Original Article

Antibiofilm activity of sodium hypochlorite against enterococcus faecalis using four irrigant activation protocols

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Abstract

Aims: The aim of the study was to compare the activity of sodium hypochlorite (NaOCl) against *Enterococcus faecalis* when used with four different irrigation protocols.

Methodology: Sixty-five single-rooted mandibular premolars with closed apex were prepared till size 35/0.04. The specimens were sterilized and infected with *E. faecalis* colonies that were cultured separately. The canals were randomly divided into four experimental groups based on irrigation activation protocol, with each group having 15 specimens each – Group 1: control, Group 2: manual dynamic agitation (MDA), Group 3: passive ultrasonic irrigation (PUI), Group 4: intracanal heating (ICH), and Group 5: passive ultrasonic irrigation followed by ICH (PUI ICH). The dentinal shavings were collected and sampled before (S1) and after (S2) the different irrigation techniques were performed. The colony-forming units were counted, and the bacterial reduction was calculated for each group.

Results: A significant reduction in the number of *E. faecalis* colonies was observed for all the experimental groups ($P < 0.001$). The groups with ICH of NaOCl showed a considerable reduction in bacterial colonies than other groups ($P < 0.001$), with Group 5 that combined ultrasonics with ICH showed the highest reduction.

Conclusion: ICH of NaOCl may be used as an adjunct to root canal irrigation to reduce the bacterial concentration from root canal spaces.

Keywords: Colony-forming units; *Enterococcus faecalis* biofilms; intracanal heating; manual dynamic agitation; passive ultrasonic irrigation; sodium hypochlorite

INTRODUCTION

The ultimate goal of endodontic treatment is the elimination of inflamed or necrotic pulp tissue and infecting microorganisms from the root canal space before three-dimensional obturation. Fulfilling this objective is more complex in infected teeth, as microorganisms have greater potential to lodge themselves within canal ramifications. It has been observed that a major cause of failure of the endodontic treatment is the survival of microorganisms in inaccessible areas, such as fins, anastomoses, apical delta, accessory canals, and isthmuses.[1-3]

A common organism associated with such failures is *Enterococcus faecalis*. This is a Gram-positive facultative anaerobe, found in 40% of primary endodontic infections. However, its presence is nine times more likely in persistent endodontic infections due to its survival strategies and ability to alter the host response.[4]

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The use of nickel–titanium files, complemented by the action of irrigating solutions, helps in shaping and disinfecting the root canal system.[5] Sodium hypochlorite (NaOCl) is the most widely used irrigant in endodontics due to its antimicrobial action and tissue-dissolving capacity.[6,7] However, manual delivery by syringe still limits its effectiveness in the apical one-third of the root canal.

Recognition of this difficulty has led to various innovative techniques to facilitate the penetration of the irrigant. Endeavors have been constantly made to develop more effective irrigant delivery and agitation systems. Methods such as manual dynamic agitation, machine-assisted rotary brushes, ultrasonic and sonic activation, and apical negative pressure have provided optimum disinfection of the root canal spaces.[8,9]

Manual dynamic agitation (MDA) has been reported to overcome the vapor lock effect and agitate the irrigant in the apical one-third of the canal.[10]

The use of ultrasonic energy to activate the irrigant has contributed vastly to enhance the cleansing of the root canal system. Passive ultrasonic irrigation (PUI) after completion of the canal preparation is the recommended technique.[11]

The efficacy of irrigants can also be amplified using additional strategies. One simple means of activation is to preheat NaOCl to a temperature of 50°C.

Extraorally heated NaOCl has been shown to have superior antimicrobial and tissue-dissolving properties.[12,13] Recently, intracanal heating (ICH) has been reported to provide more sustained temperature maintenance for enhancing bacterial elimination and tissue-dissolving capacity of NaOCl. There are, however, very few studies on this approach.[14-16]

However, there are little published scientific data comparing some of the new and emerging devices and methods for disinfection. Therefore, this study aims to compare the antibiofilm activity of NaOCl using four protocols: MDA, PUI, ICH, and a combination of PUI and ICH. The null hypothesis is that there would be no difference in the antibiofilm activity for all four protocols.

METHODOLOGY

Sixty-five freshly extracted single-rooted mandibular premolars with a closed apex were selected for the study after obtaining prior consent from the patient. The teeth were decoronated at or near the cementoenamel junction to obtain roots of a standardized length of 14 mm. Access was refined with Endo-Z bur. A size 10-K type file was inserted into each canal until its tip was visible at the apical foramen. The working length (WL) was established by subtracting 0.5 mm from this measurement. All the canals were prepared till the WL using nickel–titanium rotary instruments (NeoEndo Flex rotary files – Orikam Healthcare) using endomotor (NSK) till the size 35/0.04 to the full WL. During the cleaning and shaping procedure, the canals were irrigated with 2.5% NaOCl using a 30-gauge, side-vented endodontic irrigation needle in a syringe. A total of 6 ml of 2.5% NaOCl was used for every tooth. The root canals were then rinsed with sterile saline, followed by irrigation with 3 ml of 17% ethylenediaminetetraacetic acid for 1 min to remove the smear layer. All canals were given a final rinse of 3 ml of sterile saline. Sterile paper points were then used to dry the canals. The tooth specimens were further sterilized in an autoclave at 121°C and 15 psi for 20 min. The root surfaces and the apices of all the teeth were sealed with nail varnish.

Specimen contamination

E. faecalis (ATCC 29212 strain) was cultured anaerobically on Soyabean Casein Digest Agar (SCDA) at 37°C for 24 h. Inoculum was prepared in sterile Trypticase Soy Broth, and turbidity was set at 0.5 McFarland corresponding to approximately 1.5×10^8 colony-forming units per milliliter (CFU/ml).

All the canals were inoculated with 10 µl of the *E. faecalis* culture. The specimens were incubated for 21 days at 37°C with periodic replacement with fresh broth. After incubation, the specimens were gently rinsed with phosphate-buffered saline in order to remove the culture media and nonadherent bacteria from the root surfaces.

Experimental groups

Five specimens were selected arbitrarily to serve as a negative control (Group 1), where no activation was performed.

Sixty tooth specimens were randomly distributed into four experimental groups:

- Group 2: Manual dynamic agitation (MDA)
- Group 3: Passive ultrasonic irrigation (PUI)
- Group 4: Intracanal heating (ICH)
- Group 5: Passive ultrasonic irrigation - Intracanal heating (PUI ICH)

where each group consisted of 15 specimens ($n = 15$/group).

Group 2: Manual dynamic agitation (MDA)

Each canal was filled with room temperature 1% NaOCl (vol: 3 ml). A well-matching gutta-percha (GP) master cone whose taper is slightly less than the taper of the canal was selected (35/0.02). The length of the GP was maintained at 13 mm (WL). Manual agitation of the master cone was done with an up-and-down motion at a frequency of 50 strokes for 30 s.

Group 3: Passive ultrasonic irrigation (PUI)

Each canal was filled with room temperature (28°C) 1% NaOCl (vol: 3 ml). An ultrasonic tip with a noncutting end was mounted on an ultrasonic device (Eighteeth Medical Ultra X) and inserted passively 1 mm short of the WL and activated for 30 s.

Group 4: Intracanal heating (ICH)

Each canal was filled with room temperature 1% NaOCl (vol: 3 ml). The E and Q Plus obturation unit (Meta Biomed) was used. The plugger tip (25.4%) was passively inserted inside the root canal without contacting the walls. The tip was kept 3 mm short of the WL. The temperature was set at 150°C, and the tip was activated for 10 s bursts and left nonactivated for 10 s. The heat carrier was moved with small in-and-out movements in the canal during the procedure. Care was taken not to wedge the tip against the canal walls.

Group 5: Passive ultrasonic irrigation - Intracanal heating (PUI-ICH)

Each canal was filled with room temperature 1% NaOCl (vol: 3 ml). An ultrasonic tip with a noncutting end was inserted passively 1 mm less than the W/L and activated for 30 s. It was immediately followed by the passive placement of the heat carrier of E and Q Plus into the root canal space, 3 mm short of the WL. The temperature was set at 150°C, and the tip was activated for 10 s bursts.

Three cycles of activation were carried out for all the experimental groups, and the canals were flushed with 1 ml room temperature 1% NaOCl after each activation cycle.

All solutions were delivered into the canal using a 3-ml syringe and a Prime Dental 30-gauge side-vented endodontic irrigation needle kept around 2 mm short of the WL.

All the procedures were carried out in a laminar flow chamber to maintain sterile conditions.

At the end of each procedure, NaOCl was inactivated with 5 ml of 2M sodium thiosulfate for 30 s. The canals were further dried with sterile paper points of size 30/0.04.

Root canal sampling procedure

The first sample (S1) was taken from each canal before executing the activation protocols. The dentin debris was collected by dry filing the canals using an H-file of size 30 for 15 s to the entire WL. The H-file was immediately placed in Eppendorf tube containing saline so that the dentin shavings could disperse off. The solution was homogenized by vortex mixer (Tarson 3002 Spinix), serially diluted (1×10^4), plated on SCDA, and incubated for 48 h at 37°C. CFUs were counted.

A second sample (S2) was taken from each canal after implementing the experimental irrigation protocol. The number of CFUs was determined for the samples as described for S1. The data were entered into Excel sheet and subjected to statistical analysis [Figure 1].

RESULTS

- Mean CFU was higher in Group 1 – 745 ± 36.332 followed by Group 2 – 452.53 ± 38.972, Group 3 – 155.33 ± 42.538, Group 4 – 64.73 ± 17.79, and Group 5 – 17.47 ± 10.274 [Figure 1]
- All the test samples passed the efficacy with >1 log reduction in test organisms at 24 h of incubation [Figure 2a-e]
- Group 5 has shown enhanced efficacy against *E. faecalis* compared to the other groups [Figure 2e]
- Data were subjected to normalcy test (Shapiro–Wilk test). As data showed normal distribution, hence parametric tests (ANOVA with *post hoc* Bonferroni) were applied [Table 1]
- ANOVA test was applied to compare the CFU among the groups. ANOVA test showed a statistically significant difference among the groups ($P = 0.00$) [Table 1]
- *Post hoc* Bonferroni test showed a statistically significant difference between all the groups ($P \leq 0.05$) [Table 2].

DISCUSSION

E. faecalis is a highly resistant Gram-positive anaerobe capable of surviving harsh environmental conditions despite the use of disinfectants and intracanal medicaments during chemomechanical preparation. It can proliferate to form monospecies intracanal biofilms and has the ability to

Table 1: Mean colony forming units of the groups

Groups	*n*	Minimum	Maximum	Mean	S.D	*P*
1	5	700	783	745	36.332	0.00*
2	15	380	520	452.53	38.972	
3	15	96	220	155.33	42.538	
4	15	41	113	64.73	17.79	
5	15	4	38	17.47	10.274	

*significant

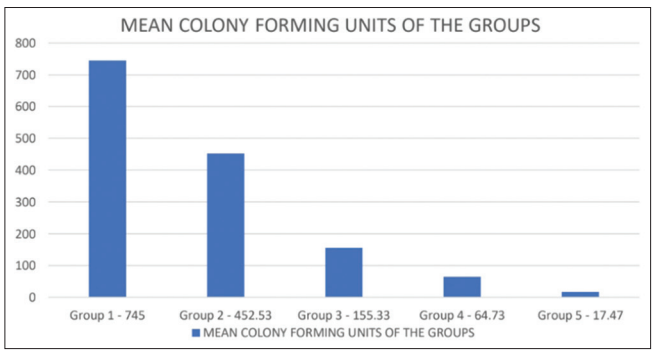

Figure 1: Mean colony-forming units of the groups

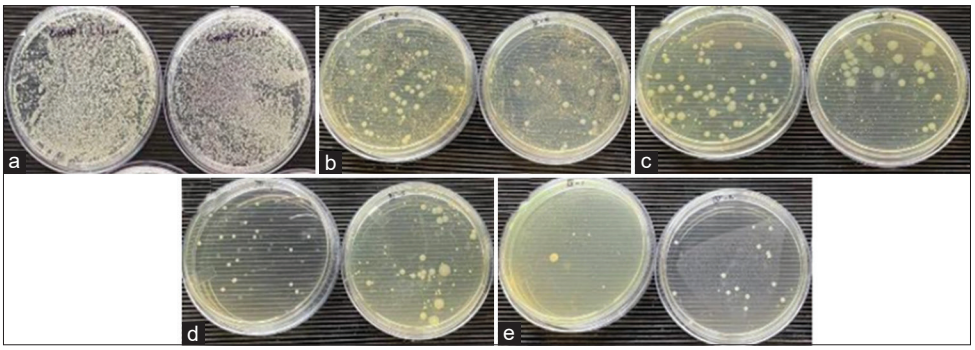

Figure 2: Groups showing *Enterococcus faecalis* colonies. (a) Group 1: Control (preactivation), (b) Group 2: Manual dynamic agitation, (c) Group 3: Passive ultrasonic irrigation, (d) Group 4: Intracanal heating (ICH), (e) Passive ultrasonic irrigation followed by ICH

Table 2: Intergroup comparison of the colony forming units using *post hoc* bonferroni

Group number	Comparison group	Mean Difference	*P*
1	2	589.667	0.000*
	4	680.267	0.000*
	5	727.533	0.000*
	2	292.467	0.000*
3	4	90.600	0.000*
	5	137.867	0.000*
	2	-297.200	0.000*
4	5	47.267	0.001*
	2	-387.800	0.000*
5	2	-435.067	0.000*

*The mean difference is significant

survive at high temperatures.[4,17] *E. faecalis* was, therefore, chosen as the test organism in this study.

NaOCl is the primary irrigant of choice for endodontic treatment due to its antimicrobial properties. However, at high concentrations of 5.25%, it demonstrates increased toxicity.[12] Warming hypochlorite increases its potency and favors the use of lower concentration with less toxicity.[18,19] Hence, in the present study, NaOCl was used at a concentration of 1%.

To enhance the penetration of the irrigant into the complexities of the root canal system as well as prolong its contact with the canal walls, irrigant activation is recommended.[2,5,20,21]

The present study used MDA, PUI, and ICH. MDA is a simple technique that generates higher hydrodynamic pressure, creating more turbulence and leading to the effective delivery of irrigant to the untouched canal surfaces. PUI produces a number of biophysical effects, such as acoustic streaming and cavitation, which disrupt the bacterial biofilms.[10,12,13] ICH of NaOCl has a number of benefits. As the temperature increases, a lower concentration of NaOCl would suffice to eliminate bacteria and dissolve pulp tissue.[13,15,16] In our study, E and Q fill plugger tip (25/0.04) was employed for intracanal warming of NaOCl. It was placed passively 3 mm short of the WL and activated at 150°C.

The colony counter was the method used to assess the CFUs of *E. faecalis*. This is a simple, rapid, and economical method although molecular methods such as polymerase chain reaction and DNA-DNA hybridization are more precise.[22] Mandibular premolars used in the study had single and straight root canals, which permitted the irrigation cannula to be inserted close to the WL.[23]

The results of this study showed that Group 2 (MDA) showed the least reduction in bacterial colonies. This was followed by Group 3 (PUI) and Group 4 (ICH). The best result was seen in Group 5 (PUI + ICH). Hence, the null hypothesis was rejected.

For Group 5, the rationale is that ultrasonic activation of NaOCl before ICH would facilitate the removal of pulpal remnants and deeper penetration of the irrigant for a more focused action of heat on the bacterial biofilms.[24] This is supported by recent studies in natural as well as artificially simulated canals, which showed that the combination helped in rendering cleaner canals compared to the use of either PUI or ICH alone.[12,13] While ultrasonic activation enhances the fluid flow of the irrigant, ICH of NaOCl enhances its breakdown, increasing the concentration of available chlorine to provide a synergistic effect. There is a significant reduction of hard tissue debris and better bacterial elimination by exposing more microorganisms to the action of the heated NaOCl.[25] Yared and Al Asmar Ramli[26] protocol was employed for ICH of NaOCl. The temperature of the plugger tip was set at 150°C. Recent studies have shown that this does not have any detrimental effect on the periodontal ligament.[12,27] During intracanal activation, the heat carrier was moved up and down in short amplitudes (2 mm) to create turbulence in the irrigant.[8] In this protocol, the irrigant was also frequently replenished to enhance the concentration of active chlorine. These two factors are probable reasons for overall improved canal disinfection.

Studies have shown that warming NaOCl within the root canal amplifies its antibacterial effect. Modified Woodmansey's technique of activating NaOCl has

been shown to disintegrate necrotic pulp tissue at an accelerated rate 210 times greater than room temperature NaOCl. *In vitro* studies have reported a higher percentage of bacterial reduction when hypochlorite was used at elevated temperatures.[12,15] This was also the case in the present study, as Group 4 (ICH) showed superior results compared to Group 3 (PUI).

As compared to conventional irrigation protocols, PUI generates eddy currents, which helps the irrigant to reach the anatomical complexities, but there is a very limited rise in temperature.[8] Previous studies have also shown that while ultrasonic agitation of NaOCl is successful in disrupting bacteria, it cannot ensure complete disruption of all microorganisms. Other researchers have also concluded that ultrasonic agitation of NaOCl without the combination of heat was not successful in the total eradication of bacterial biofilms.[11]

The primary advantage of MDA (Group 2) is the simplicity of the technique, which helps to overcome the vapor lock effect in the apical region, allowing better irrigant action in the apical portion of the canal.[10] However, in our study, it proved to be ineffective in eliminating *E. faecalis* biofilms as compared to PUI, ICH, and their combination. A previous study has attributed this to lower-frequency vibrations (3.3 Hz) generated by MDA as compared to higher-frequency vibrations produced by PUI (40–45 KHz). It is also laborious and time-consuming.[10]

Finally, our results suggest that the combination of PUI and ICH may be a viable means to eliminate *E. faecalis* biofilms from the root canals. This must, therefore, be regarded as a possible alternative to conventional irrigation protocols.

There are, however, some limitations to the present study due to its *in vitro* nature. Single-rooted teeth with relatively straight canals were employed in our study. Future studies should consider performing these irrigant activation techniques in complex root canal morphologies. Research should also be conducted to include more advanced heating tips as well as laser devices for the activation of NaOCl.

Further, *in vivo* studies and randomized control trials are necessary to encourage the use of ICH along with other agitation techniques in routine clinical practice.

CONCLUSION

- Irrigant activation at the end of the canal preparation plays a significant role in the elimination of *E. faecalis* Biofilms.
- In the present study, compared to the initial sample, all the experimental groups demonstrated a considerable reduction in the number of bacterial colonies.
- Group 5 (PUI-ICH) demonstrated the maximum reduction, while Group 2 (MDA) was the least effective. This is probably due to the fact that chemical reactions accelerate with temperature elevations.
- The ICH irrigation protocol presented here may be effective due to the elevation in kinetic energy and the boiling action of the irrigant.
- The combined use of ICH of NaOCl and ultrasonic agitation seems to be an effective, reliable, and simple solution for three-dimensional disinfection of the root canal system.

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Conflicts of interest

There are no conflicts of interest.

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