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ORIGINAL ARTICLE

Evaluation of SmearOFF, maleic acid and two EDTA preparations in smear layer removal from root canal dentin

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ABSTRACT

Objectives: To evaluate SmearOFF, 7% maleic acid (MA) and two different preparations of ethylenediaminetetraacetic acid (EDTA) in smear layer removal.

Materials and methods: Fifty single-rooted teeth were separated into five groups, instrumented and irrigated as follows: (1) SmearOFF, (2) 7% MA, (3) 18% EDTA (pH 11.4), (4) 17% EDTA (pH 8.5) and (5) 0.9% saline. Teeth samples were blinded and examined by scanning electron microscopy with Image J software.

Results: Eighteen percent EDTA was less efficient when compared to SmearOFF and MA at all thirds of the root canal system. There was no difference between SmearOFF and MA in the coronal and middle thirds. In the apical third, MA removed more smear layer. Seventeen percent EDTA was as efficient as SmearOFF and MA in coronal and middle third but not in the apical third. Eighteen percent EDTA removed smear layer less efficiently in the coronal and middle thirds than 17% EDTA; in the apical third, there was no difference observed. In the saline group, all specimens were heavily smeared. There was no significant difference between 18% EDTA and saline at all canal thirds.

Conclusions: SmearOFF and 17% EDTA (pH 8.5) had better smear layer removal capability in the coronal and middle thirds of the root canal system. In the apical third, 7% MA was superior. 18% EDTA (pH 11.4) and saline had poor smear layer removal ability.

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EDTA; maleic acid; root canal irrigation; smear layer; SmearOFF

Introduction

Root canal disinfection is usually achieved by mechanical instrumentation and irrigation [1]. During the instrumentation process, large amounts of dentin debris mixes with vital and necrotic remnants of pulp tissues, microorganisms, and microbial toxins forming the smear layer located along the root canal walls [2]. Although there is no clinical evidence relating treatment outcome and smear layer removal, it has been asserted that smear layer removal improves the fluid tight seal of the root canal system and the bonding of sealers and filling materials [2–4]. Additional studies have demonstrated that the smear layer prevents the penetration of intracanal medicaments into the dentinal tubules and prevents the killing of bacteria therein [5]. Various chemical agents have been used to remove the smear layer. To date, no single irrigating solution is capable of removing both the organic and inorganic components of the smear layer. However, the most widely accepted protocol for smear layer removal is a sequential rinse using sodium hypochlorite (NaOCl) followed by ethylenediaminetetraacetic acid (EDTA) [6]. The pH of EDTA solutions significantly affects its efficacy and calcium ion availability. As the pH increases, the excess number of hydroxyl groups will slow down the dissociation of hydroxyapatite, thus limiting the number of calcium ions available for EDTA chelation. At acidic or neutral pHs, the binding of calcium ions will tend to increase the dissociation of hydroxyapatite and calcium ion availability for chelation [7]. The optimal pH for EDTA solution seems to be between 6 and 10 [8]. Commercially, various EDTA preparations with different pH are available. Although mostly effective, EDTA has several drawbacks when used as the final rinse; EDTA has been shown to be cytotoxic [9], ineffective in removing the smear layer in the apical third of the root canal system [10–12], and reduces the bond strength of epoxy resin sealer [13].

Seven percent maleic acid (MA) is a recently proposed chelating agent that has been found to possess better smear layer removal capability when compared to EDTA [10,11]. MA is less cytotoxic when compared to EDTA [9] and has been shown to improve the bond strength of epoxy resin sealer [13].

SmearOFF (Vista Dental Inc., Racine, WI) is a novel smear layer removal agent recently introduced into endodontics. It consists of EDTA and chlorhexidine gluconate (CHX). The manufacturer claims SmearOFF yields better calcium suspension and clears more dentinal tubules compared to EDTA.

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To date, there are no published studies evaluating the effectiveness of SmearOFF and EDTA solutions of different pH. Hence, the aim of the present *in vitro* study was to evaluate the efficacy of SmearOFF, 7% MA and two EDTA preparations (17% EDTA with pH of 8.5 and 18% EDTA with pH of 11.4) in smear layer removal from root canal walls. The null hypotheses tested were: (1) there are no differences in the ability of SmearOFF, 7% MA or two EDTA preparations to remove canal wall smear layer when these solutions are used as final root canal irrigants and (2) there is no influence of pH of EDTA preparations in removal of canal wall smear layer.

Materials and methods

Specimen preparation

Ethical clearance was obtained from the institutional review board, Manipal University (IEC 121/2017). Fifty extracted human maxillary anterior teeth with a single and straight canal were selected. All were radiographed to verify the presence of a single canal with closed apex, and the absence of intra-radicular resorption or root canal filling. The teeth were stored in 0.2% sodium azide (Millipore Sigma, St. Louis, MO) at 4 °C until use. The teeth were decoronated to standardize the root length to 16 mm and then randomly divided into four experimental groups and a control group ($n = 10$). Working length was established by inserting a size 10 K file (Mani Inc., Tochigi Ken, Japan) into each root canal until it was just visible at the apical foramen (observed using magnifying loupes) then subtracting 1 mm from the recorded length. Root canals were instrumented using ProTaper® nickel titanium rotary system (Dentsply Maillefer, Ballaigues, Switzerland) per the manufacturer's instructions. All teeth were instrumented to WL with a F3. Irrigation was performed with 2 mL of 2.5% of NaOCl for 1 min between each instrument change.

Irrigation techniques

The final irrigation sequences were: (i) SmearOFF group: 5 mL of SmearOFF with pH of 7.2 (Vista Dental Products, LLC, Racine, WI) for 1 min; (ii) MA group: 5 mL of 7% MA with pH of 1.3 (KMC Pharmacy, India) for 1 min; (iii) EDTA group (pH 11.4): 5 mL of 18% EDTA (Ultradent Products, Inc., South Jordan, UT) for 1 min; (iv) EDTA group (pH 8.5): 5 mL of 17% EDTA (Vista Dental Products, LLC, Racine, WI) for 1 min; (v) negative control: 5 mL of isotonic 0.9% sterile saline for 1 min. All irrigating solutions were introduced using 29-gauge stainless steel side vented needles (Vista Dental Inc., Racine, WI). The needle tip was inserted to 1 mm short of the working length in each canal. After the irrigation protocols, each root canal was irrigated with 5 mL of deionized water. The canals were then dried with sterile paper points (Dentsply, Maillefer, Ballaigues, Switzerland) and longitudinal grooves were prepared on the buccal and lingual surfaces of each root using a diamond disc (Horico Dental, Berlin, Germany). The roots were then split into two halves and stored in deionized water at 37 °C for scanning electron microscope (SEM) examination.

Scanning electron microscopy

The specimens were dehydrated using ascending grades of ethanol (25%, 50%, 75% and 100%), mounted on metal stubs, coated with gold/palladium using an ion-sputtering machine and examined with SEM (JEOL, Tokyo, Japan). Images of the canal wall surface morphology at 1000× magnification and 20 kV were captured along the full coronal (10–12 mm from apex), middle (6–7 mm from apex) and apical (1–2 mm from apex) thirds of each specimen.

Software image analysis

Scanning electron microscope images were evaluated with ImageJ (US National Institutes of Health, Bethesda, MD) software to quantify the amount of open tubules. Prior to tubule quantification, SEM information was cropped from each image. The analysis process was performed as follows: (1) obtain the image's histogram, which summarizes the number of pixels at different intensities (0–255), (2) record the standard deviation of the image's intensity (SD) and (3) measure the mean pixel intensity of three random tubules in the image and take an average (AVE). If no tubules were visible, the AVE value was set to zero. With this data, the image's open tubule percentage (OTP) was calculated using Equation (1):

$$\text{Open Tubule Percentage(OTP)} = \left(\sum_{i=AVE+SD/2}^{AVE+SD/2} n_i / \sum_{i=0}^{255} n_i\right) \times 100 \quad (1)$$

where 'n_i' is the number of pixels at intensity 'i', which is a whole integer from 0 to 255, 'AVE' is the average intensity of the three measured tubules and 'SD' is the image's standard deviation. In essence, this calculation compares all pixels in the image to determine what percent of pixels are most similar (±one half standard deviation) to pixels in the measured dentinal tubules. The OTP metric allows for quantification of open tubules and a direct comparison of quantitative data between experimental groups.

Statistical analysis

Data were analyzed using SPSS software (PASW Statistics 18; SPSS Inc., Chicago, IL). Mann–Whitney's *U*-test was used to compare statistical significance between the experimental groups. A significance level of $\alpha = 0.05$ was used for all statistical analysis.

Results

OTP results using the various irrigants are shown in Figure 1. Eighteen percent EDTA (pH 11.4) was significantly less efficient when compared to SmearOFF and MA at all thirds of the root canal system ($p < .02$). There was no significant difference between SmearOFF and MA in removal of smear

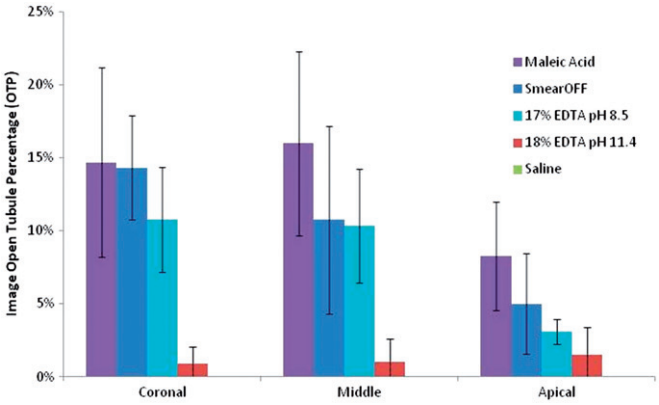

Figure 1. Open tubule percentage (OTP) of smear layer removal in the coronal, middle and apical thirds using various test irrigants.

layer from the coronal and middle thirds ($p > .10$). However, in the apical third, MA removed significantly more smear layer ($p = .04$). Seventeen percent EDTA (pH 8.5) was as efficient as SmearOFF and MA in the coronal and middle thirds but not in apical third ($p < .05$).

When 18% EDTA (pH 11.4) was compared to 17% EDTA (pH 8.5), 18% EDTA removed smear layer less efficiently in the coronal and middle thirds ($p < .001$). However, in the apical third, there was no significant difference between them ($p = .13$). In the negative control (saline) group, all specimens were heavily smeared in the coronal, middle and apical thirds of the root canal system and yielded OTP = 0%. SmearOFF, EDTA (pH 8.5) and MA performed significantly better than control group at all the thirds of the root canal system. ($p < .005$). However, there was no statistical significance when 18% EDTA (pH 11.4) and saline ($p > .10$). Figure 2 demonstrates the representative SEM images of root canal walls treated with the experimental solutions.

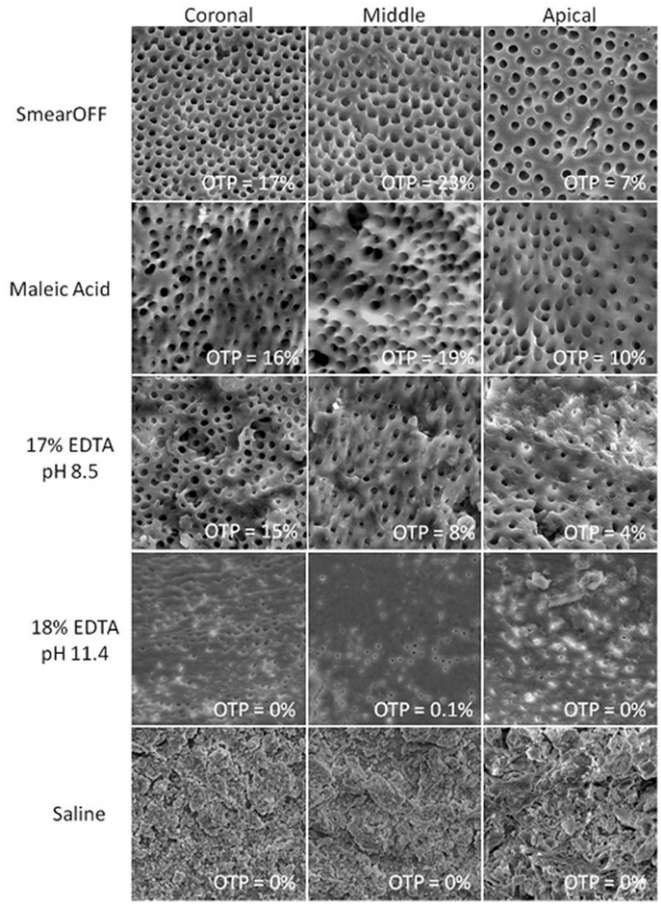

Figure 2. Representative SEM images from five experimental groups of the coronal, middle and apical thirds showing the number of open dentinal tubules.

Discussion

The present study is one of the first to evaluate the effectiveness of a novel endodontic irrigant 'SmearOFF' in smear layer removal. Both null hypotheses were rejected, that there are no differences in the ability of SmearOFF, 7% MA or EDTA preparations to remove canal wall smear layer, and also there is no influence of pH of EDTA preparations in removal of smear layer was rejected. The results of the present study showed that SmearOFF and MA, when used as a final irrigant in conjunction with 2.5% NaOCl, had similar capability in removal of smear layer which were better than EDTA preparations of pH 8.5 and 11.4. 7% MA demonstrated better removal of smear layer in apical third when compared to SmearOFF and 17% EDTA solutions. This finding is in accordance with previous studies [10–12,14], and may be attributed to the acidic pH of 7% MA (pH = 1.3) and its strong demineralizing capacity in shorter periods of time [15]. Saline which was used as a negative control in this study was found to have no smear layer removal ability which is in accordance with previous studies [10,11,16].

The superior smear layer removal effect of SmearOFF may attribute to its lower pH (7.2). Additionally, the use of SmearOFF may be clinically advantageous due to the presence of CHX, which can help provide antimicrobial substantivity following endodontic therapy. However, even though SmearOFF contains CHX, recent research has shown that SmearOFF does not form a precipitate or parachloroaniline when mixed with NaOCl [17]. Therefore, dentists and endodontists can obtain the antimicrobial benefit of CHX without the need for a separate irrigation solution (i.e. 2% CHX) while obviating the need for water/saline rinse steps between irrigants. Thus, the use of SmearOFF allows for a simplified irrigation regiment consisting of only two irrigants (NaOCl and SmearOFF).

A high pH negatively impacts EDTA's effectiveness due to excess hydroxyl ions which greatly reduce the dissociation of smear layer hydroxyapatite [7,8], thus limiting the amount of free calcium ions that EDTA can chelate. So, although the EDTA molecule becomes fully deprotonated (i.e. $EDTA^{-4}$) at pH >10.3, and as a result has the highest affinity for free calcium, the high pH significantly inhibits and reduces the dissociation of hydroxyapatite and likewise the demineralization efficacy of EDTA. Indeed, the logarithmic nature of the pH scale infers that an EDTA solution of pH 11.4 contains approximately 1000× more hydroxyl ions than an EDTA solution of pH 8.5 which explains why 18% EDTA showed poor smear layer removal ability in all canal thirds and yielded results statistically similar to saline. Other researches have shown significant decrease in EDTA performance when the

solution's pH is 9 compared to a neutral pH or slightly basic pH. Cury et al. showed that more than 2× phosphate is liberated from human dentin exposed to an EDTA solution at a pH 7 compared to the same EDTA solution at a pH of 9 [18]. Further research by Serper and Calt [19] showed that a 10% EDTA solution at a pH of 7.5 liberated about the same phosphate from dentin as a 17% EDTA solution at a pH of 9, even though the latter has almost double the EDTA concentration. Subsequently decreasing the 17% EDTA solution's pH to 7.5 then nearly doubled its phosphate liberation during the experiment and accelerated its working-time by five-fold [19]. As pH has a profound impact on EDTA performance, clinicians need to be cognizant of their EDTA solution's pH. Besides investing in a pH probe, easy-to-use pH strips may provide a quick and easy method for clinicians to check their EDTA prior-to-use to ensure solution pH and effectiveness.

In the present study, a one-min time interval for final irrigation was used which is in accordance with various other studies [20,21]. Previous studies have reported that the use of 17% EDTA for more than 1 min results in erosion of the dentinal tubules, reduction in dentine microhardness and an increase in root fragility [22,23]. The recommended amount of EDTA for the removal of smear layer varies greatly from 3 to 20 mL per canal [20,24]. In this study, a 5 mL final rinse was used as proposed by Mello et al. [25]. They showed that a final rinse with 5 mL of EDTA was as effective as 10 or 15 mL of EDTA for the removal of the smear layer.

Various techniques like SEM, micro CT, environmental SEM, atomic force microscopy, cosite optical microscopy, etc. have been used to assess the canal wall smear layer removal ability of an irrigating solution [26]. The authors suggest that image analysis using ImageJ is a preferred method as it yields quantitative results and minimizes observer bias. Conversely, standard SEM image analysis using observer scores (typically 0–3 or 0–5) yields integer results that may limit proper inter-group statistical analysis. The accuracy of the software analysis was first validated on a subset of samples of varying smear layer amounts which correlated to observer interpretation (data not shown). One limitation of the software analysis is that SEM images need to be taken perpendicular to the dentin surface to provide enough contrast between the 'tubules' and 'dentin'; if the tubules and dentin look too similar, then an erroneous software measurement will result. Further, the current software analysis method cannot distinguish between light/moderate smear layer and heavy smear layer, for example, smear layer results using EDTA (pH 11.4) and saline, respectively. However, it may be possible to further improve the accuracy of the software analysis through machine learning to more accurately quantify smear layer and tubules. Nevertheless, the present study hopes provide a foundation for further software-based analysis tools to quantify SEM imaging results.

In conclusion, the present study demonstrated that SmearOFF, 17% EDTA (pH 8.5), and MA cleaned the coronal and middle thirds of the root canal system equivalently. In apical third, 7% MA performed better than all irrigants, and SmearOFF was superior to 17% EDTA (pH 8.5). Eighteen percent EDTA (pH 11.4) and saline showed equivalently poor smear layer removal ability in all thirds of the canal.

Disclosure statement

No potential conflict of interest was reported by the authors.

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