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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