron microscopy (SEM),[49,72-74] on the external root surface near the exit of the foramen to the periapical tissue environment. It is not unreasonable to assume that organisms may be detached from these positions and occasionally congregate in the lesion *per se*.[75,76]

Hence, the question remains as to whether bacterial condensations in a biofilm structure can develop or are retained in sites of the root canal system other than near the bacteria/inflammatory interface zone, where host-derived proteins and bacterially produced adhesive substances may provide the proper prerequisites.[77]

## BIOFILMS IN ENDODONTIC INFECTIONS

Endodontic microbiota transition is more conspicuous with the progression of infection. Nutritional and environmental status within the root canal changes as infection progresses. It creates more anaerobic environment and depletion of nutrition which offer a tough ecological niche for the surviving microorganisms. The anatomical and geometrical complexities (e.g. delta and isthmus) in the root canal systems shelter the adhering bacteria from cleaning and shaping procedures.

### Intracanal microbial biofilms

Intracanal biofilms are microbial biofilms formed on the root canal dentin of the infected tooth. Identification of biofilm was earlier reported by Nair 1987 under transmission electron microscopy.[77] Major bulk of the organisms existed as loose collections of filaments, spirochetes, cocci, and rods. Apart from these, bacterial

condensations were seen as a palisade structure similar to dental plaque seen on tooth surface.[49] The extracellular matrix material of bacterial origin was also found.

### Extraradicular biofilm

Extraradicular biofilms formed on the root surface adjacent to the root apex of endodontically infected teeth are root surface biofilms.[78] In a study of cases resisting treatment (refractory endodontic cases), Tronstad *et al*.[79] examined the root tips of surgically extracted teeth under SEM and found structureless smooth biofilm with bacteria of different species and varying degrees of extracellular matrix.

*F. nucleatum*, *Po. gingivalis*, and *Tannerella forsythensis* were found to be associated with extraradicular biofilm by using polymerase chain reaction (PCR)-based 16s rRNA gene assay.[80]

### Periapical biofilm

Periapical microbial biofilms in the periapical region of endodontically infected teeth are isolated biofilms which can be seen even in the absence of root canal infections. Periapical lesions which are associated with *Actinomyces* species and *Propionibacterium propionicum* can occur when the bacteria present in such biofilms overcome host defense mechanisms. The aggregation of *Actinomyces* cells is influenced by pH, ionic strength, and cell concentration which facilitates biofilm formation.[81,82]

### Foreign body–centered biofilm

Foreign body–centered biofilm is found when bacteria adhere to an artificial biomaterial surface and form biofilm structures.[83] It is also known as biomaterial-centered infection.

It is a major complication associated with prosthesis and also in implant-supported prosthesis. Biomaterial-centered infection reveals opportunistic invasion by nosocomial organisms. Takemura *et al*.[84] reported that gram-positive facultative anaerobes colonize and form extracellular polymeric matrix surrounding gutta-percha, and serum plays a significant role in biofilm formation. Studies have suggested that extraradicular microbial biofilm and biomaterial-centered biofilm are related to refractory periapical disease.

Biofilm can be identified by various methods such as environmental SEM, confocal microscopy, and using special fluorescent stains (FISH technique).

Bacterial adherence to a biomaterial surface is also described in three phases:[85]

(1) Phase 1: Transport of bacteria to biomaterial surface,
(2) Phase 2: Initial non-specific adhesion phase, and
(3) Phase 3: Specific adhesion phase.[85]

In endodontics, e.g. biofilm on root canal obturating materials can be intraradicular or extraradicular, which depends on whether the obturating material is within the root canal space or it has extruded beyond the root apex.

*E. faecalis*, *Str. sanguinis*, *Streptococcus intermedius*, *Streptococcus pyogenes*, *Staphylococcus aureus* form biofilm on GP points.

*F. nucleatum*, *Propionibacterium acnes*, *Po. gingivalis*, and *Pr. intermedia* do not form biofilm on Gutta-Percha(GP) points.

## ROLE OF *E. FEACALIS* IN BIOFILM

One clinically important property of endodontic microorganisms is their ability to form biofilms. In treated and untreated root canals, apical periodontitis can be classified as a biofilm-induced disease.[86,87]

To the best of our knowledge, 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.[88,89]

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. As summarized by Madson *et al*.,[90] 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.[91,92] The ability of *E. faecalis* to form biofilms is advantageous in certain situations. For example, clinical strains of *E. faecalis* isolated from infective endocarditis patients were significantly associated with greater biofilm formation than nonendocarditis clinical isolates.[93] This may be attributable in part to specific virulence traits such as gelatinase production and presence of the adherence determinant; this combination was shown to be associated with the formation of thicker biofilms.[94] These virulence traits and others have also been identified in the clinical isolates of *E. faecalis* from asymptomatic, persistent endodontic infections of the root canals and the oral cavity.[95-97] Its prevalence in such infections ranges from 24 to 77%. Factors which lead to a persistent periradicular infection after root canal treatment are intraradicular infection, extraradicular infection, foreign body reaction, and cysts containing cholesterol crystals. Major cause of failure is believed to be the survival of microorganisms in the apical portion of the root-filled tooth.

Enterococci are gram-positive cocci that can occur singly, in pairs, or as short chains. 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.[97]

*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.[97]

*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.

## CURRENT THREUPATIC OPTIONS FOR 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).[98] Antimicrobial irrigating solutions and other locally used disinfecting agents and medicaments play a key role in the eradication of microbes. An ideal root canal irrigant should have high efficacy against microorganisms in biofilms while being systemically non-toxic and non-caustic to periodontal tissues.[99,100] 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-irritating effects.

Plant-derived natural products represent a rich source of antimicrobial compounds, and some have been incorporated into oral hygiene products. However, their application in endodontics is less well documented.[101-103] Berberine (BBr) is an alkaloid present in a number of clinically important medicinal plants, including *Hydrastis canadensis* (goldenseal), Coptis chinensis (coptis or golden thread), and others.[103] It possesses a broad antimicrobial spectrum against bacteria, fungi, protozoans, virus, helminthes, and chlamydia.[104-106]

The toxicity and mutagenicity of BBr to human cells are relatively low.[107,108] The antimicrobial activity of BBr against oral pathogens has been already shown.[109-111] It reduced cell–surface hydrophobicity in *Str. mutans* and *F. nucleatum* and inhibited the growth of a multispecies biofilm of *Streptococcus gordonii/F. nucleatum/Actinobacillus actinomycetemcomitans*. BBr can also synergize with miconazole in inhibiting the growth and biofilm formation of *Candida albicans*.[112]

In case of persistent apical periodontitis, *E. faecalis* is a commonly isolated species[113-115] where its long-term survival in the root canal system is due to its ability to adhere to dentin and invade the dentinal tubules,[116,117] and to form communities organized in biofilms, which may contribute to bacterial resistance and persistence after intracanal antimicrobial procedures.[118] Nowadays, most studies focus on the antimicrobial properties of the irrigating solutions, involving both forms of bacterial growth, planktonic and biofilm.

However, some studies look into the residual antibacterial activity and its influence on microbial adhesion to the dentin surface.[119] This is a relevant aspect because microbial adherence to the dentin is the first step in colonization, including tubule invasion, and the origin of biofilm infections.

Few studies have evaluated the efficacy of endodontic irrigants against microorganisms grown as a biofilm.[120] NaOCl is a frequently used irrigating solution in endodontics because of its ability to dissolve necrotic tissue as well as its potent antimicrobial action.[121] However, it has not been reported to have any residual antimicrobial activity.[122] Other irrigating solutions such as chlorhexidine (CHX) and cetrimide (CTR) are less effective than NaOCl in eradicating *E. faecalis* biofilm,[121] but CHX has substantive properties and is able to inhibit adherence of certain bacteria to dentin.[123]

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.[124,125] Maleic acid (MA), a mild organic acid, has been more recently proposed for use as a final irrigating solution, as an alternative to EDTA,[126] because of better smear layer removal from the apical third of the root canal system by MA[127] and its lower toxicity. Furthermore, its antibacterial activity has been shown *in vitro* against *E. faecalis* biofilm.[128] Different protocols and/or combinations of irrigating solutions are used in the final irrigation of the root canals, but their residual activity is not well known.

## EFFECTS OF INSTRUMENTATION ON BIOFILMS

Microorganisms that play an important role in periradicular diseases grow mostly in sessile biofilms, aggregates, and co-aggregates.[11,129,130] By mechanical instrumentation and irrigation with tissue-lytic and microbicidal solutions and antimicrobial medicaments in the root canal, the microbial load is reduced leading to disruption of biofilm.[131] NaOCl in different concentrations is used as a root canal irrigant because of its antimicrobial action and tissue-dissolving property.[132]

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.[133-135] 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 (Dentsply Tulsa Dental, Johnson City, TN, USA) has been described as a universal irrigating solution.[136] Torabinejad *et al*.[137] 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.[138] 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.[139] 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, however, very few studies have been published about the action of antimicrobial irrigants against biofilm. As a result, recent laboratory studies have attempted to evaluate the efficacy of antimicrobial agents used in root canal treatment against *E. faecalis* grown as a biofilm.[140]

## ERADICATION OF BIOFILM

### Effects of different irrigating systems on biofilms

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 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.[141]

Successful treatment of these diseases depends on biofilm removal as well as effective killing of biofilm bacteria. Because bacteria causing endodontic infections are mostly found in the main root canal, chemo-mechanical debridement plays a key role in treating endodontic

infections. However, because of the complex root canal anatomy, about 35% of the instrumented root canal area is left untouched when conventional rotary and hand instruments are used.[142] Therefore, elimination and killing of biofilm bacteria from the root canals rely to a great extent on the efficacy of endodontic irrigants.

Although bacteria rarely exist in nature in planktonic phase, most of the studies of endodontic disinfecting agents have been based on bacteria in planktonic culture.[143] However, it has been recognized that rapid killing of planktonic bacteria by various disinfecting agents does not reflect well the effect of the same agent on bacteria in *in vivo* biofilms. It has been demonstrated that biofilm bacteria can be 100–1000 times more resistant to antibacterial agents than their planktonic counterparts.[144] Because of this great difference, a growing number of studies are now focusing on the killing of biofilm bacteria instead of planktonic bacteria by the disinfecting agents.

Endodontic disease is a biofilm-mediated infection, and the primary aim in the management of endodontic disease is the elimination of bacterial biofilm from the root canal system. As eliminating surface-adherent biofilm bacteria is a challenge, different antimicrobials (ranging from antimicrobial irrigants to advanced and microbial methods such as lasers, photoactivated disinfection, and nanoparticles) are employed in the management of infected root canal systems. Many of these advanced antimicrobial strategies show tremendous inhibitory effects on most types of microbial biofilm *in vitro*.[145]

## CONCLUSIONS

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. It is foremost to understand that how the biofilm formed by root canal bacteria resists endodontic treatment measures.

## REFERENCES


1. Loesche WJ. Chemotherapy of dental plaque infections. Oral Sci Rev 1976;9:65-107.
2. Bowden GH. Microbiology of root surface caries in humans. J Dent Res 1990;69:1205-10.
3. Marsh PD. Microbial community aspects of dental plaque. In: Newman HN, Wilson M, editors. Dental Plaque Revisited. Cardiff, UK: BioLine; 1999. p. 237-53.
4. Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiology 2003;149:279-94.
5. Usha HL, Kaiwar A, Mehta D. Biofilm in endodontics: New understanding to an old problem. Int J Contem Dent 2010;1:44-51.
6. Svensäter G, Bergenholtz G. Biofilms in endodontic infections. Endod Topics 2004;9:27-36.
7. Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, *et al.* Bacterial biofilms in nature and disease. Annu Rev Microbiol 1987;41:435-64.
8. Donlan RM. Biofilms: Microbial life on surfaces. Emerg Infect Dis 2002;8:881-90.
9. Miller WD. An introduction to the study of the bacterio-pathology of the dental pulp. Dent Cosmos 1894;36:505-27.
10. Fabricius L, Dahlén G, Öhman AE, Möller AJ. Predominant indigenous oral bacteria isolated from infected root canals after varied times of closure. Scand J Dent Res 1982;90:134-44.
11. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340-9.
12. Siqueira JF Jr. Aetiology of root canal treatment failure: Why well-treated teeth can fail. Int Endod J 2001;34:1-10.
13. Costerton JW, Lewandowski Z, DeBeer D, Caldwell D, Korber D, James G. Biofilms, the customized microniche. J Bacteriol 1994;176:2137-42.
14. Caldwell DE, Atuku E, Wilkie DC, Wivcharuk KP, Karthikeyan S, Korber DR, *et al.* Germ theory vs. community theory in understanding and controlling the proliferation of biofilms. Adv Dent Res 1997;11:4-13.
15. Wingender J, Neu TR, Flemming HC. What are bacterial extracellular polymeric substances? In: Wingender J, Neu TR, Flemming HC, editors. Microbial Extracellular Polymeric Substances: Characterization, Structure and Function. Springer Verlag: Springer-Verlag; 1999. p. 1-19.
16. Nivens DE, Ohman DE, Williams J, Franklin MJ. Role of alginate and its O acetylation in formation of Pseudomonas aeruginosa microcolonies and biofilms. J Bacteriol 2001;183:1047-57.
17. Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS. Extracellular DNA required for bacterial biofilm formation. Science 2002;295:1487.
18. Donlan RM, Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15:167-93.
19. Stewart PS, Peyton BM, DruryWJ, Murga R. Quantitative observations of heterogeneities in Pseudomonas aeruginosa biofilms. Appl Environ Microbiol 1993;59:327-9.
20. de beer D, Stoodley P, Roe F, Lewandowski Z. Effects of biofilm structures on oxygen distribution and mass transport. Biotechnol Bioeng 1994;43:1131-8.
21. McLean RJ, Fuqua C, Siegele DA, Kirkland BL, Adams JL, Whiteley M. Biofilm growth and an illustration of its role in mineral formation. In: Bell C, Brylinsky M, Johnson-Green PC, Atlantic Canada Society for Microbial Ecology, editors. Microbial Biosystems: New Frontiers: Proceedings of the 8th International Symposium on Microbial Ecology. Halifax, Canada: Atlantic Canada Society for Microbial Ecology; 2000. p. 255-61.
22. Tronstad L, Sunde PT. The evolving new understanding of endodontic infections. Endo Topics 2003;6:57-77.
23. Lewis K. Riddle of biofilm resistance. Antimicrob Agents Chemother 2001;45:999-1007.
24. Spratt D, Pratten J. Biofilms and the oral cavity. Rev Environ Sci Bio Technol 2003;109-20.

25. Stoodley P, Sauer K, Davies DG, Costerton JW. Biofilms as complex differentiated communities. Annu Rev Microbiol 2002;56:187-209.
26. Grenier D, Mayrand D. Nutritional relationships between oral bacteria. Infect Immun 1986;53:616-20.
27. Al-Hashimi I, Levine MJ. Characterization of in vivo saliva-derived enamel pellicle. Arch Oral Biol 1989;34:289-95.
28. Handley PS, Carter PL, Fielding J. Streptococcus salivarius strains carry either fibrils or fimbriae on the cell surface. J Bacteriol 1984;157:64-72.
29. Handley PS, Carter PL, Wyett JE, Hesketh LM. Surface structures (peritrichous fibrils and tufts of fibrils) found on Streptococcus sanguis strains may be related to their ability to coaggregate with other oral genera. Infect Immun 1985;47:217-27.
30. Miron J, Ben-Ghedalia D, Morrison M. Invited review: Adhesion mechanisms of rumen cellulolytic bacteria. J Dairy Sci 2001;84:1294-309.
31. Cowan MM, Taylor KG, Doyle RJ. Energetics of the initial phase of adhesion of Streptococcus sanguis to hydroxyapatite. J Bacteriol 1987;169:2995-3000.
32. Bowden GH, Hamilton IR. Survival of oral bacteria. Crit Rev Oral Biol Med 1998;9:54-85.
33. Busscher HJ, van der Mei HC. Physico-chemical interactions in initial microbial adhesion and relevance for biofilm formation. Adv Dent Res 1997;11:24-32.
34. Rickard AH, Gilbert P, High NJ, Kolenbrander PE, Handley PS. Bacterial coaggregation: An integral process in the development of multi-species biofilms. Trends Microbiol 2003;11:94-100.
35. Kishen A, Haapasalo M. Biofilm models and methods of biofilm assessment. Endod Topics 2010;22:58-78.
36. Postollec F, Norde W, de Vries J, Busscher HJ, van der Mei HC. Interactive forces between co-aggregating and non-co-aggregating oral bacterial pairs. J Dent Res 2006;85:231-4.
37. Razatos A, Ong YL, Sharma MM, Georgiou G. Molecular determinants of bacterial adhesion monitored by atomic force microscopy. Proc Natl Acad Sci U S A 1998;95:11059-64.
38. Vadillo-Rodríguez V, Busscher HJ, Norde W, De Vries J, Dijkstra RJ, Stokroos I, *et al*. Comparison of atomic force microscopy interaction forces between bacteria and silicon nitride substrata for three commonly used immobilization methods. Appl Environ Microbiol 2004;70:5441-6.
39. Sum C, Mohanty S, Gupta PK, Kishen A. Influence of endodontic chemical treatment on Enterococcus faecalis adherence to collagen studied with laser scanning confocal microscopy and optical tweezers: A preliminary study. J Biomed Opt 2008;13:044017.
40. Kishen A, George S, Kumar R. Enterococcus faecalis-mediated biomineralized biofilm formation on root canal dentine in vitro. J Biomed Mater Res A 2006;77:406-15.
41. Majors PD, McLean JS, Pinchuk GE, Fredrickson JK, Gorby YA, Minard KR, *et al*. NMR methods for in situ biofilm metabolism studies. J Microbiol Methods 2005;62:337-44.
42. Grivet JP, Delort AM, Portais JC. NMR and microbiology: From physiology to metabolomics. Biochimie 2003;85:823-40.
43. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in medical intensive care units in the United States. National Nosocomial Infections Surveillance System. Crit Care Med 1999;27:887-92.
44. Katsikogianni M, Missirlis YF. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur Cell Mater 2004;8:37-57.
45. Marsh PD. Dental plaque as a microbial biofilm. Caries Res 2004;38:204-11.
46. Wilkins EM. Clinical Practice of the Dental Hygienist. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 289-303.
47. Rolla G, Rykke M. Evidence for presence of micelle-like protein globules in human saliva. Colloids Surf B Biointerfaces 1994;3:177-82.
48. Rolla G, Waaler SM, Kjaerheim V. Concepts in Dental Plaque Formation. In: Busscher HJ, Evans LV, editors. Oral Biofilms and Plaque Control: London, UK: Taylor and Francis; 1999.
49. Listgarten MA. Formation of dental plaque and other biofilms. In: Newman HN, Wilson M, editors. Dental Plaque Revisited: Oral Biofilms in Health and Disease. Cardiff, Wales: Bioline; 1999. p. 187-210.
50. Barone JP, Nancollas GH. The seeded growth of calcium phosphates. The kinetics of growth of dicalcium phosphate dihydrate on enamel, dentin, and calculus. J Dent Res 1978;57:153-61.
51. Lewis K. Riddle of biofilm resistance. Antimicrob Agents Chemother 2001;45:999-1007.
52. Gron P, Van Campen GJ, Lindstrom I. Human dental calculus. Inorganic chemical and crystallographic composition. Arch Oral Biol 1967;12:829-37.
53. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Science 1999;284:1318-22.
54. Busscher HJ, van der Mei HC. Initial microbial adhesion events: Mechanisms and implications. In: Allison DG, Gilbert P, Lappin-Scott HM, Wilson M, editors. Community Structure and Co-operation in Biofilms. Cambridge, UK: Cambridge University Press; 1998.
55. Marsh PD, Bowden GH. Microbial community interaction in biofilms. In: Allison DG, Gilbert P, Lappin-Scott HM, Wilson M, editors. Community Structure and Co-Operation in Biofilms. Cambridge: Cambridge University Press; 2000. p. 167-98.
56. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod 2002;28:689-93.
57. Hubble TS, Hatton JF, Nallapareddy SR, Murray BE, Gillespie MJ. Influence of Enterococcus faecalis proteases and the collagen-binding protein, Ace, on adhesion to dentin. Oral Microbiol Immunol 2003;18:121-6.
58. 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.
59. Lomçali G, Sen BH, Cankaya H. Scanning electron microscopic observations of apical root surfaces of teeth with apical periodontitis. Endod Dent Traumatol 1996;12:70-6.
60. Tronstad L, Barnett F, Cervone F. Periapical bacterial plaque in teeth refractory to endodontic treatment. Endod Dent Traumatol 1990;6:73-7.
61. Paster BJ, Dewhirst FE. Molecular microbial diagnosis. Periodontol 2000 2009;51:38-44.
62. Keijser BJ, Zaura E, Huse SM, van der Vossen JM, Schuren FH, Montijn RC, *et al*. Pyrosequencing analysis of the oral microflora of healthy adults. J Dent Res 2008;87:1016-20.
63. Siqueira JF Jr, Rôças IN. Exploiting molecular methods to explore endodontic infections: Part 2-Redefining the endodontic microbiota. J Endod 2005;31:488-98.
64. Saito D, Leonardo Rde T, Rodrigues JL, Tsai SM, Höfling JF, Gonçalves RB. Identification of bacteria in endodontic infections by sequence analysis of 16S rDNA clone libraries. J Med Microbiol 2006;55:101-7.
65. Munson MA, Pitt-Ford T, Chong B, Weightman A, Wade WG. Molecular and cultural analysis of the microflora associated with endodontic infections. J Dent Res 2002;81:761-6.

66. Siqueira JF Jr, Rôças IN. Uncultivated phylotypes and newly named species associated with primary and persistent endodontic infections. Clin Microbiol 2005;43:3314-9.
67. Waltimo TM, Sirén EK, Torkko HL, Olsen I, Haapasalo MP. Fungi in therapy-resistant apical periodontitis. Int Endod J 1997;30:96-101.
68. Siqueira JF, Rôças IN, Moraes SR, Santos KR. Direct amplification of rRNA gene sequences for identification of selected oral pathogens in root canal infections. Int Endod J 2002;35:345-51.
69. Vianna ME, Conrads G, Gomes BP, Horz HP. Identification and quantification of archaea involved in primary endodontic infections. J Clin Microbiol 2006;44:1274-82.
70. Siqueira JF Jr, Sen BH. Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:632-41.
71. Langeland K. Tissue response to dental caries. Endod Dent Traumatol 1987;3:149-71.
72. Lin L, Shovlin F, Skribner J, Langeland K. Pulp biopsies from the teeth associated with periapical radiolucency. J Endod 1984;10:436-48.
73. Tronstad L, Barnett F, Cervone F. Periapical bacterial plaque in teeth refractory to endodontic treatment. Endod Dent Traumatol 1990;6:73-7.
74. Leonardo MR, Rossi MA, Silva LA, Ito IY, Bonifácio KC. EM evaluation of bacterial biofilm and microorganisms on the apical external root surface of human teeth. J Endod 2002;28:815-8.
75. Tronstad L, Barnett F, Riso K, Slots J. Extraradicular endodontic infections. Endod Dent Traumatol 1987;3:86-90.
76. Tronstad L, Sunde PT. The evolving new understanding of endodontic infections. Endod Topics 2003;6:57-77.
77. Ramachandran Nair PN. Light and electron microscopic studies of root canal flora and periapical lesions. J Endod 1987;13:29-39.
78. Frank SA, Barbour AG. Within-host dynamics of antigenic variation. Infect Genet Evol 2006;6:141-6.
79. Tronstad L, Barnett F, Cervone F. Periapical bacterial plaque in teeth refractory to endodontic treatment. Endod Dent Traumatol 1990;6:73-7.
80. Conrads G, Gharbia SE, Gulabivala K, Lampert F, Shah HN. The use of 16S r DNA directed PCR for the detection of endodontpathogenic bacteria. J Endod 1997;23:433-8.
81. Medvedev AE, Sabroe I, Hasday JD, Vogel SN. Tolerance to microbial TLR ligands: Molecular mechanisms and relevance to disease. J Endotoxin Res 2006;12:133-50.
82. Jontell M, Gunraj MN, Bergenholtz G. Immunocompetent cells in the normal dental pulp. J Dent Res 1987;66:1149-53.
83. 85. Wilson M. Susceptibility of oral bacterial biofilm to antimicrobial agents. J Med Microbiol 1996;44:79-87.
84. Takemura N, Noiri Y, Ehara A, Kawahara T, Noguchi N, Ebisu S. Single species biofilm-forming ability of root canal isolates on gutta-percha points. Eur J Oral Sci 2004;112:523-9.
85. An YH, Friedman RJ. Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. J Biomed Mater Res 1998;43:338-48.
86. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: Study of prevalence and association with clinical and histopathologic findings. J Endod 2010;36:1277-88.
87. Chavez de Paz LE. Redefining the persistent infection in root canals: Possible role of biofilm communities. J Endod 2007;33:652-62.
88. Duggan JM, Sedgley CM. Biofilm formation of oral and endodontic Enterococcus faecalis. J Endod 2007;33:815-8.
89. Al-Ahmad A, Müller N, Wiedmann-Al-Ahmad M, Sava I, Hübner J, Follo M, *et al*. Endodontic and salivary isolates of Enterococcus faecalis integrate into biofilm from human salivary bacteria cultivated in vitro. J Endod 2009;35:986-91.
90. Madsen JS, Burmølle M, Hansen LH, Sørensen SJ. The interconnection between biofilm formation and horizontal gene transfer. FEMS Immunol Med Microbiol 2012;65:183-95.
91. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:86-93.
92. Sedgley C, Nagel A, Dahlén G, Reit C, Molander A. Real-time quantitative polymerase chain reaction and culture analyses of Enterococcus faecalis in root canals. J Endod 2006;32:173-7.
93. Mohamed JA, Huang W, Nallapareddy SR, Teng F, Murray BE. Influence of origin of isolates, especially endocarditis isolates, and various genes on biofilm formation by Enterococcus faecalis. Infect Immun 2004;72:3658-63.
94. Mohamed JA, Murray BE. Lack of correlation of gelatinase production and biofilm formation in a large collection of Enterococcus faecalis isolates. J Clin Microbiol 2005;43:5405-7.
95. Sedgley C, Buck G, Appelbe O. Prevalence of Enterococcus faecalis at multiple oral sites in endodontic patients using culture and PCR. J Endod 2006;32:104-9.
96. Sedgley CM, Lennan SL, Clewell DB. Prevalence, phenotype and genotype of oral enterococci. Oral Microbiol Immunol 2004;19:95-101.
97. Sedgley CM, Molander A, Flannagan SE, Nagel AC, Appelbe OK, Clewell DB, *et al*. Virulence, phenotype and genotype characteristics of endodontic Enterococcus spp. Oral Microbiol Immunol 2005;20:10-9.
98. Haapasalo M, Qian W, Portenier I, Waltimo T. Effects of dentin on the antimicrobial properties of endodontic medicaments. J Endod 2007;33:917-25.
99. Penick EC, Osetek EM. Intracanal drugs and chemicals in endodontic therapy. Dent Clin North Am 1970;14:743-56.
100. Senia ES, Marshall FJ, Rosen S. The solvent action of sodium hypochlorite on pulp tissue of extracted teeth. Oral Surg Oral Med Oral Pathol 1971;31:96-103.
101. Zehnder M. Root canal irrigants. J Endod 2006;32:389-98.
102. Zamany A, Safavi K, Spångberg LS. The effect of chlorhexidine as an endodontic disinfectant. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:578-81.
103. Radcliffe CE, Potouridou L, Qureshi R, Habahbeh N, Qualtrough A, Worthington H, *et al*. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms Actinomyces israelii, A. naeslundii, Candida albicans and Enterococcus faecalis. Int Endod J 2004;37:438-46.
104. Hwang BY, Roberts SK, Chadwick LR, Wu CD, Kinghorn AD. Antimicrobial constituents from goldenseal (the Rhizomes of Hydrastis canadensis) against selected oral pathogens. Planta Med 2003;69:623-7.
105. Quan H, Cao YY, Xu Z, Zhao JX, Gao PH, Qin XF, *et al*. Potent in vitro synergism of fluconazole and berberine chloride against clinical isolates of Candida albicans resistant to fluconazole. Antimicrob Agents Chemother 2006;50:1096-9.
106. Imanshahidi M, Hosseinzadeh H. Pharmacological and therapeutic effects of Berberis vulgaris and its active constituent, berberine. Phytother Res 2008;22:999-1012.
107. Mantena SK, Sharma SD, Katiyar SK. Berberine, a natural product, induces G1-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells. Mol Cancer Ther 2006;5:296-308.

108. Zeng XH, Zeng XJ, Li YY. Efficacy and safety of berberine for congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 2003;92:173-6.
109. Nakamoto K, Tamamoto M, Hamada T. In vitro study on the effects of trial denture cleansers with berberine hydrochloride. J Prosthet Dent 1995;73:530-3.
110. Hu JP, Takahashi N, Yamada T. Coptidis rhizoma inhibits growth and proteases of oral bacteria. Oral Dis 2000;6:297-302.
111. Chadwick LR, Wu CD, Kinghorn AD. Isolation of alkaloids from goldenseal (Hydrastis canadensis rhizomes) using pH-zone refining countercurrent chromatography. J Liq Chromatogr Relat Technol 2001;24:2445-53.
112. Wei GX, Xu X, Wu CD. In vitro synergism between berberine and miconazole against planktonic and biofilm Candida cultures. Arch Oral Biol 2011;56:565-72.
113. Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31:1-7.
114. Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. Int Endod J 2001;34:429-34.
115. Rocas IN, Siqueira JF Jr, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004;30:315-20.
116. Sedgley CM, Lennan SL, Appelbe OK. Survival of Enterococcus faecalis in root canals ex vivo. Int Endod J 2005;38:735-42.
117. Chivatxaranukul P, Dashper SG, Messer HH. Dentinal tubule invasion and adherence by Enterococcus faecalis. Int Endod J 2008;41:873-82.
118. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod 2002;28:689-93.
119. Kishen A, Sum CP, Mathew S, Lim CT. Influence of irrigation regimens on the adherence of Enterococcus faecalis to root canal dentin. J Endod 2008;34:850-4.
120. Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against Enterococcus faecalis biofilms. J Endod 2006;32:527-31.
121. Arias-Moliz MT, Ferrer-Luque CM, Espigares-García M, Baca P. Enterococcus faecalis biofilms eradication by root canal irrigants. J Endod 2009;35:711-4.
122. Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against Enterococcus faecalis biofilms. J Endod 2006;32:527-31.
123. Mohammadi Z, Abbott PV. Antimicrobial substantivity of root canal irrigants and medicaments: A review. Aust Endod J 2009;35:131-9.
124. Chávez de Paz LE, Bergenholtz G, Svensäter G. The effects of antimicrobials on endodontic biofilm bacteria. J Endod 2010;36:70-7.
125. Al-Bakri AG, Othman G, Bustanji Y. The assessment of the antibacterial and antifungal activities of aspirin, EDTA and aspirin-EDTA combination and their effectiveness as antibiofilm agents. J Appl Microbiol 2009;107:280-6.
126. Ballal NV, Kundabala M, Bhat S, Rao N, Rao BS. A comparative in vitro evaluation of cytotoxic effects of EDTA and maleic acid: Root canal irrigants. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:633-8.
127. Ballal NV, Kandian S, Mala K, Bhat KS, Acharya S. Comparison of the efficacy of maleic acid and ethylenediaminetetraacetic acid in smear layer removal from instrumented human root canal: A scanning electron microscopic study. J Endod 2009;35:1573-6.
128. Ferrer-Luque CM, Arias-Moliz MT, González-Rodríguez MP, Baca P. Antimicrobial activity of maleic acid and combinations of cetrimide with chelating agents against Enterococcus faecalis biofilm. J Endod 2010;36:1673-5.
129. Ramachandran Nair PN. Light and electron microscopic studies of root canal flora and periapical lesions. J Endod 1987;13:29-39.
130. Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G. The application of biofilm science to the study and control of chronic bacterial infections. J Clin Invest 2003;112:1466-77.
131. Siqueira JF Jr, Rôças IN, Santos SR, Lima KC, Magalhães FAC, de Uzeda M. Efficacy of Instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals. J Endod 2002;28:181-4.
132. Walker A. Definitive and dependable therapy for pulpless teeth. J Am Dent Assoc 1936;23:1418-25.
133. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg Oral Med Oral Pathol 1983;55:307-12.
134. Peters LB, van Winkelhoff AJ, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. Int Endod J 2002;35:13-21.
135. Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:231-52.
136. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, *et al*. A new solution for the removal of the smear layer. J Endod 2003;29:170-5.
137. Torabinejad M, Shabahang S, Aprecio RM, Kettering JD. The antimicrobial effect of MTAD: An in vitro investigation. J Endod 2003;29:400-3.
138. Chávez De Paz LE, Dahlén G, Molander A, Möller A, Bergenholtz G. Bacteria recovered from teeth with apical periodontitis after antimicrobial endodontic treatment. Int Endod J 2003;36:500-8.
139. Abdullah M, Ng YL, Gulabivala K, Moles DR, Spratt DA. Susceptibilities of two Enterococcus faecalis phenotypes to root canal medications. J Endod 2005;31:30-6.
140. Stewart PS, Rayner J, Roe F, Rees WM. Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. J Appl Microbiol 2001;91:525-32.
141. Peters OA, Schönenberger K, Laib A. Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography. Int Endod J 2001;34:221-30.
142. D'Arcangelo C, Varvara G, De Fazio P. An evaluation of the action of different root canal irrigants on facultative aerobic-anaerobic, obligate anaerobic, and microaerophilic bacteria. J Endod 1999;25:351-3.
143. Portenier I, Waltimo T, Ørstavik D, Haapasalo M. The susceptibility of starved, stationary phase, and growing cells of Enterococcus faecalis to endodontic medicaments. J Endod 2005;31:380-6.
144. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary Biofilm Device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol 1999;37:1771-6.
145. Kishen A. Advanced therapeutic options for endodontic biofilms. Endod Topics 2010;22:99-123.


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