

Evaluation of EDTA, apple vinegar and SmearClear with and without ultrasonic activation on smear layer removal in different root canal levels

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ABSTRACT

Objective: This *in vitro* study evaluated the efficiency of EDTA, apple vinegar and SmearClear, with and without ultrasonic activation, on smear layer removal. **Methods:** Seventy extracted canines were randomly divided into eight groups and prepared by using ProTaper instruments. The final irrigation regimens used were: Group 1 (control) (SAL) and Group 2 (control) (SALUS): saline for 3 minutes without and with ultrasonics, respectively; Group 3 (EDTA) and Group 4 (EDTAUS): 17% EDTA for 3 minutes without and with ultrasonics, respectively; Group 5 (AV) and Group 6 (AVUS): apple vinegar for 3 minutes without and with ul-

trasonics, respectively; Group 7 (SC) and Group 8 (SCUS): SmearClear for 1 minute without and with ultrasonics, respectively. Specimens were then examined under scanning electron microscope and scored for smear layer removal on the coronal, middle and apical thirds. **Results and Conclusions:** Smear layer removal was most efficient when 17% EDTA and SmearClear were used, compared to apple vinegar. Ultrasonics did not improve the smear layer removal significantly in all groups. The poorest results were observed in the apical third of the root canal, with statistical differences between the coronal third in all irrigation regimens.

Keywords: Smear layer. Chelating agents. Ultrasonics.

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Introduction

In endodontic therapy, after biomechanical preparations, an amorphous, granular and irregular layer known as the smear layer is formed and deposited on root canal walls.¹ The smear layer contains organic and inorganic substances derived from ground dentin, pulpal remnants, fragments of odontoblastic processes, necrotic materials and microorganisms in cases of infected root canals.^{1,2,3}

It appears to be prudent to remove the smear layer because it occludes the tubules and hinders effective penetration of endodontic irrigants, intracanal dressing and sealers into lateral canals and dentinal tubules and may compromise the sealing between root canal filling and the root canal wall.^{2,3,4}

No irrigating solution used in endodontic treatment is capable of acting on the organic and inorganic elements of the smear layer simultaneously. Sodium hypochlorite (NaOCl), in concentrations of 0.5% to 5.25%, is the main endodontic irrigant, but when used alone is ineffective in removing the entire smear layer.^{1,2,3} Chelating agents are used in endodontics to aid in root canal irrigation and to remove the inorganic smear layer.^{1,3,4} The ethylenediaminetetraacetic acid (EDTA) at a neutral pH has been recommended since 1957⁵ and it is the one most frequently employed for the removal of the smear layer.^{3,4,6}

Other substances have also been suggested to remove the smear layer, such as citric acid and apple vinegar.^{7,8,9} Apple vinegar is composed of 5% acetic acid and 0,35% malic acid.¹⁰ It has good cost-effectiveness and is a biocompatible substance.⁷ Its antimicrobial potential has already been demonstrated,¹¹ but little published data is available regarding its cleaning ability.

The apical region is the portion of the canal most difficult to be cleaned because of the difficult of debridement and its complex anatomy. The chelating agents, such as EDTA, has been shown to be effective in achieving smear-free walls, mainly at the middle and coronal thirds.^{12,13,14} However, the cleaning action is reduced toward the apex and is less efficient in the apical region of the root canal.^{1,12-17} This could be attributed to the narrow dimensions of the apical third, which can prevent the effective distribution of irrigants, resulting in limited contact between the canal walls and the solutions.¹⁵

Some substances or methods have been proposed to improve the penetration of irrigants into the apical

third of the root canal, such as the addition of surfactants to irrigating solutions and the use of ultrasonics,¹⁸ SmearClear (SybronEndo, Orange, CA) is a product indicated for smear layer removal, containing 17% EDTA with 2 additional surfactants. The use of ultrasonics has been suggested to improve irrigation in the root canal because it employs an acoustic streaming effect along the length of the oscillating file,¹⁹ and this effect could be beneficial in transporting irrigating solutions to the apical portion of the root canal.¹⁸

Various studies have been published on the use of ultrasonics for root canal irrigation,^{15,18,20-23} but only one study suggests the use of ultrasonic associated with SmearClear.¹⁸ There is no study evaluating the effectiveness of the combined use of apple vinegar with ultrasonics for removal of the smear layer from the root canal.

Therefore, the purpose of this scanning electron microscopic (SEM) study was to evaluate the effectiveness of 17% EDTA, apple vinegar and SmearClear, with or without ultrasonic activation in the removal of the smear layer at the coronal, middle and apical thirds of the root canal.

Materials and methods

Seventy extracted fully developed human canines with a single straight root were stored in saline solution after collection. An approval for this study was obtained from Ethics Committee of Bauru Dental School – Process n° 180/2009. The teeth were radiographed to observe the pulp chamber and root canal morphology and were selected based on uniform root canal width as determined by buccolingual and mesiodistal radiographs. The teeth were decoronated and the working length was determined by inserting a #10 K-File until the tip of the file was visible at the apical foramen and deducting 1 mm from this length. Warm wax was then used to close the apical foramen. The teeth were instrumented using a crown-down technique with ProTaper Universal rotary files (Maillefer, Ballaigues, Switzerland) with a constant speed of 300 rpm using a gentle in-and-out movement up to the F5 file corresponding to a 50/.04 size/taper. Between every instrument change, irrigation with 1 ml of 2,5% NaOCl (Rioquímica, São José do Rio Preto, São Paulo, Brazil) was performed by using a disposable syringe with a 27-G needle. After instrumentation, the teeth were randomly divided into six experimental groups (n = 10) and

two control groups ($n = 5$) to achieve different final irrigation sequences: Group 1 (control) (SAL): 3 ml of saline for 3 minutes without ultrasonics; Group 2 (control) (SALUS): 3 ml of saline for 3 minutes, activating the solution in the first minute with ultrasonics; Group 3 (EDTA): 3 ml of 17% EDTA (Biodinâmica, Ibiporã, Paraná, Brazil) for 3 minutes without ultrasonics; Group 4 (EDTAUS): 3 ml of 17% EDTA for 3 minutes, activating the solution in the first minute with ultrasonics; Group 5 (AV): 3 ml of apple vinegar (Castelo, Jundiaí, São Paulo, Brazil) for 3 minutes without ultrasonics; Group 6 (AVUS): 3 ml of apple vinegar for 3 minutes, activating the solution in the first minute with ultrasonics; Group 7 (SC): 3 ml of SmearClear for 1 minute, according to the manufacturer's instructions, without ultrasonics; Group 8 (SCUS): 3 ml of SmearClear for 1 minute with ultrasonics. After these procedures, all groups received a final flush of 5 ml of 2,5% NaOCl followed by 5 ml of saline. When the ultrasonic was used in the final irrigation sequence, it was activated using a finger spreader B (Dentsply, Maillefer, Ballaigues, Switzerland) adapted to the standard unit Jet Sonic (Gnatus, Ribeirão Preto, São Paulo, Brazil) at a power setting of 2. The finger spreader was placed in the center of the canal, avoiding the contact of the instrument to the canal walls. The root canals were then dried with absorbent paper points and the teeth were split open to expose the root interiors. Two longitudinal grooves were made in a buccolingual direction along the root surface with a carborundum disc at low-speed and a wedge was used to split the roots into two halves. The samples were dried, mounted on metallic stubs, coated with gold, and evaluated under a scanning electron microscope (JEOL, JSM T 220 A, Tokyo, Japan) at the coronal, middle and apical levels. Each radicular third of all samples was first viewed at a magnification of 500 X in order to obtain an overview of the region analyzed. Subsequently, an image acquisition on the most typical zones of the sample was performed at a magnification of 750X to assess the presence or absence of smear layer. Three pictures were obtained from each sample, one for each radicular third, for a total of 210 pictures. The amount of smear layer observed was scored as follows: 1 – no smear layer (Fig 1A); 2 – few areas covered by smear layer, with many dentinal tubule openings visible (Fig 1B); 3 – most areas covered by smear layer, with

few dentinal tubule openings visible (Fig 1C); 4 – all areas covered by smear layer, no dentinal tubule openings visible (Fig 1D).

Three examiners performed the blinded evaluations separately, after the calibration, which consisted of examining a few images together. The intra and inter-examiner's reliability was verified by using the Kappa test.

The smear layer scores were calculated between the groups using the Kruskal-Wallis and Dunn test. The use of ultrasonics was calculated and evaluated by the Mann-Whitney test. The Friedman test was used to compare the cleaning of the thirds of root canals. The level of significance was set at $p < 0.05$.

Results

Intra-examiner and inter-examiner agreements evaluated with the Kappa test showed satisfactory values of 0.74 and above for the different categories.

Table 1 shows the median and the mean rank of all groups of irrigation.

At the coronal third, there were significant differences ($p < 0.05$) between groups 3 (EDTA) and 1 (SAL); groups 4 (EDTAUS) and 1 (SAL); groups 7 (SC) and 1 (SAL); groups 8 (SCUS) and 1 (SAL).

At the middle third, the significant differences ($p < 0.05$) were observed in groups 4 (EDTAUS) and 1 (SAL); groups 4 (EDTAUS) and 2 (SALUS).

At the apical third, the results showed significant differences ($p < 0.05$) in groups 4 (EDTAUS) and 1 (SAL); groups 4 (EDTAUS) and 2 (SALUS); groups 7 (SC) and 1 (SAL); groups 8 (SCUS) and 1 (SAL).

When the irrigating solutions were analyzed without considering the use of ultrasonics, there were significant differences ($p < 0.05$) in the following comparisons: EDTA and saline; and SmearClear and saline in all thirds evaluated; apple vinegar and saline at the middle and apical thirds; and EDTA and apple vinegar only at the coronal third.

When comparing the effects of ultrasonic activation, there were no significant differences ($p > 0.05$) between the groups with and without the use of ultrasonics, in all radicular thirds.

When comparing the cleaning in the different radicular thirds, there was significant difference ($p < 0.05$) at the coronal and apical thirds, independent of the irrigation regimen used.

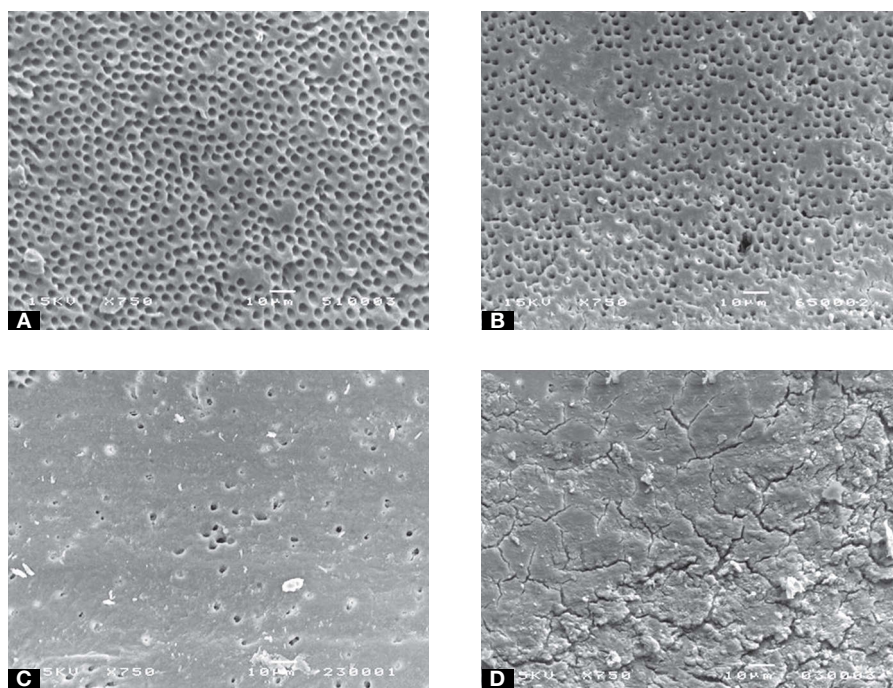


Figure 1. Representative SEM photomicrographs of specimens with score 1 (A), score 2 (B), score 3 (C) and score 4 (D) (750X).

Table 1. Median and the mean rank of all groups of irrigation.

Group	Cervical third		Middle third		Apical third		N° specimens
	Median	Mean rank	Median	Mean rank	Median	Mean rank	
Saline solution	4.0	68.00	4.0	61.70	4.0	65.50	5
Saline solution + ultrasonics	3.0	46.40	3.0	58.80	4.0	58.50	5
EDTA	1.5	22.90	2.0	28.50	2.0	32.95	10
EDTA + ultrasonics	1.5	22.90	1.0	15.20	2.0	22.80	10
Apple vinegar	2.0	41.60	2.5	38.20	3.0	35.05	10
Apple vinegar + ultrasonics	2.5	39.40	2.0	37.70	3.0	41.35	10
SmearClear	2.0	32.30	2.0	33.60	2.0	26.65	10
SmearClear + ultrasonics	2.0	32.20	2.0	35.05	2.0	27.70	10

Discussion and Conclusion

In this SEM study, we attempted to evaluate methods to improve, especially in the apical third, the removal of the smear layer of prepared root canals. The results showed that EDTA could efficiently remove the smear layer from all canal thirds, whereas saline was not able to effectively remove the smear layer from any of the root canal's portions. Some authors^{23,24,25} demonstrated that irrigation with EDTA is effective in removing the smear layer, which is in agreement with the finding of our study.

Reducing the surface tension of an endodontic solution improves its flow into narrow root canals.²⁶ The apical third is the most difficult portion of the root ca-

nal to be cleaned and this could be attributed to its narrow dimensions.¹⁵ The results of this current study demonstrated that SmearClear had a better performance compared with EDTA in the apical third, but not statistically significant. In this study, SmearClear and EDTA had similar abilities to remove the smear layer from the root canal. These findings show that the addition of surfactants in SmearClear did not enhance the cleaning ability of the EDTA, which is in agreement with the findings of other studies.^{17,18,25,27,28}

In our study we used apple vinegar as an experimental solution to possibly remove the smear layer in comparison with conventional chelators, such as EDTA. Apple vinegar has acids in its constitution,

especially acetic acid and malic acid.¹⁰ Malic acid in apple vinegar confers its therapeutic properties.¹¹ Apple vinegar also has an antimicrobial potential against the endodontic microbiota.¹¹ In this study, apple vinegar was not able to completely remove the smear layer from the root canal, with significant differences between EDTA in the coronal third, and with some dentinal tubules remaining covered by a smear layer in all thirds. Besides, the pH of apple vinegar used in this study was 2.96, and it could cause damage on the root canal walls.

In analyzing the photomicrographs of all groups, we observed a better cleaning ability in the specimens with the use of ultrasonics, although there were no statistical differences between the groups with and without the ultrasonics. In our study, ultrasonic activation of irrigants did not improve smear layer removal and dentinal tubule opening, which is in accordance with the findings presented by other authors.^{14,15,23,29} Ultrasonic activation in this study was performed dur-

ing a 1 minute period. Cameron²⁰ reported better effects with ultrasonics when used for 3 minutes. Other researchers also achieved an effective smear layer removal when ultrasonic activation was performed for a longer period than 1 minute.^{21,30} The diameter of the finger spreader used corresponded to a 25# file, and some authors²¹ recommend the use of an instrument with a smaller diameter to avoid the contact of the instrument with the root canal walls.

Our results demonstrated that the removal of the smear layer was less effective in the apical third of the root canal, with a statistical difference in the coronal third, regardless of the irrigation regimen used. Torabinejad et al⁶ attributed this fact to the inadequate penetration of the solution into the apical portion of the canal during the irrigation.

Based on the results of this experiment, we observed that the removal of the smear layer appears to be mostly influenced by the chemical action of the irrigating solution than the ultrasonic activation of irrigants.

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