A comparative evaluation of smear layer removal by using ethylenediamine tetraacetic acid, citric acid, and maleic acid as root canal irrigants: An in vitro scanning electron microscopic study

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Abstract

Introduction: Irrigants remove or dissolve smear layer formed during instrumentation. Thus it is important to study the effect of different irrigant solutions on smear layer removal.

Objectives: The aim of this study is to determine which irrigant effectively removes the smear layer from the coronal, middle, and apical third of the root canal.

Materials and Methodology: One hundred and twenty single-rooted mandibular premolars were decoronated and biomechanical preparation was done through hand instrumentation up to size 40 k file with 2.5% NaOCl irrigation between each successive filing, followed by irrigation with 5 ml of saline. The teeth were divided into Groups I, II, III, and IV containing 30 samples each and irrigated with 5 ml of 17% ethylenediamine tetraacetic acid (EDTA), 10% Citric acid, 7% Maleic acid and normal saline respectively for 1 min and final irrigation was done with 5 ml of distilled water of each sample. The canals were dried with 2% absorbent paper points. The roots were then split with a chisel and mallet. One-half of each tooth was selected and then was analyzed using a scanning electron microscope. The dentinal surfaces were observed at the cervical, middle, and apical third with ×2000 for the smear layer. The scores were attributed, according to Torabinejad et al. rating system.

Results: 7% maleic acid and 10% citric acid both are equally effective in smear layer removal from coronal and middle third, but in apical third 7% maleic acid is more effective than 10% citric acid. Between citric acid and EDTA, both are equally effective in smear layer removal from coronal and middle third, but in apical third, 10% citric acid is more efficacious than 17% EDTA.

Conclusion: Within the limitations of the study, it can be concluded that all three tested irrigants removed the smear layer from coronal, middle, and apical third. However, in apical third 7% maleic acid is the most efficacious irrigant in smear layer removal.

Keywords: Citric acid; ethylenediamine tetraacetic acid; maleic acid; scanning electron microscope; smear layer

INTRODUCTION

Root canal treatment comprises three-dimensional cleaning, shaping, and obturation of the root canal system. During cleaning and shaping, the root dentin is cut and shaped with hand or rotary instruments under constant irrigation to remove the necrotic tissue, microbes/biofilms, and other debris from the root canal space.\textsuperscript{[1]} While shaping the mineralized tissues of the root canal are not shredded or cleaved but shattered to produce considerable quantities.

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of debris. Much of this, made up of very small particles of the mineralized collagen matrix, is spread over the surface to form what is called the smear layer. The presence of the smear layer on instrumented root canals was first reported by McComb and Smith in 1975. They showed that this layer is made of remnants of dentin, odontoblastic processes, necrotic or viable pulp tissues, and bacteria. Chemically, it is composed of two phases, namely organic and inorganic. The organic phase is mainly composed of dentinal collagen residues and glycosaminoglycans. The second, inorganic phase, is the predominant phase and is mainly made of dentinal debris.

There is a controversy over whether to remove or maintain the smear layer. Some investigators believed that the presence of the smear layer helps increase the success rate of endodontic treatment. Conversely, some authors advocated the removal of the smear layer, considering that it may serve as a substrate for microorganisms to multiply and proliferate. In addition, the smear layer can minimize the ability of disinfecting agents/intracanal medicaments to penetrate deeply into the dentinal tubules. Furthermore, it also compromises the ability of the sealer to penetrate the dentinal tubules and adhere properly. The smear layer acts as a sealing barrier between the canal wall and root filling materials and may compromise the ability to form a satisfactory seal. Although controversies exist for regarding the removal or retaining of the smear layer, its removal is largely advocated as complete debridement of the root canal is essential to achieve a successful outcome.

Irrigation has a pivotal role in removing the smear layer. They not only flush out microorganisms, tissue remnants, and smear layer from the root canal but also have the advantage of reaching the complex and accessory features of root canal system such as fins, cul-de-sacs and inter-canal communications which are otherwise difficult to access. Activation of irrigants through sonics, ultrasonics, and lasers can further enhance the smear layer removal efficacy of root canal irrigants.

The review of the literature shows that various chemicals have been used as irrigants. These include Reducing agents (e.g., NaOCl, CI02), Oxidizing agents (e.g. Hydrogen peroxide), Bactericidal agents (e.g., CHX), Bacteriostatic agents (e.g., MTAD), Chelating agents (e.g., HEBP, Ethylenediaminetetraacetic acid [EDTA], MTAD, HEPB), Acids (Maleic acid, citric acid, polyacrylic acid), Combinations such as Qmix, Tetraclean, Smearclear and natural agents (e.g., Green tea, Triphala, propolis). At present, sodium hypochlorite (NaOCl) is the recommended irrigant used during biomechanical preparation, due to its potent bactericidal action and ability to dissolve organic matter and necrotic tissue. However, it lacks the ability to dissolve inorganic content, thus incapable of removing the smear layer when used alone. Hence, demineralizing agents such as ethylenediaminetetraacetic acid (EDTA) and chitosan have been recommended for smear layer removal in root canal therapy.

EDTA (17%) is the most commonly used chelating irrigant in endodontics. It reacts with the calcium ions in the dentin and forms soluble calcium chelates, promoting decalcification of dentine at depths of 20-30 µm. EDTA adversely affects the periapical tissues; thus, research continues for more biocompatible solutions as an alternative to the routine use of 17% EDTA. Weak acids, such as citric acid and maleic acid, are being evaluated at different concentrations for the removal of the smear layer. Citric acid is a demineralizing solution, available in 10-50% concentration and is used during the endodontic therapy to remove the smear layer. Maleic acid is a mild organic acid that removes the smear layer effectively at concentrations of 5% and 7%.

The review of the literature shows numerous studies comparing the efficacy of different irrigants in smear layer removal. Di Lenarda et al. 2000 reported no or a negligible difference in smear layer removal obtained by citric acid and EDTA whereas Khademi and Feizianfard 2004 claimed that EDTA is more effective in removing the smear layer when compared to citric acid, especially in apical third and middle third. Ballal et al. 2009 reported that final irrigation with 7% maleic acid for 1 min was more efficient than 17% EDTA in the removal of smear layer from the apical third of the root canal system. Kandil et al. 2014 also reported that in the apical region, maleic acid is more efficient than EDTA.

Smear layer removal is considered to be advantageous and highly desirable. However, literature shows most irrigants lack inability to remove the smear layer completely from all the parts, especially the apical third, which is crucial in determining the root canal treatment success rate. More studies are required to establish which irrigating solution has superior smear layer removal capacity and in which area of root canal the particular irrigant is more effective. Keeping this in mind, the present in vitro study was conducted to evaluate the effect of 17% EDTA, 10% citric acid, and 7% Maleic acid on smear layer removal under the scanning electron microscope (SEM). The study aims to contribute to evaluate their relevance to treatment outcome by establishing which irrigant among three has best smear layer removal efficacy and to determine how effectively the individual irrigant removes the smear layer from coronal, middle and apical third of the root canal separately.

**MATERIALS AND METHODOLOGY**

This in vitro study was conducted in the Department of Conservative Dentistry and Endodontics of Desh Bhagat
Dental College and Hospital, Mandi Gobindgarh, Punjab. The SEM study was done at SAI lab (Thapar University Patiala, Punjab).

Selection of teeth
One hundred and twenty straight single-rooted mandibular premolars with relatively similar dimension and morphology, freshly extracted, were collected from the Department of Oral and Maxillofacial Surgery of Desh Bhagat Dental College of Dental Sciences and Research, Gobindgarh to be used in this in vitro study. Teeth with the presence of a single canal were included. Teeth with previous root caries, cracks, curved canals, endodontic treatment, internal resorption or calcification were excluded.

Preparation
The selected teeth were cleaned from soft and/or hard attached tissues stored in normal saline. The samples were then autoclaved.

The teeth were then decoronated to obtain a uniform working length of 17 mm for all samples using a diamond disk (D and Z, Darmstadt, Germany). The root canals were accessed, and the biomechanical preparation was done using a standardized crown down technique. The initial coronal preparation was done with Gates-Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland) up to number 3 size. Hand instrumentation was done in step down method using K files (Dentsply-Maillefer, Ballaigues, Switzerland) in sequentially smaller sizes up to ISO size 40 with 2.5% NaOCl irrigation (Novo Dental Product, India) between each file, followed by irrigation with 5 ml of saline to continue the preparation in the apical segment until the apex was reached. The hundred and twenty specimens were randomly divided into four equal groups of thirty each.

- Group I: Irrigated with 17% EDTA
- Group II: Irrigated with 10% Citric acid
- Group III: Irrigated with 7% Maleic acid
- Group IV: Irrigated with Normal saline (control group).

Then each sample was irrigated with 5 ml of respective irrigant for 1 min with a side vented endodontic irrigating needle (RC Twents, Prime Dental Products, Mumbai, India). The depth of the irrigation needle was calculated by reducing 2 mm from the working length. The final irrigation was done with 5 ml of distilled water for each sample. The canals were dried with 2% absorbent paper points (Diadent). Diamond discs were used to cut deep grooves on the buccal and lingual surfaces of the roots, without perforating the root canals. The roots were then split with a chisel and mallet. One half of each tooth was selected and prepared for SEM examination. The specimens were dehydrated by ethyl alcohol: 30% for 10 min, 50% for 20 min, 90% for 30 min, and 100% for 30 min. The roots sections were coded and mounted on metallic stubs and were gold-sputtered to render the surface electrically conductive [Figure 1]. The coronal, middle, and apical one-third of root dentin was observed with SEM (Jeol, Tokyo, Japan) under ×2000 [Figures 2-5].

The amount of smear layer remained on the surface of the root canal or in the dentinal tubules was scored according to the criteria given by Torabinejad et al.[20] 2003

- Score 1: No smear layer (no smear layer on the surface of the root canal: All tubules were clean and open)
- Score 2: Moderate smear layer (no smear layer on the surface of the root canal, but tubules contained debris)
- Score 3: Heavy smear layer (smear layer covered the root canal surface and the tubules).

RESULTS
The data are presented as mean and standard deviation of scores obtained for smear layer removal for all the three regions of the root [Table 1]. The mean values that are presented are inversely proportional to the efficacy of irrigants for smear layer removal. Statistical analysis of the scores was performed using Kruskal–Wallis test [Table 2] for intragroup comparison along with Wilcoxon signed-rank test [Table 3] to verify the efficacy of individual irrigant in different areas of the root canal. The difference was highly significant when the coronal third was compared with the apical third of the root canal, but statistically, insignificant difference is seen between the coronal and middle third of the root canal. This signifies that in the coronal and middle one-third regions, smear layer removal was comparable for all groups, whereas irrigant was least efficacious in the apical third of the root canal. This stands true for all the irrigants tested.

Mann–Whitney test, summarized in Tables 4-6, was done for intergroup comparison of the irrigants for establishing

Figure 1: Gold sputtered samples mounted on aluminum stab for scanning electron microscope analysis
which is irrigant has better smear removal ability. In our study, the mean difference for removal of smear layer from whole root canal dentin by using EDTA, citric acid, maleic acid irrigating solution came out to be statistically significant and the following order was observed.

Maleic acid > Citric acid > EDTA > Normal Saline

**DISCUSSION**

The present study was conducted to compare the efficacy of 7% maleic acid, 10% citric acid, and 17% EDTA in smear layer removal from coronal, middle, and apical third of the root canal. Most irrigants lack in removing the smear layer completely from all the parts of the root canal. Therefore, the coronal, middle, and apical third of the canal were separately studied to test the efficacy of the irrigants in smear layer removal.

The teeth used in this study were single-rooted mandibular premolars as they are the most extracted teeth for orthodontic corrections and are easily available. The teeth were decoronated to obtain uniform working length of 17 mm. To allow adequate cleaning and penetration of irrigating solution, the apical portion of each canal was prepared up to ISO size 40 K file through hand instrumentation using crown down technique in step down method.

Irrigation by syringe can control the volume, the depth of the syringe penetration, and the resulting flow of

| Group          | n   | Minimum score | Maximum score | Mean score | SD  |
|----------------|-----|----------------|----------------|------------|-----|
| Maleic         |     |                |                |            |     |
| Coronal        | 30  | 1              | 1              | 1.00       | 0.000|
| Middle         | 30  | 1              | 2              | 1.20       | 0.407|
| Apical         | 30  | 1              | 2              | 1.87       | 0.346|
| EDTA           |     |                |                |            |     |
| Coronal        | 30  | 1              | 1              | 1.02       | 0.000|
| Middle         | 30  | 1              | 2              | 1.17       | 0.379|
| Apical         | 30  | 2              | 3              | 2.13       | 0.346|
| Citric acid    |     |                |                |            |     |
| Coronal        | 30  | 1              | 1              | 1.00       | 0.000|
| Middle         | 30  | 1              | 2              | 1.13       | 0.346|
| Apical         | 30  | 2              | 2              | 2.00       | 0.000|
| Normal saline  |     |                |                |            |     |
| Coronal        | 30  | 1              | 2              | 1.40       | 0.498|
| Middle         | 30  | 1              | 3              | 2.00       | 0.263|
| Apical         | 30  | 2              | 3              | 2.50       | 0.509|

n: Number of samples, EDTA: Ethylenediamine tetraacetic acid, SD: Standard deviation
the irrigating solution to the apical region of the root canal system. Large-volume syringes are more difficult to control for pressure, and accidents may happen. Therefore, to maximize safety, the use of 1–5-ml syringes is recommended.\textsuperscript{[21]} Hence, we used 3 ml syringe for irrigation purposes. We used RC Twents side-vented needle tips (prime dental products), which can penetrate more deeply into the apical one-third because of the small-bore size, i.e., 27 gauge.

The primary factors that govern the action of an irrigant are contact time and concentration. Optimum contact time that an irrigant solution must be kept in root canals to remove the smear layer is not yet unclear. EDTA satisfactorily remove the smear layer in 1 min, but it caused excessive erosion of peri- and intertubular dentin when applied for a longer duration 10 min.\textsuperscript{[22]} Ballal et al.\textsuperscript{[18]} reported that final irrigation with 7\% maleic acid for 1 min was more effective than 17\% EDTA in smear layer removal. Hence, in this study, we kept the irrigation time for 1 min for all irrigants.

SEM has been used in this study to evaluate the efficacy of various irrigants in the removal of smear layer. SEM allows the evaluation of the morphological details of the surfaces of the prepared root canals. Several magnifications ranging from $\times 50$ to $\times 5000$ can be chosen for observation. Lower magnification permits observation of larger fragments of dentinal or pulpal debris, whereas higher magnification identifies the smear layer and aperture of dentinal tubules openings.\textsuperscript{[23]} SEM with $\times 2000$ was used in the present study to evaluate the smear layer at the coronal, middle, and apical portion of root canals.

Kruskal–Wallis test and Wilcoxon signed-rank test were done for intragroup comparison to verify the efficacy of individual irrigants in different areas of the root canal. In the coronal and middle one-third regions, smear layer removal was comparable for all groups, with no significant differences existing between the groups. The irrigants were least efficacious in the apical third of the root canal. This stands true for all the irrigants tested. These results are in accordance with the study done by Abbott et al.\textsuperscript{[24]} which found that there is effective cleaning action of irrigant in the middle and coronal third of the root canals regardless of the nature of irrigation solutions, volume, and time. Similar results were found in the studies conducted by Charlie et al.,\textsuperscript{[25]} Kour et al.\textsuperscript{[26]} and Yamashita et al.,\textsuperscript{[27]} who reached similar conclusions in their respective studies that the dentinal walls were cleaner on the coronal third, with poorer cleaning on the apical third. In the coronal and middle third areas, a large canal diameter allows a better flow of irrigants. Efficient fluid dynamics and greater time.

\textbf{Figure 4:} (a) Scanning electron microscope showing effect of Maleic acid in coronal third. (b) Scanning electron microscope showing effect of Maleic acid in middle third. (c) Scanning electron microscope showing effect of Ethylenediamine tetraacetic acid in Apical third

\textbf{Figure 5:} (a) Scanning electron microscope showing effect of Normal saline in coronal third. (b) Scanning electron microscope showing effect of Normal saline in middle third. (c) Scanning electron microscope showing effect of Normal saline in Apical third
of contact with dentine surface facilitates the irrigant to act on the smear layer comprehensively, hence better smear layer removal efficacy. Cleaning difficulty on the apical third is explained by the reduction in root canal diameter, which impairs the access of irrigant, with the consequent reduction in its flow. At the apical third, cleanliness is critical due to the limited space, low permeability, and complex anatomical configuration. These features prevent the irrigant to reach the Working Length and exchange fluids in this area. Irfan et al. also proposed that factors which could influence the cleaning of the apical third is the difficulty of access. Paque et al. reported that dentin in the apical third of the root canal is sclerosed; hence EDTA or any such chelating agent may not have a pronounced action on sclerosed dentin in the apical third. For the intergroup comparison to verify which irrigant is more efficacious in smear layer removal when the same area of the root canal is considered, Mann–Whitney test was done.

Table 4 shows Mann–Whitney test for intergroup comparison between maleic acid and EDTA. Results showed that between maleic acid and EDTA, no statistically significant difference is observed in coronal and middle third between the two and the P value is 1 and 0.741, respectively, but in the apical third P value is 0.005 which is statistically significant. Hence, inference can be drawn that Maleic acid and EDTA, both are equally effective in removing the smear layer from coronal third and middle third, but in apical third maleic acid is more effective in removing the smear layer as compared to EDTA. These results support the findings of Ballal et al. and Kuruvilla et al. who found 7% maleic acid is more efficient than 17% EDTA in the removal of the smear layer from the apical third of the root canal. The studies conducted by Kandill et al. and Jaiswal et al. also agree with our study showing similar outcomes. Mann–Whitney test for comparison between Maleic acid and citric acid [Table 5] shows no statistically significant difference is observed in coronal and middle third, but in the apical third P value stands statistically significant. Thus, it shows that Maleic acid and citric acid are equally effective in removing the smear layer from coronal third and middle third, but in apical third maleic acid is more effective in removing the smear layer. These results are partially in accordance with the study conducted by Irfan et al. who concluded similar outcomes. This finding is contrast to the study done by Choudhary et al. who reported that both citric acid and maleic acid are equally effective in removing the smear layer from all parts of root dentin.

Similarly, Table 6 shows Mann–Whitney test for EDTA and citric acid, indicating EDTA and citric acid are equally effective in removing the smear layer from the coronal and middle third. However, in apical third citric acid is more efficient than EDTA. These findings are in agreement with the study done by Banode et al. who concluded that citric acid group is more efficient than the EDTA group in removing the smear layer. However, literature contains studies with contrary conclusions. A study by Khademi and Feizianfard proved that EDTA is better than citric acid, especially in middle and apical thirds of canals. A study done by Ahir et al. also found that there is no significant difference in smear layer removal efficacy of citric acid and EDTA in the middle and apical third of root canal. Similarly, Mann–Whitney test for intergroup comparison between maleic acid and normal saline, EDTA and normal saline, citric acid, and normal saline, respectively, showed saline was found to be most ineffective irrigant for smear layer removal. This is in accordance with studies conducted...
Table 4: Mann-Whitney test for intergroup comparison between maleic acid and ethylenediamine tetraacetic acid

| Group  | n  | Mean rank | Sum of ranks |
|--------|----|-----------|--------------|
| Coronal|    |           |              |
| Maleic acid | 30 | 30.50     | 915.00       |
| EDTA   | 30 | 30.50     | 915.00       |
| Total  | 60 |           |              |
| Middle |    |           |              |
| Maleic acid | 30 | 31.00     | 930.00       |
| EDTA   | 30 | 30.00     | 900.00       |
| Total  | 60 |           |              |
| Apical |    |           |              |
| Maleic acid | 30 | 26.77     | 803.00       |
| EDTA   | 30 | 34.23     | 1027.00      |
| Total  | 60 |           |              |

Test statistics

- Mann-Whitney U: 450.000 435.000 338.000
- Wilcoxon W: 915.000 900.000 803.000
- Z: 0.000 −0.331 −2.805
- P: 1.000 0.741 0.005*

*Grouping variable: Group. EDTA: Ethylenediamine tetraacetic acid, *Statistically significant

Table 5: Mann-Whitney test for intergroup comparison between maleic acid and citric acid

| Group  | n  | Mean rank | Sum of ranks |
|--------|----|-----------|--------------|
| Coronal|    |           |              |
| Maleic acid | 30 | 30.50     | 915.00       |
| Citric acid | 30 | 30.50     | 915.00       |
| Total  | 60 |           |              |
| Middle |    |           |              |
| Maleic acid | 30 | 31.50     | 945.00       |
| Citric acid | 30 | 29.50     | 885.00       |
| Total  | 60 |           |              |
| Apical |    |           |              |
| Maleic acid | 30 | 28.50     | 855.00       |
| Citric acid | 30 | 32.50     | 975.00       |
| Total  | 60 |           |              |

Test statistics

- Mann-Whitney U: 450.000 420.000 390.000
- Wilcoxon W: 915.000 885.000 855.000
- Z: 0.000 −0.687 −2.053
- P: 1.000 0.492 0.005*

*Grouping variable: Group, *Statistically significant

Table 6: Mann-Whitney test for intergroup comparison between citric acid and ethylenediamine tetraacetic acid

| Group  | n  | Mean rank | Sum of ranks |
|--------|----|-----------|--------------|
| Coronal|    |           |              |
| EDTA   | 30 | 30.50     | 915.00       |
| Citric acid | 30 | 30.50     | 915.00       |
| Total  | 60 |           |              |
| Middle |    |           |              |
| EDTA   | 30 | 31.00     | 930.00       |
| Citric acid | 30 | 30.00     | 900.00       |
| Total  | 60 |           |              |
| Apical |    |           |              |
| EDTA   | 30 | 32.50     | 975.00       |
| Citric acid | 30 | 28.50     | 855.00       |
| Total  | 60 |           |              |

Test statistics

- Mann-Whitney U: 450.000 435.000 390.000
- Wilcoxon W: 915.000 900.000 855.000
- Z: 0.000 −0.395 −2.053
- P: 1.000 0.720 0.040*

EDTA: Ethylenediamine tetraacetic acid, *Statistically significant

by Ahir et al.\textsuperscript{[22]} and Menezes et al.\textsuperscript{[36]} who reported that the dentin wall of the specimens irrigated with normal saline were completely covered with smear layer on the cervical, middle, and apical thirds after biomechanical preparation.

In this study, the mean difference for the removal of smear layer from root canal dentin by using EDTA, citric acid, the maleic acid irrigating solution came out to be statistically significant, and the following order was observed in the whole root canal.

MALEIC ACID > CITRIC ACID > EDTA > NORMAL SALINE

Maximum smear layer removal, especially in apical third, was seen in 7% maleic acid. The capability of maleic acid to remove the smear layer and demineralize intertubular dentin is because of its pH of 1.05. Maleic acid is more acidic and thus has a greater demineralizing effect in a shorter time duration.\textsuperscript{[37]}

**CONCLUSION**

7% maleic acid and 10% citric acid both are equally effective in smear layer removal from coronal and middle third, but in apical third 7% maleic acid is more effective than 10% citric acid. Between citric acid and EDTA, both are equally effective in smear layer removal from coronal and middle third, but in apical third, 10% citric acid is more efficacious than 17% EDTA.

Based on the results, our study concluded that all three tested irrigants removed the smear layer from coronal, middle, and apical third. However, among these irrigants 7% maleic acid is most efficacious in the apical third.

**Limitations of the study**

The conclusion of the present work is limited to in vitro conditions of the study and should be confirmed by further in vivo investigations.

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**Conflicts of interest**

There are no conflicts of interest.

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