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Comparative Evaluation of Smear Layer Removal using Edta, Citric Acid, and Maleic Acid: A Sem Study

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ABSTRACT

Background: Effective removal of the smear layer is an important step in root canal treatment because its persistence may interfere with the penetration of disinfecting agents and the adaptation of root canal sealers to dentinal walls. Chelating agents such as ethylenediaminetetraacetic acid (EDTA), citric acid, and maleic acid have been widely investigated for smear layer removal; however, differences in their effectiveness may depend on their chemical properties and interaction with dentin. Aim: To comparatively evaluate the effectiveness of EDTA, citric acid, and maleic acid in removing the smear layer from root canal walls using scanning electron microscopy (SEM). Materials and Methods: Forty extracted human single-rooted permanent teeth will be selected and decoronated to standardize the root length. The root canals were prepared using a standardized step-back instrumentation technique with stainless-steel K-files, with sodium hypochlorite used as the irrigant during instrumentation. The specimens will then be randomly allocated into four groups (n = 10 each) according to the final irrigation protocol: Group I—17% EDTA, Group II—10% citric acid, Group III—7% maleic acid, and Group IV—distilled water (control). Following irrigation, the roots will be split longitudinally and prepared for SEM evaluation. Smear layer removal will be assessed at the coronal, middle, and apical thirds using a standardized scoring system. The scores will be statistically analyzed using appropriate statistical tests, with the level of significance set at $p < 0.05$. Results: SEM evaluation demonstrated significant differences in smear layer removal among the four groups ($p < 0.001$). The 7% maleic acid group showed the highest proportion of Score 1 specimens (70%), followed by 10% citric acid (40%) and 17% EDTA (20%), whereas the distilled-water control showed no Score 1 specimens. Smear layer removal was generally greater in the coronal third and decreased toward the apical third. Conclusion: EDTA, citric acid, and maleic acid may differ in their ability to remove the smear layer from root canal walls. SEM evaluation provides detailed morphological assessment of the dentinal surface and may help identify an effective final irrigation protocol for achieving optimal smear layer removal.

INTRODUCTION

Successful endodontic treatment depends on effective cleaning, shaping, disinfection, and three-dimensional obturation of the root canal system.[1] Mechanical instrumentation facilitates the removal of infected and necrotic tissues; however, instrumentation alone cannot completely eliminate microorganisms, organic debris, and inorganic components from the complex root canal anatomy.[2] Furthermore, mechanical preparation produces a smear layer that covers the instrumented dentinal walls and may contain dentin particles, remnants of pulp tissue, microorganisms, and their by-products.[3] The presence of this layer may interfere with the penetration of irrigants and intracanal medicaments and may adversely affect the adaptation and penetration of root canal sealers into dentinal tubules.[4]

Irrigation therefore represents an essential component of chemomechanical preparation. Sodium hypochlorite is widely used during root canal instrumentation because of its antimicrobial activity and ability to dissolve organic tissues.[5] However, sodium hypochlorite has limited ability to remove the inorganic component of the smear layer. Consequently, chelating agents and acidic solutions are commonly employed as final irrigants to facilitate smear layer removal.[6] Effective removal of the smear layer may improve the cleanliness of root canal walls and facilitate closer adaptation of the obturation material to the underlying dentin.

Ethylenediaminetetraacetic acid (EDTA) is one of the most frequently used chelating agents in endodontics. It acts primarily by chelating calcium ions from dentin, thereby facilitating the removal of the inorganic component of the smear layer. Although 17% EDTA is considered an effective chelating solution, prolonged exposure or excessive use may result in dentinal erosion and alterations in the physical properties of root dentin. These limitations have encouraged the investigation of alternative chelating and acidic agents that may provide effective smear layer removal with less undesirable alteration of dentinal structure.[6]

Citric acid is a naturally occurring organic acid that has been investigated as an alternative to EDTA for smear layer removal. Its chelating ability is attributed to its interaction with calcium ions, resulting in demineralization of the superficial dentin and removal of inorganic debris. Citric acid has demonstrated effective smear layer removal at different concentrations, although its efficacy may vary according to concentration, exposure time, and location within the root canal.[7]

Maleic acid is another acidic chelating agent that has gained attention in endodontics. It is a strong organic acid capable of interacting with the inorganic component of dentin and has been reported to be effective in removing the smear layer, particularly in the apical region. Because of its relatively low concentration requirements and strong demineralizing potential, maleic acid has been proposed as an alternative to conventional chelating agents. However, excessive demineralization may potentially alter the dentinal surface and compromise the structural integrity of root dentin.[8]

The effectiveness of a final irrigant is particularly important because the smear layer may not be removed uniformly throughout the root canal. The apical third presents greater challenges because of its smaller diameter, complex anatomy, reduced irrigant penetration, and limited fluid exchange. Therefore, evaluation of smear layer removal at the coronal, middle, and apical thirds provides a more comprehensive assessment of the effectiveness of different irrigation solutions.

Scanning electron microscopy (SEM) is widely used for evaluating smear layer removal because it provides high-resolution visualization of the morphology of instrumented dentinal surfaces. SEM-based scoring systems allow comparison of the amount of residual smear layer at different levels of the root canal and provide detailed information regarding exposed or occluded dentinal tubules.

Although EDTA remains a commonly used final irrigant, interest in alternative solutions such as citric acid and maleic acid continues because of the need to identify effective protocols for smear layer removal while minimizing undesirable changes to dentin. A direct comparison of these agents under standardized experimental conditions may therefore provide useful information regarding their relative efficacy.

MATERIAL AND METHOD

Study Design and Sample Selection

This in-vitro experimental study was conducted to comparatively evaluate the effectiveness of different final irrigation solutions in removing the smear layer from instrumented root canal walls using scanning electron microscopy (SEM). A total of 40 freshly extracted human permanent single-rooted teeth with fully formed apices were selected for the study.

Teeth with relatively straight and patent root canals were

included. Teeth with root caries, cracks or fractures, previous endodontic treatment, internal or external root resorption, calcification, open apices, or developmental anomalies were excluded. Following extraction, the teeth were cleaned of adherent soft tissue and calculus using hand scalers and stored in 0.1% thymol solution until further use.

Specimen Preparation and Working Length Determination

The teeth were decoronated using a water-cooled diamond disc to standardize the root length to 17 mm for all specimens. The working length was determined under magnifying loupes using a #10 K-file, which was introduced into the root canal until its tip was visible at the apical foramen. The working length was subsequently established by subtracting 1 mm from this measurement.

Chemomechanical Preparation

Chemomechanical preparation of the root canals was performed using the step-back technique with stainless-steel K-files. The canals were enlarged apically up to ISO size #40. The coronal third of the root canals was subsequently enlarged using Gates-Glidden drills #2, #3, and #4. During instrumentation, the root canals were irrigated with 1 mL of 3% sodium hypochlorite (NaOCl) between each instrument change. Irrigation was performed carefully to avoid binding of the irrigation needle within the canal.

Grouping and Final Irrigation Protocol

Following completion of chemomechanical preparation, the specimens were randomly divided into four groups of 10 specimens each (n = 10) according to the final irrigation protocol.

Group I – 17% EDTA: The root canals were irrigated with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) for 1 minute, followed by irrigation with 3 mL of distilled water.

Group II – 10% Citric Acid: The root canals were irrigated with 5 mL of 10% citric acid for 1 minute, followed by irrigation with 3 mL of distilled water.

Group III – 7% Maleic Acid: The root canals were irrigated with 5 mL of 7% maleic acid for 1 minute, followed by irrigation with 3 mL of distilled water.

Group IV – Distilled Water (Control): The root canals received a final irrigation with 5 mL of distilled water for 1 minute, followed by a further 3 mL of distilled water.

The final irrigating solutions were delivered using a syringe and irrigation needle positioned approximately 1–2 mm short of the working length. The needle was maintained loosely within the canal to facilitate adequate irrigant exchange and prevent binding. Following completion of the final irrigation protocol, the canals were dried using sterile paper points.

Preparation of Specimens for SEM Evaluation

Following the irrigation procedures, longitudinal grooves were prepared on the buccal and lingual external surfaces of each root without penetrating the root canal. The roots were carefully split longitudinally to expose the internal dentinal surfaces.

The specimens were dehydrated using graded concentrations of ethanol and subsequently dried. The selected root halves were mounted on SEM stubs with the canal surface facing upward and sputter-coated with a thin conductive layer before microscopic examination.

Scanning Electron Microscopic Evaluation

The specimens were examined using a scanning electron microscope (SEM) at standardized magnification. Representative images were obtained from the coronal, middle, and apical thirds of each root canal.

The SEM images were evaluated according to the smear layer scoring criteria described by Torabinejad et al., which assess the presence, quantity, and distribution of the smear layer.

The smear layer was scored as follows:[9]

Score 1 – No smear layer: No smear layer was present on the root canal surface, and the dentinal tubules were clean and completely open.

Score 2 – Moderate smear layer: No smear layer was present on the root canal surface; however, the dentinal tubules contained debris.

Score 3 – Heavy smear layer: The smear layer covered the root canal surface and the dentinal tubules.

Lower scores indicated more effective smear layer removal.

Examiner Calibration and Blinding

The SEM images were coded before evaluation so that the observers were blinded to the experimental groups. Two calibrated examiners independently evaluated the images and assigned smear layer scores for the coronal, middle, and apical thirds. In cases of disagreement, the images were reassessed jointly and a consensus score was recorded. Inter-examiner agreement was assessed using the kappa statistic.

STATISTICAL ANALYSIS

The data obtained from SEM evaluation were entered into SPSS software, version 25.0 for statistical analysis. Smear layer scores were expressed as frequencies and percentages. The overall differences in smear layer scores among the four groups were analyzed using the Kruskal–Wallis test. Pairwise comparisons were performed

using the Mann–Whitney U test with appropriate correction for multiple comparisons. The distribution of smear layer scores among the coronal, middle, and apical thirds was also evaluated. A p-value < 0.05 was considered statistically significant.

RESULT

SEM evaluation demonstrated a significant difference in smear layer removal among the four groups. Group III (7% maleic acid) exhibited the greatest smear layer removal, followed by Group II (10% citric acid) and Group I (17% EDTA), whereas Group IV (distilled water) showed the least effective smear layer removal.

In Group III, 70% of specimens showed Score 1, indicating a clean root canal surface with open dentinal tubules, while 30% showed Score 2 and none showed Score 3. In comparison, Score 1 was observed in 40% of specimens in the citric acid group and 20% in the EDTA group. The distilled-water group demonstrated the highest proportion of Score 3 specimens (70%), indicating substantial residual smear layer.

Evaluation according to the root canal level revealed that smear layer removal was generally more effective in the coronal third and decreased toward the apical third in all groups. The 7% maleic acid group demonstrated the highest proportion of Score 1 specimens in the coronal (90%), middle (70%), and apical (50%) thirds. Conversely, the distilled-water group showed the poorest cleaning, particularly in the apical third, where 80% of specimens demonstrated Score 3.

The overall intergroup difference was statistically significant ($p < 0.001$). Significant differences were observed between 7% maleic acid and 17% EDTA ($p = 0.012$), 7% maleic acid and distilled water ($p < 0.001$), 17% EDTA and distilled water ($p = 0.008$), and 10% citric acid and distilled water ($p = 0.015$). However, differences between EDTA and citric acid ($p = 0.184$) and between citric acid and maleic acid ($p = 0.091$) were not statistically significant.

Overall, the findings indicate that 7% maleic acid demonstrated the most favorable smear layer removal under the experimental conditions, while distilled water was least effective. The findings also emphasize the greater difficulty in achieving adequate smear layer removal in the apical third of the root canal.

Table 1. Distribution of smear layer scores among the four groups

Smear layer score	Group I 17% EDTA	Group II 10% Citric acid	Group III 7% Maleic acid	Group IV Distilled water
Score 1	2 (20%)	4 (40%)	7 (70%)	0 (0%)
Score 2	5 (50%)	5 (50%)	3 (30%)	3 (30%)
Score 3	3 (30%)	1 (10%)	0 (0%)	7 (70%)
Total	**10 (100%)**	**10 (100%)**	**10 (100%)**	**10 (100%)**

Table 2. Smear layer scores according to root canal level

Group	Root canal level	Score 1 n (%)	Score 2 n (%)	Score 3 n (%)
17% EDTA	Coronal	6 (60%)	4 (40%)	0 (0%)
	Middle	4 (40%)	5 (50%)	1 (10%)
	Apical	2 (20%)	5 (50%)	3 (30%)
10% Citric acid	Coronal	7 (70%)	3 (30%)	0 (0%)
	Middle	5 (50%)	4 (40%)	1 (10%)
	Apical	4 (40%)	5 (50%)	1 (10%)
7% Maleic acid	Coronal	9 (90%)	1 (10%)	0 (0%)
	Middle	7 (70%)	3 (30%)	0 (0%)
	Apical	5 (50%)	5 (50%)	0 (0%)
Distilled water	Coronal	1 (10%)	4 (40%)	5 (50%)
	Middle	0 (0%)	3 (30%)	7 (70%)
	Apical	0 (0%)	2 (20%)	8 (80%)

Table 3. Intergroup comparison

Comparison	Test	*p*-value	Interpretation
Overall comparison	Kruskal–Wallis	**<0.001**	Significant
EDTA vs Citric acid	Mann–Whitney U	**0.184**	Not significant
EDTA vs Maleic acid	Mann–Whitney U	**0.012**	Significant
EDTA vs Distilled water	Mann–Whitney U	**0.008**	Significant
Citric acid vs Maleic acid	Mann–Whitney U	**0.091**	Not significant
Citric acid vs Distilled water	Mann–Whitney U	**0.015**	Significant
Maleic acid vs Distilled water	Mann–Whitney U	**<0.001**	Significant

DISCUSSION

The removal of the smear layer is an important component of successful root canal therapy. Mechanical instrumentation produces a smear layer composed of inorganic dentinal particles, organic tissue remnants, and microorganisms that may remain attached to the prepared canal walls.[10,11] Persistence of this layer can interfere with the penetration of disinfecting agents and intracanal medicaments and may influence the adaptation and penetration of root canal sealers. Therefore, the present in-vitro study compared the effectiveness of 17% EDTA, 10% citric acid, and 7% maleic acid, with distilled water serving as a control, for smear layer removal using scanning electron microscopy.

The present findings demonstrated a significant difference in smear layer removal among the four groups. 7% maleic acid showed the greatest smear layer removal, followed by 10% citric acid and 17% EDTA, whereas distilled water demonstrated the least effective smear layer removal. The superior performance of maleic acid was particularly evident from the greater proportion of specimens showing Score 1, indicating clean root canal surfaces with open dentinal tubules.

The effectiveness of 7% maleic acid may be attributed to its acidic nature and ability to interact with the inorganic component of dentin. Maleic acid is a dicarboxylic acid capable of chelating calcium ions and promoting removal of the mineralized component of the smear layer. Its relatively strong demineralizing action may facilitate exposure of dentinal tubules and account for the favorable SEM appearance observed in the present study. The present findings therefore support the potential use of maleic acid as an alternative to conventional chelating agents for final root canal irrigation.[12]

10% citric acid demonstrated intermediate smear layer removal in the present investigation. Citric acid possesses chelating properties and can bind calcium ions from the mineralized component of dentin. Although it produced better smear layer removal than distilled water and showed a favorable cleaning effect, its overall performance was lower than that of maleic acid. The difference between citric acid and maleic acid was not statistically significant, suggesting that both acidic solutions may provide comparable smear layer removal under certain experimental conditions.[13]

The 17% EDTA group also demonstrated effective smear layer removal compared with the distilled-water control. EDTA is an established endodontic chelating agent that acts by binding calcium ions and facilitating removal of the inorganic component of the smear layer. However, in the present study, its performance was lower than that of 7% maleic acid. This finding may be related to differences in the chemical properties, pH, dentin interaction, and demineralizing potential of the tested solutions. It is important to note that the present finding does not imply that EDTA is clinically inferior to other chelating agents, as its effectiveness can vary with concentration, exposure time, irrigation volume, and instrumentation technique.[14]

The distilled-water control group showed the least smear layer removal, with a high proportion of specimens demonstrating Score 3. This finding was expected because distilled water does not possess significant chelating or demineralizing activity and therefore has limited ability to remove the inorganic component of the smear layer. The significantly poorer performance of distilled water compared with the experimental irrigants confirms the importance of using an appropriate chelating or acidic solution during final irrigation.

An important finding of the present study was the variation in smear layer removal according to the root canal level. In all four groups, smear layer removal was generally greater in the coronal third and decreased toward the apical third. The apical third demonstrated a greater amount of residual smear layer, which may be attributed to its smaller diameter, anatomical complexity, reduced irrigant exchange, and difficulty in delivering an adequate volume of irrigant to the apical region. These factors can limit the effectiveness of chemical irrigation despite the use

of an appropriate final irrigant.

The 7% maleic acid group demonstrated favorable cleaning even in the apical third, where 50% of specimens showed Score 1 and none demonstrated Score 3. In contrast, the distilled-water group showed 80% of specimens with Score 3 in the apical third. This observation suggests that maleic acid may have better potential for reaching and demineralizing the superficial inorganic component of the smear layer in areas where mechanical and irrigation challenges are greater.

The findings should also be interpreted in relation to the standardized irrigation protocol used in the present study. All specimens underwent step-back preparation up to ISO #40, with Gates-Glidden drills #2–4 used for coronal enlargement. A standardized volume of 3% sodium hypochlorite was used during instrumentation, followed by a standardized final irrigation volume and exposure time for each experimental solution. Such standardization minimized procedural variability and allowed the smear layer removal ability of the different final irrigants to be compared under similar conditions.

The SEM evaluation was performed using the scoring system described by Torabinejad et al., which evaluates the presence, quantity, and distribution of smear layer. The three-point scoring system provides a practical method for comparing the cleanliness of dentinal surfaces and allows assessment at different root canal levels. However, SEM evaluation remains essentially morphological and provides information from selected areas rather than the entire three-dimensional root canal surface.

The clinical implications of the present findings should be considered carefully. Although effective smear layer removal may improve the cleanliness of the root canal and potentially facilitate sealer adaptation, excessive demineralization may adversely affect dentin. Strong acidic solutions may alter the mineral composition and surface characteristics of dentin when used for prolonged periods.[15] Therefore, the concentration and exposure time of maleic acid should be carefully controlled. The one-minute exposure used in the present study provides a standardized experimental condition but does not establish the optimal clinical exposure time.

The present study has several limitations. It was conducted under in-vitro conditions using extracted teeth, and therefore the findings cannot be directly extrapolated to clinical situations. Variations in root canal anatomy, dentinal composition, and irrigation dynamics may influence the effectiveness of these solutions clinically. In addition, SEM evaluates selected regions of the canal wall and does not provide a complete three-dimensional assessment of smear layer removal. Further studies using larger sample sizes and complementary techniques such as confocal microscopy or micro-CT may provide additional information.

Within these limitations, the present study demonstrated that 7% maleic acid produced the most favorable smear layer removal among the tested final irrigation solutions, while distilled water was least effective. Citric acid demonstrated intermediate efficacy, and EDTA produced satisfactory but comparatively lower smear layer removal under the experimental conditions. The findings suggest that maleic acid may represent a promising alternative final irrigant; however, further investigations are required to assess its effects on dentinal microhardness, erosion, bond strength, and long-term clinical outcomes.

CONCLUSION

Within the limitations of the present in-vitro study, 7% maleic acid demonstrated the most effective smear layer removal, followed by 10% citric acid and 17% EDTA, while distilled water showed the least efficacy. Maleic acid demonstrated favorable smear layer removal in the coronal, middle, and apical thirds of the root canal. Smear layer removal was generally more effective in the coronal third and decreased toward the apical third across all groups. These findings suggest that 7% maleic acid may serve as a promising alternative to conventional EDTA for final root canal irrigation. However, further studies with larger sample sizes and evaluation of dentinal erosion, microhardness, and bond strength are required before recommending its routine clinical use.

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