IEJ Iranian Endodontic Journal

Evaluation of Antimicrobial Efficacy of Calcium Hypochlorite as an Endodontic Irrigant on a Mixed-Culture Biofilm: An *Ex vivo* Study

Elham Shadmehr [a], Amin Davoudi [b*], Nima Damoon Sarmast [c], Masoud Saatchi [d]

a Preventive Restorative Dental Sciences Department, Endodontics Division, University of California San Francisco, San Francisco, USA; b Department of Prosthodontics, Dental School, Isfahan University of Medical Sciences, Isfahan, Iran; c Department of Periodontics and Dental Hygiene, School of Dentistry, University of Texas Health Science Center at Houston, Houston, Texas, USA; d Dental Research Center, Department of Endodontics, Dental Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Article Type: **Original Article**

Received: 15 Feb 2019
Revised: 01 May 2019
Accepted: 13 May 2019
Doi: *10.22037/iej.v14i3.22451*

*Corresponding author: Amin Davoudi, Dental Students' Research Committee, Department of Prosthodontics, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran.

Tel: +98-913 2949318
E-mail: amindvi@yahoo.com

ABSTRACT

Introduction: Calcium hypochlorite (CH) has been recently suggested as an endodontic irrigant. The aim of this investigation was to evaluate the antimicrobial efficacy of CH compared to sodium hypochlorite (NaOCl) and chlorhexidine (CHX) against multispecies biofilm in surface and deep dentinal tubules. **Methods and Materials:** Minimal inhibitory concentration (MIC) of irrigant agents was assessed using a microdilution method. One hundred and twenty of human maxillary incisor teeth were prepared and infected with suspension of *Entrococcus faecalis*, *Fusobacterium nucleatum* and *Prevotella intermedia* in an anaerobic jar for 7 days. Depending on irrigation solutions, specimens were divided into 4 groups (n=30); group 1: 2% CHX, group 2: 5.25% sodium hypochlorite, group 3: 5% calcium hypochlorite, group 4: positive control (normal saline (NS)). Fifteen remained specimens were used as negative control. Surviving bacteria were sampled before (S1) and after irrigation from surface (S2) and deep (S3) dentin. The medium turbidity was visualized with spectrophotometry. Data were analyzed using analysis of variance followed by Tukey post hoc test (α=0.05). **Results:** The MIC of CH against *E. faecalis*, *F. nucleatum* and *P. intermedia* was 25, 8 and 7.5 µg/mL respectively. There were no significant differences in S1 among the test groups. Moreover, 2% CHX and 5% CH had significantly lower medium turbidity at both S2 and S3, in comparison with 5.25% NaOCl (P=0.018 and 0.031, respectively). But there were no significant differences between 2% CHX and 5% CH at both S2 and S3 (P=0.862 and 0.978, respectively). **Conclusion:** Under the conditions of this *ex vivo* study, 5% CH and 2% CHX are more effective than 5.25% NaOCl in the reduction of mixed-culture biofilm.

Keywords: Calcium Hypochlorite; Chlorhexidine; Endodontics; Sodium Hypochlorite

Introduction

Complete debridement of root canal system to eliminate all bacteria and their by-products is one of the most fundamental prerequisites for predictable long-term successful endodontic therapy [1]. For this purpose, different mechanical and chemo-mechanical method has been used [1, 2]. Mechanical preparation reduces the number of bacteria, but it has been shown that regardless of the instrumentation technique 35-50% of the root canal wall often remain unistrumented [3]. Therefore, use of intracanal irrigants and medicaments are essential to make up for this drawback [4].

Adequate disinfection is hard to achieve because of the anatomical complexity of root canal system [5], types of bacterial species present [6], occurrence of smear layer [7], *etc*. On the other hand, the complex structure of the endodontics biofilm which are resistant to antimicrobial agents [8] make it more difficult; the virulence of the bacteria enhances with presence of other bacteria in a multispecies biofilm [9].

An ideal endodontic irrigant should have high and broad antimicrobial efficacy with soft-tissue dissolution while being non-toxic [1]. Sodium hypochlorite (NaOCl), chlorhexidine (CHX), ethylenediaminetetraacetic acid (EDTA), *etc.* are used as endodontic irrigants [10-12]; but there is still a need for new irrigant agent with maximum antimicrobial efficacy and less cytotoxic effect.

Calcium hypochlorite (CH) is a chlorine solutions widely used for different disinfection purposes. CH solution is prepared by adding calcium hypochlorite granules to deionized water. The reaction results in 2 units hypochlorous acid (HOCl) which is responsible for the disinfecting action of chlorine solutions [13, 14]. HOCl has been suggested to have an antimicrobial effect around 80–100 times stronger than the hypochlorite ion [15]. Moreover, it has greater available chlorine than NaOCl [16]. Therefore, it seems to have superior antimicrobial efficacy. Dutta *et al.* [16] evaluated CH for tissue-dissolving capacity and suggested its use as an endodontic irrigant.

Data about the antimicrobial efficacy of CH as an endodontic irrigant seem to be sparse. Thus, the aim of this paper was to assess the antimicrobial efficacy of 5% CH compared with 5.25% NaOCl, and 2% CHX against a multispecies biofilm in surface and deep dentinal tubules. The null hypothesis was that 5% CH has same antimicrobial efficacy as 5.25% NaOCl and 2% CHX.

Materials and Methods

Antibacterial activity and bacterial growth assay

The bacterial strains of *E. faecalis* American Type Culture Collection (ATCC) 29212, *F. nucleatum* ATCC 10953 and *P. intermedia* ATCC 25611 were prepared from Pasteur institute, Tehran, Iran. The Brain Heart Infusion medium were used as culture medium.

Teeth collection, storage and sterilization

For this *ex vivo* study, one hundred and thirty-five freshly extracted human permanent maxillary central incisors were collected and stored in 0.1% thymol solution until use. Extracted teeth were collected and enrolled in the study with the full consent of the patients in the city. Teeth with intact crowns and roots, complete root formation, round shape and no calcified canal were included in this study. External root surfaces were debrided of periodontal tissue and bone using Gracey curettes (Hu-Friedy Co., Chicago, IL, US). The experimental method used in this study was a modification of the method used by Haapasalo and Orstavik [17]. The cervical and apical portions of the roots were removed using double-faced cylindrical saw (Ref.070, D&Z, Berlin, Germany) under water-cooling. This left approximately the length 8-mm-long specimens using digital caliper (Mitutoyo digital caliper 500-714- 10, Mitutoyo Co, Tokyo, Japan). A Gates Glidden dril (GG) (Dentsply/Maillefer, Ballaigues, Switzerland) size 3 (0.9 mm in diameter) was used to enlarge each canal to create standard diameters in all specimens (Figure 1). The specimens were stored in distilled water during all procedures to prevent dehydration. To remove smear layer of the lumens, all specimens were individually placed in bottles containing 3 mL of 17% EDTA (Well-prep; Vericom Co., Anyang, Korea) and transferred to an ultrasonic bath (Bandelin, RK 102 P, Berlin, Germany) for 1 min. Specimens were then placed in bottles containing 3 mL 5.25% NaOCl (Golrang, Golrang Co.,Tehran, Iran) and ultrasonicated for 2 min. The roots were then washed with distilled water for 5 min to remove all traces of the chemicals and were then autoclaved (Dentin206 H, Farazmehr, Isfahan, Iran) at 121˚C for 30 min at 20 psi pressure. All teeth were cultured in Schaedler broth and incubated (01154, Behdad Digital incubator, Tehran, Iran) at 37˚C for 24 h before inoculation to confirm sterility.

The specimens were randomly divided into four experimental groups (*n*=30) according to the irrigation agent used as follows: Group 1: 2% CHX (Villevie, Joinville, SC, Brazil), group 2: 5.25% sodium hypochlorite (Golrang, Golrang Co., Tehran, Iran), group 3: 5% calcium hypochlorite (Aquafit, Tehran, Iran) and group 4: normal saline (positive control); and one negative group (*n*=15)(no infection).

Contamination with multispecies biofilm

The mixed culture bacterial suspension, containing *E. faecalis*, *F.* nucleatum and *P. intermedia*, were prepared at turbidity of 1.5×10^8 colony-forming units (CFU)/mL (equivalent to ≈0.5 McFarland standard) for each bacteria [18].

All the specimens were transferred to sterilized bottles containing 200 mL Schaedler broth to be inoculated by 5 mL mixed bacterial suspension. The contaminated specimens were cultured for 21 days [18] under anaerobic condition in anaerobic jar to allow biofilm formation and penetration into the canals. Meanwhile, 100 mL culture media was replaced every other day. Moreover, aliquots of cultures from each group were sampled, stained by the gram method and observed under light microscope to track the growth of all 3 tested species and to rule out contamination.

Antimicrobial assessment

After this period, each specimen was removed from the bottle under aseptic condition and the outer surfaces dried with sterile gauze pads and the canals were dried with sterile paper points (Aria dent, Asia Chemiteb Co, Tehran, Iran). The outer, apical and coronal surfaces of the specimens were covered with 2 layers nail varnish in order to prevent contact of the irrigants with the external surface. The apex end of each specimen was sealed with temporary cement (Zoliran, Tehran, Iran). The specimens were sampled for bacterial viability (S1), CFU and spectrophotometry, before irrigation to assess bacterial penetration using the protocol suggested by Xie *et al.* [18]. The root canal of each specimen was filled with phosphate-buffered saline (Cyto Matin Gene Co., Esfahan, Iran) and instrumented up and down and circumferentially with K-file ISO size #15 (Dentsply/Maillefer, Ballaigues, Switzerland) for 10 sec to disrupt the biofilm. Each file was discarded after a single use. Three consecutive sterile paper points (55-60-70) were placed into the root canal to absorb the canal contents and transferred to sterile bottles containing 2 mL BHI broth. The bottles were vortexed for 60 sec and the turbidity of the medium was visualized using spectrophotometry. The measurement of optical turbidity of the medium was proportional to the number of present bacteria. The purity of the cultures was confirmed by gram staining and colony morphology and any specimens with other contamination were excluded from the study.

The specimens in each experimental group were irrigated for 10 min with 5 mL mentioned irrigant agents. The irrigants were injected into the canals using a 27 gauge syringe (Supa, Tehran, Iran) and instrumented up and down and circumferentially for 1 min with K-file ISO size #30 for better penetration of irrigants. The files were discarded after a single use. In order to prevent carry-over effect, each specimen was additionally irrigated with 3 mL 0.5% thiosulfate for NaOCl group, 0.5 % Tween 80 (Merck, Darmstadt, Germany) for the CHX group and 0.5 % combination of 0.5% thiosulfate and 0.5% citric acid for the CH group [12]. All specimens were then irrigated with 3 mL of sterile saline and dried with sterile paper points.

Dentine samples

Dentine chips from within the lumen of all infected and non-infected specimens were collected using the GG drills to test for bacterial survival. GG drills ISO size 4 (1.1 mm in diameter) and ISO size 5 (1.3 mm in diameter) were used three times throughout the whole extension of lumen to create dentin shavings from the surface and deeper dentin of the specimens respectively. The chips from each depth were immediately collected into sterile bottles containing 3 mL of BHI broth separately and mixed for one min. For volume standardization, the bottles were weighed with a digital scale (A&D, GF600, Tokyo, Japan) before and after dentine collection. Dentine chips had to weigh approximately 4 mg. After 10 min, one mL of the upper part of the BHI was taken. Medium turbidity was visualized with spectrophtometry at 625 nm wavelengths. This was compared with BHI with uninfected dentin powder to omit a dentin powder turbidity effect. At this time another culture was made on BHI agar plus blood and the purity of the cultures was confirmed by Gram staining and CFU.

Statistical analysis

The obtained data were verified with the Kolmogorov-Smirnov test for the normality of the data distribution and the Levene test for the homogeneity of the variances. Statistical analysis was performed using the parametric one-way analysis of variance test and Tukey post hoc test (SPSS 20.0, SPSS Inc., IL, USA). Paired-samples *t*-test used to compare surface and deep dentin contamination within test groups. The statistical significance was set at a confidence level of 0.05.

Table 1. The effect of different irrigants on the total viable bacterial count before and after irrigation ($P<0.05$) **[Mean (SD)]**

Group	Baseline		Surface dentin		Deep dentin	
	Medium turbidity	CFU/mL * 10^8	Medium turbidity	CFU/mL * 10^8	Medium turbidity	CFU/mL * 10^8
CHX	0.052 (0.038) ^{A}	0.980 (0.048) ^{A}	0.027 (0.022) ^{AD}	0.512 (0.037) ^{AD}	0.037 (0.023) ^{EH}	0.706 (0.036) ^{EH}
Ca(OCl)2	0.048 (0.036) ^{A}	0.901 (0.025) ^{A}	0.033 (0.026) ^{A}	0.627 (0.030) ^{A}	0.042 (0.029) ^{E}	0.795 (0.025) ^{E}
NaOCl	0.048 (0.033) ^{A}	0.918 (0.043) ^{A}	0.052 (0.019) ^{B}	0.993 (0.043) ^{B}	0.066 (0.039) ^{F}	1.251 (0.043) ^{F}
NS (posetive)	0.060 (0.035) ^{A}	1.140 (0.035) ^{A}	0.077 (0.031) ^{C}	1.452 (0.022) ^{C}	0.095 (0.039) ^{G}	1.792 (0.026) ^{G}
Negative	0.007 (0.005) ^{B}	0.143 (0.022) ^{B}	0.010 (0.002) ^{D}	0.188 (0.051) ^{D}	0.010 (0.002) ^{H}	0.191 (0.043) ^{H}

Similar alphabetic letters show no significant differences

Table 2. The pH of tested agents

Irrigant	2% CHX	5% $Ca(OCl)_2$	5.25% NaOCl	0.5 % Tween 80	0.25% thiosulfate+0.25% citric acid	0.5% thiosulfate
pH	9.15	12.03	12.58	3.05	2.57	6.15

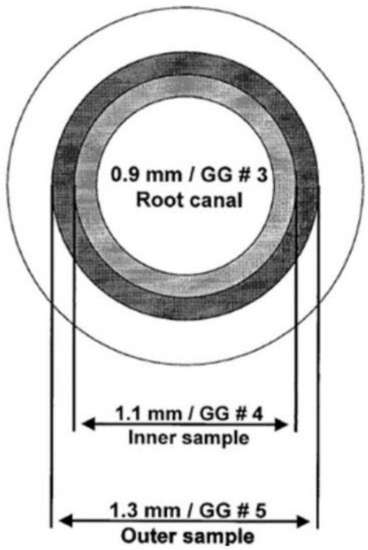

Figure1. Axial view of the diameter of each root sample and illustrating the inner sample compare to outer sample that has been collected by means of GG 4 and 5

Results

In this study baseline bacterial assessment showed no significant differences among the experimental groups (*P*=0.648). The negative group showed no infection. All test agents showed a statistically significant reduction in bacteria compared with normal saline (positive control) in both surface and deep dentine (*P*<0.001). ANOVA analysis demonstrated significant differences in both surface and deeper dentin among experimental groups (*P*<0.001). CH had a significantly lower medium turbidity compared with NaOCl and therefore lower CFU (colony-forming units)/mL in both surface (*P*=0.018) and deep dentin (*P*=0.031). Moreover, CH and CHX had the lowest medium turbidity among experimental groups and there were no significant differences among them with surface dentine (*P*=0.862) and deep dentine (*P*=0.978). Also 2% CHX did not show a significant difference compared with the negative group at the surface (*P*=0.164) and deep dentine (*P*=0.057). Comparing 2% CHX and 5.25% NaOCl, results demonstrated that CHX had significant lower medium turbidity in both surfaces (*P*=0.001) and deep dentine (*P*=0.005). The mean CFU/mL are shown in Table 1.

In all test groups, S3 had statistically significant higher medium turbidity except CH which was high but not significantly different from S2 surface dentine (Table 1). The pH of irrigants solutions are shown in at Table 2.

Discussion

The most fundamental step of root canal treatment is reducing the load of bacteria by thermomechanical preparation. In the present study, the antimicrobial efficacy CH as a fairly new irrigant were evaluated and compared with sodium hypochlorite and chlorhexidine. Our result showed that CH had the best antimicrobial efficacy against endodontics biofilm. The difference was not significant compare to CHX group in both surface and deep dentine. Both CH and CHX had better antimicrobial efficacy than the NaOCl group.

In the current study specimens were prepared as modified Haapasalo and Orstavik [17] model. We used human teeth instead of bovine teeth; based on the study by Basrani *et al.* [19]. As they showed that antimicrobial efficacy of irrigants is affected by size of the canal lumens [19]. Bovine blocks are 3 times larger than human blocks; so human dentin is more appropriate to simulate the clinical situation [19].

In this study a mixed-culture biofilm model was used, because the nature of endodontic infections is polymicrobial [20]. Many studies use single species bacteria particularly *E. faecalis* [21, 22]. *E. faecalis* is the very resistant microorganism to a wide range of disinfecting agents and may be responsible for some failures of endodontic treatment [23], other bacteria may significantly influence the properties of *E. faecalis* and its virulence, which emphasizes the importance of the multispecies model system study [9]. Complex biofilm communities are recognized as endodontic infections etiology consisting cocci, rods and filamentous [20]; so *F. nucleatum, P. intermdeia* and *E. faecalis* was utilized in the current study.

Many *in vitro* studies use a planktonic culture to investigate the antimicrobial efficacy of endodontic irrigants [24]. However, a biofilm model of bacteria reproduces approximate picture to clinical condition [8]. Biofilm mode of growth is more resistant to antimicrobial agents than planktonic form [8]. An interval of 21 days for bacterial biofilm growth was selected based on the recommendation by Haapasalo and Orstavik [17].

The majority of the root canal system bacteria have better growth at 6.5<pH<7.5 and are not able to survive at higher pH except a few of them. The pH of the irrigant solution affects the balance of HOCl and OCl^- and consequently the amount of available chlorine [13]. At pH>8.5, hypochlorite ions (OCl^-) predominate, whereas at pH<6.5 the HOCl molecule is dominant. At pH values between 6.5 and 8.5, they are in a state of equilibrium [15]. It is the hypochlorous acid (HOCl) that is responsible for the disinfecting action of the endodontic irrigant solution [13]. The pH of the solutions tested in our study is shown in Table 2.

Residual bacteria within dentinal tubules may cause reinfection and as reported previously *E. faecalis* can survive at depths of up to 300 µm within dentinal tubules [25]. Thus, penetration of the irrigant is important to achieve predictable disinfection. To evaluate this characteristic, the superficial (200 µm) and deep (400 µm) dentine was investigated as previously described [17, 26].

Recently CH was tested as an endodontic irrigant by Dutta and Saunders [16]. It is one of the chlorine solutions which is widely used for several disinfection purposes particularity for water purification treatment [27]. The CH solution has reasonably low cost. It is also safer for clinical use than NaOCl because the initial rate of tissue dissolution by CH is slower than NaOCl and less tissue irritant [16]. It also has greater available chlorine than NaOCl (up to 65% available chlorine) [16]. Its byproducts in freshly prepared aqueous solution ($Ca(OCl)_2 + 2 H2O \rightarrow 2 HOCl + Ca(OH)_2$) have both antimicrobial and tissue dissolving effect [16]. It may provide remarkable antimicrobial efficacy than calcium hydroxide. Despite all desirable properties its liberation of chlorine limits its usage; because of its effect on coronal seal and resulting symptoms [16]. Dutta and Saundres [16] concluded that CH has the potential of being a root canal irrigant. They also demonstrated the race of that tissue dissolution of NaOCl (4.65%) was faster than the CH solutions (5 and 10 %) over the first 35 min, but there were no significant differences among the solutions thereafter. CH has just been evaluated for its tissue-dissolving capacity but its antimicrobial efficacy as an endodontic irrigant has not been evaluated.

The duration for instrumentation and irrigation affects the antimicrobial efficacy of the irrigant [22], so we selected 10 min on the basis of study by Du *et al.* [22]. Also in order to avoid a carry-over effect, specific neutralizers were applied after the end of irrigation time. It means the effectiveness of each irrigant was only determined during the irrigation procedures.

There are different methods to investigate bacterial viability such as polymerase chain reaction (PCR) [28], confocal laser scanning microscopy (CLSM) [21], *etc.* We used spectrophotometer to approximate information of the bacteria recovered after the irrigation protocol. The method to determine the contamination conditions and show number of present bacteria (CFU/mL) [29].

In our study there were no significant differences in the baseline bacterial load assessment among the test groups. This indicated the consistency and reliability of the experimental design and revealed homogeneous baseline of bacteria load. The mean CFU/mL of baseline bacteria was the same as Xie *et al.* [18] study and is shown at Table 1.

All experimental solutions significantly reduced bacterial load in both surface and deep dentine compared with normal saline (positive control). This revealed their potent antimicrobial efficacy.

The presence of dentine debris because of the light absorption consume as a confounding factor. For tackling this issue, BHI medium mixed with uninfected dentin debris were used as a blank solution to compare with medium turbidity of each sample to omit dental powder turbidity effect.

In this study 5% CH had significant greater antimicrobial activity than 5.25% NaOCl in both surface and deep dentine. Sodium hypochlorite is widely used as an endodontic irrigant due to its desirable properties [30], but its noxious effects which might damage periapical tissue [1]. Its effects depend on the concentration and contact time and pH [31]. Lower concentrations are utilized to reduce this possible toxicity [32]; but with a reduction in the concentration, the antimicrobial efficacy is decreased [32]. The most efficient concentration is 5.25% [32]. There are conflicting results on the antimicrobial efficacy of NaOCl. Several investigations show that NaOCl has strong capability to reduce *E. faecalis* biofilm in an *in vitro* study [33], but it does not have such an influence in *ex vivo* [34] or *in vivo* [35] assessments. It could be because of dentine buffering effect [36]. Oliveira *et al.* [37] reported an immediate reduction in bacteria after the usage of 5.25% NaOCl; but bacteria re-colonized after 2-7 days in 80% of specimens.

In the present study 5% CH had the same antimicrobial activity as 2% CHX in both surface and deep dentine (P=0.862, P=0.978). In addition, 2% CHX was able to significantly destroy all bacteria at both depths (200 µm) (P=0.164) and deep dentine (400 µm) (P=0.057). This is in accordance with Krithikadatta *et al.* [26]; which showed that 2% CHX provided 100% inhibition of *E. faecalis* at the depths of 200 µm as well as 400 µm. Also Xie *et al.* [18] showed 100% inhibitory effect of 2% CHX against mixed-culture biofilm of *E. faecalis*, *F. nucleatum* and *P. intermedia*. Chlorohexidine has antimicrobial sensitivity against a broad spectrum of microbial species in all tested concentrations [38]. It is more biocompatible than NaOCl [38], but it has some side effects; interaction between CHX as final rinse with remaining of NaOCl produces para-chloroaniline (PCA) [39] which result in color change which may be clinically relevant and producing a precipitate which might interfere with the seal of the root filling [40]. Removal of the NaOCl before placing CHX into the canal is essential [39]. In addition, CHX is unable to dissolve organic tissue which is one of its main disadvantages [38], because organic/necrotic tissue remnants provide a source of nutrition for the surviving bacteria.

Comparing 2% CHX and 5.25% NaOCl, the present study demonstrated that CHX had significant greater antimicrobial efficacy than NaOCl in both surface and deep dentine. This result agrees with Ferraz *et al.* [41]. But Vianna *et al.* [42] showed that 5.25% NaOCl had a significantly greater antimicrobial effect than 2% CHX. It might be because of the difference in method and sample size. They used real-time quantitative-polymerase chain reaction (RTQ-PCR) for viable bacteria assessment on 32 specimens. Also Du *et al.* [22] demonstrated that the antimicrobial efficacy of 6% NaOCl was greater than 2% CHX. Maybe because they used confocal laser scanning microscopy and different exposure time (30 min). Gomes *et al.* [43] and Vianna *et al.* [44] demonstrated no significant differences between 2% CHX and 5.25% NaOCl which is in conflict with our result. They used broth dilution test and maybe it caused the different results. In their method, different microorganisms' culture (planktonic form) may affect the results. Xie *et al.* [18] also showed no significant differences between 2% CHX and 5.25% NaOCl. They used lower specimens (72 specimens) than our study and their method to assess bacterial viability was different.

Deep dentine had significant higher medium turbidity than surface dentine except with CH which demonstrates its penetration into dentinal tubules. Also 2% CHX and 5% CH had significant lower medium turbidity than 5.25% NaOCl in deep dentine (400 µm). It showed the ability of CHX in better diffusion into the dentinal tubules the depth over 400 µm. Gomes *et al.* [43] and Krithikadatta *et al.* [26] also reported this potential penetration of CHX, because of its good wettability [8]. The bactericidal activity of sodium hypochlorite may be only superficial because of it cannot remove the smear layer and its penetration into dentinal tubules is poor [45].

Notwithstanding the limits of the *ex vivo* model (its difficulty to directly correlate clinically), the null hypothesis was rejected. 5% CH and 2% CHX had greater antimicrobial efficacy than 5.25% NaOCl. Further studies are needed to assess the antimicrobial efficacy of CH and its real potential in clinical situation.

Conclusion

Under the conditions of this study, 5% CH and 2% CHX are more effective than 5.25% NaOCl in the reduction of a mixed-culture biofilm. However, to support this *in vitro* observation, further *in vivo* studies are needed.

Conflict of Interest: 'None declared'.

References

1. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J. 2014;216(6):299-303.
2. Hamama HH, Yiu CK, Burrow MF. Viability of intratubular bacteria after chemomechanical caries removal. J Endod. 2014;40(12):1972-6.
3. Peters OA, Laib A, Gohring TN, Barbakow F. Changes in root canal geometry after preparation assessed by high-resolution computed tomography. J Endod. 2001;27(1):1-6.
4. Awawdeh L, Jamleh A, Al Beitawi M. The Antifungal Effect of Propolis Endodontic Irrigant with Three Other Irrigation Solutions in Presence and Absence of Smear Layer: An In Vitro Study. Iran J Endod. 2018;13(2):234-9.
5. Nair PN. On the causes of persistent apical periodontitis: a review. Int Endod J. 2006;39(4):249-81.
6. Adcock JM, Sidow SJ, Looney SW, Liu Y, McNally K, Lindsey K, et al. Histologic evaluation of canal and isthmus debridement efficacies of two different irrigant delivery techniques in a closed system. J Endod. 2011;37(4):544-8.
7. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics: a review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;94(6):658-66.
8. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15(2):167-93.
9. Deng DM, Hoogenkamp MA, Exterkate RA, Jiang LM, van der Sluis LW, Ten Cate JM, et al. Influence of Streptococcus mutans on Enterococcus faecalis biofilm formation. J Endod. 2009;35(9):1249-52.
10. Nourzadeh M, Amini A, Fakoor F, Raoof M, Sharififar F. Comparative Antimicrobial Efficacy of Eucalyptus Galbie and Myrtus Communis L. Extracts, Chlorhexidine and Sodium Hypochlorite against Enterococcus Faecalis. Iran Endod J. 2017;12(2):205-10.
11. Zargar N, Dianat O, Asnaashari M, Ganjali M, Zadsirjan S. The Effect of Smear Layer on Antimicrobial Efficacy of Three Root Canal Irrigants. Iran Endod J. 2015;10(3):179-83.
12. Sedigh-Shams M, Gholami A, Abbaszadegan A, Yazdanparast R, Saberi Nejad M, Safari A, et al. Antimicrobial Efficacy and Cytocompatibility of Calcium Hypochlorite Solution as a Root Canal Irrigant: An in Vitro Investigation. Iran Endod J. 2016;11(3):169-74.
13. Fair GM, Morris JC, et al. Behavior of chlorine as a water disinfectant. Water Sew Works. 1949;96(5):101-4.
14. Roshani Edirisinghe EA, Anuruddhika Dissanayake DR, Abayasekera CL, Arulkanthan A. Efficacy of calcium hypochlorite and ultraviolet irradiation against Mycobacterium fortuitum and Mycobacterium marinum. Int J Mycobacteriol. 2017;6(3):311-4.
15. Rossi-Fedele G, Guastalli AR, Dogramaci EJ, Steier L, De Figueiredo JA. Influence of pH changes on chlorine-containing endodontic irrigating solutions. Int Endod J. 2011;44(9):792-9.
16. Dutta A, Saunders WP. Comparative evaluation of calcium hypochlorite and sodium hypochlorite on soft-tissue dissolution. J

Endod. 2012;38(10):1395-8.
17. Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res. 1987;66(8):1375-9.
18. Xie Q, Johnson BR, Wenckus CS, Fayad MI, Wu CD. Efficacy of berberine, an antimicrobial plant alkaloid, as an endodontic irrigant against a mixed-culture biofilm in an in vitro tooth model. J Endod. 2012;38(8):1114-7.
19. Basrani B, Santos JM, Tjaderhane L, Grad H, Gorduysus O, Huang J, et al. Substantive antimicrobial activity in chlorhexidine-treated human root dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;94(2):240-5.
20. Narayanan LL, Vaishnavi C. Endodontic microbiology. J Conserv Dent. 2010;13(4):233-9.
21. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. J Endod. 2014;40(2):285-90.
22. Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M. Effect of long-term exposure to endodontic disinfecting solutions on young and old Enterococcus faecalis biofilms in dentin canals. J Endod. 2014;40(4):509-14.
23. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006;32(2):93-8.
24. Shrestha A, Kishen A. The effect of tissue inhibitors on the antibacterial activity of chitosan nanoparticles and photodynamic therapy. J Endod. 2012;38(9):1275-8.
25. Peters LB, Wesselink PR, Moorer WR. Penetration of bacteria in bovine root dentine in vitro. Int Endod J. 2000;33(1):28-36.
26. Krithikadatta J, Indira R, Dorothykalyani AL. Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments. J Endod. 2007;33(12):1473-6.
27. Ufermann P, Petersen H, Exner M. Disinfection aboard cruise liners and naval units: formation of disinfection by-products using chlorine dioxide in different qualities of drinking water. Int J Hyg Environ Health. 2011;215(1):86-90.
28. Fouad AF, Barry J. The effect of antibiotics and endodontic antimicrobials on the polymerase chain reaction. J Endod. 2005;31(7):510-3.
29. Estrela C, Sousa-Neto MD, Alves DR, Alencar AH, Santos TO, Pecora JD. A preliminary study of the antibacterial potential of cetylpyridinium chloride in root canals infected by E. faecalis. Braz Dent J. 2012;23(6):645-53.
30. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389-98.
31. Zhang K, Kim YK, Cadenaro M, Bryan TE, Sidow SJ, Loushine RJ, et al. Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. J Endod. 2010;36(1):105-9.
32. Martin DE, De Almeida JF, Henry MA, Khaing ZZ, Schmidt CE, Teixeira FB, et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod. 2014;40(1):51-5.
33. Neglia R, Ardizzoni A, Giardino L, Ambu E, Grazi S, Calignano S, et al. Comparative in vitro and ex vivo studies on the bactericidal activity of Tetraclean, a new generation endodontic irrigant, and sodium hypochlorite. New Microbiol. 2008;31(1):57-65.
34. Siqueira JF, Jr., Machado AG, Silveira RM, Lopes HP, de Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal, in vitro. Int Endod J. 1997;30(4):279-82.
35. Oncag O, Hosgor M, Hilmioglu S, Zekioglu O, Eronat C, Burhanoglu D. Comparison of antibacterial and toxic effects of various root canal irrigants. Int Endod J. 2003;36(6):423-32.
36. Morgental RD, Singh A, Sappal H, Kopper PM, Vier-Pelisser FV, Peters OA. Dentin inhibits the antibacterial effect of new and conventional endodontic irrigants. J Endod. 2013;39(3):406-10.
37. Oliveira DP, Barbizam JV, Trope M, Teixeira FB. In vitro antibacterial efficacy of endodontic irrigants against Enterococcus faecalis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103(5):702-6.
38. Wang CS, Arnold RR, Trope M, Teixeira FB. Clinical efficiency of 2% chlorhexidine gel in reducing intracanal bacteria. J Endod. 2007;33(11):1283-9.
39. Basrani BR, Manek S, Sodhi RN, Fillery E, Manzur A. Interaction between sodium hypochlorite and chlorhexidine gluconate. J Endod. 2007;33(8):966-9.
40. Vivacqua-Gomes N, Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ. Influence of irrigants on the coronal microleakage of laterally condensed gutta-percha root fillings. Int Endod J. 2002;35(9):791-5.
41. Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ. Comparative study of the antimicrobial efficacy of chlorhexidine gel, chlorhexidine solution and sodium hypochlorite as endodontic irrigants. Braz Dent J. 2007;18(4):294-8.
42. Vianna ME, Horz HP, Gomes BP, Conrads G. In vivo evaluation of microbial reduction after chemo-mechanical preparation of human root canals containing necrotic pulp tissue. Int Endod J. 2006;39(6):484-92.
43. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. Int Endod J. 2001;34(6):424-8.
44. Vianna ME, Gomes BP, Berber VB, Zaia AA, Ferraz CC, de Souza-Filho FJ. In vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;97(1):79-84.
45. Berutti E, Marini R, Angeretti A. Penetration ability of different irrigants into dentinal tubules. J Endod. 1997;23(12):725-7.

***Please cite this paper as*:** Shadmehr E, Davoudi A, Sarmast ND, Saatchi M. Evaluation of Antimicrobial Efficacy of Calcium Hypochlorite as an Endodontic Irrigant on a Mixed-culture Biofilm: An *Ex vivo* Study. Iran Endod J. 2019;14(3): 178-84. *Doi*: *10.22037/iej.v14i3.22451*.