Assessment of the Effectiveness of Apple Vinegar and Its Constituents for Removing the Smear Layer and Calcium Ions from the Root Canal

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Abstract: The aim of this study was to assess smear layer removal, by SEM, and to quantify the calcium ion release resulting from final irrigation with different chelating solutions, by means of atomic absorption spectrometry. Fifty human canines were debrided with the rotary system K3 and irrigated with apple vinegar, 5% malic acid, 5% acetic acid, 17% EDTA and distilled water (control). The solutions were collected after final irrigation and analyzed by atomic absorption and flame emission spectrometry to quantify the concentration of calcium ions released. Removal of the smear layer in the cervical, middle and apical thirds was assessed by scanning electronic microscopy. There was a statistically significant difference (p<0.001) between 17% EDTA and the other solutions with regard to smear layer removal. Apple vinegar, 5% malic acid and 5% acetic acid promoted root canal cleaning in a similar manner among them. There was no statistical difference among the thirds studied. The highest concentrations of calcium ions were obtained with 17% EDTA (p<0.001), followed by malic acid. Apple vinegar and acetic acid removed the smallest quantity of calcium ions. The results showed that 17% EDTA enabled greater smear layer removal and promoted release of the highest concentrations of calcium ions than the other solutions tested.

Keywords: calcium, EDTA, smear layer, vinegar.

INTRODUCTION

The action of endodontic instruments associated with the irrigant solution during the biomechanical preparation stage favors cleaning and disinfection of root canal systems, eliminating aggressive and irritant agents such as microorganisms and pulp tissue residues (HÜLSMANN et al., 2003). However, the instrument in contact with the root canal walls causes formation of the smear layer (GRANDINI et al., 2002), which results in obliterating the dentinal tubules making it difficult to eliminate microorganisms and compromising the filling of the root canal systems (McCOMB; SMITH, 1975).

The literature recommends the use of an irrigant solutions combined with chelating and demineralizing agents with the purpose of removing the organic and inorganic components of the smear layer, increasing the chances of
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successful endodontic therapy (HÜLSMANN et al., 2003; BAUNGARTNER et al., 2007).

The use of ethylenediamine tetra-acetic acid (EDTA) is usually recommended to remove the smear layer. This substance is a weak acid with chelating action and concomitant protein denaturing (HÜLSMANN et al., 2003), which promotes the increase in dental permeability facilitating the action of the intracanal medication (TORABINEJAD et al., 2002) and the bond between dentin and endodontic cements (SHAHRAVAN et al., 2007), in addition to being biocompatible (SOUSA et al., 2005).

Studies have been conducted with the aim of using chelating agents that would be more efficient and biocompatible with the organic structures than EDTA. Therefore, citric acid (De DEUS et al., 2006) and apple vinegar (ESTRELA et al., 2007) have been studied. Apple vinegar has proven antimicrobial action, reduces dentinal microhardness (ESTRELA et al., 2005), in addition to removing the smear layer (ESTRELA et al., 2007; SPANÓ et al., 2009).

It is a combination of acetic, citric, formic, lactic, succinate and tartaric acids with small quantities of alcohol resulting from the fermentation process and it is responsible for reducing the surface tension of the solution. However, the highest acid concentrations of the vinegar are represented by the acetic (5%) and malic (0.35%) acids (CALIGIANI et al., 2007).

In this study, an evaluation was made by scanning electronic microscopy, of the capacity of acetic and malic acids, apple vinegar and EDTA to remove the smear layer from the root canal. The amount of calcium ions removed from the root canal by these solutions was also analyzed by atomic absorption spectrometry.

**MATERIAL AND METHODS**

**Sample Preparation**

Fifty recently extracted human teeth with completely formed roots, which had a single canal and absence of calcifications or accentuated curvatures, were selected. The crowns were removed close to the amelocemental junction with a diamond disk (KG Sorensen, Brazil) in order to obtain remaining roots 17 mm long. The working length was determined by introducing a type K file #10 (Dentsply-Maillefer, Ballaigues, Switzerland) until it was seen in the apical foramen, and the value was measured with a calibration ruler and one millimeter was subtracted from the length obtained. After this procedure, the cervical preparation of each specimen was performed with LA Axxess 20/0.06, 35/0.06 and 45/0.06 burs (SybronEndo Corporation, West Collins, Orange, CA) and then the anatomic diameter of the tooth was determined with the instrument that ran and locked at the working length. All the roots were submitted to biomechanical preparation with the K3 system (SybronEndo Corporation, West Collins, Orange, CA) starting with at least instrument 20/0.02 up to instrument 40/0.02. The motor used to drive the rotary instruments was Endo-Mate TC (NSK, Kanuma, Japan) with constant torque of 3.7 N/cm (level 5) and 500 rpm.

At every change of instrument, the canals were irrigated with 5 ml of distilled water, using a disposable plastic syringe (Ultradent Products Inc., South Jordan, Utah, USA) and a NaviTip needle (Ultradent Products Inc., South Jordan, Utah, USA), and aspirating it with a siliconized tip (Capillary tip, Ultradent Products Inc., South Jordan, Utah, USA). After this, the teeth were randomly divided into 5 groups of 10 specimens, varying the auxiliary chemical solution used during final irrigation, following the protocols described below: G1 – distilled and deionized water; GII – apple vinegar; GIII – 5% malic acid; GIV – 5% acetic acid and GV – 17% EDTA. The specimens were fixed to the top portion of a plastic collector flask, so that the all the auxiliary solution used was collected and identified for later analysis. Each solution assessed remained inside the root canal for 5 minutes, and was removed after this period with irrigation of 5 ml of distilled and deionized water. Afterwards, the excess of solution was aspirated with a silicone cannula (Ultradent Products Inc, South Jordan, USA) coupled to a Luer Lock syringe and the canals were dried with absorbent paper cones. The 5% acetic acid, 5% malic acid and 17% EDTA solutions were manipulated by the company Fórmula & Ação LTDA (São Paulo-SP, Brazil), the apple vinegar used was from the Castelo Jundiaí brand – SP, Brazil, and the water used was distilled by means of a distiller (Quimis-Aparelhos Científicos Ltda) and a deionizer (Permutio - Equipamentos e Produtos Químicos Ltda).

After irrigation with the assessed solutions, longitudinal grooves were made diametrically opposite the vestibular and palatine faces of the
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roots, using a diamond disk. During this stage, all the measurements were taken in order to avoid communication of the external medium with the hollow passage of the root canal. To prevent cut scrapings from entering into the prepared canal, a utility wax plug was made at the mouth of the canal (Polidental, Cotia-SP, Brazil). After making the grooves, the teeth were separated with the aid of a surgical hammer and a chisel with a double bevel (Quinelato, Schobel Industrial Ltda, Rio Claro - SP, Brazil), thus obtaining two proximal fragments of the root, corresponding to the tooth faces to be analyzed. The section in which the prepared canal showed the largest area was selected for analysis by scanning electronic microscopy (JSM 5410, JEOL, Tokyo, Japan). During analysis, photomicrographs at 500X magnification were made, considering each third of the root canal for qualitative-quantitative study. The photomicrographs were assessed in a double-blind study by three previously calibrated examiners, using scores that ranged from 1 to 4, in accordance with the one proposed by TAKEDA et al. (1999). Score 1 was attributed to the surface completely covered by the smear layer; 2, for the surface partially covered by the smear layer with few visible dentinal tubules; 3, surface with little smear layer and visible tubules; and 4, for a smear layer-free surface.

Calcium ion quantification was performed by means of a flame atomic absorption spectrometer – Varian - model SpectrAA-200 (Mulgrave - Victoria, Australia), equipped with a hollow calcium cathode lamp. The concentrations of the irrigant solutions obtained were determined by using a length of 422 nm and a set of standard solutions with a concentration interval ranging from 1 to 15 µg/mL. The solutions collected after final irrigation were adequately diluted so that their respective concentrations would be situated in the concentration interval of the analytical curve. Before the four irrigant solutions came into contact with the root canal, the amount of calcium ions present in each root was quantified. This measurement was taken into consideration in the calculations that determined the contents of this metal after final irrigation. As control, ultra-pure water was used in order to assure that this solvent would not be a source of calcium contamination.

The results obtained from the scores with reference to the analysis of the photomicrographs, as well as the values of calcium ion concentrations released obtained by the atomic absorption and flame emission spectrometry, were submitted to statistical analysis by means of the program InStat (GraphPad Software Inc, San Diego, USA). The results with reference to the scores were submitted to Kruskal-Wallis test with a level of significance of 1% (α>0.01), and complementary Dunn test. For the data with reference to the concentration of calcium released, the Tukey-Kramer parametric test was applied with a level of significance of 5% (α=0.05).

RESULTS

Smear Layer Removal
As regards root canal cleaning, the Dunn test showed a statistically significant difference (p<0.001) between the roots irrigated with 17% EDTA and the other groups, which showed the highest rates of areas covered by the smear layer. The roots that received final irrigation with apple vinegar, 5% malic acid and 5% acetic acid were shown to be similar among them and statistically different from the control group (p<0.001) (Figure 1). The Friedman test revealed no statistically significant differences among the cervical, middle and apical thirds of the root canals (Ho= 62.81).

Figure 1 Scanning Electronic micrographs (550X – 15KV) of the root canal after final irrigation with distilled deionized water (1); apple vinegar (2); 5% malic acid (3); 5% acetic acid (4); 17% EDTA (5); Cervical (A) Middle (B) and Apical (C)Thirds

Quantification of Ca²⁺ ions Removed from the Root Canal
The mean±SD of the quantification of calcium ions removed by the studied solutions, measured in µg/ml, were as follows: 152.20±36.47, 17% EDTA; 61.70±18.27, malic acid; 22.54±5.58,
The use of a chelating agent as an auxiliary solution in endodontic treatment diminished the inefficiency of sodium hypochlorite as regards its capacity to act on the mineral matrix of the tooth and act in removing the smear layer formed during biomechanical preparation (Hottel et al., 1999; HÜLSMANN et al., 2003; SCELZA et al., 2004; PÉREZ-HEREDIA et al., 2008; SILVA et al., 2008).

The search for a chelating agent that was more efficient and biocompatible than the EDTA has resulted in various solutions being researched over the last few years (ÇALT; SERPER, 2000; CRUZ FILHO et al., 2001; DE DEUS et al., 2006; MARQUES et al., 2006; KHEDMAT; SHOKOUHINEJAD, 2008; BARROS et al., 2008; SPANÔ et al., 2009). Among them, apple vinegar has been outstanding because of its antimicrobial action, (ESTRELA et al., 2005), and smear layer removal (ZANDIM et al., 2004; ESTRELA et al., 2007; SPANÔ et al., 2009). ESTRELA et al. (2005) assessed the antimicrobial capacity of four types of vinegars (apple, rice, red and white wine) in a mixed suspension of microorganisms (S. aureus + E. faecalis + P. aeruginus + B. subtilis + C. albicans) and a pure suspension of E. faecalis, in periods of 24, 48, 72h and 7 days. All the solutions were effective against E. faecalis, but against the mixed suspension, the apple vinegar showed the best results. The authors also verified that apple vinegar was capable of reducing the microhardness of root dentin in a similar way as done by the EDTAC solution.

Apple vinegar is constituted of acetic acid, its main component, malic, lactic, formic and citric acids (CALIGIANI et al., 2007), and malic acid is the constituent responsible for the therapeutic property of the solution (THACKER, 2000; ESTRELA et al., 2004).

In this study, the option was to compare the acetic and malic acids with the apple vinegar and EDTA, in order to verify, which of the components of apple vinegar was the main constituent responsible for removing the smear layer and the calcium ions from inside the root canal.

The method of attributing scores to the electro micrographs was used to assess the capacity of smear layer removal based on previous studies (GAMBARINI, 1999; ÇALT; SERPER, 2002; KOKKAS et al., 2004; MARQUES et al., 2006). The solutions used as final irrigation were collected and submitted to atomic absorption spectrometry, which is a widely used analytical technique, particularly in the quantification of metals (EWING, 1972).

Biomechanical preparation of specimens was performed under irrigation with distilled and deionized water and the solutions tested were applied inside the root canals with standardized volume (5 mL) and time (5 min) and after the last endodontic instrument with the aim of avoiding the interference of any other substance. Care was taken since the efficiency of the chelating solution is associated with application time, available quantity, pH and concentration of the solution (ÇALT; SERPER, 2002; HÜLSMANN et al., 2003; MARQUES et al., 2006).

CRUZ FILHO et al. (2002) studied the effect of the 1%, 3% and 5% EGTA solution on the microhardness of root dentin. They concluded that the tested solution was capable of diminishing dentinal microhardness and that the higher the concentration, the higher was the demineralization power of the solution.

ÇALT; SERPER (2002) verified the effect of EDTA on the dentinal tissue structure and the smear layer removal capacity after 1 and 10 minutes of application. The chelating agent satisfactorily removed the smear layer in 1 minute but it caused excessive erosion of peri- and intertubular dentin when applied for 10 minutes. When analyzing the results of this study, there seems to be a relationship between the capacity of the smear layer removal and the amount of calcium ions detected in the solutions resulting from the preparation. In this study it was verified that the 17% EDTA solution was more effective than the other solutions in removing the smear layer and as regards the quantity of calcium ions removed from the root canal. These findings are in agreement with those of SPANÔ et al. (2009).

These authors verified the action of different demineralizing solutions on smear layer removal.
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and the quantity of calcium ions chelated from the root canal. They concluded that the EDTA and the citric acid removed the smear layer in a statistically similar manner between them and in a more efficient way than the acetic acid, apple vinegar, malic acid, sodium citrate and sodium hypochlorite solutions; The EDTA chelated a greater quantity of calcium ions. The 5% malic acid, 5% acetic acid and the apple vinegar solutions promoted partial removal of smear layer, but it was observed that the malic acid solution resulting from the final irrigation showed a greater quantity of calcium ions when compared with the acetic acid and apple vinegar solution. The smear layer removal by EDTA was due to its high power of ionic complexation; that is, its capacity of electrostatic attraction to calcium ions. Previous studies have shown the effective action of EDTA on smear layer removal (TORABINEJAD et al., 2002; HÜLSMANN et al., 2003; SELZLA et al., 2004; LOTTANTITI et al., 2008; MELLO et al., 2009), which also explains the high concentration of calcium detected in the solution after final irrigation.

The lowest concentrations of calcium ions were observed when acetic acid and apple vinegar were used. Apple vinegar is obtained from the fruit pulp of the apple, by means of a synthetic route analogous to the one used to obtain wine vinegar. The ethyl alcohol generated in this process is oxidized and transformed into acetic acid under the action of air and specific microorganisms. This natural biological procedure is called acetification (CALIGIANI, 2007). The amount of calcium detected in the two solutions is explained by the action of H⁺ ions present in the medium. The higher the concentration of H⁺ ions, the more efficient the attack of the acid would be. The concentration of H⁺ ions present in the medium is the result of the dissociation constant (Kₐ). Because the acetic acid is a weak acid, whose Kₐ is 1.8 x 10⁻⁵ (HARRIS, 2001), that is, an acid that is little dissociated, it does not have a concentration of H⁺ ions that could produce an efficient calcium removal. The larger quantity of calcium ions detected in the malic acid solution is also due to the action of H⁺ ions. Because the malic acid is a diprotic acid, it has two dissociation constants, Kₐ₁ = 3.5 x 10⁻⁴ e Kₐ₂ = 8.0 x 10⁻⁶ (HARRIS, 2001). Kₐ₁ in fact determines the degree of acid dissociation, since the second constant is much smaller. Since the Kₐ₁ of malic acid is higher than the Kₐ of acetic acid, the malic acid dissociates more strongly, with a higher concentration of H⁺ ions, promoting removal of calcium ions more intensely.

The acetic acid and apple vinegar solutions were statistically similar between them both in removing the smear layer and in chelating calcium ions, corroborating the findings of SPANÓ et al. (2009).

ESTRELA et al. (2007) assessed the smear layer removal capacity of apple vinegar used in isolation and/or associated with EDTA and they observed that the action of apple vinegar in removing the smear layer may be increased when EDTA is associated with the solution. The results of this study showed that there were no statistically significant differences as regards the different root thirds (cervical, middle and apical) irrespective of the solution studied, being in accordance with the studies by MENEZES et al. (2003) and SELZLA et al. (2004). This is due to the methodology applied, in which the solution used had free passage through the root canal, homogeneously promoting wettability of the root dentin, differently from the studies common conducted, in which the apical foramen is sealed (SPANÓ et al., 2009) preventing the volume of the irritant solution from attaining the entire extension of the root canal in a similar manner.

Based on the results obtained and taking into account the limitations of this study, it could be concluded that among the solutions assessed, 17% EDTA promoted greater cleaning of the root canal walls and removing a larger quantity of calcium ions. Further studies must be conducted, varying the concentrations and pH of the solutions, as well as promoting the association among them, which may lead to formulating a solution with active agents that favor effective smear layer removal.

REFERENCES


