A Comparative Evaluation of Smear Layer Removal by Using Four Different Irrigation Solutions like Root Canal Irrigants: An In Vitro SEM Study

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ABSTRACT

Background: The present study aimed to evaluate and compare the efficacy of 17% ethylenediaminetetraacetic acid (EDTA), 18% etidronic acid, 10% citric acid, and 7% maleic acid in the removal of smear layer at the apical third of the root canals.

Materials and methods: Sixty single-rooted teeth were equally divided into four study groups \((n = 15)\), according to the type of irrigant used (17% EDTA, 18% etidronic acid, 10% citric acid, and 7% maleic acid) to remove the smear layer effectively from apical third of root canal. In each group, respective irrigant was used with 5.25% of sodium hypochlorite during instrumentation. Each study sample was then sectioned longitudinally and removal of smear layer was observed using a scanning electron microscope (SEM) at 2000X.

Results: A 7% maleic acid revealed better smear layer removal than all other three groups at apical third. A 10% citric acid was found to be more efficient than EDTA and etidronic acid. The intergroup comparison was performed using Mann–Whitney U test, and there was no significant difference between all the study groups, except maleic acid.

Conclusion: The present study compared the effect of various irrigants as an adjunct with 5.25% of sodium hypochlorite for root canal irrigation during and after instrumentation. The use of irrigants aids in the removal of the smear layer from the root canals, thereby increasing the success rate of endodontic therapy.

Clinical significance: This study supports the hypothesis that a thorough use of root canal irrigants can efficiently remove the smear layer which is the key for successful root canal treatment. The present study helps in choosing an appropriate irrigant that can ensure complete root canal debridement from all thirds, especially from the apical third of the root canal.

Keywords: Root canal irrigants, Scanning electron microscope, Smear layer.

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INTRODUCTION

Successful root canal treatment is dependent upon methods and quality of biomechanical preparation, irrigation, disinfection, and obturation. Adequate debridement is required for a successful endodontic treatment. Root canal preparation involves complete removal of affected dentin, necrotic pulp and debris, and microorganisms. It also prepares the root canal to receive the filling material for an efficient apical seal.

Dentin removal contributes to smear layer formation covering the entire root canal wall. It consists of organic and inorganic substances containing odontoblastic processes, necrotic materials, and microorganisms. The smear layer prevents the diffusion of intracanal medicaments into the root canal irregularities and dentinal tubules. Additionally, it also hinders the adaptation of obturating materials on the surface of the root canal. Two different layers are included in the smear layer: the superficial layer (1–2 μm thick) and the deeper smear plug (40 μm thick). Smear layer removal is essential for disinfection of the root canal system by allowing deeper penetration of medicaments and irrigants. It also helps in the penetration of the root canal sealers into dentinal tubules that aid in the close adaptation of obturating materials with root canal walls. Irrigation of root canals is imperative for debridement of infected root canals to remove necrotic tissue remnants, microorganisms, and smear layer, created during mechanical canal instrumentation.

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Smear layer removal after root canal instrumentation requires the use of irrigating solutions, either alone or in combination. The mechanical debridement efficacy of an irrigation delivery/agitation system is dependent on its ability to deliver the irrigant to the apical and non-instrumented regions of the canal space and to create a strong enough current to carry the debris away from the canal walls. Mechanical cleaning and shaping is the most time-consuming and difficult aspect of endodontic treatment.1

Proper identification of the smear layer is essential to ensure complete removal of the smear layer. For this purpose, an electron microprobe with a scanning electron microscope (SEM) is used to identify the smear layer. Smear layer removal requires a combination of sodium hypochlorite (NaOCl), an organic solvent, and acids such as citric acid, tannic, polyacrylic, phosphoric acid, or chelating agents such as EDTA or REDTA for the removal of the inorganic part. For mechanical and chemical debridement ultrasonic instruments, chelating agents and lasers are being used.3

Ethylene diaminetetraacetic acid (EDTA) is a commonly used chelating agent in endodontics. Various studies advocate the use of a combination of sodium hypochlorite (2.5–5%) and EDTA (10–17%), which is effective in organic and inorganic debris removal. EDTA is a Ca2+ chelating agent, which is capable of smear layer removal. Dentinal tubules are opened up by the final irrigation of EDTA, increasing the number of lateral canals to be filled.1

Etidronic acid is a biocompatible chelating agent used in combination with sodium hypochlorite with an adequate calcium chelating capacity. Bisphosphonates are biocompatible chelators that are administered systemically in patients with neoplastic diseases involving osteolytic bone destruction or osteoporosis.4

In adhesive dentistry, maleic acid is a mild organic acid being used as a acid conditioner. It possesses the quality of removing the smear layer. At 7%, maleic acid has a property of smear layer removal and is considered more effective than EDTA at the apical third of the root canal.6

The citric acid (CA) is also used for removal of smear layer with concentration ranging from 10 to 50%. 10% CA is advocated as an effective material in smear layer removal.6

The purpose of the present study was done to evaluate and compare the efficacy of 17% EDTA, 18% etidronic acid, 10% citric acid, and 7% maleic acid in the removal of smear layer at the apical third of the root canals.

**Materials and Methods**

The study was conducted in the Department of Conservative Dentistry and Endodontics on 60 extracted human mandibular premolars with a single root and single canal. Teeth with developmental anomalies, external resorption, vertical/horizontal root fracture, calcified canals, endodontically treated teeth, deep carious lesions, and malformed teeth were excluded from the study. The study was approved by the Institutional Ethics Committee meeting held on March 2, 2016 (Approval no. MPCDRC2016025001), and written informed consent was obtained from the participants before their enrollment. Teeth extracted due to orthodontic reasons and periodontal problems were selected for the study. All the samples were decoronated using a diamond disk to obtain a uniform working length of 17 mm. The root canals were accessed and coronal preparation was done using Gates-Glidden drills up to number 3 size. A standardized crown down technique with sequentially sized hand protapers was done. They were randomly assigned to four study groups (n = 15) based on the irrigating solutions used after being instrumented.

**Group I:** The protapers sequence was followed and concomitantly alternate irrigation was carried out with 10% EDTA and 5.25% NaOCl. **Group II:** Irrigation was carried out with 10% citric acid and 5.25% NaOCl. **Group III:** Irrigation was carried out with 18% etidronic acid and 5.25% NaOCl. **Group IV:** Irrigation was carried out with 7% maleic acid and 5.25% NaOCl.

All the irrigants were freshly prepared and standardized. According to the groups, irrigants were delivered into the root canals with a side vented endodontic irrigating needle until the working length using the manual technique. And the final irrigation was carried using 5 mL distilled water for each sample. The root canals were dried using absorbent paper points and the entrance of each canal was sealed using a cotton pellet. Deep grooves were placed on buccal and lingual root surfaces using diamond discs. The roots were then split into two halves using a chisel and mallet. One-half of each tooth was taken and prepared for SEM examination. Three examiners performed the study and two intra-observers, and inter-observer variations checked by two observers.

**SEM Analysis**

The specimens were dehydrated by ethyl alcohol: 30% for 10 minutes, 50% for 20 minutes, 70% for 20 minutes, 90% for 30 minutes, 100% for 30 minutes, and 100% for 30 minutes. Specimens were placed on coded stubs, then dried by air, placed in a vacuum chamber, sputter-coated with a gold layer of 300 A, and analyzed with SEM. The surface of dentin was observed at apical thirds at 2000x magnification for presence/absence of smear layer and visualization of the dentinal tubules entrance. Photomicrographs (2000x) of apical thirds were taken. The smear layer removal was analyzed using a single observer, who was blind to irrigation regimens used for each group. The scores were attributed using a rating system developed by Torabinejad et al.7:

- No smear layer (no smear layer on the surface of the root canal: all tubules were clean and open).
- Moderate smear layer (no smear layer on the surface of the root canal, but tubules contained debris).
- Heavy smear layer (smear layer covered the root canal surface and the tubules).

**Results**

Data were collected and analyzed statistically with a level of significance set at 0.05. All irrigants tested, removed smear layer effectively from apical third of the tooth surface. The mean scores of evaluation of smear layer removal at the apical third of the tooth surface in

**Table 1: Mean scores of smear layer removal**

| Sl. no. | Groups (n = 15 each) |
|--------|----------------------|
|        | Group I | Group II | Group III | Group IV |
| Mean ± SD | 1.355 ± 0.234 | 1.422 ± 0.332 | 1.2 ± 0.2108 | 0.488 ± 0.353 |
Comparison of Different Irrigants in Smear Layer Removal Using SEM

all groups were derived. Group IV (7% maleic acid) was found to be the most effective smear layer removal irrigant among all four used irrigants (Table 1). The order of effectiveness of smear layer removal among all the four irrigants was group IV > III > I > II (Figs 1 to 4). The intergroup comparison was conducted using the Mann–Whitney U test. There was no significant difference between group I (17% EDTA) vs group II (18% etidronic acid), group I vs group III (10% citric acid), and group II vs group III. group IV was found to be highly statistically significant ($p < 0.0001$) with all three groups (Tables 2).

**Discussion**

The objective of the present study was to evaluate and compare the efficacy of 17% EDTA, 18% etidronic acid, 10% citric acid, and 7% maleic acid in the removal of smear layer at the apical third of the root canals.

Smear layer removal, in the apical one-third of the root canal, 7% maleic acid proved to be more effective than 17% EDTA, and 18% etidronic acid. This is followed by the analysis performed by Ballal et al. and Prabhu et al. In comparison to 7% maleic acid (0.06345 N/m), this could be due to the high surface tension of 17% EDTA (0.0783 N/m). Due to a decrease in pH, the effectiveness of EDTA decreases with time, but maleic acid is highly acidic with a stronger demineralizing effect in a shorter span. In smear layer removal at apical third, EDTA was less effective than citric and maleic acid. This may be because at the apical third of the root channel, dentin is sclerosed and EDTA on sclerosed dentin is less efficient.11

**Table 2:** Intergroup comparison using Mann–Whitney U test

| Groups | Mann–Whitney | Z score | $p$-value* | Significance |
|--------|--------------|---------|------------|--------------|
| I vs II | 102          | −0.41478| 0.6818     | Not significant |
| I vs III| 72.5         | 1.63833| 0.101      | Not significant |
| I vs IV | 4.5          | 4.45889| 0.00001    | Significant   |
| II vs III| 70.5        | 1.72134| 0.8544     | Not significant |
| II vs IV| 6            | 4.39668| 0.00001    | Significant   |
| III vs IV| 10.5         | 4.21002| 0.00001    | Significant   |

*p-value < 0.05 is significant; **p-value > 0.05 is insignificant
Statistically, the difference between EDTA and citric acid was negligible. Similar results were found in a study by Takeda et al.\textsuperscript{12} Etidronic acid was the least effective compared to other irrigants in smear layer removal at apical third. This is attributed to reduced chelating activity and decreased sclerosed dentin efficacy. In research conducted by De-Deus et al., similar results were found.\textsuperscript{13}

It was found to be much higher with EDTA than with etidronic acid, but less effective than with other irrigants. For effective outcomes, EDTA requires an implementation period of approximately 15 minutes. Several studies have shown that a neutral EDTA solution can reduce the dentin portion of mineral and non-collagenous proteins (NCPs).\textsuperscript{14–16} Thus, not only does EDTA eliminate loose calcium ions but also calcium is linked to NCPs. As the content of NCPs in the apical third decreases, the degree of EDTA decalcification in this portion is low. Thus, EDTA was found to be less potent in the apical third.\textsuperscript{14}

As the principal irrigant between each instrument, 5.25% sodium hypochlorite was used in the current chemomechanical preparation research. Different studies have shown that sodium hypochlorite is successful in removing the smear layer’s organic material.\textsuperscript{17} In our analysis, it was found that all four irrigants (17% EDTA, 18% etidronic acid, 10% citric acid, and 7% maleic acid) effectively eliminated the smear layer with a statistically insignificant difference from the apical third layer. Due to their capacity to dissolve inorganic debris from the smear layer, these irrigants were used.\textsuperscript{18}

To avoid any anatomical variation and preserve standardization, straight single canal and single-rooted mandibular premolars were selected according to requirements provided by Schneider et al.\textsuperscript{19} with a curvature of fewer than 5°. Mandibular premolars are readily accessible from orthodontic treatment extraction. Most single-rooted teeth have single-canal anatomy that assists in the SEM analysis of root canal instrumentation, irrigation, and sectioning. For root canal irrigation, 27-gauge needle tips were used after instrumentation, as they penetrate deep into the apical one-third due to the small-bore scale. They have a special side vent that increases the irrigant’s contact with the walls of the canal, thereby preventing irrigants from moving through the apical foramen by force. This increases the irrigant’s efficacy in eliminating the smear coating from root canals.\textsuperscript{20}

For smear layer removal, the suggested amount of EDTA ranges from 3 to 20 mL per canal. A high-volume delivery is a challenging task that requires a longer time and allows the operator to fatigue from using a fine needle.\textsuperscript{21} To evaluate the smear layer on the dentinal surface, various techniques are available like SEM, digital image analysis, etc. But in the present study, we opted for SEM as it is a commonly available tool to evaluate the smear layer.

**Limitations of the Study**

The present research was performed only with curvatures of less than 5° in vitro. Also, the results from this in vitro research need to be validated in vivo conditions and this warrants further research. The root canal system has different factors that can influence the behavior of these agents, such as blood, tissue remnants, etc. The removal of the smear layer from curved canals can be more challenging and complicated. Since our study was done in a single canal system that is wider and allows deeper penetration of the needle, therefore, further studies and long-term clinical trials will be required to assess the effectiveness of irrigants in narrow canals of posterior teeth, to validate these findings, and to evaluate their application to treatment outcomes.

**Conclusion**

The present study supports the use of various irrigants as an adjunct with 5.25% of sodium hypochlorite for irrigating the root canals during and after instrumentation. Usage of irrigants helps to remove the smear layer from the root canals, which can subsequently increase the success rate of endodontic therapy. Within the limitations of the study, we conclude that 7% maleic acid is a highly effective irrigant at the apical third of the root canal, followed by 10% citric acid, 17% EDTA, and 18% etidronic acid.

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