Efect of different surfactant addition to EDTA on microhardness of root dentin. An in vitro study.

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Abstract: The aim of study was to evaluate and compare the effect of different surfactant addition to EDTA on microhardness of root dentin in vitro. Forty recently extracted single rooted human teeth were selected. The crowns were sectioned at cemento enamel junction. The roots were split longitudinally into two parts. The specimens were randomly divided into 4 groups and were treated with 17% EDTA, 17% EDTA + 10% carbamide peroxide, 17% EDTA + 0.75% cetrimide, 17% EDTA + 0.5% Tween 80(Polyisorbate 80, Detergent). Reference microhardness value of untreated specimen was initially measured with the Vickers Indenter under a 50-g load and 10 sec dwell time at the midroot and apical third level of root dentin. After the baseline microhardness measurement a standardized volume of 50 ml of each solution was used for 1 minute. Post treatment microhardness values were obtained in the same manner as initial value. The decrease in microhardness was tabulated and calculated as percentage. Data was analyzed statistically using one-way analysis of analysis of variance (ANOVA) with Post-Hoc Tukey’s correction for multiple group comparisons. The EDTA + 0.5% Tween 80 solution group showed the highest change in microhardness. The plain 17% EDTA and 17% EDTA + 10% carbamide peroxide solution groups had similar values. Overall, Tween 80 showed highest reduction in microhardness of root canal dentin. 17% EDTA and carbamide peroxide showed moderate reduction in microhardness and least was cetrimide.

Keywords: carbamide peroxide, cetrimide, dentin microhardness, EDTA, Tween 80.

INTRODUCTION

It is important to emphasize that chemo-mechanical preparation of root canal instrumentation cannot be separated in any manner because the objective is to obtain the trinomial: cleaning, shaping, and disinfection of root canal1.

Endodontic instrumentation using either manual or mechanized techniques, produces a smear layer and smear plugs which contains organic and inorganic particles of calcified tissue and organic elements such as pulp tissue debris, odontoblastic processes, microorganisms, and blood cells on dentinal tubules2. The use of chelating agents and acids have been recommended to remove the smear layer from the root canal, because the components of this loosely bound structure are very small particles with a large surface-mass ratio that makes them highly soluble in acids15,38,39.

Chelation is a physico-chemical process which involves the uptake of multivalent positive ions by specific chemical
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substances. In the specific case of root dentin, the agent reacts with the calcium ions in the hydroxyapatite crystals. This process can cause changes in the microstructure of the dentin and changes in the Calcium: Phosphorus ratio³.

Initially, the use of EDTA solution in Endodontics was proposed by Ostby (1957) who recommended the use of 15% EDTA to assist with the instrumentation of calcified, narrow or blocked canals, because of its ability to foster the chelation of the calcium ions at a pH close to neutral (Hill 1959)³. Hill and Goldberg and Abramovich reported that addition of a quaternary ammonium bromide (Cetavlon) to 15% EDTA increased the action by reducing its surface tension, because EDTA solutions act only through direct contact with the substrate. Guerisoli et al ⁴⁰ stated that the association of EDTA with a wetting agent enhances its bactericidal effectiveness.

It has been reported that some chemicals used for endodontic irrigation are capable of causing alterations in the chemical composition of dentin ⁴²,⁴³,⁴⁴. Any change in the Calcium/Phosphorus ratio may modify the original proportion of organic and inorganic components, which in turn change the microhardness, permeability, and solubility characteristics of dentin ⁴²,⁴³,⁴⁴,⁵².

Panighi and G’Sell ⁴⁵ reported a positive correlation between hardness and the mineral content of the tooth. It has been indicated that microhardness determination can provide indirect evidence of mineral loss or gain in dental hard tissues ⁴⁶. So as microhardness of root canal dentin is sensitive to its composition and surface changes.

The present study aims to demonstrate that microhardness tests, being a simple and effective method to evaluate and compare the demineralization power of different chelating agents, given that the tests are carefully calibrated.

MATERIALS AND METHODS
Preparation of samples
Forty intact freshly extracted single rooted human teeth were selected and stored in physiological saline until they are used. Teeth were sectioned transversely at the cemento-enamel junction with diamond disc, roots were split longitudinally into two parts and the crowns were discarded. The root halves were embedded in acrylic resin followed by polishing with different grades of sand paper and finally with alumina suspension on felt cloth. Then the samples were randomly divided into four groups (n=10) based on test solution used. Group A- 17% EDTA, Group B- 17% EDTA + 10% carbamide peroxide, Group C- 17% EDTA + 0.75g cetrimide, Group D- 17% EDTA + 0.5% Tween 80 (polysorbate 80 detergent).

Determination of microhardness
At the beginning of experiment, reference microhardness values (MHVs) were obtained for samples prior to the application of solutions (Before Application), so that the same samples can act as their own controls. A MicroVicker’s Hardness Tester (Fuel Instruments and Engineers Pvt. Ltd.) was used. The diamond-shaped indentations were carefully observed in an optical microscope.
with a digital camera and image analysis software, allowing the accurate digital measurement of their diagonals. The average length of the two diagonals was used to calculate the microhardness value (MHV). All experiments were completed under the same conditions: 50 g load and 10 s dwell time, following the suggestions by Cruz-Filho et al. (2001). In each sample, two indentations were made each in the middle and apical third of the root canal dentin sample.

**Specimen treatment**

The specimens were randomly divided into 4 groups (n = 10) and were treated with the irrigation solutions immediately after the initial baseline microhardness measurements. The application time for all solutions was 1 minute. The specimens in each group were soaked in the test solutions (50 mL), rinsed in distilled water, and blotted dry. Indentations were made on each specimen adjacent to the initials in the same manner, and the microhardness values were recorded (V2). For each specimen, the decrease in microhardness was calculated.

**RESULTS**

Data was analyzed statistically using one-way analysis of analysis of variance (ANOVA) with Post-Hoc Tukey’s correction for multiple group comparisons. The decrease in Vickers microhardness values (mean standard deviation) for the tested agents is summarized in Table 1. All solutions significantly decreased the microhardness of root dentin (P < .05). Although there was no significant difference among the solutions (P > .05), Fig. 1 clearly illustrates that the specimens in EDTA + 0.5% Tween 80 solution group showed the highest change in microhardness.

Comparison of reduction in microhardness between each group can be seen in Table 2. The plain EDTA and plain EDTA + 10% carbamide peroxide solution groups had almost similar values.

**Table 1.** Descriptive statistics of percentage change in hardness across four groups at each level.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of samples (n)</th>
<th>Percentage change in Hardness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
</tr>
<tr>
<td>Group A</td>
<td>10</td>
<td>10.1</td>
</tr>
<tr>
<td>Group B</td>
<td>10</td>
<td>9.8</td>
</tr>
<tr>
<td>Group C</td>
<td>10</td>
<td>4.4</td>
</tr>
<tr>
<td>Group D</td>
<td>10</td>
<td>12.2</td>
</tr>
</tbody>
</table>

**Table 2.** Statistical comparison of percentage change in hardness across four groups at each level.

<table>
<thead>
<tr>
<th>Group Comparison</th>
<th>Middle Level</th>
<th>Apical Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-value</td>
<td>Significance</td>
</tr>
<tr>
<td>Group A v/s Group B</td>
<td>0.999</td>
<td>Non-Significant</td>
</tr>
<tr>
<td>Group A v/s Group C</td>
<td>0.588</td>
<td>Non-Significant</td>
</tr>
<tr>
<td>Group A v/s</td>
<td>Group D</td>
<td>0.961</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>Group B v/s</td>
<td>Group C</td>
<td>0.627</td>
</tr>
<tr>
<td>Group B v/s</td>
<td>Group D</td>
<td>0.945</td>
</tr>
<tr>
<td>Group C v/s</td>
<td>Group D</td>
<td>0.325</td>
</tr>
</tbody>
</table>

**DISCUSSION**

EDTA solution induces the decrease in dentin microhardness. EDTA bonds to calcified component of dentin through chelating action and causes demineralization and softening of dentin.

Surfactant is a compound that lowers the surface tension of liquid, the interfacial tension between two liquid, or that between a liquid and a solid. They may act as detergents, wetting agents, emulsifiers, foaming agents and dispersants. The effect of root canal therapy would depend at least in part on the wetting properties of the medicament. Therefore, researchers suggested using medicaments with a high wetting capacity. The efficiency of an endodontic irrigant could be improved by reducing its surface tension. Briefly, if an irrigant can easily spread over dentin surface, irrigation efficiency may improve. EDTA solutions with surfactant were reported to be very effective in smear layer removal. However, Zehnder et al. reported that it might not be necessary to add a wetting agent to the chelating solution to improve its effectiveness in removing inorganic smear layer components. The current findings suggest that root canal irrigation with surfactant added EDTA solutions leads to structural changes as evidenced by the reduction of dentin microhardness.

17% EDTA was added with various substances like cetrimide, Tween 80, carbamide peroxide. Tween 80 (polysorbate 80), surfactant substance is a non-ionic hydrophilic type, which promotes the diffusion of the solution into the root canal irrigant into the dentinal tubules and decreases the surface tension which promotes increased EDTA chelation property. RC-Prep introduced by Stewart et al. in 1969, contains 15% EDTA, 10% urea peroxide (UP), and glycol. Oxygen is set free by the reaction of RCPrep with NaOCl irrigant so that pulpal remnants and blood coagulates can be easily removed from the root canal wall.

It is accepted today that using chemical agents is essential to modify the smear layer during root canal therapy. However, these chemicals can lead to structural changes on the dentin surface, which may also modify its physical properties. Consequently, microhardness determination can provide indirect evidence of mineral loss or gain in dental hard tissues. Furthermore,
a positive correlation between hardness and the mineral content of the tooth was reported.  

The Vickers test is less sensitive to surface conditions among the microhardness measurement methods and more sensitive to measurement errors when equal loads are applied.  

Dentin hardness is related to location, and its value decreases as the indentations tested are made closer to the pulp. In the present study, to measure the Vickers hardness values for dentin, indentations were made from the middle third of the root canal and were done at a depth of 100 mm from the pulp-dentin interface for standardization, each using a 50-g load and a 10-second dwell time. The reason for choosing a lighter load and less of a dwell time was the inverse correlation between dentin microhardness and tubular density. In addition, the contact time of the solution needs to be taken into account as another causal factor in the microhardness of dentin. 

In the present study, the effect of 17% EDTA solution alone and with different surfactants on the microhardness of human root dentin was evaluated in vitro with a 1-minute contact time. 17% EDTA has the potential for causing excessive dentinal erosion if the application time exceeds 1 minute.

The degree of softening and demineralization in root canal dentin may have an influence on its physical and chemical properties. There seem to be many questions relating to whether these alterations can affect the prognosis of root canal treatment. According to the researchers, changes in mechanical properties of intertubular dentin are unlikely to be causative for fractures after endodontic treatment.

CONCLUSION

Within the limitation of this study and methodology it can be concluded that, all tested solutions reduced the microhardness of dentin of root canal lumen. There was no statistically significant difference in the microhardness reduction of root canal dentin by 17% EDTA, carbamide peroxide, and cetrimide.

Overall, Tween 80 showed highest reduction in microhardness of root canal dentin. 17% EDTA and carbamide peroxide showed moderate reduction in microhardness and least was cetrimide.

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