ORIGINAL ARTICLE

THE EFFECT OF 17% ETHYLENEDIAMINETETRAACETIC ACID AND 10% CITRIC ACID SOLUTIONS WITH MANUAL AND ULTRASONIC AGITATION ON SMEAR LAYER REMOVAL AND INTRARADICULAR DENTINE EROSION: A SCANNING ELECTRON MICROSCOPE EVALUATION

EFEITO DAS SOLUÇÕES DE EDTA 17% E ÁCIDO CÍTRICO 10% ATRAVÉS DA AGITAÇÃO MANUAL E ULTRASSÔNICA NA REMOÇÃO DO SMEAR LAYER: ANÁLISE EM MICROSCÓPIO ELETRÔNICO DE VARREDURA

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Resumo

Este estudo ex vivo avaliou a eficácia de diferentes protocolos de irrigação final na remoção da smear layer e na erosão da dentina intrarradicular. Trinta e cinco caninos humanos extraídos foram instrumentados e divididos aleatoriamente, de acordo com as técnicas de irrigação final utilizadas, em 7 grupos: 1 (ED3M) e 3 (ED3US), EDTA a 17% por 3 minutos com agitação manual e ultrassônica, respectivamente; 2 (CA30M) e 4 (CA30US), ácido cítrico a 10% por 30 segundos com agitação manual e ultrassônica, respectivamente; 5 (CA3M) e 6 (CA3US), ácido cítrico a 10% por 3 minutos com agitação manual e ultrassônica, respectivamente; e 7 (Na3), NaOCl a 5,25% por 3 minutos sem agitação (controle). Todas as amostras foram irrigadas com NaOCl a 5,25%, divididas longitudinalmente e examinadas em microscopia eletrônica de varredura (MEV) nos terços apical, médio e coronal. Os dados foram analisados pelos testes de Kruskal-Wallis e Mann-Whitney. A eficácia do EDTA 17% e do ácido cítrico 10% na remoção da smear layer foi significativamente maior que NaOCl 5,25% (controle). Não houve diferenças significativas entre os protocolos finais de irrigação na remoção da smear layer ou nos efeitos erosivos. Entretanto, ao comparar os terços dos grupos 1 (ED3M) e 2 (CA30M), a menor remoção da camada de smear layer e erosão foram observadas no terço apical, mas o procedimento do grupo 4 (CA30US) foi mais eficaz que 1 (ED3M) (p = 0,0004), 2 (CA30M) (p = 0,0018) ou 3 (ED3US) (p = 0,0003) na remoção da smear layer no terço apical. Concluiu-se que os protocolos utilizados neste estudo foram semelhantes na remoção da smear layer e efeitos erosivos.

Palavras-chave: EDTA. Ácido cítrico. Microscopia eletrônica de varredura. Erosão dentária. Smear layer.

Abstract

This study evaluated the ex vivo effectiveness of different final irrigation protocols in smear layer removal and intraradicular dentine erosion. Thirty five extracted human canines were instrumented and randomly divided, according to final rinse techniques used, into 7 groups: 1 (ED3M) and 3 (ED3US), 17% EDTA for 3 minutes with manual and ultrasonic agitation, respectively; 2 (CA30M) and 4 (CA30US), 10% citric acid for 30 seconds with manual and ultrasonic agitation, respectively; 5 (CA3M) and 6 (CA3US), 10% citric acid for 3 minutes with manual and ultrasonic agitation, respectively; and, 7 (Na3), 5.25% NaOCl for 3 minutes without agitation (control). All specimens then were irrigated with 5.25% NaOCl, split lengthwise, and examined under scanning electron microscopy (SEM) in apical, middle and coronal thirds. Data were analyzed with Kruskal-Wallis and Mann-Whitney tests. The effectiveness of 17% EDTA and 10% citric acid in removing smear layer was significantly greater than 5.25% NaOCl (control). There were no significant differences among final irrigation protocols in smear layer removal or erosive effects. However, when comparing the thirds in groups 1 (ED3M) and 2 (CA30M), the least smear layer removal and erosion was seen in the apical third, but the group 4 procedure (CA30US) was more effective than 1 (ED3M) (p=0.0004), 2 (CA30M) (p=0.0018) or 3 (ED3US) (p=0.0003) in smear layer removal for the apical third. It was concluded that protocols used in this study were similar in smear layer removal and erosive effects.

Keywords: *EDTA. Citric acid. Scanning electron microscopy. Dental erosion. Smear layer*

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INTRODUCTION

Scanning electron microscopy (SEM) investigations have shown that the chemo-mechanical instrumentation of root canals leaves a smear layer covering the dentinal walls. This layer contains inorganic and organic materials derived from ground dentine and predentin; pulpal remnants; odontoblast processes; and, in cases of infected root canals, bacteria and their by products (1-3). Its removal aids penetration of disinfectant agents (including irrigants and intracanal medication) into the dentinal tubules (4), provides a better adaptation of filling materials (5), and reduces apical and coronal leakage (6,7).

Mechanical instrumentation and irrigation reduce the number of bacteria (8). Sodium hypochlorite (NaOCl) is the most widely used irrigating solution, presenting several properties. It reacts with organic debris in the root canal (9), but its capacity to remove smear layer from instrumented walls is insufficient. Therefore, sequential use of NaOCl to remove the organic component of the smear layer, and ethylenediaminetetraacetic acid (EDTA), the inorganic component, has been recommended (10). No single irrigant simultaneously eliminates both of its organic and inorganic components (4).

EDTA is the most widely recommended chelating agent for endodontic therapy. It is thought to soften the root canal dentin chemically, dissolve the smear layer, and increase dentin permeability (11).

Another irrigant solution used is citric acid, a weak organic acid effective in removing the superficial smear layer (12). It also is a more biocompatible (13) and a more effective demineralizing substance (14,15).

Studies have demonstrated that for maximum effect after instrumentation, chelating agents must be used followed by a tissue solvent. Therefore, in removing the smear layer from dentinal walls, final irrigation with EDTA or citric acid should be followed by NaOCl (16,17). In two studies however, detailed examination of the dentinal tubules revealed erosion of dentin, not only on the surface of the canal wall but also inside the dentinal tubules after irrigation with EDTA followed by NaOCl. This tubule enlargement also may change the sealing ability of the root canal filling material (18,19).

Lopes et al. (1996) obtained the best result in removing smear layer by mechanically stirring the EDTA when it was inside the root canals. The authors attributed this to clearing air bubbles, mainly present in the middle and apical thirds of the root canal, thereby allowing chelating agents to contact dentinal walls (20). Ultrasonic agitation also has been advocated, to "accelerate chemical reactions, create cavitational effects, and achieve a superior cleansing action" (21) It appears important to apply the ultrasonic instrument after completing the canal preparation. A freely oscillating instrument causes more ultrasonic effects in the irrigating solution than one which binds to canal walls (22).

The choice of a final irrigation solution, one which removes a larger amount of smear layer without promoting intraradicular dentine erosion, the length of time it is used, and the agitation method, all are issues yet to be resolved. Therefore, the purpose of this ex vivo study was to evaluate the effectiveness of different final irrigation protocols using 17% EDTA (ED) and 10% citric acid (CA) solutions in smear layer removal and intraradicular dentine erosion.

METHODS

Sample Selection

Thirty five fully developed human canines with a straight single root canal extracted from 35- to 60-year-old patients were selected. The teeth were devoid of caries, cracks, endodontic treatments or restorations. Buccolingual and mesiodistal radiographs were used to select teeth with intact and mature root apices and uniform root canal widths. After extraction, teeth were stored in 2% thymol at room temperature and used within 1 week. Teeth were decoronated to a standardized root length of 12 mm.

Canine Preparation

Specimen working length (WL) was determined by subtracting 1 mm from the length recorded when the tip of a #15 K-file (Dentsply Malleifer, Ballaigues, Switzerland) was visible at the apical foramen. Next, the outside of the apical third of the root was

covered with utility wax to prevent irrigation through the apical foramen. The specimens were shaped with #3-#2 Gates-Glidden drills (Dentsply Malleifer, Ballaigues, Switzerland) using a crown down technique with apical preparation prepared by K-files to size #40 and step-back technique to #55. After using each file and before proceeding to the next, irrigation with 2 mL of 5.25% NaOCl (Crystalpharm, Niterói, RJ, Brazil) at 37°C, was performed with a disposable syringe (Injex Industrias Cirúrgicas Ltda., Ourinhos, SP, Brazil) and a 24-gauge needle (BD Precision Glide®, Curitiba, SC, Brazil) at a distance of 1 mm from the WL.

After instrumentation, teeth in different groups underwent different final irrigating sequences. When used in the final irrigating sequence, Ultrasonics (US) (ENAC, Osada Electric, EUA) was used with a #15 K-file at a distance of 1 mm from the WL, with a power setting of 2. For manual agitation (M), a #15 K-file was moved up and down gently in short 4- to 5-mm strokes.

The final irrigation sequences were: groups 1 (ED3M) and 3 (ED3US), 17% EDTA for 3 minutes with manual and ultrasonic agitation, respectively; groups 2 (CA30M) and 4 (CA30US), 10% citric acid for 30 seconds with manual and ultrasonic agitation, respectively; groups 5 (CA3M) and 6 (CA3US), 10% citric acid for 3 minutes with manual and ultrasonic agitation, respectively; and group 7 (Na3) (control), 5.25% NaOCl for 3 minutes without agitation. Next, all specimens were irrigated with 5 mL of 5.25% NaOCl at 37°C. Finally, root canals were irrigated with 10 mL of saline (Frenesius Kabi Brasil Ltda, Campinas, SP, Brazil) and dried with sterile paper points (Endopoints, Manacapuru, AM, Brazil).

Teeth were opened in a buccolingual direction to expose root interiors. A longitudinal groove was made along the root surface with a diamond disc at low-speed and a wedge was used to split the root in half. For each root, the half containing the more visible apical portion was coded. Specimens were dried, mounted on metallic stubs, gold sputtered, and evaluated under SEM (JEOL - JSM-T330A, Tokyo, Japan).

SEM Evaluation

After a general survey of the canal, 12 scanning electron microscopy images were taken at magnifications of 750X and 2,000X at the coronal (10 mm to apex), middle (6 mm to apex), and apical (2 mm to apex) thirds of each specimen. Blind evaluation was performed independently by two observers after joint examination of 20 specimens for calibration. Interexaminer reliability for SEM assessment was verified by the Kappa test.

The amount of smear layer remaining on the surface of the root canal or in the dentinal tubules was scored according to the following criteria (23): 0 = no smear layer, dentinal tubules open and free of debris; 1 = smear layer present only in the apertures of the dentinal tubules; 2 = thin smear layer covers the surface, outline of the dentinal tubules indiscernible, tubular apertures covered by debris; the location of the tubule indicated by a crack; and 3 = heavy smear layer, indiscernible tubule apertures. Other investigators scored erosion of dentinal tubules as follows (24): 1 = no erosion, all tubules looked normal in appearance and size; 2 = moderate erosion, the peritubular dentin was eroded; 3 = severe erosion, the intertubular dentin was destroyed, and tubules were connected with each other (Figure 1).

The Kruskal-Wallis and Mann-Whitney tests were used to analyze the data with statistical significance at the $p = 0.05$ level.

RESULTS

Kappa test results, with significance set at 0.5, showed good intraexaminer and interexaminer agreement with values of 0.90 and above for the different groups. The effectiveness of 17% EDTA and 10% citric acid in removing smear layer were significantly greater than NaOCl (control). There were no significant differences between irrigants in removing smear layer or erosive effects (Figure 2). However, when comparing the thirds in groups 1 (ED3M) and 2 (CA30M), the least smear layer removal and erosion occurred in the apical third. The technique used for group 4 (CA30US) was more effective than the techniques used for groups 1 (ED3M), 2 (CA30M), and 3 (ED3US) in smear layer removal in the apical third (Table 1).

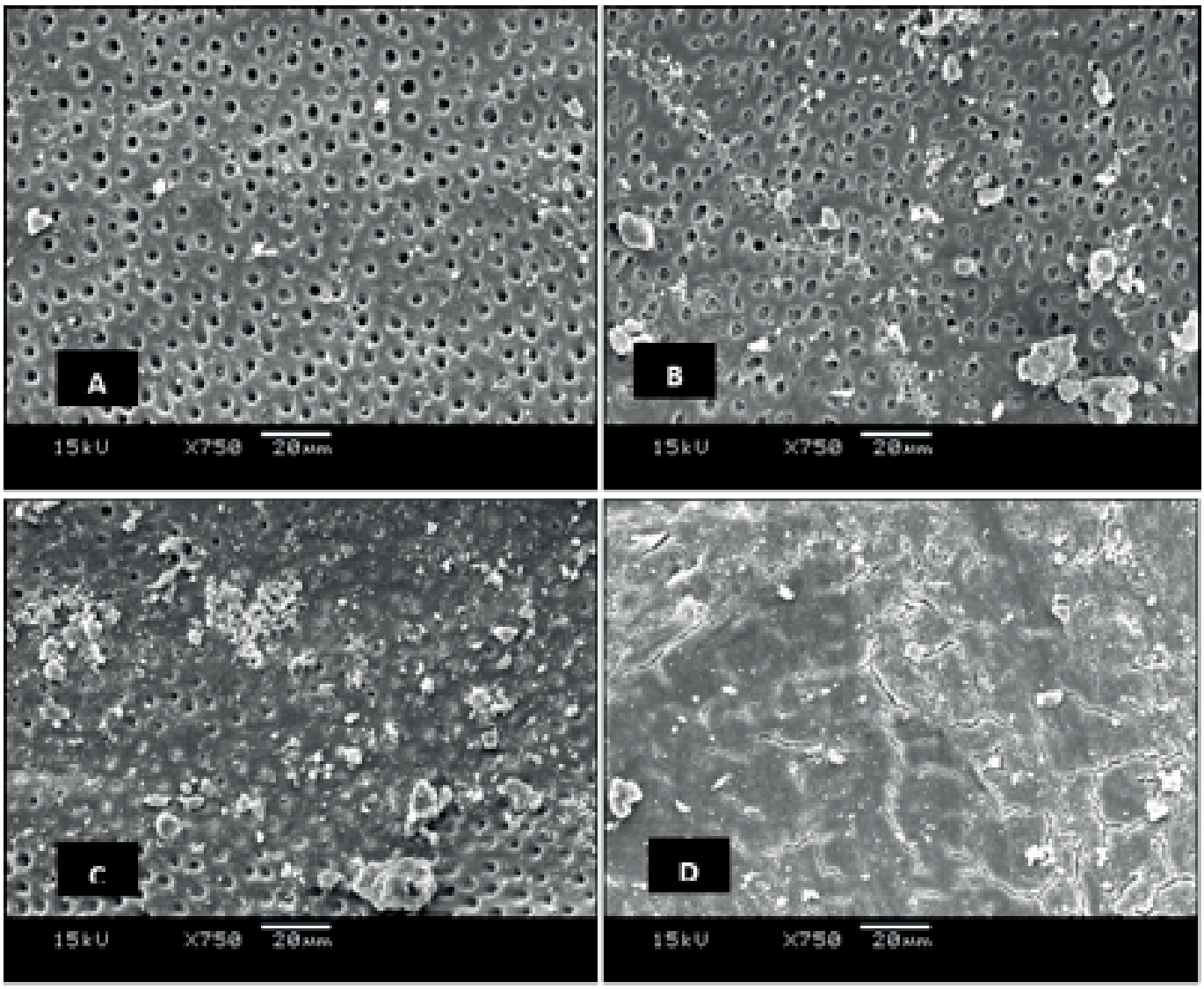

Figure 1 - Images (750X) representative of the scores regarding the removal of the smear layer: A - score 0; B - score 1; C - score 2 and D - score 3.

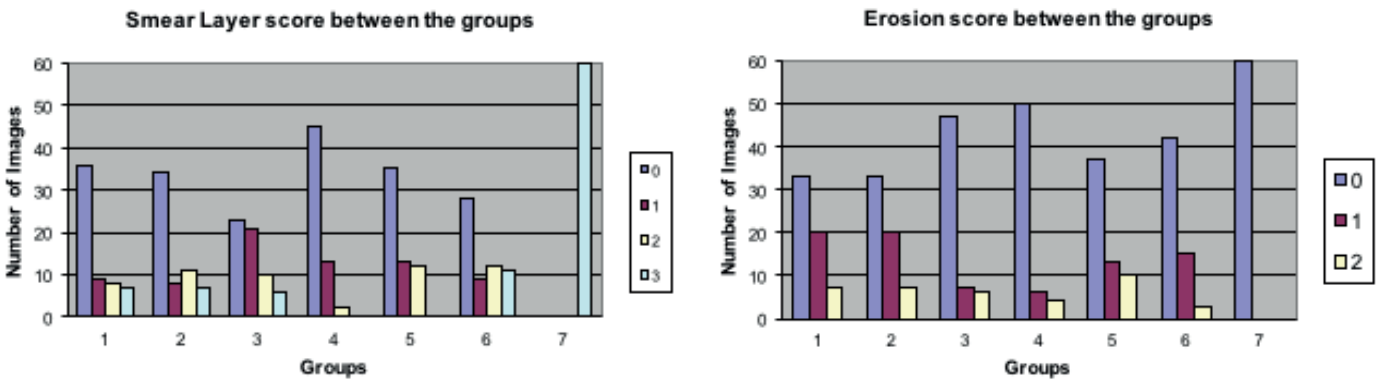

Figure 2 - Analysis of the removal of smear layer and formation of erosion between the groups.

Table I - MEAN SCORES AND STANDARD DEVIATION VALUES (SD) OF SMEAR LAYER REMOVAL IN THE APICAL THIRD.

Group	Mean	SD
ED3M	1.75†	1.07
CA30M	1.70‡	1.17
ED3US	1.60*	.94
CA30US	.50	.69

Different symbols indicate significant means difference ($p < .05$) with the group 4 (CA30US).

DISCUSSION

SEM has been used to determine the effectiveness of various irrigants to remove smear layer since first described (3). Most SEM operators select clean canal areas with open dentinal tubules rather than areas with large amounts of debris (25), however, SEM allows an examination of the morphologic details of prepared root canal surfaces (24).

Sodium hypochlorite remains the most widely recommended irrigant in endodontics on the basis of its unique capacity to dissolve necrotic tissue remnants and excellent antimicrobial potency (17,26). Findings of this study agree with other studies showing that NaOCl is not effective in removing the inorganic part of the smear layer (17, 23, 24, 27).

Smear layer removal requires a combination of NaOCl and chelating agents or acids to remove both organic and inorganic components (28). In the present study, both 17% EDTA and 10% citric acid followed by a final flush of 5.25% NaOCl showed similar results on smear layer removal in instrumented root canals. However, on the apical third, treatment with 17% EDTA was less effective than 10% citric acid except when the citric acid is used with manual agitation for 30 seconds, which appears to be insufficient for smear layer removal.

There is no consensus on the time a decalcifying agent must be in contact with the root canal wall surface to remove smear layer adequately (18, 29). In our study the different protocols showed similar results, although citric acid solutions had greater chelating effects compared to 17% EDTA for several experimental times (30). Our results indicated that 10% citric acid was faster on smear layer removal and since it is more biocompatible than 17% EDTA (13), it may be more suitable for clinical use, consistent with the findings of Ramachandran et al. which showed that 10% citric acid released the greatest amount of calcium ions and removed the largest smear layer among all irrigants (31).

Although a continuous rinse with 5 mL of 17% EDTA for 3 minutes can remove smear layer efficiently from all areas of root canal walls (32), the use of ultrasonic agitation on final irrigation has demonstrably greater effectiveness in smear layer removal (33, 34). Consistent with the findings of Tinaz et al. (35), the present study showed similar results between manual and ultrasonic agitation for all protocols on all thirds of the instrumented canals. Saber and Hashem (36) demonstrated that manual agitation resulted in better removal of smear layer in the apical third, however in our results ultrasonic agitation was more effective.

Cleaning the apical third of root canals is a major challenge in clinical endodontics. That difficulty is explained by the reduction in root canal diameter which impairs access of the irrigant with consequent reduction in its flow (23). In the present study, a fine irrigating needle was used very close (1 mm) to the working length, as described by Sedgley et al. (37). Therefore, access of the irrigant was not an important fac-

tor in apical third cleaning.

Smear layer removal by final irrigation with EDTA or citric acid followed by NaOCl may cause dentinal erosion when used for longer periods of time (38). Erosive effects have been observed on inter- and peri-tubular dentin when citric acid was used for more than 60 seconds (30). In the present study, erosion was similar among groups. However, when the thirds in each group were compared among themselves, there was significantly less erosion and smear layer removal in the apical third in groups 1 (ED3M) and 2 (CA30M), possibly due to the manual agitation.

The specimens in this study were single-rooted canines with straight canals. In order to extend the applicability of the findings, further studies should be conducted on this subject without such limitations.

Irrigation activation techniques improve smear layer removal when compared to conventional irrigation and, therefore, its use is recommended during root canal treatment. However, current data is too heterogeneous to compare and subsequently recommend individual techniques. Despite the abundance of literature reporting the effectiveness of these techniques, the results are often conflicting (4).

CONCLUSION

The combination protocols used in this study resulted in similar smear layer removal and erosive effects.

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