Disinfecting Oval-shaped Root Canals: Effectiveness of Different Supplementary Approaches

Flávio R.F. Alves, PhD, Bernardo M. Almeida, DDS,* Mônica A.S. Neves, MSc,* Jaime O. Moreno, DDS,*† Isabela N. Rôças, PhD,* and José F. Siqueira, Jr., PhD**

Abstract

Introduction: This study compared the ability of different approaches to supplement the antibacterial effects of chemomechanical preparation in oval-shaped root canals. **Methods:** Long oval canals from extracted teeth infected with *Enterococcus faecalis* (ATCC 29212) were chemomechanically prepared up to a 40/04 rotary BioRaCe instrument using 2.5% NaOCl irrigation and then subjected to two supplementary protocols. In the passive ultrasonic irrigation (PUI)/chlorhexidine (CHX) group, canals were subjected to PUI for the activation of NaOCl followed by a final rinse with 0.2% CHX digluconate solution. In the Hedström group, canals received additional Hedström filing directed towards the buccal and lingual canal recesses. Bacteriological samples were taken before and after preparation, after PUI or Hedström instrumentation, and after CHX final rinsing. **Results:** Chemomechanical preparation and the supplementary steps promoted a highly significant bacterial reduction ($P < .001$). Quantitative (reduction in levels) and qualitative (frequency of negative cultures) analyses showed that PUI alone or Hedström filing did not significantly increase bacterial reduction ($P > .05$). Further rinsing with CHX also failed to significantly increase bacterial elimination when compared with post-PUI samples. However, the cumulative antibacterial effects of PUI and CHX final rinse were effective in significantly reducing bacterial counts to levels below those achieved after preparation ($P = .03$). This combined PUI/CHX approach also resulted in a significant increase in the incidence of negative cultures ($P = .04$). **Conclusions:** Findings suggest that there may be a benefit of using the PUI for the activation of NaOCl followed by a final rinse with CHX as supplementary steps in the treatment of infected oval-shaped root canals. *(J Endod 2011;37:496–501)*

Key Words

Chlorhexidine, endodontic treatment, oval root canals, passive ultrasonic irrigation, sodium hypochlorite

Microbial control is paramount in clinical endodontics (1, 2). Among the treatment steps, chemomechanical procedures play a pivotal role in eliminating or reducing bacterial populations from the main root canal, but the disinfecting effects of instruments and irrigants may be somewhat hampered in cases with complex anatomy. A clear example includes the cross-sectional root canal configuration, which has been classified as round, oval, long oval, flattened, or irregular (3). Oval, long oval, and flattened canals are those presenting a ratio between the maximum and minimum cross-sectional diameter of less than 2:1, 2 to 4:1, and greater than 4:1, respectively (3).

Numerous studies have reported that hand and rotary instrumentation of oval-shaped canals leaves unprepared buccal and lingual extensions or recesses (4–9), which can harbor remnants of necrotic pulp tissue and bacterial biofilms. Moreover, recesses can be packed with dentin debris generated and pushed therein by rotating instruments (10). Residual biofilms and infected debris can serve as a potential source of persistent infection and treatment failure (11).

Some approaches have been suggested to deal with the problem of cleaning and disinfecting oval canals. Ultrasonic instrumentation (12) and a combination of rotary nickel-titanium (NiTi) instruments and hand instrumentation with a modified Hedström file were reported to improve the preparation (13), but no technique completely cleaned oval-shaped canals. A histologic study (8) reported that preparation with hand Hedström files and another two techniques (anatomic endodontic technology and rotary NiTi instruments) failed to completely prepare and clean oval canals. Another recent study (7) evaluated the prepared surface areas of oval-shaped canals using four different instrumentation techniques: Hedström files in circumferential filing, ProTaper NiTi rotaries considering the oval canal as 1 canal, ProTaper considering buccal and lingual aspects of the oval canal as 2 individual canals, and ProTaper in a circumferential filing motion. They reported that the amounts of treated surface areas were statistically similar in the apical 4 mm for all techniques examined and concluded that preparations of oval-shaped root canals left a variable portion of surface area unprepared regardless of the instrumentation technique.

There are not many studies consistently investigating the ability of different approaches to disinfect oval-shaped canals. In a recent study, Siqueira et al (14) compared the *in vitro* capability of a newly developed instrument, the self-adjusting file, and rotary NiTi instrumentation to eliminate *Enterococcus faecalis* populations from long oval root canals. They observed that rotary NiTi instrumentation used with syringe/needle irrigation failed to predictably disinfect root canals and was significantly less effective than the self-adjusting file. The difficulty of effectively cleaning and disinfecting oval-shaped canals open perspectives to the use of alternative or supplementary approaches.

Postinstrumentation supplementary approaches have been proposed to improve and/or expedite root canal disinfection. For instance, to take advantage of the benefits of both NaOCl and chlorhexidine (CHX) as irrigants, it has been recommended to use

From the *Department of Endodontics, Faculty of Dentistry, Estácio de Sá University, Rio de Janeiro, Rio de Janeiro, Brazil; and †Department of Endodontics, Santo Tomas University, Bucaramanga, Colombia.

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Address requests for reprints to Dr José F. Siqueira Jr, Faculty of Dentistry, Estácio de Sá University, Av Alfredo Baltazar da Silveira, 580/cobertura, Recreio, Rio de Janeiro, RJ, Brazil 22790-710. E-mail address: jf_siqueira@yahoo.com

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NaOCl during preparation and to supplement disinfection by a final rinse with CHX (15, 16). Activation of the irrigant solution has also been recommended, and among the methods available, passive ultrasonic irrigation (PUI) is probably the most used (17). PUI refers to either intracanal placement of an irrigant with a syringe followed by ultrasonic activation or continuous delivery of irrigant through an ultrasonic handpiece (18). PUI has been shown to be more effective than other irrigation systems in removing tissue remnants and dentinal debris from the main root canal as well as from irregularities (19–22). Based on these reports, it seems interesting to test the effects of PUI and the CHX final rinse on oval-shaped root canal disinfection.

The present study was undertaken to investigate the ability of different approaches to supplement the intracanal antibacterial effects of rotary NiTi instrumentation against *E. faecalis* populations in long oval root canals of extracted human teeth.

Materials and Methods

Specimen Selection and Preparation

This study used 54 extracted teeth (single-rooted and single-canaled mandibular incisors and maxillary second premolars) with long oval root canals obtained from an existing collection of extracted teeth at Estácio de Sá University. These teeth were extracted for reasons not related to this study, and approval for the study protocol was obtained from the Ethics Committee of the Estácio de Sá University. Teeth were selected on the basis of radiographs taken in both buccolingual and mesiodistal directions. Selected teeth had root canals presenting a greater than 2.5:1 ratio between the buccolingual and mesiodistal dimensions at a level 5 mm from the root apex. Pairs of teeth were selected on the basis of similar radiographic root canal morphology, and each tooth from each pair was randomly assigned to each experimental group.

Conventional access cavities were prepared using round burs and Endo-Z burs (Dentsply Maillefer, Ballaigues, Switzerland). All root canals were instrumented at the apical foramen up to a hand #25 K-type file in alternating rotation motions under continuous irrigation with running water. The smear layer was removed by using 17% EDTA for 3 minutes followed by 2.5% NaOCl irrigation. Irrigation was performed using a NaviTip needle (Ultradent, South Jordan, UT) placed as much apically as possible to ensure that the irrigants reached the entire extent of the canal. After the inactivation of residual NaOCl with 10% sodium thiosulfate, the teeth were immersed in trypticase soy broth (TSB) (Difco, Detroit, MI), ultrasonicated for 1 minute to release entrapped air and allow penetration of culture media into root canal irregularities, and then sterilized in an autoclave for 20 minutes at 121°C. Each flask contained 10 teeth immersed in 200 mL TSB. The experiment was planned so that 10 specimens could be prepared and the respective bacteriological samples processed per day.

Bacterial Biofilm Formation

E. faecalis strain ATCC 29212 was used to infect the root canals. A suspension was prepared by adding 1 mL of a pure culture of *E. faecalis* grown in TSB for 24 hours to 5 mL of fresh TSB. One milliliter of this suspension was used to inoculate each of the flasks. *E. faecalis* was allowed to grow for 30 days at 37°C under gentle shaking. Culture media was replenished every week.

Afterwards, all teeth had the excess of culture medium dripped off and their external root surface wiped with sterile gauze. Four teeth were processed for scanning electron microscopic (SEM) analysis to confirm bacterial colonization and biofilm formation. These 4 teeth were fixed in 10% buffered formalin, longitudinally split, dried in ascending ethanol concentrations, dehydrated to their critical point in CO_2, and then sputter-coated with gold under vacuum. SEM analysis was performed using a JEOL microscope (model JSM-5800LV; JEOL, Tokyo, Japan).

The other 50 teeth had their apical foramen sealed with a fast set epoxy resin in order to prevent apical bacterial leakage and also to create a closed-end channel that produces the vapor lock effect (23). To make both handling and identification easier, 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 tooth crown, including the pulp chamber walls, and the silicone surface were disinfected with 2.5% NaOCl followed by inactivation of this substance with 10% sodium thiosulfate. Next, the working length (WL) was determined by introducing a #20 K-file in the canal until it reached the apical foramen. The initial (S1) sample was then taken from each canal (see later).

Root canals were instrumented using BioRaCe instruments (FKG Dentaire, La Chaux-de-Fonds, Switzerland). Canals were prepared at the WL by using the BR2 instrument (25/04; size/taper) up to the BR5 instrument (40/04) with 2.5% NaOCl as the irrigant. Irrigation was performed with disposable 5-mL syringes and 30-G NaviTip needles taken up to 3 mm short of the WL. After preparation was complete, the canal was rinsed with 5 mL 17% EDTA followed by 5 mL 2.5% NaOCl. The total volume of NaOCl was 15 mL per canal (Fig. 1). After preparation in both groups, each root canal was washed with 1 mL 10% sodium thiosulfate to inactivate NaOCl, dried, and refilled with the same solution, which remained in the canal for 5 minutes. Postpreparation (S2) samples were taken.

Supplementary Antibacterial Procedures

Six teeth that showed no bacterial growth in S1 samples were excluded from the study. In group PUI/CHX (20 teeth), the root canal was irrigated with 2 mL 2.5% NaOCl, and then this solution was ultrasonically activated in the canal for 1 minute by using a stainless steel #15 K-type file mounted in a piezoelectric ultrasonic device (Enac-Osada, Tokyo, Japan). The ultrasonic instrument was used at 1 mm short of the WL. The canal was again irrigated with 2 mL NaOCl. After washing the canal with 1 mL 10% sodium thiosulfate, this substance was left for 5 minutes filling the canal, and then S3 sample was taken. Eventually, sample S4 was taken from root canals of this group after rinsing the canal with 2 mL 0.2% CHX digluconate for 1 minute (Fig. 1). Irrigation was always performed with 30-G NaviTip needles taken up to 3 mm of the WL.

After chemomechanical preparation in the Hedström group (24 teeth), the root canal was irrigated with 2 mL 2.5% NaOCl, and then Hedström files to size #40 were used in filing motion along the buccal and lingual recesses of the oval canal. Three short strokes were used per face, and the canal was again irrigated with 2 mL NaOCl. This substance was inactivated with 1 mL 10% sodium thiosulfate, which was left for 5 minutes in the canal, and then S3 sample was taken (Fig. 1).

Sampling Procedures and Processing

S1 sample was taken as follows. The root canal was gently rinsed with 1 mL sterile saline solution to remove unattached cells, and an initial sample was taken by the sequential use of three to five paper points placed to the WL. Each paper point remained in the canal for 1 minute. Paper points were transferred to tubes containing 1 mL sterile 0.85% saline solution and immediately processed.

S2, S3, and S4 samples were taken using an approach to maximize recovery of bacteria from oval canals (14). Initially, the root canal flooded with 10% sodium thiosulfate was sampled by agitating the fluid in the canal with a sterile #35 or #40 gutta-percha point used in

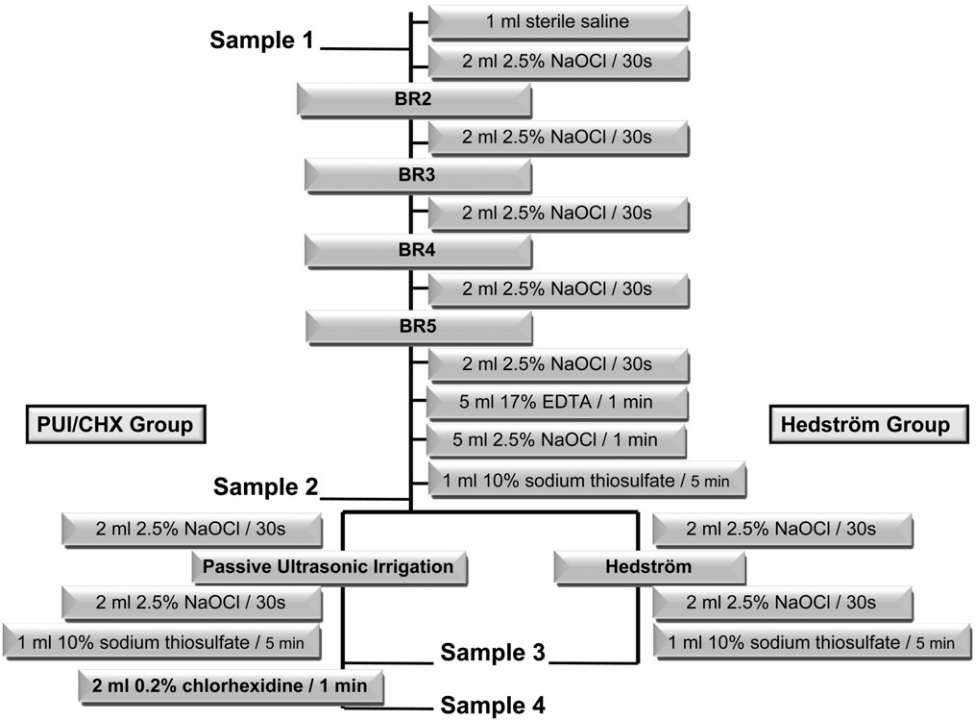

Figure 1. A flowchart of the experimental procedures.

a pumping motion. Next, a sterile precurved stainless steel hand #20 K-file was inserted in the canal up to the WL. The curvature applied to the instrument was gentle and involved approximately the last 3 mm near the instrument's tip. The precurved instrument was turned so that its tip faced the buccal recess and then moved three times with a pulling motion. This motion was repeated after turning the file so that its tip faced the lingual recess. This approach was intended to disrupt and dislodge biofilm remnants and dentinal debris packed or unaffected in the recesses. Root canal contents were then absorbed with sterile paper points until the canal was dry. Paper points were transferred to tubes containing 1 mL sterile saline and immediately processed. Specifically for S4 samples (PUI/CHX group), saline contained a mixture of 0.07% lecithin, 0.5% Tween 80, and 5% sodium thiosulfate to neutralize CHX.

Sample processing involved agitation in vortex for 1 minute followed by 10-fold serial dilutions in saline. Afterwards, aliquots of 100 μL 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. Two parameters were evaluated per sample: qualitative (positive vs negative culture) and quantitative (number of CFUs).

To confirm the identification of *E. faecalis* in all positive samples, species-specific polymerase chain reaction (PCR) was performed as described previously (24). PCR amplicons were separated by electrophoresis in a 1.5% agarose gel in Tris-borate-EDTA buffer, and positive reactions were determined by the presence of the predicted 310-bp amplicon.

Statistical Analysis

The Mann-Whitney *U* test was used for all quantitative analysis. Intragroup quantitative analysis compared the reduction in the number of CFU counts from S1 to S2, S3, or S4; S2 to S3 or S4; and S3 to S4. Data for intergroup quantitative comparisons consisted of either the absolute counts in S3 and S4 or the reduction values in CFU counts from S1 to S3 and from S1 to S4. Intergroup analysis served to compare the effects of Hedström filing (S3, Hedström group) with PUI alone (S3, PUI/CHX group) or PUI plus CHX final rinse (S4, PUI/CHX group). The incidence of negative cultures after S2, S3, and S4 was compared within and between groups using the two-tailed Fisher exact test or the chi-square test. Significance level for all analyses was set at $P < .05$.

Results

The root canal walls of the four specimens subjected to SEM analysis were densely colonized by *E. faecalis* cells, very often resembling biofilm-like structures. Successful root canal colonization was further confirmed by bacterial growth in baseline (S1) samples of 44 teeth used in the antibacterial study. PCR analysis confirmed the identification of *E. faecalis* in all positive samples.

Table 1 reveals the mean, median, and range of CFU counts observed for the two groups. Intragroup quantitative analyses evaluating the reduction in CFU counts from S1 to S2, S3, or S4 showed that chemomechanical preparation and the supplementary steps promoted a highly significant bacterial reduction ($P < .001$). In the PUI/CHX group, the comparison of S2 with S3 revealed that PUI did not significantly increase bacterial reduction ($P = .17$). Further rinsing with CHX also failed to significantly decrease the bacterial counts (S3 and S4 comparison, $P = .31$). However, when evaluating the effects of the combined approach (PUI plus CHX rinse, S4 data) in reducing bacterial counts after preparation (S2), the results were statistically significant ($P = .03$). In the Hedström group, S2 and S3 data comparison showed that additional filing with Hedström instruments did not succeed in significantly enhancing bacterial reduction ($P = .65$).

Intergroup quantitative analysis of S1 samples revealed no significant difference ($P = .37$). This indicates that the method of experimental contamination provided a homogeneous and reliable baseline of bacterial load. Further intergroup analysis served the intent to compare if additional Hedström filing was better than additional PUI

followed or not by CHX rinsing in eliminating *E. faecalis* cells from the root canal. Data used for these analyses consisted of either the absolute counts in S3 and S4 or the differences from S1 to S3 or S4. Whatever the dataset used, there were no significant differences between the groups ($P > .05$).

Qualitative analyses involved frequency of negative cultures in S2, S3, and S4. In the PUI/CHX group, 9 of 20 (45%) canals were rendered culture negative after preparation, 13 of 20 (65%) after PUI, and 16 of 20 (80%) after CHX rinsing (Table 2). In the Hedström group, 15 of 24 (62.5%) canals were culture negative after preparation and 14 of 24 (58%) after filing the canal recesses with Hedström instruments (Table 2). Intragroup qualitative analysis revealed that PUI did not significantly increase the incidence of negative cultures when compared with S2 ($P = .34$). A comparison between S3 and S4 also revealed that a final rinse with CHX did not contribute any further to significantly increase the incidence of negative cultures after PUI. However, PUI plus CHX rinse significantly increased the incidence of negative cultures when compared with postinstrumentation samples (S2 and S4 comparison, $P = .04$). In the Hedström group, no increase in negative cultures after additional Hedström filing was observed. In fact, one negative case reverted to positive. Intergroup qualitative comparisons showed no significant differences ($P > .05$).

Discussion

Oval-shaped canals represent a great challenge for proper cleaning, shaping, and disinfection. Because in most current preparation techniques hand or engine-driven instruments are usually worked with reaming motion, the final preparation is usually round in cross-section and leaves uninstrumented recesses in oval, long oval, and flattened canals. These recesses have the potential to harbor persistent bacteria that may jeopardize the treatment outcome. This *in vitro* study investigated the ability of different approaches used after chemomechanical procedures to supplement disinfection of long oval canals. Canals prepared by a rotary NiTi technique were additionally subjected to either Hedström filing of buccal and lingual recesses or PUI with 2.5% NaOCl for 1 minute followed by 0.2% CHX rinsing.

Quantitative analyses showed that chemomechanical preparation, regardless of the supplementary steps, was highly effective in reducing the baseline bacterial load. This is in agreement with several previous *in vitro* and *in vivo* studies and confirms the critical role of chemomechanical procedures in microbial control (14, 25–28). However, like most previous studies, many cases still harbored detectable bacteria after preparation. These findings confirm the previous observations that chemomechanical preparation alone may not suffice to predictably disinfect root canals and that oval-shaped canals pose a problem for proper cleaning, shaping, and disinfection (4–8, 14, 29). Attempts to supplement the antibacterial effects of preparation by performing PUI or an additional Hedström filing were ineffective in significantly reducing bacterial counts or rendering more canals culture negative. Remaining bacteria are conceivably lodged in buccal and/or lingual root canal recesses and persist unaffected by instruments (because of physical limitations) and irrigants (because of time constraints).

Although PUI alone was not significantly effective, the best effects observed in this study were for the sequential use of PUI and CHX final rinse. The cumulative antibacterial effects of this combined approach were able to reduce the bacterial counts to levels significantly lower than those observed immediately after chemomechanical procedures. The higher efficacy of the PUI/CHX combined approach over PUI alone might suggest a synergistic antibacterial effect, with the PUI approach leading to disorganization of biofilms in recesses and making them

TABLE 1. Counts of *E. faecalis* CFUs before (S1), after Chemomechanical Procedures (S2), and after Different Supplementary Approaches (S3 and S4)

Groups	S1			S2			S3			S4		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
PUI/CHX	2.05×10^6	1.00×10^5	$2.58 \times 10^3 - 2.67 \times 10^7$	1.17×10^4	3.00×10^1	$0 - 2.10 \times 10^5$	3.73×10^2	0	$0 - 2.00 \times 10^3$	7.60×10^1	0	$0 - 6 \times 10^2$
Hedström	4.46×10^5	1.60×10^5	$8.32 \times 10^3 - 4.40 \times 10^6$	3.07×10^2	0	$0 - 3.40 \times 10^3$	2.90×10^2	0	$0 - 3.36 \times 10^3$	—	—	—

TABLE 2. The Incidence of Negative Cultures after Chemomechanical Preparation (S2) and Supplementary Approaches (S3 and S4) for Disinfection of Oval-shaped Canals

Groups	S2	S3	S4
PUI/CHX	9/20 (45)*	13/20 (65)	16/20 (80)
Hedström	15/24 (62.5)	14/24 (58)	—

*The number of cases with a positive culture/number of cases examined (%).

more susceptible to the effects of CHX. Because there was no significant difference between PUI (S3) and CHX rinse (S4), a better explanation might be an additive antibacterial effect.

The incidence of negative cultures in clinical studies has been considered an important parameter to define adequate antimicrobial protocols with the potential to provide a predictable treatment outcome (25). In the present *in vitro* study, the incidence of negative cultures after chemomechanical preparation in the two groups was very similar to that reported in clinical studies (45% in the PUI/CHX group and 62.5% in the Hedström group) (2). The number of negative cultures remained unaltered after additional Hedström filing, except for one tooth that reversed to positive. This may have occurred because of limitations in the sampling technique and/or because the additional filing may have exposed bacterial biofilms deep into recesses and facilitated sampling.

The most interesting qualitative finding was also observed in the PUI/CHX group. Although PUI did not significantly increase the incidence of negative cultures (65%) when compared with S2, the sequential effects of PUI and CHX final rinse led to a significant increase in the frequency of negative cultures (80%). Therefore, this study suggests that there might be a benefit to include PUI followed by CHX rinse to significantly increase the incidence of negative cultures after chemomechanical procedures. If this approach works similarly in the clinical setting including to the point of being able to circumvent the need for an interappointment intracanal medication still needs to be shown by clinical trials.

Although studies have revealed that PUI may enhance cleaning of root canal irregularities, many of these studies also showed that along with other tested irrigation approaches, PUI was not able to completely remove debris in the apical part of the root canal (21, 22). As for disinfection, *in vitro* findings about the effectiveness of PUI in reducing bacterial populations have been somewhat inconclusive. One study showed that it was superior to syringe irrigation (30), and another one found no significant difference between the two techniques (31). PUI was not superior than syringe irrigation or passive sonic activation, all using 5.25% NaOCl, in eliminating *E. faecalis* from root canals of extracted teeth (32). The present findings with PUI alone corroborate those from studies showing no significant additional antibacterial effects. However, when combined with a final rinse with CHX, the whole approach was significantly effective. A variation in PUI with the irrigant being pumped under a high flow rate through a needle attached to an ultrasonic handpiece has been proposed (19, 33) and shown to improve cleaning (19) and disinfection (33, 34). The antibacterial effects of the PUI approach with constant irrigation remain to be evaluated in oval-shaped canals.

In conclusion, the present *in vitro* study showed that PUI followed by CHX rinsing significantly reduced the bacterial counts and the incidence of positive cultures after chemomechanical preparation of oval-shaped root canals. Therefore, there seems to be a benefit of using this combined approach as supplementary steps in the treatment of infected root canals. Further clinical studies are required to confirm these results. Also, the search for effective alternative or supplementary measures to predictably disinfect oval-shaped canals should be encouraged.

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The authors deny any conflicts of interest related to this study.

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