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Research Article

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Smear layer removal by passive ultrasonic irrigation and 2 new mechanical methods for activation of the chelating solution

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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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ABSTRACT

Objectives: The aim of this study was to compare smear layer removal by conventional application (CA), passive ultrasonic irrigation (PUI), EasyClean (EC), and XP-Endo Finisher (XPF), using 17% ethylenediaminetetraacetic acid (EDTA) after chemomechanical preparation, as evaluated with scanning electron microscopy (SEM).
Materials and Methods: Forty-five single-rooted human mandibular premolars were selected for this study. After chemomechanical preparation, the teeth were randomly divided into 5 groups according to the protocol for smear layer removal, as follows: G1 (control): CA of distilled water; G2 (CA): CA of 17% EDTA; G3 (PUI): 17% EDTA activated by PUI; G4 (EC): 17% EDTA activated by EC; and G5 (XPF): 17% EDTA activated by XPF. SEM images (×1,000) were obtained from each root third and scored by 3 examiners. Data were evaluated using the Kruskal-Wallis and Dunn tests ($p < 0.05$).
Results: In the apical third, there were no statistically significant differences among the groups ($p > 0.05$). In the cervical and middle thirds, the experimental groups performed better than the control group ($p < 0.05$); however, G2 presented better results than G3, G4, and G5 ($p < 0.05$), which showed no differences among one another ($p > 0.05$).
Conclusions: No irrigation method was able to completely remove the smear layer, especially in the apical third. Using CA for the chelating solution performed better than any form of activation.

Keywords: Cleaning; Irrigation; Root canal

INTRODUCTION

The main objectives of root canal treatment are to promote a significant reduction in the microbes present in the root canal system and to prevent recontamination [1]. Although mechanical debridement significantly reduces the bacterial load, complete disinfection is impossible due to the complex anatomy of the root canal system. Therefore, the need to use irrigating solutions is uncontestable [2]. However, the use of files associated with irrigation solutions causes the production of a smear layer. This is a 1- to 2-mm-thick amorphous structure containing both inorganic dentin debris and organic substances, including fragments of the odontoblastic process, microorganisms, and necrotic pulp tissue [3].

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Smear layer removal after chemomechanical preparation and before root canal filling has been recommended, since the smear layer can interfere with the diffusion of antimicrobial agents from intracanal medications into the root dentin [3], block the tubular entry of endodontic sealers, and act as a barrier between obturation materials and canal walls [4], thereby compromising root canal sealing and increasing the risk of reinfection [5].

The most common chelating solutions for smear layer removal are based on ethylenediaminetetraacetic acid (EDTA), which reacts with the calcium ions in dentin to form soluble calcium chelates [3]. Its conventional application (CA) with a needle and syringe does not seem to be able to remove the smear layer efficiently. Thus, different activation methods for enhancing the action of EDTA have been proposed and studied [6].

Passive ultrasonic irrigation (PUI) was initially described by Weller *et al.* [7]. The protocol is based on the passive insertion of a metal tip/file attached to an ultrasonic device oscillating at a frequency of 30 kHz into a canal filled with irrigating or chelating solution [7]. When the instrument is activated, it is surrounded by acoustic streaming to boost the performance of solution agitation and to enhance debris and smear layer removal [6]. Although many articles have been published on smear layer removal, there is no consensus in the literature on the efficiency of PUI in smear layer removal versus conventional irrigation. Some studies have reported that PUI enhanced canal cleaning [8,9], whereas others have found that it did not have significant differences from other methods [10,11].

The introduction of mechanical agitation of the irrigant using electric motor–driven instruments with reciprocating and rotary motion has provided new options for smear layer removal. Two new instruments recently launched in the market adopt the same principles of optimizing the action of chemical agents using instruments unaffected by contact with canal walls, by the space in which they operate, or by the dispersion of forces within the canal [12-15].

EasyClean (EC) is an acrylonitrile butadiene styrene plastic instrument (Easy Equipamentos Odontológicos, Belo Horizonte, Brazil [US patent pending 61/849,608]). The instrument has a size of 25/0.04 and an “aircraft wing”–shaped cross-section; it operates using reciprocating or rotary motion [14,15].

XP-Endo Finisher (XPF) (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) is an ISO 25/0.00 instrument produced with a special NiTi alloy known as MaxWire (Martensite-Austenite Electropolish-Flex, FKG). According to the manufacturer, the file is straight in its M phase (room temperature and when cooled), and changes into the A phase when exposed to body temperature, at which it takes on a unique spoon shape, 10 mm long from the tip and 1.5 mm deep, fashioned by its molecular memory. Its recommended operating speed with irrigating solutions is 800 rpm after root canal preparation to size #25 or larger [12,13].

The aim of this study was to compare smear layer removal by CA, PUI, EC, and XPF, using 17% EDTA as a chelating solution after chemomechanical preparation, by scanning electron microscopy (SEM). The null hypothesis tested was that there would be no significant differences among the protocols studied.

MATERIALS AND METHODS

Forty-five single-rooted human mandibular premolars with single straight canals and fully formed roots, free from cracks and previous endodontic treatment, were selected for this study after approval was received from the University Research Ethics Committee (CAAE. 09457419.3.0000.0109). Mesiodistal and buccolingual radiographs were performed to confirm that the teeth satisfied these requirements. The external root surfaces of the teeth were cleaned by ultrasound (Profi NEO – US, Dabi Atlante, Ribeirão Preto, SP, Brazil), and kept in receptacles containing 0.2% thymol solution until use. Immediately before the experiment, they were washed in running water for 24 hours for disinfection. The crowns of the teeth were then sectioned close to the amelocemental junction, using a double-faced disc (KG Sorensen, Barueri, SP, Brazil) to standardize the root segments to a length of 15 mm.

Biomechanical preparation

The root canal entrances were prepared with Largo #2 (Dentsply/Maillefer, Ballaigues, Switzerland) and #3082 burs (KG Sorensen). The cervical and middle thirds were prepared with Gates Glidden #3, #2, and #1 drills (Dentsply/Maillefer), according to the crown-down technique. The working length (WL) was determined by inserting a K-type #15 instrument (Dentsply/Maillefer) until it could be visualized at the apical foramen, and subtracting 1 mm from this measurement. Clinical conditions were simulated by sealing the apical region of each root with a layer of OpalDam Green gingival barrier (Ultradent Products, South Jordan, UT, USA), avoiding extravasation of the irrigating solution [9,16]. A #15 K-file was inserted before the layer was applied, to prevent the gingival barrier from entering the canal [16].

Reciproc R25 and R40 instruments (VDW, Munich, Germany), powered by an electric motor (VDW Silver; VDW), were used for mechanical preparation of the specimens, according to the manufacturer's instructions. Briefly, an R25 file was directed to the apical region until reaching the WL. During this procedure, the instrument was used in a reciprocating motion, with slight apical pressure and a slow in-and-out pecking motion, at an approximate amplitude of 3 mm. Then, an R40 instrument was used in the same manner up to the WL. Each instrument was used in 1 tooth and then discarded [15].

Irrigation was performed with a NaviTip 31-gauge double sideport needle (Ultradent Products), inserted 1 mm short of the WL, using a total volume of 40 mL of 2.5% sodium hypochlorite (NaOCl) per canal [15].

Smear layer removal

The teeth were randomly divided into 1 control ($n = 5$) and 4 experimental ($n = 10$) groups according to the protocol for smear layer removal that was used.

- G1 (control): The root canals were filled with 2.5 mL of distilled water using a 31-gauge NaviTip double sideport needle (Ultradent Products) calibrated to reach 1 mm short of the WL.
- G2 (CA): The root canals were filled with 2.5 mL of 17% EDTA using a 31-gauge NaviTip double sideport needle (Ultradent Products) calibrated to reach 1 mm short of the WL.
- G3 (PUI): The root canals were filled with 2.5 mL of 17% EDTA using a 31-gauge NaviTip double sideport needle (Ultradent Products) calibrated to reach 1 mm short of the WL. PUI was performed with a special tip having no cutting power, with a #20 and 0.01 apical diameter and taper, respectively (Irrisonic E1; Helse, Santa Rosa de Viterbo, Brazil), calibrated to 1 mm short of the WL, activated by ultrasound (Profi Neo

– US, Dabi Atlante, Ribeirão Preto, SP, Brazil) at a power of 40%, as indicated by the manufacturer. Care was taken to avoid contact with the walls of the root canal for more than 20 seconds.
- G4 (EC): The root canals were filled with 2.5 mL of 17% EDTA using a 31-gauge NaviTip double sideport needle (Ultradent Products) calibrated to reach 1 mm short of the WL. EC was introduced 1 mm short of the WL, and operated at low rotary speed for 20 seconds.
- G5 (XPF): Root canals were filled with 2.5 mL of 17% EDTA using a NaviTip 31-gauge double sideport needle (Ultradent Products) calibrated to reach 1 mm short of the WL. XPF was placed 1 mm short of the WL and activated for 20 seconds.

In each group, the solution used was renewed and/or activated for 9 cycles of 20 seconds each, totaling an irrigation/activation time of 3 minutes. The canals were then irrigated with 2.5 mL of 2.5% NaOCl (Fórmula & Ação), aspirated, and dried with absorbent paper points (R40, Reciproc. VDW).

Analysis by SEM

Initially, a gutta-percha cone with the same size as the last instrument (Reciproc R40 gutta percha, VDW) was introduced into the canal of all the specimens. Longitudinal grooves were made on the mesial and distal external surfaces of each tooth to facilitate fracturing the specimen into halves, using double-sided diamond discs (KG Sorensen), operated at low rotary speed until the presence of the gutta-percha cone was seen, thus avoiding accidental contamination and invasion of the canal by sharp debris [10,16].

The hemi-sections were then fixed on circular metal stubs to sputter-coat the surface with a 30-nm-thick coat of gold (Quorum Q150R ES, Ashford Kent, UK). Images of each hemi-section were captured for each root canal third at ×1,000 magnification using a scanning electron microscope (Tescan VEGA 3, Tescan, Brno, Czech Republic) (**Figure 1**).

Three examiners were calibrated by analyzing 15 images randomly selected from each third of 1 specimen from each group, using the scoring system proposed by Torabinejad *et al.* [17]: 1) absence or small quantity of smear layer (**Figure 1**: G2/cervical and middle thirds); 2) moderate presence of smear layer (**Figure 1**: G3/cervical third); and 3) dense smear layer covering practically all the dentinal tubule entrances (**Figure 1**/apical third). During this process, communication among examiners was allowed in order to establish only 1 final score for each image. The same methodology was used for the definitive process of scoring the images, but with no communication among examiners. The analysis was performed with the examiners blinded [4].

Statistical analysis

Initially, the kappa test was used to establish reproducibility among the examiners, and thereby to validate the findings. The Kruskal-Wallis test was used to compare the cleaning efficacy scores. The Dunn test was then applied for pairwise multiple comparisons. All statistical calculations were performed using Minitab 17® software (Minitab LLC, State College, PA, USA), with a 5% level of significance.

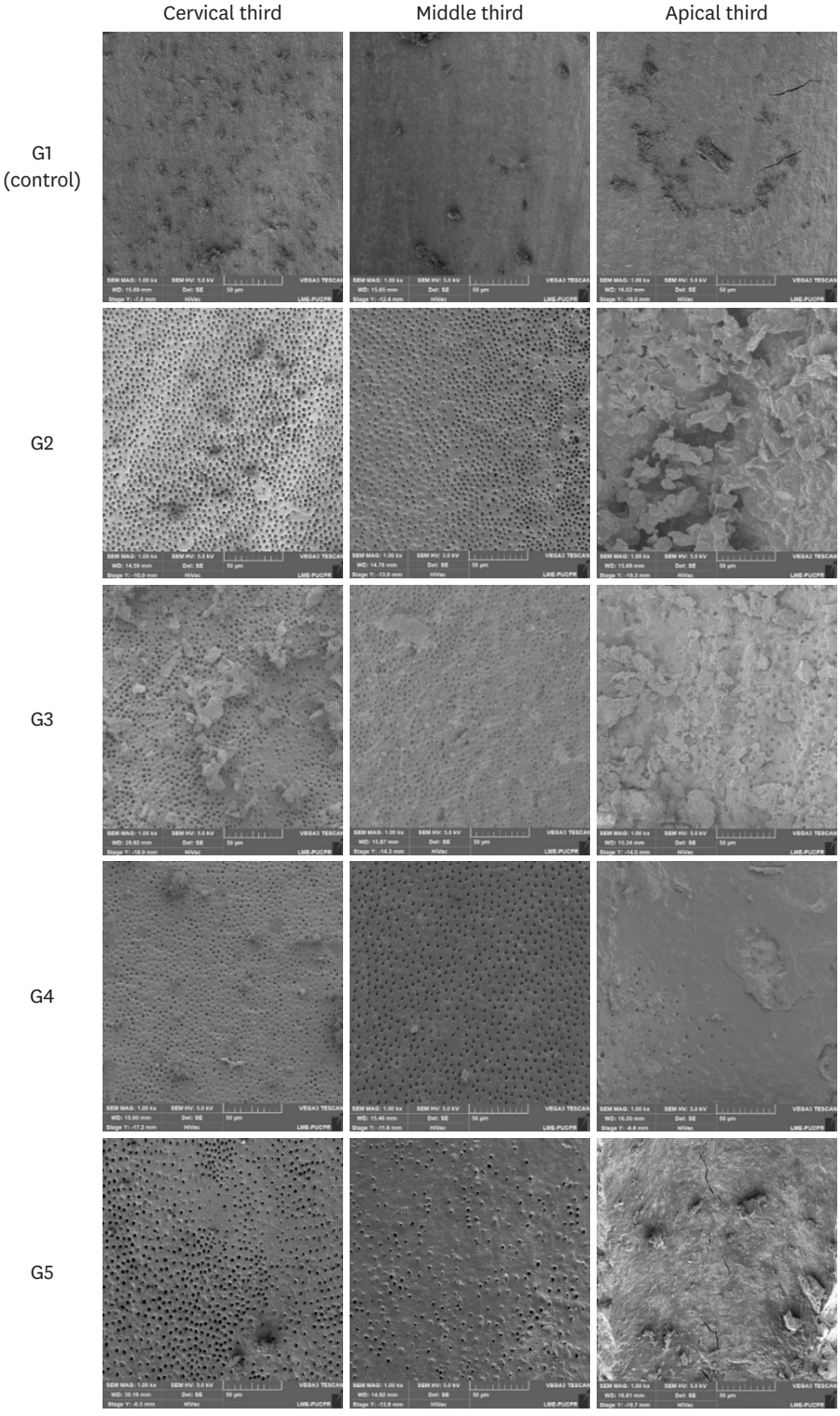

Figure 1. Scanning electron microscope images representative of the root canal walls according to groups and thirds.

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Table 1. General analysis of the root thirds, regardless of the group

Third	*n*	Median	Quartile deviation	*p* value
Cervical	45	2.00[a]	0.50	$p < 0.05$
Middle	45	2.00[a]	0.50	
Apical	45	3.00[b]	1.00	
Total	135	-	-	

Different superscript letters indicate statistically significant differences ($p < 0.05$).

Table 2. Results obtained for groups according to the root thirds

Group	Root third			*p* value
	Cervical ($n = 45$)	Middle ($n = 45$)	Apical ($n = 45$)	
G1: control group ($n = 5$)	$3.00 \pm 0.00^{A,a}$	$3.00 \pm 0.0^{A,a}$	$3.00 \pm 0.00^{A,a}$	1.0000
G2: CA ($n = 10$)	$1.00 \pm 0.00^{B,b}$	$1.00 \pm 0.37^{B,b}$	$3.00 \pm 0.37^{A,a}$	0.0002
G3: PUI ($n = 10$)	$2.00 \pm 0.00^{A,ab}$	$2.00 \pm 0.37^{A,ab}$	$2.50 \pm 0.50^{A,a}$	0.1220
G4 EC ($n = 10$)	$1.50 \pm 0.50^{B,ab}$	$2.00 \pm 0.50^{AB,ab}$	$3.00 \pm 0.37^{A,a}$	0.0104
G5: XPF ($n = 10$)	$2.50 \pm 1.00^{A,ab}$	$2.00 \pm 0.37^{A,ab}$	$3.00 \pm 0.37^{A,a}$	0.1433
p value	0.002	0.005	0.807	

Different superscript letters indicate statistically significant differences ($p < 0.05$). Considering thirds (columns): uppercase letters; considering rows: lowercase letters.
CA, conventional application; PUI, passive ultrasonic irrigation; EC, EasyClean; XPF, XP-Endo Finisher.

RESULTS

Kappa values of 0.90 and above were obtained, demonstrating excellent agreement among examiners for the scores given. In the apical third, there were no statistically significant differences among the groups ($p > 0.05$) (**Table 1**). In the cervical and middle thirds, the experimental groups (G2, G3, G4, and G5) performed better than the control group (G1) ($p < 0.05$); however, G2 presented better results than G3, G4, and G5 ($p < 0.05$), which showed no difference among one another ($p > 0.05$) (**Table 2**).

DISCUSSION

The smear layer may interfere with diffusion of antimicrobial agents from intracanal medications into the root dentin [3], block tubular entry of endodontic sealers, and act as a barrier between obturation materials and canal walls [4], thereby compromising root canal sealing and increasing the chances of reinfection [5]. Therefore, several methods have been proposed for its removal [14,18-21]. The aim of this *in vitro* study was to evaluate smear layer removal by CA, PUI, EC, and XPF, using 17% EDTA as a chelating solution after chemomechanical preparation, as evaluated with SEM. The null hypothesis tested was rejected because there were significant differences among the groups.

SEM is the most widely used method to evaluate smear layer removal [22,23]. However, this methodology has been criticized, because the areas analyzed are limited relative to the entire space of the root canal. Therefore, the practice of attributing scores to classify the degree of cleanliness may be a very subjective parameter, contingent on the different interpretations of each examiner [24,25]. In an endeavor to decrease the negative impacts of subjectivity, the images in this study were acquired at smaller magnifications (×1,000) than in other studies – (×2,000 [26] and (×1,500 [27], thus allowing a more comprehensive analysis of each specimen in each root third. In addition, an appropriate calibration process was performed before classifying the images [26]. Kappa values of 0.90 and above were

obtained, demonstrating excellent agreement among the examiners and supporting both the importance of the calibration process and the reliability of the results obtained [4].

Machado *et al.* [28] compared the amount of residual smear layer after root canal instrumentation using different instrumentation systems (WaveOne, Reciproc, Unicone, ProTaper Next, Mtwo, and HyFlex). The systems tested showed similar performance in terms of their ability to remove the smear layer, irrespective of the alloy composition. However, considering only the systems with reciprocating motion, the performance of WaveOne was superior to that of Reciproc. Since the present study sought to analyze only smear layer removal by different methods, the Reciproc system was selected, because it produces a greater amount of residual smear layer during chemomechanical preparation.

A closed apex model was used to simulate clinical situations more accurately. In this model, the canal behaves like a closed-end space, and *in vivo* cleaning and shaping in a space where the root is enclosed by the bone socket could result in gas entrapment inside the root canal, creating a vapor-lock effect [9,15,16,29,30].

No statistically significant differences were found among the groups in the apical third ($p > 0.05$). This finding confirms the difficulties encountered in smear layer removal regarding the final millimeters of the root canal, as reported by several other authors who used different irrigation methods [9,10,16]. Considering only the CA group, this result might be explained by the accentuated constriction of the apical third, which hinders the flow and backflow of both irrigating and chelating solutions, compromising smear layer removal [4,31]. Regarding the other groups, it is important to clarify that, in general, the activation of a chelating solution within the root canal by an instrument aims to enhance the cleaning process by moving the solution against the dentinal walls without the instrument touching them. For this, a certain amount of space is needed. In the apical third, this space is invariably smaller than the space found in the cervical and middle thirds; therefore, cleaning capacity can be diminished. If the instrument used for the chelating solution activation touches the root canal walls, a new smear layer may even form during this process [32].

In the cervical and middle thirds, there were no statistically significant differences in smear layer removal among the groups that used some form of chelating solution activation (G3, G4, and G5) ($p > 0.05$). EC performed similarly to PUI, unlike previous findings, which showed more effective debris removal either using the former [14] or using the latter [30]. These contradictory results may be explained by differences in the methodological designs of the studies. Kato *et al.* [14] performed chemomechanical preparations using the ProDesign Logic rotary system (Easy Equipamentos Odontológicos) up to a 30/0.05 file in the mesiobuccal root canals of mandibular molars with 3 mL of distilled water at each change of file. The chelating solution (17% EDTA) was activated for 3 cycles of 20 seconds each. Afterwards, 6 round indentations created on the apical third at 1-mm intervals were cleaned and analyzed by environmental SEM. In the study of Prado *et al.* [30], chemomechanical preparations were performed by using a K3 rotary system up to a 25/0.06 file in single-rooted anterior teeth with 1 mL of 6% NaOCl at each change of file. In the PUI and EC groups, another chelating solution (QMix) was activated for 1 minute (no cycles were reported). In the present study, mandibular premolars were instrumented with the Reciproc System up to a size 40/0.06 file and irrigated with 2.5 mL of 2.5% NaOCl, and 17% EDTA was activated for 9 cycles of 20 seconds each. The root canal anatomy [33], the instrumentation system [28], the apical preparation size [34], and the irrigation method [16] all play important roles in

smear layer formation or removal. Considering these factors, Marques *et al.* [35] evaluated the smear layer after different final irrigation protocols, and used a methodological design very similar to that of our study. Mandibular premolars standardized to a length of 15 mm were instrumented with WaveOne Primary (25/0.08) or Large (40/0.08) files, using 2 mL of 2.5% NaOCl as the irrigant. In the final chemomechanical preparation, 17% EDTA was used for 3 minutes with and without agitation by EC, in rotary or reciprocating motion, and by PUI. Smear layer removal was analyzed by SEM images and scores. Corroborating our results, EC, with both reciprocating and rotary motion, showed similar results to those of PUI in the middle and cervical thirds.

XPF showed similar results to those of PUI and EC for smear layer removal. To the best of our knowledge, this is the first study to compare EC and XPF in terms of this variable; therefore, there is no way of discussing it directly or comparatively. Regarding the lack of significant differences between XPF and PUI for smear layer removal, Leoni *et al.* [8] found the same result when analyzing accumulated hard tissue debris in the mesial root canals of mandibular first molars. This may be explained by the highly flexible proprietary alloy used in the XPF, combined with its small core size and zero taper. These attributes allow it to expand its reach when in rotation. It can be inferred that this unique property produced agitation of the chelating solution, thereby allowing disruption of the smear layer within the root canal, followed by its removal through the final flushing action of the syringe/needle irrigation, similarly to PUI [8].

The central point of this study was that the static use of EDTA showed better results for removing the smear layer from the cervical and middle thirds than found in the groups where different forms of activation were applied. This finding is in contrast with those of most studies on the subject [8,9,16]. Corroborating the results reported by Schimidt *et al.* [10], the present study also found a larger number of samples showing dentin erosion in the groups where different forms of activation were applied [10,36]. According to these authors, the eroded dentin surface showed significant irregularities, making it harder to identify dentinal tubules. However, they used a software that considered grayscale images to perform an automatic analysis and; therefore, the risk of bias was greater. In the present study, a conventional SEM analysis was performed and enabled a safe investigation of the removal of the smear layer in different root canal thirds, regardless of the presence of dentin erosion. The degree of agreement among examiners corroborates this fact. Furthermore, Kanaan *et al.* [37] evaluated whether ultrasonic activation, EC, or EDDY systems, used to promote agitation of the irrigating solutions during the final irrigation step, could lead to smear layer formation in the apical third of the root canal. Within the limitations of the methodology employed, it was concluded that all the tested irrigant agitation systems were associated with smear layer formation during application of the final irrigation step, even if no previous instrumentation procedure was performed on the root canal walls.

The experimental groups showed significant smear layer removal, meaning that the root canal system demonstrated improved cleanliness [15]. However, none of the systems was able to leave root canals completely clean. This finding corroborates those of several previous studies [8,15], and underscores the conclusion that chemomechanical preparation invariably creates a dense accumulation of the smear layer, which cannot be removed with currently available techniques [15]. For this reason, it is imperative that this deficiency be resolved by developing new protocols and instruments to optimize and enhance the cleaning of root canals before filling them [9,15].

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CONCLUSIONS

No irrigation method was able to completely remove the smear layer, especially in the apical third. Using CA for the chelating solution performed better than any form of activation.

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