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Abstract
This invitro study was conducted to evaluate the efficiency of removal of smear layer from apical, middle & coronal part of the root canal system using three chelating agents. Sixty extracted single-rooted human teeth were randomly divided into 4 groups (n -15) and instrumented using protaper nickel-titanium rotary instruments. The following solutions were used to irrigate each canal subsequently: 5.25% NaOCl (control), SmearClear, 17% EDTA and 10% citric acid. Then the specimens were subjected to final irrigation with 5.25% NaOCl. Following de-coronation and splitting of roots, one-half of each root was examined under scanning electron microscope (SEM) at 1000X and 2000X magnification for smear layer at coronal, middle, and apical thirds. The results showed that there was difference in the effectiveness of three chelating agents in smear layer removal however the result was not clinically significant (p<0.0005). The protocol used in this study showed better smear layer removal in the coronal and middle third when compared to apical third.

Keywords: Rotary instrumentation, Citric acid, EDTA, SmearClear, Smear layer removal, Chelating agents.

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Introduction
Smear layer is a layer of organic and inorganic material formed due to endodontic instrumentation which contain bacteria and their by-products. A study conducted by Ostavik and Haapasalo showed decreased time to attain disinfection is possible when the smear layer is removed and dentinal tubules should be patent¹. It is very much necessary to remove the smear layer for better penetration of intracanal medicaments , sealers into the dentinal tubules and adaptation of obturating materials to the canal wall. Chemical, ultrasonic and laser techniques are various methods of smear removal currently used but none are universally accepted ².

Endodontic therapy should be aimed at smear layer removal for achieving complete disinfection of the root canal system than only limiting to the removal of pulp remnants and the widening of the root canal. Chemomechanical preparation using various chemical irrigants helps to achieve this. For effective removal of both organic and inorganic components of the smear layer, combined application of NaOCl and a chelating agent, such as EDTA, is recommended ³-⁵. The smear layer was efficiently removed using 1 ml of 17% EDTA for 1 minute followed by 3 ml of 5.25% NaOCl as final irrigant by a study conducted by Crumpton et al.⁶ However EDTA has shown to cause erosion of the dentinal tubules⁷. Hence there is a search of chemicals to remove smear layer efficiently from the canal without causing any harm to the dentinal tubules. Citric acid is a chelating agent that reacts with metals to form nonionic soluble chelate. The literature reports have shown citric acid and EDTA there is negligible difference in smear layer removal and in when 10% citric acid and 2.5% NaOCl combination was very effective...
approach for the smear layer removal. SmearClear (Sybron Endo, Orange, CA) is a product recently introduced for removing the smear layer. It contains 17% EDTA, cetrimide (cationic surfactant), polyoxyethylene isooctylcyclohexyl ether (anionic surfactant) and water; having a pH of 8.0. Lui JN et al compared SmearClear with EDTA in removing the smear layer in the apical and middle thirds of the root canal they found that the surfactants within the SmearClear did not improve its efficiency. Apical part of the canal system usually contains dentin which is sclerotic and EDTA is proved to be less efficient in the apical 3rd of the canal in smear layer.

Hence this study was conducted to compare the efficacy of SmearClear, 17% EDTA, and 10% citric acid in combination with 5.25% sodium hypochlorite as final irrigants in the removal of the smear layer in the coronal, middle, and apical thirds of the instrumented root canal.

**Materials and methods**

In this in-vitro study sixty freshly extracted human teeth with single root were collected from the Department of oral and maxillofacial surgery of Yenepoya Dental College, Deralakatte, Mangalore, India. Inclusion criteria was Non carious, Non fractured, Non restored, straight single rooted and fully developed apices teeth were used which was extracted due to periodontal reason. Carious, restored, fractured and multi-rooted teeth were not included in the study. The teeth were cleaned of superficial debris and stored in 0.1% thymol solution.

The teeth were then decoronated with a diamond disc to standardize the root length to 12mm. The working lengths were measured by deducting 1mm from lengths recorded when tips of #15 K-files were visible at the apical foramina.

The specimens were prepared using Protaper Ni Ti rotary instruments. Instruments were used at the working length in each canal according to the manufacturer’s instructions in the following sequence: Sx, S1, S2, F1, F2 and F3 in a crown down technique to a standardized master apical file #30. Each instruments were used for only for the preparation of five teeth.

After using each file and before proceeding to the next, canals were irrigated with 2ml of 5.25% sodium hypochlorite by means of a 26 gauge needle which penetrates 1 to 2mm from the working length. Teeth were divided into 4 groups of 15 teeth each and canals were irrigated with one of the following irrigants to remove the smear layer and finally irrigated with 5.25% of NaOCl (Reachem Laboratory Chemicals private limited).

**Materials used:**

After instrumentation all teeth were irrigated with following irrigants using 26 gauge needle which penetrated 3mm short of the working length. The groups were irrigated as follows:

- **Group A:** Control-1ml of 5.25% NaOCl for 1min followed by 3ml of 5.25%NaOCl
- **Group B:** 1ml of SmearClear for 1min followed by 3ml of 5.25% NaOCl
- **Group C:** 1ml of 17% EDTA for 1min followed by 3ml of 5.25% NaOCl
- **Group D:** 1ml of 10%citric acid for 1min followed by 3ml of 5.25%NaOCl

After this the root canals were irrigated with 5ml of distilled water and dried with paper points. Diamond disc were used to prepare two deep longitudinal grooves on lingual/palatal surfaces of each root without penetration into the canal. Chisel was used to split the tooth into two halves. Then each half root with the most visible part of the apex is chosen for the study and coded. The coded specimens were dried with a critical point dryer and then mounted on metallic stub with a double sided adhesive, with the canal surface facing upwards. Then the specimens mounted on it were placed in the ion vacuum dried and then coated with gold. All the specimens were then viewed under Scanning Electron Microscope. After general survey of the canal wall, scanning electron photomicrographs were taken at magnifications of 1000x and 2000x at the coronal(10mm to apex), middle (6mm to apex), and apical (2mm to apex) thirds of each specimen in order to determine the relative efficacy of various irrigants. The amount of smear layer remained on the surface of the root canal or in the dentinal tubules were scored accordingly at the coronal, middle and apical portion of each canal according to the following criteria used by Torabinejad et al and the smear scores for all the groups were tabulated.

1 – No smear layer, no smear layer was detected on the surface of the root canals and all tubules were clean and open.

2 – Moderate smear layer, no smear layer...
was observed on the surface of the root canal, but tubules contained debris.

3– Heavy smear layer, the smear layer covered the root canal surface and the tubules.

**Statistical analysis**

Data collected was statistically analyzed using Chi square, Kruskal-Wallis, Mann-Whitney U test and Wilcoxon signed ranks tests to determine any significant difference between the groups using SPSS (Statistical Package for Social Sciences) version 11. A p-value of <0.05 was considered as statistically significant.

**Results**

Descriptive statistics of the groups is given in Table/Fig 1 with mean and standard deviation of smear scores for all the groups. Chi square test done showed there is significant difference between the smear scores between all the groups at coronal, middle and apical 3rd (p<0.0005). None of the chelating agent showed capacity to remove smear layer completely (100%) from the root canal system. Control group showed score 3 at coronal, middle and apical 3rd for all the samples whereas among experimental groups the smear layer was cleared very well by 17% EDTA at coronal and middle 3rd.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronal (10mm to apex)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>3.00</td>
<td>.00</td>
</tr>
<tr>
<td>Smear Clear</td>
<td>15</td>
<td>1.67</td>
<td>.498</td>
</tr>
<tr>
<td>17% EDTA</td>
<td>15</td>
<td>1.40</td>
<td>.507</td>
</tr>
<tr>
<td>10% Citric Acid</td>
<td>15</td>
<td>1.33</td>
<td>.498</td>
</tr>
<tr>
<td>Middle (6mm to apex)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>3.00</td>
<td>.00</td>
</tr>
<tr>
<td>Smear Clear</td>
<td>15</td>
<td>1.67</td>
<td>.488</td>
</tr>
<tr>
<td>17% EDTA</td>
<td>15</td>
<td>1.47</td>
<td>.640</td>
</tr>
<tr>
<td>10% Citric Acid</td>
<td>15</td>
<td>1.53</td>
<td>.640</td>
</tr>
<tr>
<td>Apical (2mm to apex)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>3.00</td>
<td>.00</td>
</tr>
<tr>
<td>Smear Clear</td>
<td>15</td>
<td>2.20</td>
<td>.676</td>
</tr>
<tr>
<td>17% EDTA</td>
<td>15</td>
<td>1.87</td>
<td>.743</td>
</tr>
<tr>
<td>10% Citric Acid</td>
<td>15</td>
<td>2.00</td>
<td>.655</td>
</tr>
</tbody>
</table>

Table 1. Descriptive statistics.

![Figure 1](https://example.com/figure1.png)  
Figure 1. Photomicrographs showing intact smear layer when treated with 5.25% NaOCl.

![Table 2](https://example.com/table2.png)  
Table 2. Kruskal-Wallis test - between groups for coronal, middle & apical.

![Figure 2](https://example.com/figure2.png)  
Figure 2. Photomicrographs showing partial smear layer and partial dentinal tubules when treated with Smearclear + 5.25% NaOCl.

Statistical analysis of the results indicated that there was a significant difference in the effectiveness of different irrigation regimens in removing smear layer at the coronal, middle and apical thirds (p<0.0005) of the root canals using Kruskal Wallis (Table/Fig 2).

Mann-Whitney U test (Table/Fig 3) was done to evaluate intergroup comparison between four groups showed SmearClear, EDTA and citric acid differs significantly from the control group (p<0.0005) in coronal, middle and apical thirds. But there was no significant difference among SmearClear, 17% EDTA and 10% Citric Acid groups in the smear layer removal at the coronal, middle & apical thirds (p>0.05).

![Table 3](https://example.com/table3.png)  
Table 3. MANWHITNEY TEST.

Control differs from Smear clear, 17% EDTA, and 10% citric acid groups significantly in the Coronal, Middle & Apical thirds of the root canal (p<0.0005). Smear clear, 10% citric acid and 17% EDTA group do not differ significantly in the Coronal, Middle & Apical (p>0.05).
Smear Layer Removal using various chelating Agents

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Figure 3. Photomicrographs showing smear free surface and open dentinal tubules when treated with 17% EDTA + 5.25% NaOCl.

Figure 4. Photomicrographs showing partial smear layer and partial open dentinal tubules when treated with 10% CITRIC ACID + 5.25% NaOCl.

Figure 5. Comparison of mean efficacy in smear layer removal of different groups.

Table 4. Wilcoxon Signed Ranks Test- Between Coronal, Middle & Apical.

<table>
<thead>
<tr>
<th></th>
<th>Middle (6mm to apex) Vs Coronal (10mm to apex)</th>
<th>Apical (2mm to apex) Vs Coronal (10mm to apex)</th>
<th>Apical (2mm to apex) Vs Middle (6mm to apex)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Z 0.00 p 1.000ns</td>
<td>0.00 p 1.000ns</td>
<td>0.00 p 1.000ns</td>
</tr>
<tr>
<td>Smear Clear</td>
<td>Z 0.00 p 1.000ns</td>
<td>-2.828 p 0.009ns</td>
<td>-2.828 p 0.009ns</td>
</tr>
<tr>
<td>17% EDTA</td>
<td>Z -1.000 p 0.377ns</td>
<td>-2.646 p 0.008ns</td>
<td>-2.446 p 0.014ns</td>
</tr>
<tr>
<td>10% Citric Acid</td>
<td>Z -1.732 p 0.083ns</td>
<td>3.162 p 0.002ns</td>
<td>-2.646 p 0.008ns</td>
</tr>
</tbody>
</table>

But in SmearClear, EDTA and citric acid groups showed significant difference in middle and coronal 3rd with the apical 3rd. There was no difference between coronal and middle 3rd. The results show that Control group is not capable of removing the smear layer from all the levels of root canal system. Smear clear, 17% EDTA and 10% citric acid are capable of removing the smear layer from the coronal and middle 3rd better than apical 3rd with statistically significant difference (Table/Fig 1 to Table/Fig 5). No irrigating solutions used in this study could remove the smear layer completely from three the levels of root canal system.

Discussion

Different irrigant solutions have been tried in removing smear layer from the root canals. Present study used 17% EDTA, Citric acid and SmearClear for canal irrigation along with NaOCl to evaluate their smear layer capacity.

Russell S. Yamada and Melvin Goldmann showed that the most efficient final irrigant for removing superficial debris was 5.25% NaOCl. The results of the present study showed that irrigation with 5.25% NaOCl alone which is used as control in this study was not able to remove the smear layer from the canal. These results are consistent with those of other authors who stated that this solution is not capable of effectively removing the smear layer from the root canal walls if used alone.

According to Berutti and Ricardo Mariní use of chelating agent is important to prepare the canal surface, for the NaOCl to exert its action at a depth, within the accessory canals and within the dentinal tubules. Baumgartner and Mader reported that the combination of NaOCl and EDTA caused a progressive dissolution of the dentin at the expense of peritubular and intertubular areas, so that the diameter of tubular orifice on the instrumented root canal wall were enlarged to 2.5 to 4 micron. The erosion of the exposed globular surface of the calciospheres and the enlargement of the orifices of the dentinal tubules probably resulted from the alternating action of NaOCl, which dissolved the organic component of dentin and EDTA that demineralised the inorganic component.

Since smear layer contains organic and inorganic components and to remove the smear layer, irrigating solutions should dissolve both
shown an effective cleaning action in the coronal with the results of various studies that have third of the canals. This finding is in agreement and middle thirds when compared with the apical layer removal was more effective in the apical one third.[Table/Fig 2]

Smear Clear (Sybron Endo, Orange, CA, USA) contains 17%EDTA and cetrimide (surfactant) which decreases the viscosity and the surface tension. This formulation of Smear Clear claimed to reduce the contact angle of the EDTA solution when placed on dentin surface and enhanced cleaning efficacy. Studies have confirmed that reduction of surface tension of endodontic solutions improved their flow into narrow root canals. Therefore, it may be speculated that the addition of two surfactants to EDTA should improve its penetration ability into narrow apical region of the root canal.

The finding in the present study showed that EDTA with surfactants containing SmearClear compared with the surfactant-free EDTA did not improve the effect of smear layer removal. This result is in agreement with the findings of Lui et al who used 5ml of SmearClear for 1 minute followed by 5 ml of 1% NaOCl as final irrigants showed that the addition of surfactants to EDTA in SmearClear did not result in better smear layer removal compared with EDTA alone. Also, other studies have shown that the reduction of surface tension of endodontic chelators did not improve their calcium chelating ability.

In the current study, 1ml of 10% citric acid was used for 1 minute. There was significant difference between the coronal, middle and apical thirds of the root canals in citric acid group may be related to the volume and/or application time of citric acid. There is no difference when citric acid is compared with EDTA, which in agreement with those studies that reported a minor or no difference in smear layer removal with citric acid and EDTA. Studies have shown that the application of higher volumes of citric acid over 1 minute improves its efficacy in removing the smear layer.

The results of this study showed smear layer removal was more efficient in the coronal and middle thirds when compared with the apical third of the canals. This finding is in agreement with the results of various studies that have shown an effective cleaning action in the coronal and middle thirds of the canals even when different irrigation times and volumes of solutions were investigated. The removal of the smear layer is better in the coronal and middle thirds because of the larger canal diameter which allows more volume of irrigants to reach the dentinal tubules. But reduction in the root canal diameter in the apical third, decrease the access of irrigant to reach thus making it difficult to clean.

In the present study, the addition of surfactants to EDTA in SmearClear did not result in better smear layer removal capacity when compared with EDTA alone and citric acid. This could be due to lesser flow of the irrigant in the apical 3rd or may be due to more sclerotic dentin at the apical end of the root canals.

**Conclusions**

For clinical relevance based under the tested conditions, the following conclusion can be drawn that canal irrigation with SmearClear, 17% EDTA, and 10% citric acid for 1 minute followed by 3ml of 5.25%NaOCl effectively removed smear layer from the coronal and middle but was less effective in the apical third. The addition of surfactants to EDTA in SmearClear did not result in improvement of smear layer capacity of experimental solution.

**Declaration of Interest**

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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