**EFFECT OF VARIOUS IRRIGANTS ON DENTAL BIOFILM: A REVIEW**

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Received: 11 April 2015, Revised and Accepted: 12 May 2015

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**ABSTRACT**

**Objective:** The objective of the article is to describe in brief the various endodontic irrigants on dental biofilm.

**Method:** Articles based on various endodontic irrigants and dental biofilm were searched in an iterative manner from journals, books, and sites such as PubMed.

**Result:** It was found out that sodium hypochlorite is more effective on dental biofilms than chlorhexidine, MTAD, EDTA, herbal irrigants and new irrigants such as Qmix and tetraclean.

**Conclusion:** Disinfection of root canal system is done using mechanical instrumentation, chemical irrigation along with medication. Sodium hypochlorite an excellent antibacterial agent is the most commonly used and is more effective than other irrigants on dental biofilm.

**Keywords:** Irrigants, Dental biofilm, Antimicrobial resistance, Sodium hypochlorite, Chlorhexidine, Tetraclean, Herbal irrigants.

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**INTRODUCTION**

The effectiveness of biomechanical preparation underpins the success and longevity of root canal treatments [1]. Elimination of microorganisms from a root canal is a complicated task. Bacteria within a biofilm have increased resistance to the host defense mechanisms, antibiotics, and shear forces compared with isolated bacterial cells [2].

A favorable outcome with root canal treatment is high when the infection is eradicated before obturation. However, if microorganisms persist at the time of obturation or penetrate the canal after obturation, there are high chances of treatment failure [3]. This article describes in brief, the various endodontic irrigants used in root canal treatments and its effect on the endodontic biofilm.

**BIOFILM**

Microbial biofilm is defined as a sessile, multicellular microbial community characterized by cells that are firmly attached to a surface and enmeshed in a self-produced matrix of extracellular polymeric substances [4]. Endodontic biofilms are divided as intracanal, periapical, and external root (cementum) biofilms [5].

**Intracanal biofilm**

Using transmission electron microscopy Nair described intracanal biofilm. He examined the root canal content of teeth with gross coronal caries and to which periapical inflammatory tissue was attached upon extraction. He observed that the major bulk of organisms existed as a loose collection of cocci, rods, filaments, and spirochetes. Most of these organisms appeared suspended in a moist canal space while dense aggregates were also found sticking to canal walls and forming thin to thick layers of bacterial condensations. Amorphous material filled the inter-bacterial spaces and this was interpreted as an extracellular matrix of bacterial origin [6].

George et al. observed that when Enterococcus faecalis cells were grown under the aerobic nutrient rich condition, they produced irregularly shaped amorphous macro-structures of 500-1000 µm in dimension. These structures were identified as aggregates of bacterial cells [7].

Distel et al. found that pure cultures of E. faecalis inoculated to calcium hydroxide medicated or non-medicated root canals were able to form a biofilm structure on the canal walls [8].

**External root surface (cementum)**

These biofilms were reported in teeth with asymptomatic apical periodontitis and in teeth with chronic apical abscesses associated with sinus tract [5].

Tronstad et al. identified cocci and rods with the presence of fibrillar forms in the apex of roots [9]. Lomc ál et al. reported that lacunar resorption zones were frequently observed on the apical root surfaces of teeth with chronic apical periodontitis. Bacteria and yeast cells were detected in some lacunae. Periapical bacterial plaques, which had microorganisms embedded in an extracellular matrix was found. This coating was considered to be a combination of bacterial by products and local inflammatory components. These findings suggest that host defense mechanisms may be unable to hold back the microorganisms outside the apical foramen may not be eliminated by conventional endodontic procedure and systemic antibiotic usage [10].

**Periapical biofilms**

These biofilms are found in the periapical region of endodontically involved teeth and may or may not be dependent on root canal infections [5]. Members of the genus Actinomyces and species Propionobacterium propionicum have been found in asymptomatic periapical lesions refractory to endodontic treatment [11].

Actinomycotic colonies may live in equilibrium with the host tissues without necessarily inducing an acute response, but rather maintains a chronic periapical inflammation. Very high numbers of Actinomyces cells are usually necessary to form persistent infections [12].

The perpetuation of the chronic periapical lesion may be due to the low pathogenicity of these microorganisms and the consequent minimal host response.

**MECHANISM OF ANTIMICROBIAL RESISTANCE**

Biofilm formation is a stepwise process that includes deposition of conditioning film, adhesion, and colonization of planktonic microorganisms in a polymeric matrix, coadhesion of other organisms and detachment of biofilm microorganisms into their surrounding [13].

Biofilm is made of numerous microcolonies of organisms in an aqueous solution surrounded by a matrix of glycolax, this type of growth...
provides resistance to antimicrobial agents, communicate between the bacterial population, exchange of genetic material, increase in the local concentration of nutrients and production of growth factors.

This unique type of growth gives antimicrobial resistance and makes the organisms to grow in tough environmental conditions [14].

**IRRIGANTS**

**Sodium hypochlorite (NaOCl)**

NaOCl (household bleach) is the most commonly used irrigant in root canal treatment. It is used in dilutions ranging from 0.5% to 5.25%. It is an antiseptic and a lubricant. Free chlorine in NaOCl dissolves vital and necrotic tissues by breaking down proteins into amino acids.

Increasing its volume increases its effectiveness and decreasing the concentration of the solution reduces its toxicity, antibacterial effect, and ability to dissolve tissues [15].

Its advantages are its ability to dissolve organic substances present in the root canal system and its cheap. However, it is cytotoxic when it is injected into periradicular tissues, foul smell, and taste; and causes corrosion of metal objects [16]. It does not kill all bacteria and does not remove smear layer [17,18].

Spratt *et al.* showed that NaOCl was the most effective antimicrobial irrigant [19]. Ozok *et al.* compared the growth and susceptibility of mono and dual species biofilms of *Pseudomonas nucleatum* or *Peptostreptococcus micros* at 24 or 96 hrs, *in vitro* to different concentration of NaOCl [20].

It was revealed that although at 24 hrs the dual species biofilms had similar viable counts to those of mono species, they were more resistant to NaOCl. At 96 hrs, both microorganisms had higher viable count, and the dual species were more resistant to NaOCl than mono species. Time-dependent synergy in growth and resistance to NaOCl were showed by mixed species biofilms of *F. nucleatum* and *P. micros*.

In 2007, Giardino *et al.* did a comparative evaluation of antimicrobial efficacy of NaOCl, a mixture of tetracycline isomer, an acid, and a detergent (MTAD), and tetraclean against *E. faecalis* biofilm generate on cellulose nitrate membrane filters. They reported that only 5.25% NaOCl can disintegrate and remove the biofilm every time [21].

A similar study conducted by Dunavant *et al.* in 2006 showed that 1% NaOCl and 6% NaOCl were more efficient in eliminating *E. faecalis* biofilm than other endodontic irrigants [22]. Lundstrom *et al.* conducted a study to assess the bacterial efficacy of 0.04% stabilized chlorine dioxide, 3% NaOCl, 2% chlorhexidine (CHX), and sterile distilled water in a polymicrobial biofilm model. He reported that NaOCl possessed higher bacterial activity than that of stabilized chlorine dioxide against *Streptococcus sanguinis*, *Actinomyces viscosus*, and *Prevotella nigrescens* [23].

A study of the effect of exposure to irrigant solutions on apical dentin biofilms in vitro with different concentrations of NaOCl, 2% CHX, and BioPure MTAD on intracanal contents from 10 patients diagnosed with chronic apical periodontitis revealed that 6% NaOCl was the only irrigant capable of both rendering bacteria non-viable and physically removing the biofilm [24].

Since extrusion of NaOCl into periapical tissues can result in severe injury to the patient, it should be used carefully [25].

**Ethylenediaminetetraacetic acid (EDTA)**

Since hypochlorite is active only against organic material, other irrigants must be used for the complete removal of smear layer and dentin debris.

EDTA is commonly used as 17% neutralized the solution. Although it is biocompatible, it is immediately reduces the available chlorine in solution making NaOCl ineffective in bacteria and necrotic tissues [26]. EDTA has little or no antibacterial effective [27].

Yoshida *et al.* conducted a clinical evaluation of the efficacy of EDTA solution as an endodontic irrigant in 189 single rooted infected teeth using 15% EDTA solution with ultrasonic agitation without antibacterial intracanal medicaments in between appointments. They reported that 15% EDTA was more effective than a saline solution as root canal irrigant [28].

Calt and Serper showed that 10 ml irrigation with 17% EDTA for 1 minute was effective in the removal of smear layer [29]. Longer exposure of 10 minutes can cause excessive peritubular and intertubular dentin [30].

In a study conducted by Paul *et al.* in 2013, comparison of efficacy of different irrigants with EDTA, EDTA with ultrasonics, citric acid, MTAD, and mixture of tetracycline isomer revealed that none of the irrigants were completely effective [31].

**CHX**

CHX has a broad spectrum antibacterial action, sustained action, and low toxicity, so it is widely used in dentistry [15].

Its advantages over NaOCl that it does not have a foul smell and does not cause injury to the surrounding tissues, but it is unable to kill all bacteria and cannot remove the smear layer. It is used in concentration of 0.2-2% [32].

In 2007, an *in vitro* study conducted by Oliveira *et al.* on the antibacterial efficacy of endodontic irrigants against *E. faecalis* revealed that 2% CHX gluconate gel, and 5.25% NaOCl were effective in eliminating *E. faecalis* even after 7 days after instrumentation [33].

In another *in vitro* assessment between 2% CHX gel and liquid against 5.25% NaOCl it demonstrated that although 5.25% reduced *E. faecalis* colony forming units (CFU) immediately after instrumentation but was not able to keep root canal free of detectable *E. faecalis* in the final sample whereas 2% CHX liquid reduced CFU in post-treatment and final microbiological samples [34].

Studies by Delany *et al.*, Shah *et al.*, Vahdaty *et al.*, and Heling *et al.* demonstrated 0.2% CHX was effective in removing aerobic bacteria in 80% of the cases and anaerobic bacteria in 76% of the cases [35-38].

**MTAD**

Biopure MTAD is a mixture of tetracycline isomer, acetic acid, and a detergent (MTAD). and mixture of tetracycline isomer revealed that none of the irrigants were completely effective [31].

MTAD Biopure MTAD is a mixture of tetracycline isomer, acetic acid, and a detergent [39]. It is superior to CHX in antimicrobial activity [18]. It is also biocompatible, has a sustained bacterial activity and enhances bond strength [40].

Giardino *et al.* showed that MTAD was not able to remove bacterial biofilms [41]. Stojicic *et al.* demonstrated that MTAD was unable to kill all plaque bacteria in 30 seconds, and some *E. faecalis* cells were able to survive even after 3 minutes exposure [42].

A comparative study by Paul *et al.* revealed that MTAD showed excellent results in the removal of the smear layer in the apical third as compared to EDTA, EDTA with ultrasonication, citric acid [31]. Effectiveness of EDTA and MTAD on debris and smear layer removal using self-adjusting file revealed no significant difference whereas with ultrasonication, MTAD appeared to cause less dentinal erosion while allowing proper removal of smear layer and debris [43,44].

Tong *et al.* said that adding nisin to MTAD enhanced its effectiveness against *E. faecalis* biofilm [45].

**OTHER IRRIGATING SOLUTIONS**

The other irrigating solutions used are sterile water, physiologic saline, iodine compounds, urea peroxide, hydrogen peroxide, citric acid.
Spratt et al. concluded that iodine and NaOCl were more effective than CHX against Streptococcus intermedius, F. nucleatum, and E. faecalis but not against Prevotella intermedia and P. micros. Iodine and NaOCl showed 100% bacteria elimination after 1 hr incubation for all used strains [19].

Water and saline can get contaminated and does not have antimicrobial activity. A study to evaluate, through scanning electron microscopy, the micromorphology of dentinal walls of primary anterior teeth with focus on the presence of smear layer after endodontic debridement and final irrigation with different systems revealed that NaOCl promoted formation of smear layer during shaping and the use of EDTA and citric acid facilitated smear layer removal [46].

NEWER IRRIGATING SOLUTIONS

Tetraclean
It is a mixture of antibiotic, an acid and a detergent-like MTAD, but the concentration of antibiotic, doxycycline (50 mg/ml), and type of detergent is different [47].

Giardino et al. compared the antimicrobial efficacy of MTAD, tetraclean, cloroexim (CHX digluconate and cetrimide) and NaOCl on E. faecalis, Porphyromonas gingivalis and F. intermedias; and concluded that 5.25% NaOCl showed a high antimicrobial activity against anaerobic bacteria, MTAD, and tetraclean showed a high action against strictly anaerobic and facultative anaerobic bacteria while chloroexim (CHX + cetrimide) showed the lowest antibacterial activity [21].

Qmix
It is a mixture of EDTA, CHX, and a detergent. In a study conducted in 2013, it was revealed that Qmix™ 2 in 1 was less toxic to rat subcutaneous tissue than 3% NaOCl, 2% CHX, and 17% EDTA [48]. In another study, using Qmix™ 2 in 1 revealed that Qmix was superior to EDTA in smear layer removal and exposure of dentinal tubules in root canal system in single-rooted teeth [49].

Ozonated water
It is a chemical compound containing three oxygen atoms. It is a powerful antimicrobial agent against bacteria, fungi, protozoa, and virus but rarely used as irrigant [4]. Huth et al. demonstrated that the efficacy of ozonated water and 2.5% NaOCl were about the same when the specimen was irrigated with sonication [50]. However, another study by Hems et al. found that NaOCl was superior to ozonated water in killing E. faecalis in biofilm and broth culture [51].

HERBAL IRRIGANTS

Herbs such as green tea, Azadirachta indica, Salvadora persica solution (miswak-siwalk), triphala, German chamomille, tea tree oil, Psidium guajava can be used as irrigants. Triphala and green tea polyphenols (GTP) contains a beneficial physiological effect, as well as it was being an antioxidant, anti-inflammatory, and radical scavenging activity [52]. In an in vitro study based on herbal irrigants, it was evaluated that 5% NaOCl showed maximum antibacterial activity against E. faecalis biofilm formed on tooth substrate. Triphala, GTP’s, and MTAD showed statistically significant antibacterial activity [53].

REFERENCES


