Effect of EDTA-Urea Peroxide combination at different time periods on smear layer and root dentin surface: A SEM study

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\textbf{ABSTRACT:} \textit{Aim} - To evaluate the effect of EDTA-Urea peroxide combination on smear layer and root dentin surface at different time intervals.  
\textit{Methodology} - 24 specimens were prepared from 12 freshly extracted non-caries intact human maxillary central incisors. These 24 specimens were randomly divided into 2 groups. Group-A: Control group having 4 specimens. Group-B (15\% EDTA-10\% Urea peroxide combination) 20 specimens – samples were divided (3 minutes, 6 minutes, 9 minutes and 12 minutes) into four subgroups of 5 specimens each. The specimens were examined under SEM. The presence of smear layer and erosion of dentinal surface was evaluated using a four step scoring criteria. Mean scores were calculated & statistically analyzed for significance (p<0.01) using Student’s unpaired ‘t’ test.  
\textit{Result} - 12 minutes of application of EDTA-Urea peroxide combination (Group B) caused severe erosion with incomplete removal of the smear layer.  
\textit{Conclusion} - Within the limitation of the present study, EDTA-Urea peroxide combination (Group B) when used for 12 minutes, cannot remove the smear layer completely, but caused severe erosion of dentinal surface.

\textbf{KEYWORDS}: EDTA, Urea peroxide, Cleaning & Shaping, Smear layer, SEM.

\section{INTRODUCTION}

When root canals are instrumented during endodontic therapy, a layer of material composed of dentin, remnants of pulp tissue and odontoblastic processes and sometimes bacteria are also formed on the canal walls. This layer is called as the “\textit{smear layer}”.\textsuperscript{1,3} The presence of smear layer on root canal walls was first reported by McComb and Smith (1975).\textsuperscript{4} The removal of smear layer is necessary to achieve disinfection of the root canal system, by deeper penetration of the root canal...
medicaments and irrigants. It also allows greater penetration of the root canal sealers into the dentinal tubule openings aiding in an intimate adaptation of the obturating materials with the prepared canal walls.\textsuperscript{1-3, 5-9}

Over the years various chemical agents like Sodium hypochlorite (0.5\% to 5.25\%), Urea peroxide, Hydrogen peroxide, Organic acids like Citric acid (6\%, 10\%, 50\%), Chelating agents like EDTA at 15-17\% have been used to remove the smear layer. However none of the above is totally effective or has received universal acceptance for eradicating smear layer. EDTA and NaOCl combination is the most tried and tested combination during the chemico-mechanical preparation of root canals to disinfect the canal, to lubricate the canal and to remove the smear layer.\textsuperscript{7} Another documented reagent for removing the smear layer is EDTA-Urea peroxide combination which is a potent bactericidal agent and increases the permeability of dentin significantly.\textsuperscript{10, 11}

This study attempts to assess the effect of EDTA-Urea peroxide combination at various time periods, on the smear layer and root dentin surface.

2 AIM & OBJECTIVES

The aim of the study was to evaluate the effect of EDTA-Urea peroxide combination on smear layer and root dentin surface at different time intervals

The objectives of the study were:

i) To evaluate the efficiency of EDTA-Urea peroxide combination in removing smear layer from the root canal walls at 3 minutes, 6 minutes, 9 minutes and 12 minutes of application.

ii) To evaluate the effect of EDTA-Urea peroxide combination on root dentin surface at 3 minutes, 6 minutes, 9 minutes and 12 minutes of application.

3 MATERIAL & METHOD

3.1 MATERIALS

i) Twelve freshly extracted non-carious intact human maxillary central incisors.

ii) Normal saline (0.9 \% w/v, Nirma Ltd., India).

iii) 15 \% EDTA-10 \% Urea peroxide gel (RC-Prep, Premier Dental Products Co, King of Prussia, PA).


3.2 METHOD

Twelve freshly extracted non carious intact human maxillary central incisors with single canals were chosen. Endodontic access cavity was prepared using # 012 round diamond point and # 017 non-end cutting tapered fissure. Root canals were instrumented with H and K files upto # 70, using step-back technique. Intermittent copious irrigation using normal saline solution was done. The teeth were cut at cemento-enamel junction, to separate the roots from the crowns using diamond disc on micromotor contrangle handpiece. Each root was then cut longitudinally into two halves, thus resulting in 24 hemisections. These 24 specimens were randomly divided into 2 groups as follows:-

Group-A: Control group having 4 specimens which were not treated with any test solutions.

Group-B: 20 specimens – the exposed dentin of the root canals were treated with 15\% EDTA-10\% Urea peroxide combination. Samples were divided into four subgroups of 5 specimens each. The combination gel was kept on each specimen for 3 minutes, 6 minutes, 9 minutes and 12 minutes respectively.

Once stipulated time was over for each of the subgroup, the specimens were washed with 10 ml of normal saline and air-dried. The specimens were now ready for viewing under a SEM.

The photomicrographs were evaluated by five observers in a blind manner, as per the following scoring criteria:

For smear layer-

\begin{itemize}
  \item 0 – No smear layer seen with all the dentinal tubules opened (100\% distinguishable tubular outline).
  \item 1 – Little smear layer seen with more than 50 \% distinguishable tubular outline.
\end{itemize}
Effect of EDTA-Urea Peroxide combination at different time periods on smear layer and root dentin surface: A SEM study

2 – Moderate smear layer seen with less than 50 % distinguishable tubular outline or with more than 50 % indistinguishable tubular outline.

3 – Heavy smear layer seen with indistinguishable tubular outline.

For erosion of root dentin surface

0 – No erosion of dentin surface.

1 – Little erosion of dentinal surface not involving more than 50 % of the surface.

2 – Moderate erosion of dentinal surface involving more than 50 %, but not the entire surface.

3 – Severe erosion of dentinal surface involving almost entire surface.

The data was analyzed statistically using Student's unpaired ‘t’ test.

4 RESULTS

The results of this study can be summarized as follows:

i) Group A: Normal saline was unable to remove the smear layer (mean score-3). Heavy smear layer was noted in all the specimens (Figure 1).

ii) Group B:

- EDTA-Urea peroxide combination was unable to remove smear layer when used for 3 minutes (Figure 2.A) and 6 minutes (Figure 2.B). All the samples showed presence of heavy smear layer (mean scores-3).
- EDTA-Urea peroxide combination when used for 9 minutes (Figure 2.C), partially removed smear layer but major part of dentinal surface was covered with smear layer (mean score-2) with mild erosion (mean score-1).
- EDTA-Urea peroxide combination when used for 12 minutes (Figure 2.D), smear layer was partially removed with only some part of dentinal surface still covered by smear layer (mean score-1). It also caused severe erosion of dentinal surface, making the surface irregular (mean score-3).

Figure 1: Exposed dentin of the root canals when treated with normal saline

Figure 2: Exposed dentin of the root canals when treated with EDTA-Urea peroxide combination for 3 minutes (A), 6 minutes (B), 9 minutes (C), 12 minutes (D).
Table 1: Comparison of the observations of EDTA-Urea peroxide combination (Group B) on removal of smear layer at various time intervals

<table>
<thead>
<tr>
<th>Time (mean scores)</th>
<th>‘t’ value</th>
<th>‘p’ value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mins (3 ± 0.0) Vs 6 mins (3 ± 0.0)</td>
<td>0</td>
<td>p&gt;0.05</td>
<td>Not significant</td>
</tr>
<tr>
<td>3 mins (3 ± 0.0) Vs 9 mins (2 ± 0.4)</td>
<td>5.73</td>
<td>p&lt;0.01</td>
<td>Highly significant</td>
</tr>
<tr>
<td>3 mins (3 ± 0.0) Vs 12 mins (1 ± 0.3)</td>
<td>15.22</td>
<td>p&lt;0.01</td>
<td>Highly significant</td>
</tr>
<tr>
<td>6 mins (3 ± 0.0) Vs 9 mins (2 ± 0.4)</td>
<td>5.73</td>
<td>p&lt;0.01</td>
<td>Highly significant</td>
</tr>
<tr>
<td>6 mins (3 ± 0.0) Vs 12 mins (1 ± 0.3)</td>
<td>14.92</td>
<td>p&lt;0.01</td>
<td>Highly significant</td>
</tr>
<tr>
<td>9 mins (2 ± 0.4) Vs 12 mins (1 ± 0.3)</td>
<td>4.54</td>
<td>p&lt;0.01</td>
<td>Highly significant</td>
</tr>
</tbody>
</table>

Table 2: Comparison of the observations of EDTA-Urea peroxide combination (Group B) on erosion of root surface dentin at various time intervals

<table>
<thead>
<tr>
<th>Time (mean score)</th>
<th>‘t’ value</th>
<th>‘p’ value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mins (0 ± 0.15) Vs 6 mins (0 ± 0.15)</td>
<td>0</td>
<td>p&gt;0.05</td>
<td>Not significant</td>
</tr>
<tr>
<td>3 mins (0 ± 0.15) Vs 9 mins (1 ± 0.8)</td>
<td>2.74</td>
<td>p&lt;0.01</td>
<td>Highly significant</td>
</tr>
<tr>
<td>3 mins (0 ± 0.15) Vs 12 mins (3 ± 0.16)</td>
<td>35.71</td>
<td>p&lt;0.01</td>
<td>Highly significant</td>
</tr>
<tr>
<td>6 mins (0 ± 0.15) Vs 9 mins (1 ± 0.8)</td>
<td>2.74</td>
<td>p&lt;0.01</td>
<td>Highly significant</td>
</tr>
<tr>
<td>6 mins (0 ± 0.15) Vs 12 mins (3 ± 0.16)</td>
<td>35.71</td>
<td>p&lt;0.01</td>
<td>Highly significant</td>
</tr>
<tr>
<td>9 mins (1 ± 0.8) Vs 12 mins (3 ± 0.16)</td>
<td>2.77</td>
<td>p&lt;0.01</td>
<td>Highly significant</td>
</tr>
</tbody>
</table>

5 Discussion

Smear layer is a very thin microscopic layer, soluble in acid, hence it is not apparent on routinely processed specimens examined under the light microscope. Therefore samples were viewed under SEM.

SEM was used for the evaluation of samples in this study for the following reasons –

i) With SEM, much higher magnification can be achieved.
ii) It allows simple direct examinations of the specimens.
iii) Details of each specimen can be seen clearly and are well defined.
iv) It has ability to examine large number of samples.

A combination of EDTA and Urea peroxide acts as an effective chelating and irrigating agent for root canals and allowed deeper penetration of the medicament into the dentin (Stewart and colleagues 1969) 11.

Not much research has been carried out on EDTA-Urea peroxide combination. When EDTA-Urea peroxide combination was used for 3 minutes (Figure 2.A) and 6 minutes (Figure 2.B), it showed that homogenous smear layer was present on the canal wall which obliterated the surface of dentinal tubules completely. However when this combination was used for 9 minutes, most of the canal wall was covered by non homogenous smear layer and very few tubular openings with indistinguishable outlines were visible (Figure 2.C).

The combination of EDTA and Urea peroxide when used for 12 minutes, opened most of the tubules (Figure 2.D). But the 12 minutes application of EDTA and Urea peroxide caused the severe deterioration of dentinal surface. This might be the reason for maximum leakage into filled canals as reported by Cooke et al (1976) 12. Deterioration of dentinal surface might be because of residue of RC-Prep which remained on the canals inspite of thorough irrigation and cleansing. This has also been reported by Zubriggen et al (1975) 12. Viscosity of the material may have a role to play.

Thus in this present study it was found that even after 12 minutes of application of EDTA-Urea peroxide combination, smear layer was not removed completely. This finding is in agreement with Rane (1980), who stated that RC-Prep left a smear layer after canal instrumentation rather than removing it. 1
CONCLUSION

From the present study, it is concluded that:

i. Normal saline is unable to remove smear layer.

ii. Smear layer cannot be removed completely, even with 12 minutes using EDTA-Urea peroxide combination (Figure 2.D).

iii. Severe erosive changes on dentinal structure are seen with 12 minutes application of EDTA-Urea peroxide combination (Figure 2.D).

Thus, within the limitation of the present study, EDTA-Urea peroxide combination cannot remove smear layer completely even after 12 minutes of application and it caused severe erosive changes on dentinal surface.

REFERENCES


