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# Effectiveness of Sodium Hypochlorite plus EDTA Compared with Peracetic Acid in Removing Smear Layer and Killing *Enterococcus faecalis*

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**ABSTRACT**

**Introduction:** The aim of this study was to evaluate the effectiveness of 2.5% sodium hypochlorite associated with 17% Ethylenediaminetetraacetic acid (NaOCl-EDTA), *versus* that of 1% peracetic acid (PA), in removing the smear layer, as assessed by scanning electron microscopy (SEM), and in exerting bactericidal action against *Enterococcus faecalis* (*E. faecalis*), as assessed by the real-time polymerase chain reaction (real-time PCR). **Methods and Materials:** Fifty-five extracted mandibular single-rooted premolars were selected, and divided into two experimental groups (NaOCl-EDTA and PA; *n*=25) and one control group (0.9% saline; *n*=5). Pre- and post-instrumentation samples were collected and assessed for the presence of *E. faecalis* using real-time PCR. The teeth were instrumented using hand files and the ProTaper Universal system (hybrid technique) for a standardized time of 7 min. A total of 20 mL of NaOCl followed by 5 mL of EDTA were applied during instrumentation in the NaOCl-EDTA group, whereas 20 mL of PA and 20 mL of saline were applied in the PA and control groups, respectively. An additional 5 mL of saline was applied in all the groups to neutralize the environment. A scoring system was used to conduct the SEM assessment. The results were submitted to the Kruskal-Wallis test, complemented by Dunn's test (SEM analysis) ($P<0.05$). **Results:** A significant microbial reduction was observed in both the PA and the NaOCl-EDTA groups ($P<0.05$). In the PA group, the presence of a smear layer in the apical third was significantly greater than in the cervical third ($P<0.05$); no significant differences were observed between the middle and cervical thirds, or between the middle and apical thirds ($P>0.05$). In the NaOCl-EDTA group, the smear layer scores were significantly higher in the apical third than in the cervical and middle thirds ($P<0.05$). **Conclusion:** This *in vitro* study showed that there was no significant difference between PA and NaOCl-EDTA irrigation regimens regarding either antimicrobial action against *E. faecalis* or removal of the smear layer, except for greater removal in the middle third by the NaOCl-EDTA group.

**Keywords:** Endodontic Irrigation; *Enterococcus faecalis*; Peracetic Acid; Real-Time Polymerase Chain Reaction; Sodium Hypochlorite

## Introduction

During root canal instrumentation, organic and inorganic remnants of dentinal tissue are deposited on the canal walls [1, 2]. These remnants contribute to the formation of a smear layer [3]. They adhere to the instrument and are compacted against the canal walls, obliterating the dentinal tubule entrances [4]. It has been argued that the presence of a smear layer may lead to treatment failure. On the other hand, its removal increases dentinal permeability, thus enabling the penetration of microorganisms and their sub-products into the dentinal tubules, which, in turn, may jeopardize the treatment outcome [5, 6]. Other authors consider this increase in permeability as beneficial, in that it contributes to increasing the interface between the dentin and the materials applied inside the root canal during endodontic procedures [7]. Despite this controversy, it is widely accepted that

endodontic instrumentation should be combined with the application of an irrigation solution capable of cleaning and reducing the number of microorganisms effectively [8].

Several irrigating substances have been tested to improve disinfection and removal of the smear layer in the root canal system, including propolis, chlorhexidine, *Eucalyptus galbie, Myrtus communis L.*, and citric acid [9-12]. Furthermore, several methods have been developed with the aim of agitating these solutions to render their action more effective [2].

Sodium hypochlorite (NaOCl) solution is the endodontic irrigant most widely used in root canal cleansing, because of its ability to promote tissue dilution and to exert strong bactericidal action. Ethylenediaminetetraacetic acid (EDTA), is a chelating agent that promotes the removal of the inorganic components of the smear layer, acting as an adjunct to irrigation [13, 14].

Another irrigant that has been tested is peracetic acid (PA). According to Arias-Moliz *et al.* [15], it is not only able to remove the smear layer, but also contributes toward disinfecting the root canal system. The use of PA instead of EDTA may be clinically interesting, because of its potential to improve disinfection of root canals [16, 17]. Cord *et al.* [16] concluded that the bactericidal effect of irrigations performed with PA and with 17% EDTA associated to 2.5% sodium hypochlorite were equivalent. This equivalence could potentially allow a simplification of the irrigation procedure and save time, in that a single substance could provide the same disinfection effectiveness as that provided by a NaOCl+EDTA association. According to Teixeira *et al.* [18], another advantage of PA is its low cytotoxicity.

*Enterococcus faecalis* (*E. Faecalis*) is a facultative anaerobic bacterium associated with persistent endodontic infections. It may cause endodontic treatment failure, particularly in cases where there is secondary infection [19]. An important feature of this bacterium is its ability to penetrate dentinal tubules [20]. Studies on the effectiveness of PA used for endodontic irrigation are scant in the literature. Thus, the aim of the present study was to compare the effectiveness of 2.5% NaOCl associated to 17% EDTA *versus* that of 1% PA, in terms of smear layer removal, as assessed by scanning electron microscopy (SEM); and bactericidal action against *E. faecalis*, as assessed by the real time polymerase chain reaction method (Real-time PCR).

## Materials and Methods

This study was approved by the Research Ethics Committee of the São Leopoldo Mandic Dental Research Center (CAAE: 41157115.2.0000.5374) and that of the Federal University of Rio Grande do Sul, a research partner in this study (CAAE: 41157115.2.3001.5347). The sample size was calculated using the Cochran method [21].

Fifty-five permanent human mandibular premolars obtained from the tooth bank of the São Leopoldo Mandic Dental Research Center were selected. The study groups were composed as follows: PA Group (*n*=25): irrigation performed with 1% PA; NaOCl-EDTA Group (*n*=25): irrigation performed with 2.5% NaOCl associated to 17% EDTA; and S Group (*n*=5, control): irrigation performed with 0.9% saline solution.

***Selection and preparation of specimens***

After extraction, the teeth were cleaned with ultrasound and stored in 0.1% thymol until the time of the experiment. The roots were washed in running water for one h, and then dried with an air jet and gauze. The teeth were then selected based on radiographs performed in the buccolingual and mesiodistal directions. Inclusion criteria were teeth with straight, single fully formed roots, and with a single, oval-shaped canal (buccolingual length=twice the mesiodistal length) [22]. Teeth with fractures, calcifications, dilacerations, or previous endodontic treatment were excluded.

The crowns of the teeth were sectioned at the cementoenamel junction to produce specimens with a standard length of 16 mm. The working length (WL) was determined by inserting a #10 K-type file until it was visible at the apical foramen with an operatory microscope (MC-M12MF; DFVasconcellos, Valença, RJ, Brazil) under 12.5× magnification, and then subtracting 1 mm from the resulting length. Whenever canal patency could not be obtained or when a #10 K-type file did not fit perfectly, the root was replaced by another specimen [23]. Two longitudinal grooves were then made on the buccal and lingual surfaces of the roots with a diamond disc (KG Sorensen, Cotia, SP, Brazil) [16, 24], as a preparation step for the cleavage procedure to be performed prior to the SEM evaluation. Subsequently, the canal was enlarged to a diameter corresponding to a #20 Flexofile instrument (Dentsply-Maillefer, Ballaigues, Switzerland) to allow contamination by *E. faecalis*.

Initially, two layers of an epoxy adhesive (Araldite; Brascola Ltda, Taboão da Serra, SP, Brazil) were applied to root apices, which were then covered with a pellet of #7 wax (Asfer Indústria Química, São Caetano do Sul, SP, Brazil). Next, the roots were inserted into polyvinychloride (PVC) tubes, and the space between the roots and the tubes was filled with condensation silicone. Finally, the roots were covered with a thin layer of ethyl-cyanoacrylate (Super Bonder, Loctite-Henkel, Itapevi, SP, Brazil) to provide stability. The specimens were then randomly numbered from 1 to 55 (www.random.org), sterilized in ethylene oxide (ACECIL Comércio de Esterilização e Indústria Ltda, Campinas, SP, Brazil), and contaminated with *E. faecalis* after 14 days. The specimens were stored in an incubator during this time.

***Contamination of specimens***

The microorganism targeted in the PCR test was *E. faecalis* (American Type Tissue Collection, USA, ATCC 29212), cultured and stored in brain-heart infusion (BHI) liquid medium with 20% glycerol. The inoculum was prepared by mixing 100 µL of *E. faecalis* stock in 2 mL of BHI, kept in an incubator at 37°C for a maximum of five days. After this period, the broth turbidity was compared to that of the MacFarland 1 scale (Nefelobac, Probac, Brazil), equivalent to a count of 3.0×108 bacteria/mL. Three teeth from each group were contaminated with *E. faecalis* and kept at 37°C for 21 days. Every three days, more BHI was added when necessary, and the effectiveness of the contamination was observed. The root canal was rinsed with 1 mL of sterile 0.9% saline to remove unbound bacteria, and an initial collection of material from inside the canals was performed with a #20 sterile absorbent paper point for 1 min. The paper points were stored in polypropylene Eppendorf tubes containing 1 mL of sterile phosphate buffered saline (PBS) at pH 7.2.

The teeth were instrumented using the ProTaper Universal rotary system (Dentsply Sirona, Ballaigues, Switzerland) and the X-Smart electric motor (Dentsply Sirona, Ballaigues, Switzerland). A hybrid instrumentation technique was applied for a standardized time of 7 min, as follows: Flexofile instruments #10 and #15 up to the WL, S1 and SX files operated at a speed of 300 rpm and torque of 3 N/cm and applied with a brushing motion; Flexofile instrument #15 up to the WL, S1 and S2 files to prepare the middle and apical thirds with a brushing motion; Flexofile instrument #25 applied with alternating clockwise/counter-clockwise motion, F2 file at 300 rpm and 2 N/cm; Flexofile instrument #30 with alternating clockwise/counter-clockwise motion; and F3 file at 300 rpm and 3 N/cm with a brushing motion up to the WL.

Irrigation was performed at each instrument change with a disposable 5-mL plastic syringe coupled to a Navitip 31-G needle, introduced to a level 2 mm short of the WL [25]. A total of 20 mL of 2.5% NaOCl followed by 5 mL of 17% EDTA were applied during instrumentation in the NaOCl-EDTA group, whereas 20 mL of 1% PA and 20 mL of 0.9% saline were applied in the PA and control groups, respectively. The EDTA was agitated with a hand file for 3 min in the NaOCl-EDTA group, and an additional 5 mL of saline was applied in all the groups to neutralize the environment.

After instrumentation, a second collection was performed with a #30 sterile paper point, held in position for 1 min. The point was then placed in an Eppendorf tube containing the transport medium plus 1 mL of sterile PBS. The tube was vortexed for 30 sec, and the specimens were then kept at -20°C until DNA extraction.

***DNA extraction***

Samples were centrifuged and the supernatant, discarded. A lysis buffer (2% Triton X-100, 1% sodium dodecyl sulfate, 100 mM NaCl, 20 mM Tris-HCl, pH 7.5) was added, and the samples were kept at 65°C for 30 min. The samples were treated with a 25:24:1 v/v phenol, chloroform and isoamyl alcohol solution (UltraPure™; Life Technologies, Carlsbad, CA, USA), centrifuged under 10000 g for 5 min, and the aqueous phase was transferred and treated with isopropanol. After a new centrifugation, the supernatant was removed, and the pellet was washed with a 70% ethanol solution. Ethanol was then eliminated, and the DNA was purified in 25 µL of ultra-pure water. The concentration and quality of DNA were determined by a nano-spectrophotometer at 260 and 280 nm wavelengths. The standard curve was calculated in a previous pilot study, using a pure culture of *E. faecalis*.

***Real-time PCR***

Quantitative (q) PCR was performed using a 7500 Fast Real Time PCR System (Life Technologies, Carlsbad, CA, USA) with SYBR Green as the detection dye. Cycling conditions were 10 min at 95°C followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. The primer set for *E. faecalis* was F 5′-CCAATCAAATGGCGGCTTCTACG -3′ and R 5′-GCGATCAGGGAAATGATCGATTCC -3′. The quantification data were analyzed with SDS System Software (Life Technologies, Carlsbad, CA, USA), and the relative expression levels were calculated.

***Scanning electron microscopy (SEM) evaluation***

The instrumented teeth were cleaved, and both halves were taken to a desiccator containing silica, and subsequently metallized using a vacuum coating unit (Denton Vacuum Desk II; Moorestown, NJ, USA). Only the fragments free of cracks were selected for SEM analysis. Each root third was initially located under 50× magnification, and the image of the canal wall surface was gradually enlarged up to 500×. The most representative image of each third was then selected using a visual criterion (subjective). A single operator selected the images and demarcated the root thirds with a ballpoint pen.

Three examiners with a PhD in endodontics assessed the images using a scoring system modified from that proposed by Hülsmann [26]. Inter-examiner agreement was assessed using the Kappa test and was considered adequate (k=0.8). The 165 digital images obtained from the 55 specimens were numbered and displayed in a PowerPoint presentation (Microsoft Corp., Redmond, WA, USA). No identification of the experimental group or root third displayed was visible (blind test). Simultaneously, the examiners received a second presentation comprising four SEM images in descending order of cleanliness to serve as a reference for attributing scores of 0 to 4 to the study images, as follows: Score 0, completely clean surface

with all the dentinal tubules clean or with the rare presence of a smear layer; 100% clean walls; Score 1, Surface with less than 50% of dentinal tubules exposed; Score 2, Surface covered by a thin smear layer, with half of the dentinal tubules exposed; 50% clean walls; Score 3, Surface with more than 50% of the walls with dentinal tubules exposed; and Score 4, Surface completely covered by a thick smear layer, with no visible dentinal tubules; 100% dirty walls.

### *Statistical analysis*

The results obtained in the real-time PCR test were converted into log10. In the SEM analysis, the coincident scores between the 3 examiners were used. When the scores assigned to a specimen by the examiners did not coincide, the highest score was used. The results were analyzed using the Kruskal-Wallis test complemented by Dunn's test. BioEstat 4.0 software (BioEstat; Belém, PA, Brazil) was used in the analyses. The level of significance was set at 0.05.

## Results

### *Bactericidal action*

The real-time PCR test revealed significant microbial reductions ($P<0.05$) in the PA and NaOCl-EDTA groups, contrasting with the S group. There was no significant difference between the PA and NaOCl-EDTA groups ($P>0.05$; Table 1).

### *Smear layer removal*

In the intra-group analysis performed for the PA group, the scores in the apical third were significantly higher than in the cervical third ($P<0.05$); there was no significant difference between the middle and cervical thirds, nor between the middle and apical thirds($P>0.05$). In the NaOCl-EDTA group, scores were significantly higher in the apical third than in the cervical and middle thirds ($P<0.05$) (Table 2).

***Table 1.*** Microbial counts ($log_{10}$), obtained by the real-time PCR method, before and after irrigation with peracetic acid (PA), sodium hypochlorite associated to EDTA (NaOCl-EDTA), and saline solution (S)

| | PA | | NaOCl-EDTA | | S | |
|---|---|---|---|---|---|---|
| | B | A | B | A | B | A |
| **Median** | 4.42$^{A}$ | 2.07$^{B}$ | 6.24$^{A}$ | 3.13$^{B}$ | 5.14$^{A}$ | 2.82$^{A}$ |
| **Interquartile range** | 1.57 | 1.75 | 0.68 | 0.92 | 0.39 | 0.49 |
| **Arithmetic mean** | 4.55 | 2.03 | 6.15 | 3.30 | 4.92 | 2.80 |
| **Standard deviation** | 1.16 | 1.11 | 0.50 | 1.10 | 0.66 | 0.30 |
| ***P*-value** | <0.0001 | | <0.0001 | | 0.0679 | |
| **% reduction** | 98.22%$^{A}$ | | 97.99%$^{A}$ | | 71.58%$^{B}$ | |

*B: before instrumentation; A: after instrumentation; different letters represent significant differences (P<0.05; Kruskal-Wallis test complemented by Dunn's test)*

***Table 2.*** Intra-group comparison of the frequency distribution (*n*, %) and mean values for the smear layer scores assigned to each root canal third after irrigation with peracetic acid (PA) and sodium hypochlorite associated to EDTA (NaOCl-EDTA).

| | PA | | | NaOCl-EDTA | | |
|---|---|---|---|---|---|---|
| | C | M | A | C | M | A |
| **Score 0** | 5.20% | 1.4% | 1.4% | 8.32% | 2.8% | 0.0% |
| **Score 1** | 6.24% | 4.16% | 2.8% | 8.32% | 7.28% | 0.0% |
| **Score 2** | 8.32% | 10.40% | 7.28% | 9.36% | 11.44% | 7.28% |
| **Score 3** | 6.24% | 10.40% | 15.60% | 0.0% | 5.20% | 18.72% |
| **Arithmetic mean** | 1.6$^{A}$ | 2.16$^{AB}$ | 2.44$^{B}$ | 1.04$^{A}$ | 1.76$^{A}$ | 2.72$^{B}$ |
| ***P*-value** | 0.01 | | | 0.00 | | |

*C: cervical third; M: middle third; A: apical third; different letters represent significant differences between thirds within the same group (P < 0.05; Kruskal-Wallis test complemented by Dunn's test)*

***Table 3.*** Inter-group comparison of the frequency distribution (*n*, %) and mean values for the smear layer scores assigned to each root canal third after irrigation with peracetic acid (PA), sodium hypochlorite associated to EDTA (NaOCl-EDTA), and saline solution (S)

| | C | | | M | | | A | | |
|---|---|---|---|---|---|---|---|---|---|
| | PA | NaOCl-EDTA | S | PA | NaOCl-EDTA | S | PA | NaOCl-EDTA | S |
| **Score 0** | 5.20% | 8.32% | 0.0% | 1.4% | 2.8% | 0.0% | 1.4% | 0.0% | 0.0% |
| **Score 1** | 6.24% | 8.32% | 0.0% | 4.16% | 7.28% | 0.0% | 2.8% | 0.0% | 0.0% |
| **Score 2** | 8.32% | 9.36% | 0.0% | 10.40% | 11.44% | 0.0% | 7.28% | 7.28% | 0.0% |
| **Score 3** | 6.24% | 0.0% | 25.100% | 10.40% | 5.20% | 25.100% | 15.60% | 18.72% | 25.100% |
| **Arithmetic mean** | 1.6$^{A}$ | 1.04$^{A}$ | 3$^{B}$ | 2.16$^{AB}$ | 1.76$^{A}$ | 3$^{B}$ | 2.44$^{A}$ | 2.72$^{A}$ | 3$^{A}$ |
| ***P*-value** | 0.0008 | | | 0.0067 | | | 0.1617 | | |

*C: cervical third; M: middle third; A: apical third; different letters represent significant differences between groups considering the same root third (P<0.05; Kruskal-Wallis test complemented by Dunn's test)*

The inter-group analysis revealed that, in the cervical third, the scores observed in the S Group were significantly higher than in the PA and NaOCl-EDTA groups ($P<0.05$). In the middle third, the highest scores occurred in the S Group, which were significantly higher than in the NaOCl-EDTA group ($P<0.05$). In the apical third, there was no significant difference between the groups ($P>0.05$), and the worst results were observed in this region (Table 3).

## Discussion

The 1% PA solution performed as well as the 2.5% NaOCl+17% EDTA solution regarding bactericidal action; however, there were significant differences between these groups and the control group, leading to a partial rejection of the null hypothesis. Cord *et al.* [16] showed that the efficacy of 1% PA was similar to that of 17% EDTA+2.5% NaOCl in cleaning root canals contaminated with *E. faecalis,* while Arias-Moliz *et al.* [15] showed that the total biovolume decrease observed after irrigating with 2.5% NaOCl alone, 2.5% NaOCl + 1% etidronic acid, or 1% PA solutions was significantly greater than the decrease obtained after using chlorhexidine or a control solution [15]. These studies confirm the results found in the present study that showed that the effectiveness of 1% PA against *E. Faecalis* was similar to that of 2.5% NaOCL+EDTA.

Significant microbial reductions were observed in all the experimental groups after instrumentation, with the exception of the control group. This finding confirms the results obtained by Baldasso *et al.* [27]. In none of the groups were the bacteria in the root canals totally eliminated, confirming the results obtained by Dornelles-Morgental *et al.* [28], who stated that irrigating solutions may present antimicrobial activity, but cannot eradicate *E. faecalis* from the root canal system. According to Siqueira Junior and Rôças [29], the presence of detectable levels of persistent bacteria in a great number of cases indicates that the continuous search for more effective antimicrobial treatment strategies should be encouraged [29].

PA could be advantageous compared to NaOCl, because it does not produce toxic residues when it decomposes. Teixeira *et al.* [18] found that 1% PA was less cytotoxic than 2.5% NaOCl and 17% EDTA [18]. PA can be used over a broad spectrum of temperatures (0°C to 40°C), and no microbial resistance to it has been reported to date. However, it is yet unknown whether its effectiveness in destroying microorganisms on surfaces is similar to that of NaOCl [30].

Over the past few years, more modern microbiological techniques, such as PCR, have allegedly allowed bacteria to be identified with greater precision, sensitivity and specificity than those using bacterial cultures [31]. Cogulu *et al.* [32], however, reported results indicating that both methods-PCR and bacterial culture-were equally sensitive in detecting *E. Faecalis* in root canals.

In the present study, the smear layer buildup observed in the apical third was significantly higher than in the cervical and middle thirds in the NaOCl-EDTA group. A possible explanation for this could be related to the surface tension of these irrigants, which would hamper their action in the apical third. This interpretation could also explain the less effective removal of smear layer in the PA group, considering that PA has a greater surface tension than NaOCl and EDTA. There was no significant difference between root thirds in the saline group, confirming the results found by Mello *et al.* [33]. The greater presence of dentin mud in the apical third also confirms the findings of Yang *et al.* [34], Rocha *et al.* [35], Haapasolo *et al.* [36], Zarei *et al.* [37], and Baldasso *et al.* [27]. These authors stated that the apical third is the most critical, because irrigation solutions display poorer cleaning action in this area.

According to Taneja *et al.* [38], PA was the irrigant that produced the highest level of calcium removal from the dentinal structure, thus lowering its microhardness. However, in their study, only samples from the cervical third of the root canal were analyzed, and the specimens were ground prior to their assessment, which may have interfered in the results.

De-Deus *et al.* [39], concluded that, after 60 sec, removal of the smear layer promoted by 0.5% and 2.25% PA was significantly greater than that promoted by 17% EDTA. However, this study was carried out on dentin discs in an experimental condition far-removed from clinical reality. This is because horizontal application of the solutions to the test specimens precluded gravitational force from being exerted on the irrigants within the root canals. In addition, the dentin discs were obtained from the coronal portion of the tooth.

This could explain the difference between these results and those of the present study, where similar results were obtained for both experimental groups in the apical and cervical thirds, and where NaOCl promoted even greater removal than PA in the middle third. Kuga *et al.* [40] concluded that both EDTA and PA associated to NaOCl failed to contribute significantly to increase the removal of dentin mud. Although that study also used immersed dentin discs, the considered areas were from the middle third of the root, therefore imparting greater reliability to the results, compared to those of De-Deus *et al.* [39]. On the other hand, the Kuga *et al.* [40] study was conducted on bovine teeth.

Tartari *et al.* [41] investigated the effects of decalcifying agents alone and in combination with NaOCl on organic and inorganic components of dentin. These authors found that diphosphonic acid and EDTA-$Na_4$ caused minor dentin

demineralization, whereas EDTA-$HNa_3$ and PA caused greater dentin demineralization; both effects were time-and concentration-dependent. Combinations of NaOCl and decalcifying agents can be used to create dentin surfaces with different compositions aiming at different interactions with endodontic cements. Tartari *et al.* [41] also observed that PA is capable of promoting tissue dilution, but to a lesser degree than that promoted by sodium hypochlorite. In the present study, PA was used alone in order to establish its isolated antibacterial and smear layer removal capacity. Nevertheless, new studies are warranted to assess new PA associations as well as its tissue dilution power.

Hartmann *et al.* [17] observed that passive ultrasonic irrigation contributed to a higher bactericidal efficiency of the irrigating solution. Thus, new protocols using sonic or ultrasonic agitation of PA in order to increase the tissue dissolving power of this solution should also be investigated.

De-Deus *et al.* [39] reported in a literature review that one of the major problems involved in using traditional SEM is the lack of standardization of the site evaluated, and the variation between specimens in terms of anatomy, curvature and dentin characteristics. In the present study, we sought to compensate for the lack of standardization by selecting the region of interest in sequential steps, namely, by initially assessing the entire surface of the canal wall under 50× magnification, then delimiting the root third, then progressively magnifying up to 500× , and, finally, selecting the most representative image of the root third considered.

According to Shahravan *et al.* [7], removal of the smear layer improved endodontic sealing, whereas other factors such as obturation technique or cement type had no significant effect on sealing. Carvalho *et al.* [42] found that the use of different chelating agents did not influence the adhesion strength of endodontic sealers. Their study, however, was also performed on dentin discs from the middle root third of the extracted teeth. This may have interfered in the results, owing to its far-removal from clinical reality. Kuga *et al.* [40] agreed with Carvalho *et al.* [42] and conclude that the association of NaOCl to acid solutions does not increase the penetration depth of the solution in root dentin.

## Conclusion

The irrigations performed with 1% peracetic acid and with 2.5% NaOCl associated to 17% EDTA showed similar bactericidal action against *E. faecalis*. The two irrigation regimens yielded equivalent smear layer removal results, except regarding the middle third of the root canal, where the NaOCl plus EDTA association was superior to PA. New *in vitro* and mainly *in vivo* studies with PA are warranted to provide more robust scientific evidence to support new root canal irrigation and cleaning protocols.

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