An in-vitro evaluation of effect of EDTAC on root dentin with respect to time
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Abstract:
Background: The present study was devised to evaluate the effects of 17% EDTAC on smear layer removal and on the dentin structure after irrigation with 1 minute and 10 minutes.

Materials & Methods: One hundred extracted mandibular molars with two separate mesial canals were selected; mesiobuccal canal was instrumented to size 30 file with crown down technique. One half of each root (either mesial or distal) was randomly selected and prepared for scanning electron microscopic (SEM) evaluation that was then cut longitudinally into two equal segments. Using 10 ml of 17% EDTA solution, halves belonging to the same root were irrigated for 1 and 10 min, respectively. All specimens were subjected to irrigation with 10 ml of 5% NaOCl. Then all the specimens were prepared for SEM evaluation.

Results: The results showed that 1 min EDTA irrigation is effective in removing the smear layer. However a 10 min application of EDTA caused excessive peritubular and intertubular dentinal erosion.

Conclusion: Therefore we suggest that this procedure should not be prolonged >1 min during endodontic treatment.

Key Words: EDTA, intertubular dentin, peritubular dentin

Introduction
The main objectives of endodontic therapy are cleaning and shaping, and then obturating the root canal system in three dimensions, to prevent reinfection.¹ Card² have shown that mechanical aspect of the endodontic instrumentation may remove majority of the bacteria found in the root canal microflora. Nevertheless Grossman³(1943), Tucker⁴ (1975), Moodnik⁵ (1976) confirmed that due to anatomical complexities of root canals, such as fins, prolongations, isthmi, accessory canals and apical deltas, even after meticulous mechanical procedures organic residues and bacteria located deep in the dentinal tubules cannot be reached.

Therefore the use of chemical agents is highly desirable during and immediately after the mechanical preparation of the root canals to remove debris and necrotic pulp tissue and to eliminate the microorganism that cannot be removed by mechanical instrumentation.⁶ Disodium salt of Ethylenediaminetetraacetic acid (EDTA) is generally accepted as the most effective chelating agent with prominent lubricant properties and is widely used in endodontic therapy. It is used to enlarge root canals, remove the smear layer, and to prepare the dentinal walls for better adhesion of filling materials.⁷⁻¹⁰ For effective removal of both the organic and inorganic components of the smear layer, irrigating root canals with 10 ml of 17% EDTA, followed by 10 ml of 5% sodium hypochlorite is recommended.¹¹,¹² It is widely recommended that, under clinical conditions, EDTA and sodium hypochlorite should be applied in 10 ml volume each; however there is no consensus on the duration of irrigation with EDTAC.

Because EDTAC solution has a strong demineralizing effect, it causes enlargement of the dentinal tubules, softening of the dentin, and denaturation of the collagen fibers.¹³ These effects may cause difficulty in adaptation of the root canal filling materials to the root canal wall. Thus, the present study was devised to evaluate the effects of 17% EDTAC on smear layer removal and on the dentin structure after irrigation with 1 minute and 10 minutes.
and apex open over size 20K file in diameter, were excluded from study.

**Specimen preparation:**
Standard conventional endodontic access cavity was established using Endo access kit (DentsplyMalliferBallalgues Switzerland). Size 10K file was used to establish patency of each canal by gently inserting, until the tip emerged from the apical foramen. This length was noted and the working length was calculated by subtracting 1 mm from the apical foramen. The root end of each specimen was covered with sticky wax to prevent visualization of instrumentation and irrigation while chemomechanical preparation of the canal.

**Preparation of the root canal:**
Single operator carried out instrumentation in the mesiobuccal canal of each specimen with crown down technique using profile instrument system (DentsplyMalliferBallalgues Switzerland) and hand Nitiflex K-file (DentsplyMalliferBallalgues Switzerland). Each canal of the specimen was instrumented to size 30 file. Irrigation was performed with 30 gauge 1 ½ inch irrigating needle (ProRinse, Dentsply) placed as far as possible in the canal without binding.

**Specimen Grouping:**
One hundred specimens were divided into two experimental groups Group ‘A’ and ‘B’ of 50 each, Each specimen in experimental groups received irrigation with 2ml of 5% sodium hypochlorite (Hyposept, ups hygienes Pvt. Ltd.) after each instrumentation.

**Group ‘A’** each canal of the specimen received final irrigation of 10 ml of 17% EDTAC (Canalarge, Ammdent) for 1 minute followed by 10 ml of 5% sodium hypochlorite for 5 minutes.

**Group ‘B’** each canal of the specimen received final irrigation of 10 ml of 17% EDTAC (Canalarge, Ammdent) for 10 minutes followed by 10 ml of 5% sodium hypochlorite for 5 minutes.

Canal of each specimen were finally irrigated with 10ml of distilled water for 5 minutes to remove any precipitate that might have formed from the test irrigants.

With slow speed carbide disc under saline irrigation two grooves on buccal and lingual surface of the mesial root was created. The groove created followed the root

<table>
<thead>
<tr>
<th>Specimens</th>
<th>BO Group 1</th>
<th>BO Group 2</th>
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BO1 – Blind Observer 1 BO2 – Blind Observer 2
Sub- Group ‘A’ irrigated with 17% EDTAC for 1 minute.
Sub- Group ‘B’ irrigated with 17% EDTAC for 10 minutes.
1 - No erosion. All tubules looked normal in appearance & size
2 - Moderate erosion. The peritubular dentin was eroded
3 - Severe erosion. The intertubular dentin was destroyed and tubules were connected with each other.

curvature and did not enter into the canal. Roots were then split longitudinally in a buccolingual direction with a chisel and mallet resulting in the mesial and distal half of the root. One half of each root (either mesial or distal) was randomly selected and prepared for scanning electron microscopic (SEM) evaluation.

Preparation for Scanning Electron Microscopic evaluation:
The specimens were placed in 2% Glutaraldehyde solution for 24 hours. The fixed specimens were dehydrated using ascending concentration of ethyl alcohol (30 – 100%).

After dehydration, the specimens were left in dessicator containing calcium chloride (which acts as moisture absorber) for 24 hours. Finally, each specimen were coded and mounted on aluminium stubs which were placed into sputter coater (Platinum Auto Fine Sputter Coater - JEOL-JFC 1600) and coated with 20μm thick film of Platinum. The coated and mounted specimens were placed in the vacuum chamber of the Scanning Electron Microscope (SEM).

Photomicrographs of the apical third of each canal were obtained at various magnifications ranging from X 1000
to X 5000 and were taken for final evaluation.
The photomicrographs taken were qualitatively evaluated blindly by two observers and scored the degree of erosion of dentinal tubules as follows:

**Score 1** - No erosion. All tubules looked normal in appearance & size

**Score 2** - Moderate erosion. The peritubular dentin was eroded

**Score 3** - Severe erosion. The intertubular dentin was destroyed and tubules were connected with each other.

After scoring the photomicrographs, the information was recorded and analyzed using Kruskal – Wallis and Wilcoxon rank Sum (Mann Whitney U) Test.

**Results**

Highly significant difference is noted in amount of erosion of peritubular and intertubular dentin among Group ‘A’ and Group ‘B’

**Group ‘A’** – (received irrigation with 17% EDTAC for 1 minute) Showed surface devoid of smear layer with no erosion of peritubular and intertubular dentin.

**Group ‘B’** – (received irrigation with 17% EDTAC for 10 minute) Showed surface devoid of smear layer with excessive erosion of peritubular and intertubular dentin.

Comparison of Group ‘A’ and Group ‘B’ highly significant (p < 0.001). Suggesting irrigation with 17% EDTAC for 1 minute is effective for smear layer removal, whereas as irrigation with 17% EDTAC for 10 minute causes excessive erosion of peritubular and intertubular dentin (Table 1 & 2).

The bar diagram illustrates the comparative mean scores of erosion in peritubular and intertubular dentin. No erosion of peritubular and intertubular dentin was observed with Group ‘A’ (received irrigation of 17% EDTAC for 1 minute), excessive erosion of peritubular and intertubular dentin was observed in specimens of Group ‘B’ (received irrigation with 17% EDTAC for 10 minute).

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**Table 2: Wilcoxon rank Sum (Mann Whitney U) Test.
Comparison of Group ‘A’ and Group ‘B’**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
<th>Mann Whitney U</th>
<th>Wilcoxon W</th>
<th>Z-value</th>
<th>p-value</th>
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<tr>
<td>Group A</td>
<td>50</td>
<td>9.07</td>
<td>136.00</td>
<td>16.00</td>
<td>136.00</td>
<td>4.27</td>
<td>0.001</td>
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<tr>
<td>Group B</td>
<td>50</td>
<td>21.93</td>
<td>329.00</td>
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<td>P&lt;0.001</td>
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</table>

Wilcoxon rank Sum (Mann Whitney U) Test Comparison of Group ‘A’ and Group ‘B’ of Group IV is highly Significant (p < 0.001).
irrigation of 17% EDTAC for 10 minute).

**Discussion**

**Baumgartner and Mader (1987)** reported that the combination of EDTA and sodium hypochlorite caused a progressive dissolution of dentin at the expense of peritubular and intertubular areas, and they suggested that this effect may have resulted from the alternating action of sodium hypochloride, which dissolved the organic component of the dentin, and EDTA, which demineralized the inorganic component.

Peritubular dentin is highly mineralized and therefore harder than intertubular dentin. The hardness of peritubular dentin may provide added structural support for the intertubular dentin. Lower collagen content makes peritubular dentin more quickly dissolvable in acid than is intertubular dentin.

**Hill (1959)** concluded that 15% solution of EDTAC soften the walls of the canal in 3 – 5 minutes. McComb and Smith (1975) reported to produce cleanest dentin wall after use of REDTA for 24 hours. Goldberg and Spielberg (1982) reported that the optimum working time for EDTAC is 15 minutes.

With the background of review of literature, scope still exist in the dental literature; concerning effect of 17% EDTAC on root dentin with respect to time. The present study was carried out to compare the structural effects of 17% EDTAC (Canalarge, Ammdent) on root dentin with respect to duration of application i.e irrigation for 1 minute and 10 minutes.

Under the conditions of the present study irrigation with 17% EDTAC for 1 minute followed by 5% sodium hypochloride completely removed the smear layer and open the dentinal tubules with no erosion of the peritubular and intertubular dentin. This is in accordance with Meryon SD, Tobias RS and Jakemen KJ (1987), Crumpton BJ, Goodwell GG and McClanahan SB (2005) and when 17% EDTAC for 10 minutes followed by 5% sodium hypochloride is applied, excessive erosive effects were observed under scanning electron microscope with dissolution of peritubular and intertubular dentin.

Thus the finding of this present study suggests; that 1 minute irrigation with 17% EDTAC removes optimum smear layer with no erosion of the peritubular and intertubular dentin. If irrigated for 10 minutes 17% EDTAC causes excessive erosion of the peritubular and intertubular dentin, which ultimately may reduce the hardness of the dentin.

**References**