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The antimicrobial efficiency of endodontic irrigation solutions on bacterial biofilm. A literature review

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ABSTRACT

The purpose of endodontic treatmentis the prevention or the removal of endodontic system infection, namely the chronic apical periodontitis, either by cutting down the access of the microorganisms in the root canal or by removing them completely. However, despite all the efforts for the correct enlargement and irrigation of the root canal, several studies have shown that microorganisms from the endodontic system cannot be entirely eliminated due to the biofilm characteristics at this level, which are different from those of planktonic cells and single cells, but also due to the morphology of the endododontic area, which features several microspaces such as side channels, isthmi, apical ramifications, anastomoses, all of them favouring the development and persistance of bacterial biofilm. Various medical substances have proved to be effective in eradicating planktonic bacteria *in vitro*. But since the internal environment of the tooth reduces the activity of irrigants substantially, thereare also failure rates in obtainingpredictable results in this aspect. The purpose of this review is to assess the efficiency of chlorhexidine and sodium hypochlorite, used as irrigant solutions, against the endodontic biofilm development.

Keywords: *endodontic treatment, antimicrobial, bacterial biofilm, review.*

1. INTRODUCTION

The purpose of endodontic treatmentis the prevention or the removal of endodontic system infection, namely the chronic apical periodontitis. This is done either by cutting down the access of the microorganisms in the root canal or by removing them completely [1-4]. This objective is achieved by an appropriate, accurate handling of the root canal [1, 5, 6], which is completed by the cleansing effect of strong antibacterial washing solutions.But despite all the efforts for the correct enlargement and irrigation of the root canal, several studies have shown that microorganisms from the endodontic system cannot be entirely eliminated [5, 7-12]. This bacterial presence is due to the biofilm characteristics at this level, which are different from those of planktonic cells and single cells [13], but also due to the morphology of the endododontic area, which features several microspaces such as side channels, isthmi, apical ramifications, anastomoses, all of them favouring the development and persistance of bacterial biofilm[9, 14-18].

Various medical substances have proved to be effective in eradicating planktonic bacteria *in vitro*. But since the internal environment of the tooth reduces the activity of irrigants substantially, thereare also failure rates in obtainingpredictable results in this aspect [19-23].

A strategy to reduce the amount of microorganisms is represented by using simultaneously endodontic treatment components, namely the instrumentation, the chemical disinfection using irrigation solutions and the placement of medicated dressing, but the results obtained are controversial [24-29].

The irrigation is a fundamental component of the chemomecanical endodontic disinfection procedure [30, 31]. An optimal amount of solution is necesarry to reach all branches of this space and to be periodically refreshed. Its role is to chemically inactivate bacteria, biofilm, metabolic products or their endotoxins, to remove smear layer and to dissolve local tissue debris. The removal of biofilm by irrigating solutions of endocanalicular usage is of a major importance, since a considerable amount of the endodontic internal area is not accessible to direct instrumentation [32].

The properties that are looked for when asessing and choosing an irrigant are: action on endodontic microbiota, tissue dissolution capacity, low toxicity, and last but not least, its effect on the adhesion of composite resins.

2. SODIUM HYPOCHLORITE

The sodium hypochlorite (NaOCl) is a first choice lavage solution, being used in concentrations between 0.5 and 6%. Sodium hypochlorite has the extremely important property of dissolving necrotic organic tissue [33, 34]. It also has a strong antibacterial effect, and acts on the essential bacterial enzymes, causing their irreversible inactivation. Hypochlorite's high pH alters the heat shock proteins and damages the phospholipids of the bacterial cell leading to cell membrane deterioration, cellular metabolism alteration [35], and later producing the death of microorganisms [36].

The sodium hypochlorite is the most frequently used irrigant due to its excellent antibacterial effects and also due to being less expensive and easy to obtain. However, it can only partially remove microbiota components from the endodontic space [37].

The studies on hypochlorite utilisation as a disinfectant liquid were started in the nineteenth century by Koch, Pasteur [38], and later by Dakin [39], who also studied the effecton the necrotic tissue of other irrigant solutions. From 1920, it became one of the main components of the lavage procedure [8, 38, 40].

Sodium hypochlorite has a dissolving action, especially on necrotic tissue, compared to vital pulp [41]. It is also used on microorganisms as a broad spectrum virucide and sporicide [42]. The hypochlorite acts at bacterial level but also on dentin debris and collagen fibers, having a strong oxidative action. It is a powerful antimicrobial agent against gram positive and gram negative bacteria, fungi, spores, viruses and HIV.

However, from a biological perspective, the hypochlorite may pose some problems. It is not clinically toxic, if used properly and within the endodontic space only. But if it happens to accidentally leave this space, the severity of reactions will depend on the solution's concentration, pH and exposure time. In that case, it may cause inflammation, severe pain, extensive swelling, necrosis and cell destruction in almost all exposed tissues, except for the epithelium, which is strongly keratinized. Grossman, 1941 [43] studied the effect of sodium hypochlorite on pulp tissue dissolution, noting that the required dissolution time was from 20 minutes to 2 hours. Previous studies on bovine pulp tissue showed that the solubilization efficiency is directly dependent on the following factors: irrigant higher concentration level, irrigant higher temperature [44], and the absence of a surfactant [44-46]. Along with a highly concentrated irrigation solution, the decreasing rate of the pH value isdiminished [46].

Due to its above listed properties and its negative effect on the resin adhesion to dentin, sodium hypochlorite should not be used as a final irrigant, the recommended replacement being sterile saline solution (neutral).

After the action of sodium hypochlorite, it is recommended to use 17% EDTA (ethylenediaminetetraacetic acid). This acts on inorganic tissue and removes smear layer, but has no effect on collagen fibers. EDTA is a chelating agent and also a demineralization agent. It softens the dentin and improves the permeability of calcified channels. EDTA acts sometimes as a lubricant, allowing the opening of dentinal tubules and reducing the risk of instrument fracture. In this way, it improves the penetration of medicinal substances and materials for root canal filling in the dentinal tubules.

3. CHLORHEXIDINE

Chlorhexidine acts directly on specific bacterial species, having bacteriostatic effect when used in low concentrations, and bactericidal effect when used in high concentrations.It is a broad spectrum antimicrobial agent which inhibits spore germination, and it is effective on vegetative forms of bacteria, mycobacteria, fungi, viruses, gram positive cocci and *Enterococcus faecalis,* the latter being resistant to calcium hydroxide. However, it is less effective on gram positive bacilli and gram negative bacilli.

Chlorhexidine binds either on the dentinal wall or at mucosa level, adhering to the hydroxyapatite component of the dentin. The clorhexidine is thus released at therapeutic levels for 8-12 hours, during this time preserving a prolonged antimicrobial action and providing an favourable environment to healing, property named substantivity [47-50]. It also presents a minimal toxicity on the host tissues and it has a positive effect on the adhesion to dentin.When absorbed inside the dentinal canaliculi, it facilitates the penetration of dentin bonding agents at this level. It has no tissue dissolving properties and does not remove the remaining dentine detritus.

Its properties give the indication to be used as final irrigant, after sodium hypochlorite and EDTA [51]. The chlorhexidine interaction with the bacterial cell membrane causes the precipitation of membrane's plasma content [47, 52].

Chlorhexidine 2% produces an incomplete dissolution of biofilm and organic substances, having a limited chemical effect during instrumentation, especially in less accessible areas (isthmuses and lateral channels) [32].

The effect of 2% chlorhexidine by direct contact on biofilm dissolution is lower than the effect of NaOCl solution;2% chlorhexidine shows limited effectiveness during instrumentation, especially in low accessible areas such as isthmuses, side channels, complex endodontic anatomy[25, 53, 54].This fact refers to the dissolution on biofilms of 30-50μm thickness. On those of greater thickness or biofilm within anatomical irregularities, the necessary action time *in vivo* will increase [32]. The effects of irigants and other endodontic disinfecting agents have started to be studied on biofilms recently, because there are limitations in the area of planktonic bacterial cultures, which behave differently. Researchers conducted so far have used a variety of biofilm models, and the results obtained are difficult to compare and integrate with clinical data [26, 55, 56]. A standardized biofilm model having clear features has not been established yet.

4. EFFICIENCY OF CHLORHEXIDINE AND SODIUM HYPOCHLORITE ON ENDODONTIC BIOFILMS

The removal of microorganisms from the endodontic space is still the subject of numerous studiesmadeboth *in vivo* and *in vitro.*Thedisadvantages of *in vivo* studies are the variations in anatomy, shape, size of root canals, different types and quantities of microorganisms found locally in each case, and also the difficulty to obtain standardized biological samples from representative root canal areas. On the other hand, *in vitro* studies use experimental models that are too simplified, and most frequently utilize planktonic cells in the research on endodontic disinfection [57].

In vitro studies used bacterial biofilms grown on microscopic slides [58], porcelain [59], polystyrene microtiter plates [55], nitrocellulose membranes [60], dentin [61] and hydroxyapatite discs [23, 62].

Early research related to endodontic disinfection was focused generally on removing planktonic microorganisms. Later on, it was proved the role of biofilms in root canal treatments [64, 65] and the capacity of *Enterococcusfaecalis*to form biofilms [63]. Studies began touse biofilms as experimental models for testing the efficiency of different methods for disinfection and irrigation.

Utilisation of experimental biofilmdeveloped*in vitro* or *in situ* helps us to identify the factors (such as thickness or biofilm age) that allow microorganisms survival, even in the presence of strong antibacterial substances [32].

Biofilm develops a resistance towards antimicrobial agents, including antibiotics and disinfectants.Also, it can not be removed using only the biomechanical preparation [66, 67]. Biofilm formation is a dynamic process, the adhering of free bacterial cells to a surface being followed by the development of a colony of mature and complex microorganisms, and later by the detachment of certain bacteria in the environment [68]. The mechanism of resistance of biofilm microorganisms to medical substances is caused by factors such as: the polymeric matrix, which slows the penetration of therapeutic agents [69]; reduced antimicrobial effects on microorganisms located in the depth of the biofilm; phenotypic changes suffered by bacteria from the aggregated community, with a lower growth rate [70-72]; a subpopulation of "persistent" bacteria [73]; the physiological state of biofilm cells, different from the planktonic organisms; phenotypic changes and accommodation to environments that are low in nutrients [69].

Oral microbiota differs from subject to subject, and as a result each individual dental plaque biofilm is likely to contain different species [74].

The studies *in vitro* may reveal excellent results concerning the antimicrobial efficiency of endodontic irigants on young biofilms, but these data are not always confirmed by the studies *in vivo*[75, 76]. Most studies with regard to the action of irigants upon biofilms use these bacterial communities only at one moment in their development, and without determining their exact degree of maturation. Thus, the results of researches on biofilms *in vitro,* compared to those*in situ* are inconsistent, with different results.This creates the demand for observing the whole necessary maturation period for biofilms, when studying experimental models, and also for comparing the antimicrobial efficiency of endodontic disinfectants on biofilms of different ages [77].

Research of this kind is few, only one study is evaluating the action of chlorhexidine-based irrigation products on bacteria belonging to biofilms of different stages of development [78]. The author reveals acorrelation between the amount of microorganisms removed from endodontic space and the type of lavage fluid, as well as the biofilm age. The biofilm becomes more resistant to chlorhexidine action from 2 to 3 weeks and beyond this age, compared to younger biofilms (from 2 days to 2 weeks).

The efficiency of removing endodontic microorganisms during chemomecanical disinfection is correlated with the type of the irrigant and its duration of action, the age of development and the nutrition state of the biofilm.Previous studies were conducted on biofilms that were younger than seven days [25, 58], therefore only some of them might have been mature enough at the contact with disinfectants.

The resistance to different antimicrobial agents is more influenced by the development and maturation of biofilm, and less influenced by the type of the irrigant, or the type of bacteria in the endodontic system.

When assessments of this kindare done, it is important to use mature biofilms, since most endodontic or periodontal infections are produced by biofilms of several weeks, months or even several years old. Testing antimicrobial agents on biofilms that are too young, not fully developed yet, can give results that are too optimistic [77].

Research studies were conducted upon the antimicrobial activity of sodium hypochlorite and chlorhexidine, using different concentrations and different action times, but the results are controversial[47, 79]. Many of these studies have shown that the concentration of hypochlorite does not affect itsantimicrobial effect *in vivo*.There are many adjacent factors for the assessments made *in vivo*, such as root canal anatomy, apical diameter, penetration level of the irrigation needle, composition and quantity of endodontic microbiota [57]. The author shows that 6% sodium hypochlorite and the new irrigant Qmix have stronger antibacterial action in dentin, compared to 1% and 2% sodium hypochlorite and 2% chlorhexidine.

For the cases of 1% and 2.5% NaOCl irrigant solutions, it has been observed that a short action time of the irrigant does not dissolve the biofilm properly. But 30 minutes of irrigation with sodium hypochlorite proved to be effective to clean the dentin and dissolve the biofilm, even when the concentrations used was as low as 1% and 2.5%. Also, after 30 minutes of action, it was found that 1% NaOCl solution exhibited the same effect as 5.25% NaOCl solution. Previous studies showed that in terms of antimicrobial power and dissolution of organic tissue, there were no differences between the concentrations mentioned [7, 80].

A lower concentration of the irrigant has the advantage thattoxicity will lower as well [81].

However, some studies have shown that a high concentration of sodium hypochlorite enhance collagen degradation processes and loss of dentin proteins, thus reducing dentin flexural strength [82, 83].

In clinical conditions, a shorter contact time in the apical third of the root may be insufficient for a complete removal of intraradicular biofilm [32].

Compared to other disinfectants such as chlorhexidine digluconate 2%, BioPure MTAD ™ ™, and TetracleanTM, the most effective solution for removing endodontic biofilm and *Enterococcusfaecalis* is 1% - 6% sodium hypochlorite solution. Studies show that only a 5-minute exposure to 5.25% NaOCl would remove the biofilm completely [27, 59]. Jiang *et al.* achieve the removal of *Enterococcusfaecalis* in proportion of90%, after 5 minutes of exposure to 5.25% NaOCl, slightly lower than results reported by other studies (> 99%)[27, 59, 84]. Chlorhexidine 2% does not seem to have a noticeable effect on biofilm structure.

5. NON-THERMAL PLASMA

Using a single-bacterium biofilm model, *in vitro*, Jiang *et al* simply and reproducibly demonstrated the bactericidal effect of non-thermal plasma on biofilm containing *Enterococcusfaecalis* [84]. But natural endodontic biofilmis pluri-specific and pluri-layered[9, 85]. Although dentin is a better factor for evaluating the action of irigants, the authors used hydroxyapatite discs(major mineral component of sintered dentin) covered with saliva [14]. The hydroxyapatite discs were used as biological substrates for the proper growth of biofilm, since they could be easily standardized and had properties that were easier to control and adjust.Shen *et al,* 2009 showed that best substrate for multispecific growth of biofilm was the synthetic hydroxyapatite, made with or without type I collagen coating [23].

Jiang *et al* used biofilms that had been grown on discs for 6 days [84], while other researchers used different substrates and different bacteria growth times: one day, on pieces of porcelain [59], or two days, on filtering membrane [27]. *Enterococcus faecalis* sensitivity to the action of disinfectants depended on the substrate interactions as well as on the physiological state of bacteria [56, 86]. Plasma removed microorganisms from hydroxyapatite discs, whereas NaOCl kept both living and dead bacteria on the substrate. Experiments performed at room temperature on biofilms with *Enterococcus faecalis* that had been grown on hydroxyapatite discs showed that plasma had an antimicrobial effect that was comparable to that of sodium hypochlorite 5.25%. Plasma proved to be safer than traditional disinfectants, as the oxidation processes could be located more accurately, and thus with an increased antibacterial efficiency [84].

6. N-ACETYLCYSTEINE

In order to determine biofilm susceptibility to N-acetylcysteine, Yiling Quah *et al* used biofilmsof*Enterococcus faecalis* grownon dentine discs*,* aged 21 days [87]. These simulated the clinical situation of *in vivo*infected canals, in contrast to previous studies, in which the biofilm had been examined *in vitro,* using models developed on porous membranes [53].

N-acetylcysteine has a better efficiency concerning the antimicrobial activity and removing the planktonic cellsand biofilm.Itcan be used for the irrigation and treatment of the infected canals, but its long-term effects on the dentin are unknown and must be tested [57].

An important factor that causes the development of resistance mechanism in microorganisms is the lack of nutrients in the environment where they are living. Liu *et al.* described an enhanced resistance to the action of NaOCl for the nutrient-deprived biofilm with *Enterococcusfaecalis*, compared to the biofilm with stationary bacteria [88]. Distel *et al*showed that *Enterococcusfaecalis*formedcolonies that penetrated deep into the dentinal walls and on the root canal surface [63]. Apart from the *Enterococcusfaecalis*, which is a well-known cause of endodontic failures, the number of bacteria resistant to the chemomechanical treatment or alkaline stress is much higher, including here several species of streptococci and gram-positive bacilli [54].

One problem of the endodontic disinfection *in vivo* is theneutralization of antimicrobial action of the irrigant by the organic pieces of waste [89].

Many of the studies on bacterial survival were based on microorganisms growth within the endodontic spaces of extracted teeth or on dentin blocks. The dentin blocks allowed bacteria to penetrate up to 500 mm deep in the main channel [90]. Recent researches showed that microorganisms could be visualized in the dentinal tubules, with the help of confocal laser microscopy (CLSM); this enables the identification of living and dead bacteria in the infected dentin [91,93]. A challenge that still remains is that one of getting similar quantitative bacterial specimens. The difficulty is to reproduce clinical situations of dentin infection using methods of microorganism cultivation*in vitro*[94]. It is almost impossible or at least very difficult to assess disinfectants action using cultivation methods of microorganisms or CLSM. Even after a long incubation period, the bacteria from the dentinal tubules invade them only to a small extent [95, 96].

6. COMBINATIONS OF ANTIMICROBIAL SOLUTIONS

Stojicic *et al,* 2013 used a mixed biofilm model, showing that 1% hypochlorite, 0.2 / 0.4% iodine potassium iodide(IPI) and also 2% chlorhexidine removed 7-14 days old biofilms [77]. Those biofilms that were older than 3 weeks showed resistance to these endodontic irrigants. The age of the biofilms that were tested were one to two weeks old for young biofilms, and two to three weeks old for mature biofilms. All three solutions used were effective for younger biofilms, whereas NaOCl 1% was the most effective for both young and mature biofilms. Biofilms over 2-3 weeks became resistant to all antimicrobial agents utilised.

IPI mechanism of action involves multiple effects on cells such as proteins, nucleotides and fatty acids binding [97].

7. ENHANCEMENT OF THE ANTIMICROBIAL EFFICIENCY OF ENDODONTIC IRRIGANTS

The antimicrobial efficiency of endodontic irrigants may be enhanced by sonic and ultrasonic agitation. However, the results of studies that have been published so far on this topic are contradictory, as they depend on sample models and variables utilised. Therefore a clear conclusions on this subject has not been established yet. Several antibiofilm models were tested, such as resin blocks [98], extracted teeth [99] and hydroxyapatite coated collagen discs [94]. Antibacterial activity has been evaluated on

biofilms of different ages, ranging from several hours [99] up to several weeks [94].

Several irigants were used, such as sterile saline solution [100], chlorhexidine [94] and sodium hypochlorite [101]. Different values for the liquid agitation were experimented such as: the distance relative to the working length, the level of energy transmitted to the irigant and the action time, which ranged from 15 seconds [102] up to 5 minutes [103]. The effect of passive ultrasonic irrigation was attenuated when the needle reached the canal walls. The attenuation was higher when the contact occurred at cathode compared to that one occuring at anode [104, 108]. The acoustic waves obtained inside curved channels were made more active by previously bending the instruments [105, 107].

4. CONCLUSIONS

In conclusion, these studies reveal the need for a more efficient chemomecanical disinfection procedure, to eliminate the endodontic biofilm. They also highlighted the difficulty of designing a standardized experimental model for testing anti-biofilm irrigation solutions. This model should include as many variables as possible associated with the endodontic infection, so that the results should be predictable *in vivo*.

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