

# A comparative scanning electron microscopy evaluation of smear layer removal with apple vinegar and sodium hypochlorite associated with EDTA

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## ABSTRACT

**O**bjective: The purpose of this study was to evaluate by scanning electron microscopy (SEM) the removal of smear layer from the middle and apical root thirds after use of different irrigating solutions. Material and Methods: Forty roots of permanent human teeth had their canals instrumented and were randomly assigned to 4 groups (n=10), according to the irrigating solution: apple vinegar (group A), apple vinegar finished with 17% ethylenediaminetetraacetic acid (EDTA) (group B), 1% sodium hypochlorite (NaOCl) finished with 17% EDTA (group C) and saline (group D - control). After chemomechanical preparation, the roots were cleaved longitudinally and their middle and apical thirds were examined by SEM at  $\times 1,000$  magnification. Two calibrated examiners ( $\kappa=0.92$ ) analyzed the SEM micrographs qualitatively attributing scores that indicated the efficacy of the solutions in removing the smear layer from the surface of the dentin tubules (1 - poor, 2 - good and 3 - excellent). Data from the control and experimental groups were analyzed by the Kruskal-Wallis and Dunn's test, while the Wilcoxon test was used to compare the middle and apical thirds of the canals within the same group ( $\alpha=0.05$ ). Results: The middle third presented less amount of smear layer than the apical third, regardless of the irrigant. There was statistically significant difference ( $p=0.0402$ ) among the groups in the middle third. In the apical third, the apple vinegar/EDTA group showed the greatest removal of smear layer ( $p=0.0373$ ). Conclusion: Apple vinegar associated or not with EDTA was effective in removing smear layer when used as an endodontic irrigant.

**Key words:** EDTA. Smear layer. Scanning electron microscopy.

## INTRODUCTION

Sodium hypochlorite (NaOCl) is one of the most widely used endodontic irrigants for the chemomechanical preparation of root canals because of its excellent antimicrobial action and capacity of dissolving organic materials<sup>15</sup>, which increase directly with the increase of the concentration<sup>21</sup>. However, the optimal organic

tissue-dissolving property of NaOCl is non-selective, which means that, especially at high concentrations, this chemical agent may dissolve both vital and necrotic pulp remnants indistinguishably and have high toxicity to the periapical tissues in case of inadvertent extrusion through the apical foramen to the periradicular space<sup>13</sup>. Another disadvantage of NaOCl is that it decreases the mechanical resistance of dentin<sup>15,22</sup> by causing deterioration of

collagen and proteoglycans<sup>14</sup>. There are also reports of accidents and allergic reactions to the use of NaOCl during root canal therapy<sup>3,17,18,29</sup>. Therefore, research has been done to find an irrigating solution that may have better biocompatibility than NaOCl while maintaining its properties of tissue solving capacity and high bactericidal action.

The use of different chemical irrigants for smear layer removal during the root canal treatment has been proposed, such as EDTA<sup>23,28</sup>, citric acid<sup>23</sup>, maleic acid<sup>19</sup> and apple vinegar<sup>9,24,30</sup>.

Vinegar has been indicated as an antiseptic agent due to its medicinal properties and has been used for the treatment of infected wounds<sup>26</sup>. Distilled white vinegar and wine vinegar are composed mainly of acetic acid, whereas apple vinegar is composed mainly of malic acid, which has therapeutic properties<sup>2</sup>. More recently, the use of apple vinegar as an auxiliary solution in the chemomechanical preparation of root canals has also been investigated and deserves attention due to the promising results obtained when compared to traditional endodontic irrigants, such as NaOCl and EDTA<sup>4</sup>. Pioneering studies evaluating the efficacy of apple vinegar on the endodontic microbiota, its physicochemical properties and its role on the periapical tissue healing have been done<sup>8,9,10,30</sup>. It has good cost-effectiveness and its principle of action in mineralized tissue is similar to that of EDTA<sup>4</sup>. Apple vinegar associates a good capacity to remove smear layer from the dentinal tubule entrances<sup>8,9,30</sup> with bactericidal action against microorganisms that are frequently associated with endodontic infections, such as *Staphylococcus aureus*<sup>7</sup> and *Enterococcus faecalis*<sup>10</sup>. The high biocompatibility of apple vinegar is mainly attributed to the high concentration of malic acid in its composition<sup>2</sup>.

The purpose of this study was to evaluate by scanning electron microscopy (SEM) the removal of smear layer from the middle and apical thirds of root canals irrigated with 1% NaOCl and apple vinegar associated or not with EDTA.

## MATERIAL AND METHODS

Forty extracted human maxillary and mandibular molars were selected from a random collection and were checked for absence of root caries, cracks and structural defects. Soft debris were eliminated with hand cures. Crowns were removed at the cemento-enamel junction with a carborundum disc under distilled water cooling, and the palatal roots of the maxillary molars and distal roots of mandibular molars were trimmed coronally to a uniform length of 16 mm and stored in saline until use to prevent dehydration.

The working length was checked with a #10 K-file (Dentsply/Maillefer, Ballaigues, Switzerland)

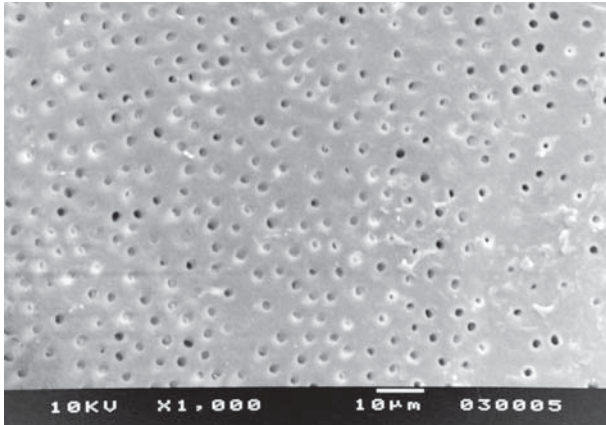
introduced into the root canal of each tooth up to the point when it was visualized at the apex and then pulled back 1 mm. All canals were instrumented up to a #45 K-file (Dentsply/Maillefer, Ballaigues, Switzerland). The roots were randomly assigned into 4 groups in each 10 specimens, each according to the irrigating solution used during chemomechanical preparation: A- Apple vinegar (Minhoto, Grupo Raymundo da Fonte, Paulista, PE, Brazil); B- Apple vinegar (Minhoto) and 17% EDTA as a final rinse (Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brazil); C- 1% NaOCl (Biodinâmica Química e Farmacêutica Ltda.) and 17% EDTA as a final rinse; D- Saline (control group). The canals were irrigated with 2 mL of each solution at every change of file followed by fluid content aspiration.

After chemomechanical preparation, grooves were prepared along the long axis of the roots with a water-cooled diamond disk (KG Sorensen Ind. Com., São Paulo, SP, Brazil) and a surgical chisel was used to cleave the roots longitudinally in a buccolingual direction to expose the entire canal extension. The middle and apical thirds of the canals were examined with a scanning electron microscope (JSM-T220A; JEOL, Tokyo, Japan) at  $\times 1,000$  magnification. Two calibrated examiners ( $\kappa=0.92$ ) examined the SEM micrographs qualitatively attributing scores that indicated the efficacy of the solutions in removing the smear layer from the dentinal walls surface and from dentin tubules entrance, according to Vale, et al.<sup>28</sup> (2003): Score 3 (Excellent) - no smear layer, total extension of dentin with open dentin tubules (Figure 1); Score 2 (Good): few areas covered by smear layer, and several dentin tubules visible (Figure 2); and Score 1 (Poor): all areas covered by smear layer, and no dentin tubules visible (Figure 3).

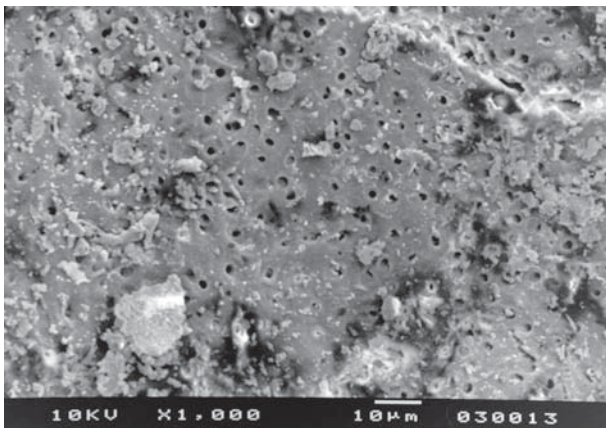
Data from the control and experimental groups were analyzed by the Kruskal-Wallis and Dunn's test, while the Wilcoxon test was used to compare the middle and apical thirds of the canals within the same group. A significance level of 5% was set for all analyses.

## RESULTS

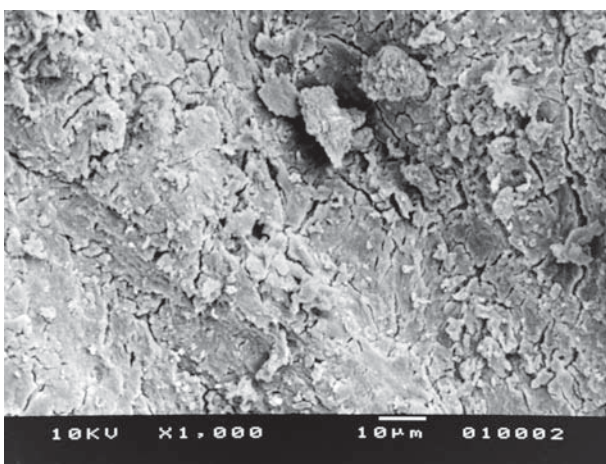
A significantly large number of specimens in the groups irrigated with apple vinegar (A and B) showed greater removal of smear layer from the middle third compared to the apical third ( $p < 0.05$ ), while no statistically significant difference ( $p > 0.05$ ) was found between the middle and apical thirds in the C and D groups. The B group (apple vinegar plus EDTA) showed the greatest efficacy in removing the smear layer from both the middle and apical thirds, but this difference was significant only in relation to groups C and D. (Figures 4 and 5) ( $p < 0.05$ ).



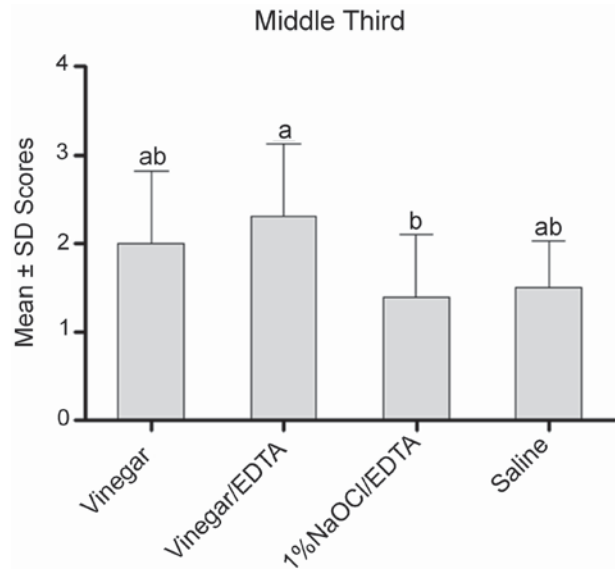
**Figure 1-** Scanning electron microscopy (SEM) micrograph of root dentin surface showing absence of smear layer and fully exposed dentin tubules, corresponding to score 3 (Excellent) ( $\times 1,000$  magnification)



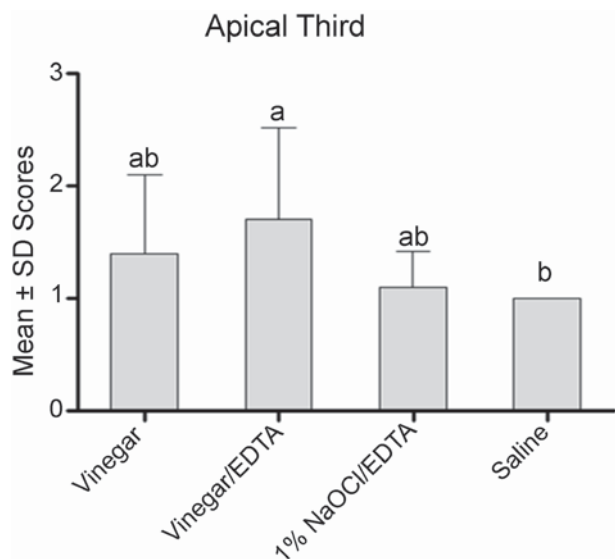
**Figure 2-** Scanning electron microscopy (SEM) micrograph of root dentin surface showing partial closure of the dentin tubules entrances by smear layer, corresponding to score 2 (Good) ( $\times 1,000$  magnification)



**Figure 3-** Scanning electron microscopy (SEM) micrograph of root dentin surface showing total closure of the dentinal tubule entrances by smear layer, corresponding to score 1 (Poor) ( $\times 1,000$  magnification)



**Figure 4-** Mean scores  $\pm$  Standard deviation recorded for each group ( $n=10$ ) in the middle root third. The groups with a different superscript letter are statistically different ( $p < 0.05$ )



**Figure 5-** Mean scores  $\pm$  Standard deviation recorded for each group ( $n=10$ ) in the apical root third. The groups with a different superscript letter are statistically different ( $p < 0.05$ )

The B group produced significantly cleaner canals in both middle and apical thirds ( $p=0.0402$  and  $p=0.0373$ , respectively). The sequence of the irrigating solutions in a decreasing order of efficacy in smear layer removal was as follows: B-apple vinegar/EDTA > A-apple vinegar > D-saline > C-NaOCl/EDTA, representing the middle third (Figure 4) and B-apple vinegar/EDTA > A-apple vinegar > C-NaOCl/EDTA > D-saline, representing the apical third (Figure 5).

## DISCUSSION

The main goals of the chemomechanical preparation are to eliminate bacteria and their byproducts from the root canal system, remove pulp tissue remnants and contaminated organic and inorganic debris that are formed during instrumentation and compacted into the dentin tubules and produce a continuously tapered shape in the crown-apex direction to allow effective irrigation and three-dimensional obturation of the canal space. Chemical endodontic irrigants must have some important properties such as biocompatibility, dissolution of organic tissues, bactericidal action and capacity to remove smear layer from the canal walls. Different solutions, such as NaOCl at several concentrations, chlorhexidine and more recently apple vinegar, have been used as endodontic irrigants.

The biocompatibility of apple vinegar is attributed to the presence in its composition of malic acid<sup>2</sup>, which has therapeutic properties. It increases the organism resistance because it is one of the acids of the Krebs cycle, which is a set of reactions responsible for production of energy in the cells. In addition, apple vinegar has a remarkable medicinal potential due to its high mineral content (potassium, phosphorus, magnesium, sulfur, calcium, fluoride and silicon)<sup>8</sup>, and contains other elements, such as pectin, beta-carotene, enzymes and amino acids, which attack free radicals that affect the immune system<sup>8,10</sup>, and may have some beneficial role the periapical repair process. Therefore, it may be assumed that apple vinegar has some antiinflammatory activity, which is an important characteristic for an endodontic irrigating solution. In addition to the biocompatibility, it has been demonstrated that apple vinegar has bactericidal activity against *E. faecalis*<sup>10</sup>, which is one of the main microorganisms associated with endodontic treatment failure.

The 1% NaOCl solution has considerable antibacterial activity and tissue-dissolving property<sup>1,6,16,20,21</sup>, and do not cause significant decrease of the structural resistance of dentin<sup>4</sup>.

In line with the findings of previous investigations<sup>25,27</sup>, in the present study, the apical third was more challenging for smear layer removal compared to the middle third. This can be explained by the fact that the access of instruments and chemical solutions to the middle third is easier and the exposed dentin tubules orifices are larger.

Failure to remove smear layer from the root canal walls is considered as one of the main reasons of endodontic therapy failure<sup>5,11,12</sup>. Removal of the smear layer can allow intracanal medicaments to penetrate the dentin tubules in infected root canals more readily and consequently cause a better

disinfection procedure<sup>11</sup>. The lack of adherence between the filling material and the smear-covered canal walls compromise the apical seal, which may result in apical leakage, favoring the survival and multiplication of bacteria that were not eliminated during the chemomechanical preparation<sup>5,11,12,20</sup>.

In a recent SEM evaluation, Spanó, et al.<sup>24</sup> (2009) verified smear layer removal with several root canal chelators (15% EDTA, 10% citric acid, 10% sodium citrate, apple vinegar, 5% acetic acid, 5% malic acid, and 1% NaOCl) and found that EDTA and citric acid were the most efficient solutions. However, those authors did not use apple vinegar as an irrigating solution but rather as a chelating agent for 5 min after chemomechanical preparation with 1% NaOCl, which makes it difficult to establish a comparison with the findings of the present study.

In the middle third, the groups irrigated with apple vinegar, especially when associated with EDTA, showed greater efficacy than NaOCl in smear layer removal, which is in agreement with the results of Prabhu, et al.<sup>19</sup> (2003). In the apical third, the association of apple vinegar and EDTA was more effective than the use of NaOCl associated with EDTA, in the same way as reported by Estrela, et al.<sup>9</sup> (2007) and Zandim, et al.<sup>30</sup> (2004). Since malic acid is present in the composition of apple vinegar, the results of the present study are consistent with those of Prabhu, et al.<sup>19</sup> (2003), who investigated the smear layer removal capacity of malic acid and found significantly better results than EDTA associated with 5.25% NaOCl.

Hence, the choice for the irrigating solutions used during endodontic therapy must take into account some important properties such as biocompatibility, bactericidal action and efficacy in eliminating organic and inorganic debris from the canal walls in order to promote greater elimination of microorganisms from the root canal system and improve the seal between the filling materials and the dentin walls.

## CONCLUSIONS

It may be concluded that the apple vinegar associated or not with EDTA was more effective in removing smear layer from the root canals than NaOCl associated with EDTA. Clinical investigations are needed to corroborate the use of apple vinegar in endodontics, since it is a biocompatible substance and which produces an effective elimination of smear layer *in vitro*, being potentially considered as an alternative for root canal cleaning.

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