

Comparison of the Effectiveness of Three Irrigation Techniques in Reducing Intracanal *Enterococcus faecalis* Populations: An *In Vitro* Study

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Abstract

Introduction: Several irrigation techniques have been recently introduced with the main objective of improving root canal disinfection. This *in vitro* study aimed at comparing the intracanal bacterial reduction promoted by chemomechanical preparation with 3 different irrigation techniques. **Methods:** Root canals from extracted teeth were contaminated with *Enterococcus faecalis* ATCC 29212 for 7 days and then randomly distributed into 3 experimental groups of 20 teeth each: group 1, conventional irrigation with NaviTip needles inserted up to 3 mm short of the working length; group 2, same as group 1, but supplemented with final irrigant activation by the EndoActivator system; and group 3, irrigation with the EndoVac system. NaOCl and ethylenediaminetetraacetic acid (EDTA) were the irrigants used in all experimental groups. The overall preparation time was kept constant for the groups, but the total volume ranged from 20 mL (groups 1 and 2) to 43 mL (group 3). The control group was irrigated with saline solution (total volume, 43 mL). Samples taken before and after chemomechanical procedures were cultured, and the colony-forming units (CFUs) were counted. **Results:** Reduction in the bacterial populations was highly significant for all groups. The 3 experimental groups with NaOCl and EDTA as irrigants were significantly more effective than the control group with saline in reducing CFU counts. There were no significant differences between the 3 techniques tested. **Conclusions:** There was no evident antibacterial superiority of any of the irrigation techniques evaluated in the present *in vitro* model. (*J Endod* 2009;35:1422–1427)

Key Words

Endodontic treatment, *Enterococcus faecalis*, irrigation, root canal infection, sodium hypochlorite

The main microbiologic goals of the chemomechanical preparation of infected root canals are to completely eliminate intracanal bacterial populations or at least to reduce them to levels that are compatible with periradicular tissue healing (1). Bacteria persisting after chemomechanical procedures at levels detectable by culturing techniques might influence negatively the treatment outcome (2, 3). Therefore, efforts should be driven to establish chemomechanical protocols that predictably promote negative cultures (1). Studies have demonstrated that the incidence of negative cultures ranges from 40%–60% of the cases after chemomechanical preparation with different instrumentation techniques and instruments and conventional irrigation with different irrigants (3–9). On the basis of these reports, it becomes apparent that strategies are required to maximize root canal disinfection before filling. In this regard, many different irrigation protocols, solutions, and delivery systems have been recently introduced in endodontics, with the promise of optimizing root canal disinfection (10). Two recent systems that have received substantial attention because of their alleged properties include the EndoVac System (Discus Dental-Smart Endodontics, Culver City, CA) and the EndoActivator system (Dentsply Tulsa Dental, Tulsa, OK).

The EndoVac system is regarded as an apical negative pressure irrigation system composed of 3 basic components: a master delivery tip, which delivers and evacuates the irrigant concomitantly; the macrocannula, which is made of plastic with an open end of 0.55 mm in diameter and a 0.02 taper, used to suction irrigants up to the middle segment of the canal; and the microcannula, which is made of stainless steel and has 12 microscopic holes disposed in 4 rows of 3, laterally positioned at the apical 1 mm of the cannula. Each hole is 0.1 mm in diameter, the first one in the row is located 0.37 mm from the tip of the microcannula, and the distance between holes is 0.2 mm (authors' unpublished observations). The microcannula has a closed end with external diameter of 0.32 mm and should be taken to the working length (WL) to aspirate irrigants and debris. As the macrocannula and microcannula are placed in the canal, negative pressure forces the irrigant supplied in the pulp chamber by the master delivery tip down the canal to the tip of the cannula. *In vitro* studies have demonstrated that the EndoVac system can provide better cleaning at the most apical part of the prepared canal (11), presents reduced risk of apical extrusion of irrigants (12), and promotes a better intracanal disinfection than conventional irrigation (13).

The EndoActivator is a cordless, battery-operated sonic handpiece that uses non-cutting polymer tips to quickly and vigorously agitate irrigant solutions during treatment. The activator tips are available in 3 sizes (yellow 15/02, red 25/04, and blue 35/04) and can be activated in 3 speeds: 2,000, 6,000, and 10,000 cpm. Ruddle (14) recommends this device be used after completion of chemomechanical preparation to activate

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ethylenediaminetetraacetic acid (EDTA) and NaOCl. No study has so far investigated the supplemental antibacterial effects of this system.

The present study compared the *in vitro* intracanal reduction of *Enterococcus faecalis* populations promoted by instrumentation and irrigation with NaOCl/EDTA in 3 different regimens: the EndoVac system and conventional irrigation with NaviTip needles followed or not by final irrigant activation by the EndoActivator device.

Material and Methods

Specimen Preparation and Contamination

The study protocol was approved by the ethics committee of the Estácio de Sá University. Seventy single-rooted and single-canal canines exhibiting a total length of 23–27 mm were selected for this study. Presence of a single canal was determined by radiographs taken in both the mesiodistal and buccolingual directions. Conventional access cavities were done by using round burs and Endo-Z burs (Dentsply/Maillefer, Ballaigues, Switzerland). The WL was established by introducing a K-file #10 or #15 in the canal until its tip was visualized at the apical foramen through a stereoscopic microscope. Teeth exceeding 25 mm in length were adjusted to 25 mm by incisal reduction. To standardize the apical constriction size, root canals were instrumented at the apical foramen up to a K-type file #25 in reaming action, under irrigation with running water. Afterwards, the foramen was sealed with a fast set epoxy resin to prevent bacterial leakage. To make both handling and identification easier, the teeth were mounted vertically up to the cervical region in blocks made of a silicone impression material (President Jet; Coltène AG, Cuyahoga Falls, OH). The blocks containing the teeth were sterilized in autoclave for 20 minutes at 121°C.

A suspension was prepared by adding 1 mL of a pure culture of *E. faecalis* (ATCC 29212), grown in trypticase soy broth (TSB) (Difco, Detroit, MI) for 24 hours, to 5 mL of fresh TSB. Each root canal was completely filled with the *E. faecalis* suspension by using sterile 1-mL insulin syringes without overflowing. Sterile K-type files #15 were used to carry the bacterial suspension to the entire root canal length. Blocks were then placed inside a metallic box and incubated at 37°C for 7 days in 100% humidity. Fresh culture medium was added to the canal at 1, 4, and 6 days after the initial inoculum. After 7 days of experimental contamination, 66 teeth followed in the study and 4 teeth were subjected to scanning electron microscopy to allow visualization of the pattern of colonization. These 4 teeth were fixed in 10% buffered formalin, longitudinally split, and then dried in ascending ethanol concentrations. They were then dehydrated to their critical point in CO₂ and sputter-coated with gold under vacuum. Specimens were examined by using a scanning electron microscope (JEOL, JSM-5800LV, Tokyo, Japan).

Testing Procedures

Teeth were randomly divided into 3 experimental groups of 20 teeth each according to the irrigation technique used and a control group consisting of 6 teeth. Groups were as follows. In group 1, root canals were irrigated by using 30-gauge NaviTip needles (Ultradent, South Jordan, UT) placed in the canal up to 3 mm from the WL (Fig. 1A). Group 2 was similar to group 1, except for the final activation of irrigants by using the EndoActivator device (Fig. 1B and C). In group 3, root canals were irrigated by using the EndoVac system (Fig. 1D-F). Positive control group was similar to group 1, but with 0.85% saline solution as the irrigant.

Root canals from all groups were instrumented as follows. The coronal and middle segments of the canal were prepared with a LA Axxess bur #35 (SybronEndo, Glendora, CA). Then rotary ProTaper Universal instruments F3 and F4 (Dentsply/Maillefer) were used to prepare the canal up to the WL.

Groups 1 and 2

Canals were initially irrigated with 2 mL of 2.5% NaOCl for 30 seconds. After each instrument used, the canal was irrigated with 2 mL of NaOCl by using a 30-gauge NaviTip needle adapted to a disposable plastic syringe. The needle was placed up to 3 mm short of the WL. After the F4 instrument was used, NaOCl was left undisturbed in the canal for 60 seconds, and then a final irrigation procedure was performed as follows. In group 1, the canal was rinsed with 2.5 mL of 2.5% NaOCl, followed by 5 mL of 17% EDTA, and again with 2.5 mL of 2.5% NaOCl. In group 2, the canal was rinsed with 5 mL of 17% EDTA, followed by sonication of this solution with EndoActivator blue tip size #35/0.04, placed up to 2 mm of the WL, at 10,000 cpm for 60 seconds. Finally, the canal was irrigated with 5 mL of 2.5% NaOCl, followed by sonication of the substance with the EndoActivator blue tip for 30 seconds, the same way as above. The protocol for the EndoActivator device was as suggested by Ruddle (14). Overall, 20 mL of irrigants was used per canal in both groups for approximately the same time period.

Group 3

Before instrumentation, the root canal was irrigated with 6 mL of 2.5% NaOCl by using the EndoVac master delivery tip placed above the access opening to constantly deliver the irrigant. The canal and pulp chamber were kept full of irrigant throughout the procedures. After each instrument used, the canal was irrigated with 6 mL of NaOCl by using the master delivery tip. Specifically after apical preparation with the ProTaper F4 instrument, “macroirrigation” with 6 mL of NaOCl was accomplished during a 30-second period while the irrigant was delivered coronally by the master delivery tip. For this step, the macrocannula was constantly moved up and down in the canal from a point just below the canal orifice to 4 mm short of the WL. NaOCl was then left undisturbed in the canal for 60 seconds. In sequence, 3 cycles of “microirrigation” were accomplished. During each cycle, the pulp chamber was maintained full of irrigant, while the microcannula was placed at WL for 6 seconds. In sequence, the microcannula was positioned 2 mm from the WL for 6 seconds and then moved back to WL for 6 seconds. This up-down motion continued for 30 seconds, allowing 18 seconds of active irrigation directly at WL. After 30 seconds of irrigation, the microcannula was withdrawn from the canal in the presence of sufficient irrigant in the pulp chamber to ensure that the canal remained totally filled with irrigant and that no air was drawn into the canal space. This completed 1 microirrigation cycle. The first cycle used 2.5% NaOCl (6 mL) as the irrigant, the second cycle used 17% EDTA (5 mL), and the third cycle used 2.5% NaOCl (6 mL) once again. At the end of the third cycle, the microcannula was left at WL to remove excess irrigant. The EndoVac protocol was very similar to that used by Nielsen and Baumgartner (11). The overall volume of irrigants for this group was 43 mL.

Positive Control

In this group, instrumentation was performed as for the other groups. Irrigation was conducted with 30-gauge NaviTip needle, and saline was used as the irrigant, totaling 43 mL per canal.

The time of irrigation for all groups was standardized to approximately 4 minutes and 30 seconds.

Sampling Procedures

The root canals were sampled before (S1) and after (S2) chemomechanical procedures. Canals were filled with sterile 0.85% saline solution, and the S1 sample was taken by the sequential use of 3 paper points placed to the WL. Each paper point remained in the canal for 1 minute. Paper points were transferred to tubes

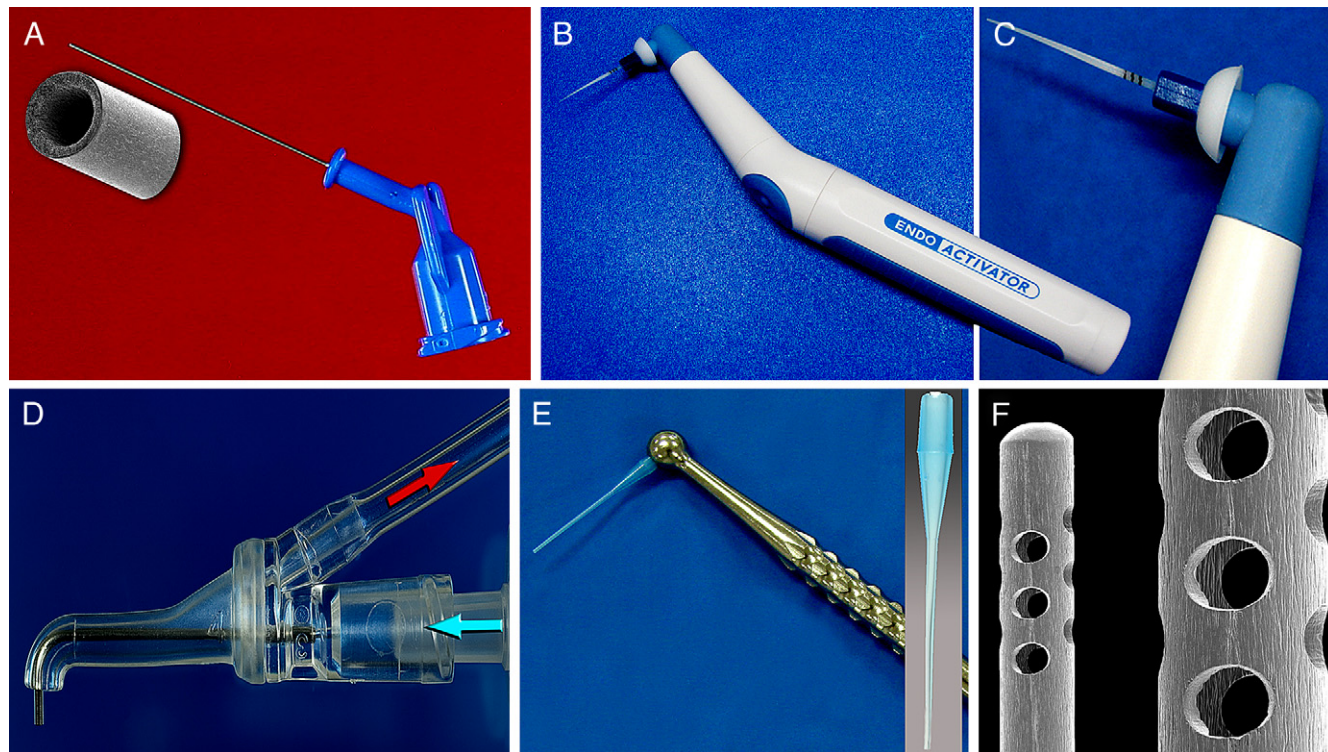


Figure 1. Components of the irrigation techniques used in the present study. (A) NaviTip needle; (B) EndoActivator handpiece; (C) EndoActivator blue tip adapted to the handpiece; (D) EndoVac master delivery tip; (E) EndoVac macrocannula; (F) electron micrograph of the EndoVac microcannula, showing its array of small holes near the end.

containing 1 mL of 0.85% saline solution and agitated in vortex for 1 minute. After 10-fold serial dilutions in saline, aliquots of 0.1 ml were plated onto Mitis-Salivarius agar plates (Difco) and incubated at 37°C for 48 hours. The colony-forming units (CFUs) grown were counted and then transformed into actual counts based on the known dilution factors.

Before S2 sample taking, the root canal was flushed with 1 mL of 10% sodium thiosulfate to neutralize the NaOCl. Each canal was then rinsed with saline, and a Hedström instrument #40 was used to file vigorously the dentinal walls. Afterwards, the canal contents were aspirated with a 1-mL disposable plastic syringe and then placed into tubes containing 1 mL of sterile saline. Two paper points #40 were placed at

the WL and also used to soak up the canal contents. Paper points were transferred to the same tubes containing 1 mL of saline. After agitation in vortex, samples were treated the same way as S1 samples.

The volumes of both sodium thiosulfate and saline before S2 (2 mL) were all included in the total volume calculation for each group.

Statistical Analysis

S1 data were submitted to the normality test of D’Agostino and Pearson omnibus. Because it revealed a normal distribution for all groups (experimental and control), the Student *t* test was used to compare the initial infection between groups. The Mann-Whitney test was used for intragroup analyses (reduction from S1 to S2). Percent reduction in the number of CFUs was calculated on the basis of quantitative data obtained from S1 and S2. The Kruskal-Wallis test was used for the intergroup comparative analysis of S2 data. Dunn multiple comparison test was used to isolate the differences between the experimental and the control groups. The χ^2 test with Yates correction was used to compare the occurrence of positive and negative cultures in S2 between the experimental groups. The significance level was set at $p < .05$. Analyses were performed by using SPSS 11.0 (SPSS, Chicago, IL) and Origin 6.0 (Microcal Software, Northampton, MA).

Results

The root canal walls of all 4 specimens analyzed by scanning electron microscopy were densely colonized by *E. faecalis* cells (Fig. 2). In some instances, cells were also seen penetrating the dentinal tubules.

Table 1 reveals the mean, median, range, and percent reduction of CFUs observed for all groups. Intragroup analyses revealed that in all groups (experimental and control), the reduction in the number of CFUs from S1 to S2 was highly significant ($p < .001$).

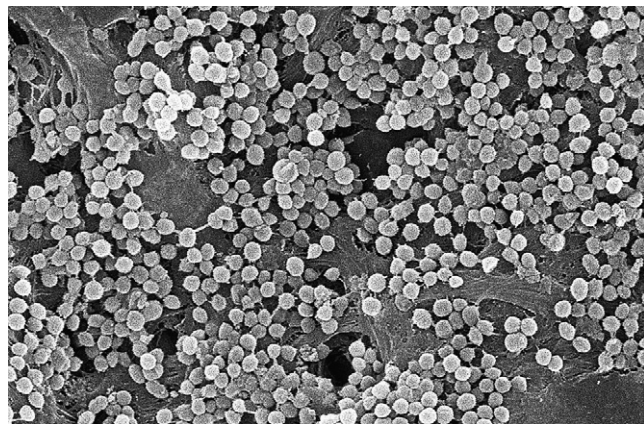


Figure 2. Scanning electron micrograph showing colonization of the root canal walls by *E. faecalis* ATCC 29212 (original magnification, $\times 3300$).

TABLE 1. Counts of *E. faecalis* CFUs before and after Chemomechanical Preparation by Using 3 Different Irrigation Techniques

Group	S1				S2				% Reduction (S1 to S2) Mean (range)
	Mean	Median	Range	Mean	Median	Range	Mean	Range	
Conventional (Navitip)	6.38×10^6	5.54×10^6	1.11×10^5 to 1.86×10^7	1.49×10^3	3.50×10^1	0 to 9.04×10^3	99.98 (99.78–100)		
Conventional (Navitip) plus EndoActivator	6.71×10^6	4.76×10^6	9.70×10^5 to 3.28×10^7	1.48×10^3	7.50×10^1	0 to 1.58×10^4	99.97 (99.86–100)		
EndoVac	4.85×10^6	5.50×10^6	3.00×10^5 to 9.44×10^6	7.43×10^2	3.00×10^1	0 to 3.78×10^3	99.98 (99.23–100)		
Control	6.63×10^6	5.63×10^6	1.52×10^5 to 1.30×10^7	3.26×10^4	3.52×10^4	3.38×10^3 to 6.69×10^4	99.51 (99.02–99.89)		

Intergroup analysis of S1 samples revealed no significant difference, indicating that the method of experimental contamination was capable of providing a homogeneous and reliable baseline of bacterial load. Consequently, data from S2 could be used for direct intergroup comparisons, which were carried out by using the nonparametric Kruskal-Wallis test. All experimental groups with NaOCl/EDTA as the irrigants were significantly more effective than the positive control group with saline in reducing *E. faecalis* populations ($p < .001$) (Fig. 3). However, no significant differences were observed between the experimental groups (Fig. 3). Frequency of negative cultures in S2 was 7 of 20 (group 1), 5 of 20 (group 2), and 9 of 20 (group 3). The χ^2 test failed to reveal any significant differences in the occurrence of negative cultures between the experimental groups. All specimens of the control group yielded positive cultures in S2.

Discussion

The present *in vitro* study was conducted to compare the antibacterial efficacy of 3 irrigation techniques used during chemomechanical preparation in reducing intracanal *E. faecalis* populations. All techniques showed a significant reduction in the bacterial populations in intragroup analyses and were significantly more effective than the control group with a high volume of saline as the irrigant. These findings confirm the important role of mechanical instrumentation in reducing bacterial populations, which, however, needs to be supplemented by the use of antibacterial irrigants in a chemomechanical approach (15–19). However, there were no significant differences between the test techniques.

The EndoVac system has been introduced with the claim of being safer and able to maximize cleaning and disinfection of the root canal, especially in its apical third (20, 21). At the time of writing this article, only one previous study had investigated the antibacterial effects of the EndoVac system (13). In an *in vitro* experiment, Hockett et al (13) concluded that the apical negative pressure created by the EndoVac system can produce a better microbial control than traditional irrigation delivery system. This is not in line with our present findings, and such divergence might be explained by critical methodologic differences in the experimental models; in the present study, the volume of NaOCl was standardized for all experimental groups, whereas it was not taken into account by Hockett et al. Even so, here the EndoVac system used twice as much volume of irrigant than the other experimental groups. Perhaps even more importantly, in the present study the effects of the EndoVac system were evaluated in association with instrumentation, whereas in the study by Hockett et al the effects of EndoVac were evaluated in isolation, with no concomitant instrumentation, because the canals were instrumented before contamination with *E. faecalis*.

Nielsen and Baumgartner (11) found significantly better results with EndoVac than with conventional irrigation in terms of cleaning the most apical part of the canal (1 mm short of the WL). No differences were observed for the canal area 3 mm short of the WL. Our study investigated the antibacterial effects in the entire canal, and no significant differences were observed. At this time it is unknown whether there would be differences in bacterial elimination if only the apical third had been investigated.

Although the EndoVac microcannula effectively aspirates irrigants in the most apical area of the canal, this effect on disinfection might not be so pronounced, given the small size of its perforations, which might become even smaller as a result of clogging by debris, reducing the fluid flow in the apical canal. Also, the concomitant and more potent coronal aspiration with the master delivery tip competes with the microcannula for fluid evacuation. Even so, the microcannula allows the irrigant to

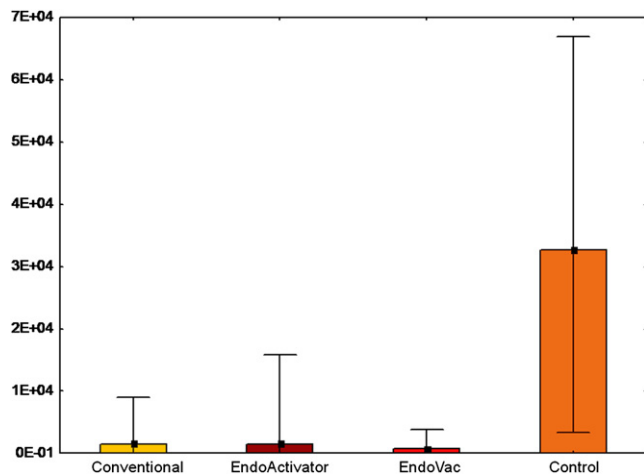


Figure 3. Mean and range (minimum-maximum) of *E. faecalis* CFU counts in samples taken after chemomechanical procedures by using different irrigation techniques.

effectively reach the apical canal and has been reported to suction nearly 50% of the fluid delivered by the master delivery tip (12). This might be an advantage when compared with conventional irrigation with large needles placed at a point just slightly below the canal orifice. However, when compared with small NaviTip needles taken up to 3 mm short of the WL, there was no significant difference in antibacterial performance. This might have been because such deep penetration of the irrigation needle allowed the irrigant to reach the apical segment of the canal to exert its effects. It has been demonstrated that the deeper the insertion of the irrigation needle, the higher the efficacy of the irrigation procedure in cleaning and disinfecting the root canal (22–24). However, it must be pointed out that although the antibacterial effectiveness was similar between the 2 techniques, the EndoVac system has been claimed to be safer when it comes to the risks of apical extrusion of NaOCl (12, 21).

Noteworthy was the absence of significant difference in antibacterial efficacy in spite of the great difference in the total volume of irrigants when comparing the EndoVac system with the other 2 groups (43 mL versus 20 mL). It has been shown that as far as bacterial elimination is concerned, the frequency and volume of irrigation can compensate for the effects of NaOCl concentration (17). Although the volume of irrigant has been demonstrated to exert mechanical influence on bacterial elimination (22, 25), our findings suggest that there might exist a point of saturation, above which the increase in volume will no longer affect bacterial elimination. Indeed, it is reasonable to realize that the increase in volume of the irrigant beyond a certain threshold will not improve distribution of the irrigant to the point of reaching irregularities and areas distant to the main canal. The time the irrigant remains in the canal would be more important in this regard. However, time was not a variable in the present study.

Sonic activation of EDTA and NaOCl with the EndoActivator device after chemomechanical procedures did not result in improved disinfection. Previous studies have demonstrated that sonic and ultrasonic instrumentation is not better than hand instrumentation in disinfecting (26) or cleaning (27) root canals. Similar to the results of sonic activation observed in this study, ultrasonic activation of NaOCl for 1 minute has not been shown to be better than conventional irrigation in an *in vitro* study (28). Results from a recent clinical trial have revealed that addition of ultrasonic irrigation for 1 minute after chemomechanical procedures significantly reduced the CFU counts and the occurrence of positive cultures (29). Actually, the results of NaOCl

activation by ultrasonics as compared with irrigation alone remain contradictory (30). Sonic activation in turn is expected to be less effective than ultrasonic as a result of lower frequency, which might result in less acoustic stream (31). Although the size of the EndoActivator tip used in this study was compatible enough with the root canal diameter prepared to a ProTaper F4 instrument to generate strong visible agitation of the irrigants in the canal, further studies are required to evaluate whether smaller sizes can enhance the antibacterial results.

In conclusion, the present *in vitro* study demonstrated that all 3 irrigation protocols performed similarly as to the reduction of *E. faecalis* populations in the root canals. Trials investigating the effects of these regimens on a mixed bacterial community in the clinical set are required to determine the method that best provides predictable disinfection of infected root canals of teeth with apical periodontitis.

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