

Smear Layer Removal and Chelated Calcium Ion Quantification of Three Irrigating Solutions

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The purpose of this study was to evaluate, by scanning electron microscopy (SEM), smear layer removal and quantify, by atomic absorption spectrophotometry, the amount of calcium ion present in the chelating solutions after their use. Sixteen extracted canines were instrumented using the step-back technique and were assigned to 3 groups according to the irrigating solution used: G1: 1 mL 17% EDTAC between each file; G2: 1 mL 17% CDTA; G3: 1 mL 17% EGTA. The solutions were collected after use. The teeth were cleaved longitudinally, evaluated under SEM and assessed for smear layer by blinded examiners and scored from 1 to 4. In order to quantify calcium ion release, the collected solutions were examined by atomic absorption spectrophotometry. Friedman's test was used for statistical analysis of SEM values and showed that canals irrigated with 17% EDTAC and 17% CDTA had significantly less smear layer throughout the canals than 17% EGTA ($p < 0.01$). For analysis of the collected solutions, Tukey's test was used and showed that EDTAC and CDTA had a greater amount of calcium ions (22.8 ± 7.54 and 60.6 ± 20.67 $\mu\text{g/mL}$, respectively) compared to EGTA (70.5 ± 14.2 $\mu\text{g/mL}$) ($p < 0.01$). The association both methodologies may contribute to the understanding of how these solutions act in the root canal.

Key Words: EDTA, CDTA, EGTA, smear layer, calcium ions.

INTRODUCTION

During biomechanical instrumentation, an amorphous mass known as smear layer is formed and deposited on the root canal walls. The cutting debris forced into dentinal tubules form the so-called smear plugs. The smear layer has an amorphous, irregular and granular aspect and is composed of inorganic material (dentin chips containing hydroxyapatite) and organic material (necrotic or vital pulp tissue, odontoblastic remnants, coagulated proteins, blood cells, nerve fibers, collagen, tissular fluid, saliva, and bacteria and their by-products). Smear layer is formed regardless of the instrument or instrumentation technique used during biomechanical preparation (1).

For smear layer removal, a chemical chelating solution should be used associated with sodium hy-

pochlorite to overcome its inefficacy on the mineral matrix and remove debris (2,3). Chelating solutions can be either liquid or gel. The liquid solutions EDTA (ethylenediaminetetraacetic acid) and EDTA-C [EDTA associated with Cetavlon (cetyltrimethylammonium bromide)] are the most commonly used (4-6). Recently, EGTA (ethyleneglycotetraacetic acid) and CDTA (trans-1,2-cyclohexanediaminetetraacetic acid) at different concentrations have also been investigated for the same purpose (3,7).

Several studies have evaluated the improvement of final cleaning and sealing obtained by the association of chelating solutions in root canal therapy. Cruz-Filho et al. (8) investigated the action of 15% EDTAC, compared to 1% CDTA and 1% EGTA on root dentin microhardness and reported that, despite the difference in concentrations, the decrease in microhardness was

the same when applied for 5 min. Cruz-Filho et al. (9) also evaluated the influence of different EGTA concentrations (1, 3 and 5%) on dentin microhardness and found that there was no statistically significant difference between the solutions at 15 s. However, other authors (10,11) reported that EGTA can be used as an option for smear layer removal because it does not cause erosion of dentin or at the canaliculi junction, which occurs when EDTA is used. A previous work (12) evaluated the effect of 17% EDTA, 1% EGTA and 1% CDTA on adhesion and apical microleakage on human dentin using different endodontic cements (Sealer 26, Sealapex, N-Rickert, and Endofill) and found that EDTA associated with Sealer 26 showed the best adhesion and less apical microleakage when compared to the other solutions.

Therefore, the purpose of this study was to evaluate, by SEM, the capacity of CDTA and EGTA compared to EDTAC to remove smear layer and quantify, by atomic spectrophotometry absorbance, the chelated calcium ions released in these solutions after use in root canal therapy.

MATERIAL AND METHODS

Sixteen human maxillary canines were obtained from laboratory stock and stored in 0.1% thymol at 9°C before use. The teeth were radiographed to confirm the presence of a single root canal.

The teeth were sectioned transversely at the cemento-enamel junction and the crowns were discarded. A size 10 K-type file (Kerr Corporation, Orange, CA, USA) was introduced into the canal until its tip appeared at the apical foramen. The working length was established by subtracting 1 mm from this measurement.

Biomechanical preparation was performed using the step-back technique with K-type files (Kerr Corporation) up to a size 40 file at the apex. EDTAC, EGTA and CDTA (Sigma Chemical Company, St. Louis, MO, USA) were prepared at the Laboratory of Chemistry and Hydric Resources of the University of Ribeirão Preto at 17% concentration and pH 7. One mL of each chelating solution was used for irrigation between files, as follows: G1: 17% EDTAC (total 8 mL); G2: 17% EGTA (total 8 mL); and G3: 17% CDTA (total 8 mL). In all groups, a Luer-Lok syringe was used for irrigation and the total volume of irrigating solution used was collected in plastic recipients. One tooth was used as a negative

control and was irrigated only with distilled and deionized water.

After treatment, the teeth were cleaved longitudinally and the roots were measured to provide 3 sections of similar size (cervical, middle and apical). Specimens were examined using a scanning electron microscope (model JSM 5410; JEOL, Tokyo, Japan) with X750 magnification and the amount of smear layer was assessed by 3 independent, calibrated and blinded examiners. The scoring system ranged from 1 (no smear layer) to 4 (all areas covered by smear layer) using the Photoscore software (13).

The collected solutions were evaluated at the Laboratory of Chemistry and Hydric Resources of the University of Ribeirão Preto to quantify calcium ion release using an atomic absorption spectrophotometer (Model A. Analyst 700; Perkin/Elmer Applied Biosystems, Foster City, CA, USA). Results were recorded as µg/mL.

Statistical analysis was performed by Kruskal-Wallis test (for smear layer analysis), Friedman's test (for root canal third analysis) and Tukey's test (for chelating quantification analysis). Significance level was set at 1%.

RESULTS

Data relative to the evaluation of smear layer removal are reported on Table 1. Due to the non-normality of distribution, the Kruskal-Wallis test was applied and showed statistically significant differences ($p < 0.01$) for smear layer removal: 17% EGTA (greater amount of smear layer) > 17% EDTAC = 17% CDTA 17% (no smear layer) (Fig. 1). No significant differences were found among the root canal thirds (Friedman's test; $p > 0.01$).

There was statistically significant difference

Table 1. Means of scores for smear layer removal with the irrigating solutions at each root canal third.

Third	17% CDTA	17% EGTA	17% EDTAC
Cervical	1.2	2.4	1.4
Middle	1.0	2.4	1.2
Apical	1.6	3.8	1.6

Scores: 1 = no smear layer; 4 = completely covered by smear layer.

($p < 0.01$) between 17% EGTA ($22.8 \pm 7.54 \mu\text{g/mL}$) and the other solutions [17% CDTA ($60.6 \pm 20.67 \mu\text{g/mL}$) and 17% EDTAC ($70.5 \pm 14.2 \mu\text{g/mL}$)], which were similar to each other and chelated more calcium ions.

DISCUSSION

Based on the literature, many aspects were considered for the methodology applied in this study: standard concentration (17%), similar molarities (17% EDTAC: 0.457 mols/L; 17% CDTA: 0.466 mols/L; 17% EGTA: 0.447 mols/L) and neutral pH for all solutions.

This study quantified the calcium ions chelated from the root canal by atomic absorption spectrophotometry because the capacity of a chelating solution to remove smear layer can be associated with its capacity to remove calcium ions. The results obtained from the quantification of chelated calcium ions showed that the root canals irrigated with 17% EDTAC and 17% CDTA were statistically similar to each other and both were different from the specimens irrigated with 17% EGTA, which chelated the least amount of calcium ions. The same relation was found for smear layer removal, with 17% EGTA presenting the highest smear layer scores (high scores indicate less smear layer removal). The analysis of these results suggests a relationship between smear layer removal and the quantity of calcium ions present in the irrigating solution after use.

The use of 17% EGTA did not show satisfactory results in either smear layer removal or calcium chelating probably because of its pH. The explanation is that EGTA is more effective at a higher pH due to an increase

in the dissolution of the salt in water (14), which enhances its ability to remove calcium ions and consequently to remove smear layer. Çalt and Serper (10) and Viswanath et al. (11) observed different results for the capacity of EGTA to remove smear layer, however, these authors associated EGTA with 5% sodium hypochlorite. We believe that this association alters the pH of the medium, which justifies their better outcomes.

Similar results with higher pH were also observed for EDTA in a previous study (15). It was reported that an increase in pH from 4 to 7.5 increased calcium chelating, and that above pH 7.5 chelating still occurs but with no increase. Above 10.5, the demineralization rate is lower than at neutral pH due to a decrease in solubility at this pH (15). Other authors (16,17) also showed that a small increase in pH by the association of EDTA and Dakin solution (from 7.3 to 9.0) led to an increase in dentin permeability. However, there was a decrease in dentin microhardness.

The increase in CDTA concentration to 17% has been proved efficient for smear layer removal and calcium removal, differently from what occurred when it was used at 1% (10,18,19). The results of this study also showed that smear layer was equally removed in all thirds by the 3 irrigating solutions, which is consistent with the results of other investigations (3,20).

The findings of the present study showed that the association of SEM and atomic absorption spectrophotometry can contribute for the understanding of how chelating solutions act in the root canal because these methodologies were able to indicate the particular chemical action and determine what volume should be used to remove smear layer from all the canal walls.

RESUMO

O objetivo do presente estudo foi avaliar a remoção de smear layer por meio da microscopia eletrônica de varredura (MEV) e quantificar a liberação de íons cálcio resultante da irrigação com as soluções quelantes estudadas, por meio da espectrofotometria de absorção atômica. Dezesesseis caninos extraídos foram instrumentados com a técnica *step-back* e divididos em 3 grupos de acordo com a solução irrigadora utilizada: G1: 1 mL de EDTAC a 17% entre cada lima; G2: CDTA a 17%; e G3: EGTA a 17%. As soluções foram coletadas após o uso. Os dentes foram seccionados longitudinalmente e as raízes examinadas por MEV para verificação de smear layer nos terços por meio de escores (variando de 1 a 4), e avaliadas por três examinadores calibrados "cegos". Para quantificar a liberação de íons cálcio, as soluções coletadas foram avaliadas por espectrofotometria de absorção atômica. Com relação ao smear layer, o teste de Friedman

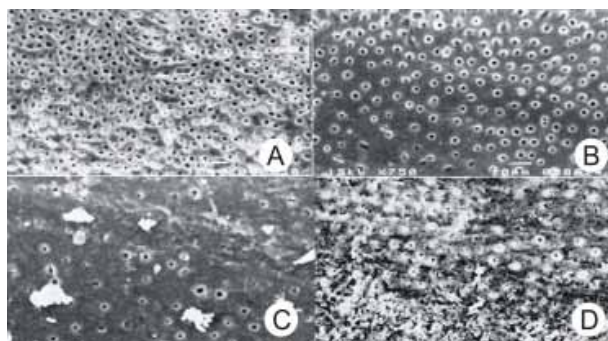


Figure 1. SEM micrographs (750X) of the middle third of the root canal after use of each chelating solution (A: EDTAC; B: CDTA; C: EGTA and D: water).

evidenciou diferença estatisticamente significativa ($p < 0,01$) comparando-se o EGTA 17% ao EDTAC 17% e ao CDTA 17%, sendo que os canais irrigados com estas duas soluções apresentaram menor quantidade de smear layer que aqueles irrigados com EGTA. As soluções de EDTAC 17% ($70,5 \pm 14,2 \mu\text{g/mL Ca}$) e CDTA 17% ($60,6 \pm 20,67 \mu\text{g/mL Ca}$) apresentaram maiores quantidades de íons cálcio ($p < 0,01$) quando comparadas ao EGTA 17% ($22,8 \pm 7,54 \mu\text{g/mL Ca}$). Desta forma, pode-se concluir que a associação destas metodologias pode contribuir para o entendimento da ação das soluções quelantes no interior dos canais radiculares.

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