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Comparisons among three supplementary irrigation techniques and a calcium hydroxide dressing for bacterial elimination after chemomechanical preparation using the self-adjusting file

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ABSTRACT

Introduction: Bacterial elimination from the root canal is the ultimate goal of endodontic treatment. Many supplementary systems and substances have been introduced to improve root canal disinfection. This study aimed to compare the effectiveness of sonic and ultrasonic-activated irrigation, a chlorhexidine (CHX) final rinse, and a calcium hydroxide [$Ca(OH)_2$] dressing in eliminating bacteria after chemomechanical preparation of root canals using the self-adjusting file (SAF).
Methods: Eighty maxillary and mandibular premolars were inoculated with Enterococcus faecalis for 4 weeks, instrumented with SAF, and randomly distributed into four test groups ($n = 15$) according to the supplementary approach used for bacterial elimination: EndoActivator (EA) irrigation, passive ultrasonic irrigation (PUI), CHX final rinse, and $Ca(OH)_2$ dressing. Two groups ($n = 10$) used as a positive and negative controls. Bacteriological samples were obtained from the canals before and after SAF preparation and after the supplementary approaches. The number of bacteria in each sample was determined by plate count.
Results: The bacterial population significantly decreased after SAF preparation ($P < 0.001$). EA irrigation and PUI were significantly more effective than the CHX rinse and $Ca(OH)_2$ dressing in decreasing bacterial colony-forming units ($P < 0.05$).
Conclusions: EA irrigation and PUI after chemomechanical preparation using SAF were more effective than the CHX final rinse and $Ca(OH)_2$ dressing in decreasing root canal infection.

1. Introduction

The ultimate goal of chemomechanical preparation of infected root canals is complete eradication of intracanal bacteria or their reduction to levels that create a favorable environment for the healing of periradicular tissue [1]. This goal is not always achieved for several reasons, including anatomical complexities and limitations of instruments and medicaments [2–8]. Therefore, alternative strategies have been developed to overcome the limitations of current instrumentation, including alternative instrument design, supplementation of irrigation by sonic or ultrasonic energy, and the use of final MTAD (a mixture of a tetracycline isomer, an acid, and a detergent) or chlorhexidine (CHX) rinses [9].

The self-adjusting file (SAF) has proven superior in terms of disinfection in ex vivo and in vivo models [10–12]. Nevertheless, root canals can still harbor bacteria after SAF instrumentation [10,11]. For enhanced disinfection, a supplementary step is required after chemomechanical preparation. Commonly recommended supplementary approaches include a final rinse with CHX or sonic and ultrasonic irrigation. A final rinse with CHX after chemomechanical preparation has the advantage of the prolonged residual antimicrobial effects provided by CHX [13] and has shown promising results in terms of enhanced root canal disinfection [14,15]. However, in many cases, detectable levels of bacteria persist in the main root canal [16]. Sonic and ultrasonic energy reportedly enhances disinfection through cavitation, acoustic streaming, and sodium hypochlorite (NaOCl) warming, although findings from previous antibacterial studies have been inconclusive [14,17–20].

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This study aimed to compare the in vitro supplementary antibacterial effectiveness of EndoActivator (EA) irrigation, passive ultrasonic irrigation (PUI), a final CHX rinse, and a calcium hydroxide $Ca(OH)_2$ dressing after chemomechanical preparation using SAF.

2. Materials and methods

2.1. Specimen selection and preparation

Periapical radiographs of 80 human extracted teeth (maxillary second premolars and mandibular first and second premolars) with mature apices were obtained in both the buccolingual and mesiodistal directions to confirm the presence of a single oval canal. After access cavity preparation, patency was confirmed with a #10 K-file, the working length (WL) of each canal.

was determined with a #10 K-file by subtracting 1 mm from the lengths of the files when they extruded just beyond the apical foramen and verified with 3.0 × magnification loupe. The root canals were instrumented up to a #20 K-file, and the root apices were sealed with flowable composite. The teeth were then sterilized in an autoclave for 20 min at 121 °C.

All teeth were inoculated with *Enterococcus faecalis* ATCC 29,212 and incubated for 4 weeks under anaerobic conditions at 37 °C. The media were changed every 7 days. At the time of replacement, random samples from the root canals were cultured to confirm the growth of *E. faecalis*. The teeth were then mounted vertically up to the cervical region in a customized model made of a silicone impression material. The tooth crown, including the pulp chamber walls, and the silicone surface were disinfected with 2.5% NaOCl, followed by its inactivation with 10% sodium thiosulfate.

Initial bacteriological samples were obtained from all canals before preparation (S1). The root canals were filled with phosphate-buffered saline (PBS), and their walls were subjected to gentle circumferential filing with a #20 K-file such that the canal contents were suspended in the saline solution. Sterile paper points were consecutively placed in the canal to a level approximately 1 mm short of the working length and were transferred to a microcentrifuge tube containing 1 mL PBS after soaking up the fluid in the canal. All collected samples were vortexed for 10 s. After 10 fold serial dilutions in saline to 10^{-5}, aliquots of 0.01 mL were plated onto blood agar in triplicates and the plates were incubated at 37 °C for 24 h. The colony forming units of each sample were counted according to the dilution factor used.

The teeth were randomly assigned to four groups ($n = 15$) according to the final supplementary antibacterial regimens used after SAF instrumentation: group I, involving 1-min agitation of 2.5% NaOCl by EndoActivator (EA); group II, involving 1-min PUI with 2.5% NaOCl; group III, involving a final rinse with 5 mL of 2% CHX; and group IV, involving packing of the root canal with $Ca(OH)_2$ dressing for 7 days. Ten infected root canals were dried with paper points and not instrumented to act as a positive control. Where Ten sterile root canals were not contaminated to confirm the absence of any bacterial growth throughout all the test procedures (negative control).

2.2. SAF instrumentation

The SAF system was used according to the manufacturer instructions, with instrumentation involving in-and-out movements by a vibrating handpiece (GENTLE power; KaVo, Bieberach a.d. Riβ, Germany) with an RDT3 head (ReDent-Nova) at a speed of 5000 rpm and an amplitude of 0.4 mm. Each root canal was instrumented with a single SAF, and each instrument was used to prepare only one canal. A special irrigation device (VATEA; ReDent-Nova) was used for the continuous delivery of 2.5% NaOCl at a flow rate of 5 mL min^{-1} (total of 20 mL per canal). After preparation, NaOCl was inactivated using 10% sodium thiosulfate and a post-instrumentation (S2) sample was obtained and CFUs was counted as described in the initial sample.

2.3. Group I: EA irrigation

After preparation using SAF, the canals were dried with sterile paper points and irrigated with 1 mL of 17% EDTA using a 27-gauge needle. The EA system was used to activate this solution for 30 s using a size 15, 0.02-taper polymer tip. Each canal was then flushed with 3 mL of 2.5% NaOCl, which was activated using the same EA polymer tip for 30 s. The EA tip was inserted 1 mm short of the working length and was activated at 10,000 cycles per minute using pumping actions in short, 2–3-mm vertical strokes, as recommended by the manufacturer. NaOCl was inactivated using 10% sodium thiosulfate.

2.4. Group II: PUI

Ultrasonic activation was performed using a size 15, 0.02-taper stainless steel ultrasonic file (Irrisafe; Satelec Acteon Group, Merignac Cedex, France) mounted on the Suprasson P5 Booster ultrasonic unit (Satelec Acteon Group). The file was inserted 1 mm short of the working length and passively activated using a power setting of 4; it was passively inserted into the canal without any filing motion. The file was then used to agitate 17% EDTA and 2.5% NaOCl solutions using the same procedure described for group I.

2.5. Group III: CHX final rinse

The instrumented root canals were rinsed with 5 mL of 2% CHX using NaviTip needles inserted up to 1 mm short of the working length. For the inactivation of residual CHX, the canals were irrigated with 3% Tween 80 and 0.3% lecithin for 1 min.

2.6. Group IV: $Ca(OH)_2$ dressing

The instrumented root canals were packed with the UltraCal XS $Ca(OH)_2$ paste (Ultradent, South Jordan, USA) for 7 days. After 7 days, the temporary filling was removed and the $Ca(OH)_2$ paste was rinsed out of the canal using sterile saline solution and a hand file. The root canal walls were filed lightly to remove loose $Ca(OH)_2$ remnants.

Third bacterial sample (S3) was obtained after procedure completion in all groups as described in the initial sample (S1) and the post instrumentation sample (S2).

2.7. Statistical analysis

The Wilcoxon matched pairs test and the Mann–Whitney U test were used for intragroup and intergroup comparisons, respectively. The significance level was set at 5% ($P < 0.05$).

3. Results

None of the negative control samples showed growth. All positive control samples showed growth. Intragroup quantitative analyses evaluating the bacterial reduction from S1 to S2 in all groups demonstrated that SAF instrumentation promoted a highly significant bacterial reduction ($P < 0.001$). Analysis of quantitative data revealed that the number of colony forming units (CFUs) in S2 and S3 was significantly lower than that in S1 ($P < 0.001$). There was no significant difference in quantitative bacterial reduction between the S2 and S3 samples, except in groups I and II ($P = 0.017$ and

Table 1
Enterococcus faecalis counts before (S1) and after (S2) chemomechanical preparation using the self-adjusting file and after (S3) the different supplementary approaches.

		S1				S2				S3			
		Mean	Median	Min.	Max.	Mean	Median	Min.	Max.	Mean	Median	Min.	Max.
Group	EA	6030606.67	341000.00	4000.00	33000000.00	902186.67	4300.00	.00	13000000.00	56.67	.00	.00	400.00
	PUI	5337726.67	370000.00	5900.00	33000000.00	27549.33	440.00	.00	179000.00	76.00	.00	.00	600.00
	CHX	1627040.00	549000.00	4000.00	11400000.00	37326.67	10300.00	.00	320000.00	18873.33	.00	.00	132000.00
	$Ca(OH)_2$	4106353.33	630000.00	16300.00	33000000.00	98380.00	11900.00	.00	1100000.00	24726.67	.00	.00	191000.00

EA: EndoActivator, PUI: passive ultrasonic irrigation, CHX: chlorhexidine, $Ca(OH)_2$, calcium hydroxide.

0.046, respectively). Table 1 reveals the mean, median, and range values for the number of CFUs observed in all groups. When the four groups were compared (intergroup analysis), quantitative data (CFU counts) revealed that EA irrigation and PUI were significantly more effective than the CHX final rinse and $Ca(OH)_2$ dressing. There was no significant difference in effectiveness between EA irrigation and PUI ($P > 0.05$).

4. Discussion

Several studies have demonstrated the effectiveness of SAF instrumentation in eradicating microorganisms from infected root canals; however, complete eradication of microorganisms using only SAF instrumentation was not possible in most cases [10,12,21–23]. Furthermore, a study demonstrated the inability of SAF to control apical enlargement, thus limiting the ability of the irrigants to achieve effective and predictable disinfection [24]. A clinical study [10] highlighted the need for a supplementary step after chemomechanical preparation using SAF to enhance disinfection on the basis of the finding that almost 50% teeth in that study had detectable bacteria after instrumentation.

Till date, and to our best knowledge, no studies have evaluated the effectiveness of supplementary bacterial eradication procedures after SAF instrumentation. Therefore, we conducted this study to evaluate the effectiveness of different supplementary approaches in eliminating residual bacteria from root canals prepared using SAF.

The quantitative data obtained in our study showed that SAF instrumentation was effective in promoting a significantly high decrease in intracanal bacterial populations ($P < 0.001$). In total, SAF instrumentation resulted in negative bacterial culture in 46.6% (28/60) teeth. This finding is consistent with those of several previous reports on the antibacterial efficacy of chemomechanical preparation using SAF [10–12,21,22]. No significant difference in quantitative bacterial reduction was observed between the S2 and S3 samples obtained from groups III and IV, wherein the CHX final rinse and $Ca(OH)_2$ dressing were used ($P = 0.134$ and 0.280), respectively.

The ability of $Ca(OH)_2$ as a temporary dressing to decrease the infection burden below the levels achieved by chemomechanical debridement has been the subject of previous studies [25–27]. However, the findings from these studies have been inconsistent, with some studies showing enhanced disinfection [25,26] and others showing limited or decreased effects [27]. In the present study and a previous study, a $Ca(OH)_2$ dressing placed for 7 days did not significantly enhance disinfection after chemomechanical preparation [28].

The results of this study also confirm the findings of Pavia et al. [16], who reported insignificant quantitative bacterial reduction after a final rinse with 2% CHX. This may be explained by the insufficient volume and contact time to expand the area of action for the substance. Despite the frequency differences between sonic (10 KHz) and ultrasonic (35 KHz) irrigation used in this study, both approaches significantly decreased the bacterial counts to a level lower than that achieved by chemomechanical preparation using SAF. This may be explained by the mode of agitation used in this study, which was proven effective in several previous studies [29–32]. PUI was used to agitate the irrigation solutions by inserting the tip 1 mm short of the complete working length with no further movements; this induced acoustic cavitation, acoustic microstreaming, and heat, which disrupts and kills any bacteria within root canals. The positive effects of EA irrigation may be explained by the increased number of bubbles exiting along the EA file during irrigation. The vertical pumping motion used as part of the protocol promotes the increased formation of microbubbles that gradually increases in diameter until they collapse, provoking very effective small implosions that produce irregular agitation of the irrigant [20,30]. Another important factor was the agitation of EDTA for 30 s before final agitation of NaOCl for another 30 s; this may allow better disinfection by NaOCl because of more effective removal of the smear layer [30,33].

This study has some limitations. First, only the main canal was sampled. Second, preparing the canal walls with a file size corresponding to the size of the master apical file for collecting dentinal shavings may not be adequate to detect viable bacteria in the deepest portion of the canal. Therefore, no definitive conclusions can be derived with regard to disinfection of the entire root canal. It would be appropriate to develop a method that can predictably assess the antibacterial efficacy of endodontic treatment regimens in the entire root canal system.

5. Conclusions

In conclusion, EA irrigation and PUI were more effective than the CHX final rinse and $Ca(OH)_2$ dressing in eliminating bacteria from infected root canals after SAF instrumentation. The presence of remnant bacteria after chemomechanical preparation using SAF and the supplementary effects of EA or PUI suggest that further modifications are required to enhance disinfection.

Conflicts of interest

None.

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