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ORIGINAL ARTICLE

Effect of manual dynamic activation with citric acid solutions in smear layer removal: A scanning electron microscopic evaluation

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Abstract *Background/purpose:* Chelating agents have been used for the removal of the smear layer on teeth. However, due to inadequate volume and/or penetration of the solutions during irrigation, smear layer removal is less effective in the apical third. The purpose of this study was to compare the efficacy of three chelating solutions with and without manual dynamic irrigation in smear layer removal.
Materials and methods: Sixty-six single-root canal teeth were decoronated, instrumented, and divided into six experimental groups ($n = 10$) and two control groups ($n = 3$). The groups received a final rinse with 1 mL of 17% EDTA and 5% or 10% citric acid (CA) for 1 minute, with or without manual dynamic activation, followed by a final 3-mL rinse with 4.2% NaOCl (5 minutes). The teeth were then longitudinally split and prepared for environmental scanning electron microscopy analysis. Digital images (500×) were taken for smear layer removal evaluation at 2 mm, 6 mm, and 10 mm from the working length.
Results: The most effective smear layer removal occurred with 5% and 10% CA combined with manual dynamic activation (Groups 7 and 8), where significant differences were observed when compared with the EDTA groups (Groups 2 and 6; $P < 0.05$). We found no significant differences between manual dynamic activation with 5% and 10% CA (Groups 7 and 8) in smear layer or debris removal ($P > 0.05$).
Conclusion: Manual dynamic activation of CA improves smear layer removal, and a reduction in CA concentration to 5% does not compromise smear layer removal in comparison with higher concentrations.

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Introduction

Mechanical instrumentation of the root canal creates an irregular layer of debris on dentinal walls, known as the smear layer.[1] It has been defined as an amorphous, irregular entity containing inorganic dentin debris and organic materials such as vital pulp tissue, odontoblastic processes, necrotic debris, and microorganisms and their metabolic products.[2]

It has been demonstrated that the smear layer itself prevents the access of intracanal solutions into dentinal tubules[3] and thus, protects the bacteria within the dentinal tubules.[2] Bacteria can remain in this layer, survive and multiply,[4] and can grow into the dentinal tubules.[5] In addition the presence of a smear layer promotes adhesion and colonization of microorganisms.[6] Yoshida et al[7] demonstrated in a clinical study that removal of the smear layer significantly reduces the number and presence of microorganisms in the root canals. Smear layer also may delay the effect of disinfectants,[8] and may interfere with the adaptation and penetration of root canal sealers reducing adhesion and affecting sealing negatively.[1,9] Moreover, in a systematic review and meta-analysis of leakage studies from 1975—2005, Shahravan et al[10] concluded that removal of the smear layer improves the fluid-tight seal of the root canal system.

Various chelating agents have been used for the removal of the smear layer. These solutions have shown to be time dependent. Irrigation times <1 minute can significantly decrease efficiency in smear layer removal,[11] and produce a high decalcifying effect in the dentin surface when contact time is prolonged,[12] with a denaturation of the fibers of collagen and weakening of the root dentin.[13]

EDTA solutions, with or without surfactants such as cetrimide, are most commonly used for smear layer removal.[14] Crumpton et al[15] showed that using 1 mL of 17% ethylene diamine tetra-acetic acid (EDTA) for 1 minute followed by 3 mL of 5.25% NaOCl removed the smear layer with efficient results. Citric acid (CA) has also been proposed for smear layer removal.[16] Concentrations ranging from 10% to 50% have been evaluated,[17—19] and 10% CA has proven to be an effective approach in smear layer removal.[16] Di Lenarda et al[19] reported similar results in smear layer removal with CA and EDTA during canal shaping.

When different chelating agents are used with NaOCl, the smear layer is removed in the middle and coronal thirds of canal preparations, however, this combination is less effective in the apical third.[20] This is probably due to inadequate volume and/or penetration of the solution into the apical portion of the canal during irrigation. Consequently, it is important to use other methods to improve the efficiency of chelating agents used for a short irrigation time.[21]

For an effective smear layer removal, irrigation solutions must come into contact. However, root canal anatomy and the vapor lock effect make access to root canal irregularities and the apical one-third a challenge. Gentle push—pull movements with a well-fitting master cone inside the root canal have proven to improve effectiveness in stained collagen removal,[22] and to produce better smear layer removal results when compared with static irrigation.[23]

Increased contact time has been shown to produce erosion in intertubular and peritubular dentin.[24] Several studies have reported dentin erosion when chelating agents were used for more than 1 minute.[14] Surface erosion also occurs due to the acid nature itself, the higher the concentration the more aggressive the effect on the canal wall surface. In addition, cytotoxicity of both EDTA and CA are also proportional to the concentration of the solution,[25] and when dilutions of 10% CA were tested, it resulted in a higher biocompatibility when compared with dilutions of 17% EDTA.[26] Using less harmful substances may be necessary, especially when cell survival is crucial, such as in revascularization protocols. However, an excessive dilution of the concentration may alter its ability to remove the smear layer and may impede the reported release of entrapped growth factors from dentin.[27]

To our knowledge, there are no studies evaluating the effectiveness of manual dynamic activation for smear layer removal with CA. This study aimed to evaluate the effect of a low CA concentration solution (5%) combined or not with manual dynamic activation for smear layer removal.

Materials and methods

Sixty-six single-root extracted teeth with straight root canals were selected for this study and stored in a saline solution until use. All teeth were radiographed to verify the presence of a single canal with mature apex and absence of root resorption. The working length was determined by placing a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) in the canal until the tip of the instrument was visibly adjusted to the apical foramen. The canal length was measured, and the working length was calculated by subtracting 1 mm from this measurement. The teeth were then decoronated at 15 mm using a low-speed saw (Isomet 1000; Buehler, Illinois, USA) under water-cooling and the working length was established at 14 mm for all teeth.

All the samples were then longitudinally grooved using a diamond disk and mounted in silicone (Dupliflex; Protechno, Girona, Spain) with the apical portion coated with wax (Periphery wax: ENTA B.V., Bergen op Zoom, The Netherlands) to ensure a closed-end channel behavior.

Each canal was prepared with a manual glide path up to a #20 K-file before rotary canal shaping. Root canals were then prepared using the ProTaper Universal rotary system (Dentsply Maillefer) up to an F3. Apical enlargement was continued up to a 40.04-file using ProFile instruments (Dentsply Maillefer). The teeth were irrigated with 1 mL of 4.2% NaOCl after every file during instrumentation.

After root canal preparation, the teeth were randomly divided into six groups of 10 teeth ($n = 10$) and two control groups of three teeth ($n = 3$) according to the final irrigation protocol as follows:

- Group 1 (Control Group 1): 1 mL of 4.2% NaOCl for 1 minute followed by 3 mL of 4.2% NaOCl 37°C (5 minutes). Irrigation time was counted from the start of the solution delivery until the next change of irrigant.
- Group 2: 1 mL of 17% EDTA for 1 minute followed by 3 mL of 4.2% NaOCl 37°C (5 minutes).

- Group 3: 1 mL of 5% CA for 1 minute followed by 3 mL of 4.2% NaOCl 37°C (5 minutes).
- Group 4: 1 mL of 10% CA for 1 minute followed by 3 mL of 4.2% NaOCl 37°C (5 minutes).
- Groups 5 (Control Group 2), 6, 7, and 8: the same irrigation protocol as Groups 1, 2, 3, and 4 respectively, with the addition of 75 intracanal push—pull motions of a well-fitted tapered gutta-percha point (Autofit Analitic; Sybron Endo, Glendora, CA, USA) to the working length between the 20-second and 45-second period of the total 1 minute of chelating irrigation time.

All irrigation was accomplished using a 30-gauge Max-i-Probe (Dentsply Maillefer) irrigation tip placed 1 mm short of the working length, with no soaking time beyond the group-specified time of delivery. Finally, the root canals were irrigated with 3 mL physiologic saline solution and were immediately prepared and examined under the environmental scanning electron microscope (ESEM). All the procedures were performed by the same operator.

Smear layer removal evaluation

All the samples were split buccolingually and were immediately mounted on aluminum stubs and examined under the ESEM. Images at 500× magnification were taken at 2 mm, 6 mm, and 10 mm from the apical mayor foramen. Smear layer was defined as a surface film of debris retained on dentine and other surfaces. The presence of smear layer (500×) was evaluated by measuring visible tubules applying the scale proposed by Chopra et al.[27] Representative ESEM images of smear layer scores are presented in Figure 1. Microphotographs were evaluated and scored by two blind observers, previously calibrated. In case of disagreement, evaluation of a third observer was registered.

Statistical analysis

Results were analyzed using the Statgraphics Centurion XV software (Statpoint Technologies, Warrenton, VA, USA). Interexaminer reliability for the ESEM assessment was verified by the Spearman's rank correlation coefficient. Debris and smear layer score results were analyzed using the analysis of variance test and the Fisher's exact test at a significance level of $P < 0.05$.

Results

Spearman's results showed a high interexaminer agreement with an overall value of 0.9305 for debris and smear layer evaluation.

The results in smear layer removal in the apical, middle and coronal aspects of the root canals are shown in Table 1. Samples in the two control groups (Groups 1 and 5) failed to completely remove any smear layer in the apical, middle, and coronal thirds. For all eight categories, higher values of smear layer appeared to be visible in the apical third followed by the middle and coronal aspects with significant differences ($P < 0.05$).

The most effective smear layer removal occurred with 5% and 10% CA combined with a manual dynamic activation (Groups 7 and 8), where significant differences were observed when compared with Groups 2, 4, and 6 ($P < 0.05$). We found no significant differences between 5% and 10% CA (Groups 7 and 8) in smear layer or debris removal ($P > 0.05$).

Discussion

Efficacy of irrigating solutions is dependent on several factors including the final apical instrument size,[28] the volume used,[29] and the time spent on irrigation.[30] Apical enlargement was performed up to a 40.04-file. This is in accordance with other studies that have concluded that larger apical preparations produce a greater reduction in remaining bacteria and dentin debris when compared with smaller preparations.[31,32]

There is no gold standard recommendation as to the optimal time period of chelating agents. To minimize destructive effects on dentin reported by some researchers,[12,33] we opted for a low volume (1 mL) of chelating agents for a short application time (1 minute).[12,15] When chelating solutions are used for more than 1 minute it causes erosion of dentinal tubules, which could affect the adhesion, decrease dentin microhardness, and weaken root dentin.[34] Although all specimens were irrigated with distilled water after the final irrigation protocols, ESEM evaluation was performed immediately without any storage time, to avoid any possible alteration.

Because the root is enclosed in the bone socket it behaves as a closed-end channel, producing a vapor lock effect during the delivery of irrigating solutions, which hampers access to the apical third.[35] Our study was

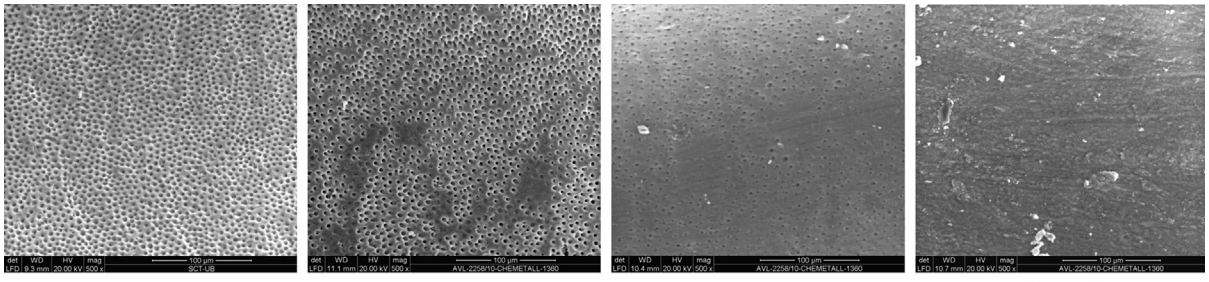

Figure 1 Representative environmental scanning electron microscopy (ESEM) images of different scores (×500).

Table 1 Comparison of smear layer removal scores.

Group	*n*	Apical	Middle	Coronal	Total
1 (NaOCl)	3	$3.0^{b} \pm 0.47$	$3.0^{a} \pm 0.48$	$3.0^{a} \pm 0.40$	$3.0^{a} \pm 0.33$
2 (17% EDTA)	10	$3.0^{ab} \pm 0.26$	$2.0^{c} \pm 0.26$	0.9 ± 0.22	$1.4^{bc} \pm 0.18$
3 (5% CA)	10	$1.6^{cd} \pm 0.26$	$1.2^{b} \pm 0.26$	0.5 ± 0.22	$1.1^{b} \pm 0.18$
4 (10% CA)	10	$2.1^{abd} \pm 0.26$	$1.0^{b} \pm 0.26$	0.6 ± 0.22	$1.3^{b} \pm 0.18$
5 (NaOCl + MDA)	3	$3.0^{b} \pm 0.47$	$3.0^{a} \pm 0.48$	$3.0^{b} \pm 0.40$	$3.0^{a} \pm 0.33$
6 (17% EDTA + MDA)	10	$2.7^{ab} \pm 0.26$	$1.3^{b} \pm 0.26$	0.3 ± 0.22	$1.87^{c} \pm 0.18$
7 (5% CA + MDA)	10	$0.9^{c} \pm 0.26$	$0.8^{b} \pm 0.26$	0.3 ± 0.22	0.67 ± 0.18
8 (10% CA + MDA)	10	$1.0^{c} \pm 0.26$	$0.5^{b} \pm 0.26$	0.4 ± 0.22	0.63 ± 0.18

Data are presented as the mean ± standard deviation.
Values that share the same superscript letter within each column are not statistically significantly different at each level ($P < 0.05$).
CA = citric acid; MDA = manual dynamic activation.

designed to simulate such conditions by embedding the root in wax and silicon.

The two control groups (Groups 1 and 5) showed a smear layer-covered surface, which corroborates the requirement for a chelating agent to remove the inorganic components.[36] It is has been proposed that removing the smear layer may dissolve attached microbiota and their toxins from root canal walls and dentin tubules reducing the risk of bacterial survival and reproduction.[2] However, in agreement with previous studies,[12,19] the results from the present study showed that all of the irrigation protocols used failed to completely remove smear layer remnants, especially in the apical third. In addition, Paque et al[37] reported the presence of sclerotic dentin in the apical third. This can also explain part of the difficulty in obtaining open dentinal tubules in the apical region.

The specimens irrigated with CA solutions revealed a more effective smear layer removal in the apical and middle thirds than 17% EDTA. These results are in agreement with Di Lenarda et al[19] who found that in the apical third the best results were obtained using a CA solution. However, significant differences in this study were only found when combined with manual dynamic activation ($P < 0.05$). Differences with other studies that reported minor or no difference between EDTA and CA could be explained by the difference in experimental conditions, and irrigation times and volumes used.[17,18]

In our study the push–pull motion of a well-fitting gutta-percha point in the canal has shown to improve smear layer removal for the three irrigants tested. This could be explained by the generation of higher intracanal pressure changes, leading to a more effective contact to canal surfaces and avoiding the vapor lock effect.[22] Moreover, it is a feasible and inexpensive method of irrigant activation, and is especially promising in curved canals,[23] with respect to root canal preparation without any risk of ledging or new smear layer formation as in ultrasonic activation.

Calt and Serper[12] reported a direct relationship between EDTA concentration and dentin erosion. If the chelating agents cause excessive erosion of the inorganic compound, they will also cause major exposure of the collagen fibers to a final contact with NaOCl, which will produce an alteration of dentin properties.[38] In addition, cytotoxic activity of both CA and EDTA is also directly proportional to their concentration.[24] Chan et al[24] found increased cell death related to CA pH. However, dilutions of 10% CA resulted in a higher percentage of viable fibroblast cells and the maintenance of the cells' self-renewal capacity when compared with 17% EDTA dilutions.[25] Thus, lower concentrations have proven to be less harmful to the root dentin with a reduced cytotoxicity. Moreover, according to the results of this study, a reduction in CA concentration to 5% does not compromise smear layer removal in comparison with higher concentrations.

Within the limitations of this study, a 1-minute application of 5% CA with manual dynamic activation followed by 3 mL of 4.2% NaOCl is an effective final irrigation protocol for the removal of the smear layer from the root canal.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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