Evaluation of decalcifying effect of maleic acid and EDTA on root canal dentin using energy dispersive spectrometer

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Objective. The aim of this study was to evaluate mineral contents of root canal dentin after treatment with 7% maleic acid (MA) or 17% EDTA.

Study design. Thirty pieces of teeth were divided into 3 groups: 1) 17% EDTA; 2) 7% MA; and 3) saline. All specimens were treated for 0, 1, 5, 10, 15 minutes. Levels of calcium, phosphorus, magnesium, sodium, sulfur, and oxygen were measured using energy dispersive spectrometer. Data were analyzed using 1-way analysis of variance. Tukey honest significant difference and Bonferroni test were used for comparison between the groups and time periods.

Results. MA reduced maximum amount of calcium and phosphorus at all time intervals, but was significant only up to 5 minutes \((P < .001)\). Oxygen, sulfur, and magnesium were decreased more with saline and least with MA \((P < .001)\). Sodium was decreased more with MA and least with EDTA \((P < .001)\).

Conclusions. MA decalcifies the root dentin, with most calcium and phosphorus extracted during the first 5 minutes, compared with EDTA. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:e78-e84)

The success of endodontic treatment depends on the eradication of microbes from the root canal system and prevention of reinfection. At present, irrigation is considered to be one of the best methods for the successful debridement of the root canals after mechanical shaping procedure.1 Mechanical instrumentation of the root canal produces a smear layer that covers the instrumented root canal walls.2 For the complete removal of smear layer alternating use of EDTA and sodium hypochlorite (NaOCl) has been commonly recommended, which can dissolve both organic and inorganic components.3,4 Studies have reported that some of the endodontic irrigants are capable of altering the chemical composition of dentin5,6 by removal of calcium (Ca\(^{2+}\)) and phosphorus (PO\(_4^{3-}\) ) ions present in the hydroxyapatite crystals. Calcium and phosphorus in hydroxyapatite crystals are the major inorganic components of dental hard tissue. The \(\text{Ca}^{2+}/\text{PO}_4^{3-}\) ratio of hydroxyapatite in dentin was shown to be 1.67 mol/L, depending on the crystal type, availability of Ca\(^{2+}\), anatomic location, and the technique of determination.7 Any change in the \(\text{Ca}^{2+}/\text{PO}_4^{3-}\) ratio may change the original proportion of organic and inorganic components, which in turn changes the permeability, microhardness, and solubility of root dentin7,8 and may also adversely affect the sealing ability and adhesion of resin-based cements and root canal sealers to root dentin.8,9 Dentin adhesion depends on the presence of the residual Ca\(^{2+}\) on the bonding surface.9,10 Studies have reported that partial depletion of surface Ca\(^{2+}\) may significantly reduce the bond strength of certain adhesive materials.9,11

Studies have demonstrated that EDTA when used as an irrigating solution decreases the \(\text{Ca}^{2+}/\text{PO}_4^{3-}\) ratio of root dentin significantly.5,6 Maleic acid (MA) is a mild organic acid used as an acid conditioner in adhesive dentistry.12 It has been found to possess a smear layer–removing quality when used as an acid etchant in restorative dentistry.12,13 It has also been shown to remove the smear layer significantly better than EDTA when used as a root canal irrigant in endodontics.14 Ballal et al.15 reported MA to be less cytotoxic than EDTA. To date, there are no studies reported evaluating the demineralizing effect of MA on root canal dentin. The purpose of the present in vitro study was to evaluate the mineral contents of human root canal dentin after the treatment with 7% MA or 17% EDTA solutions using energy-dispersive spectrometric analysis.

MATERIAL AND METHODS

Fifteen maxillary central incisors, extracted for periodontal reasons, were used in this study. The teeth which were selected were non carious and had no restorations on their root surfaces. The teeth were stored in distilled water with 0.2% sodium azide until use. Ethical clearance was obtained from the Ethical

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Committee of Manipal University, Manipal, Karnataka, India. Before the experiment, the soft tissue covering the root surfaces was removed using a soft brush. The crowns of the teeth were removed at the cementoenamel junction, and the apical portions of the teeth were removed using high-speed diamond disk (Horico, Berlin, Germany) under water cooling. The length of the specimens was standardized to 10 mm. Thereafter, the roots were split longitudinally to obtain 30 root halves and the pulp tissue was removed with a fine brush. All the specimens were dehydrated in a sterilizer at 120°C and weighed on a precision balance (AT 261; Mettler, Greifensee, Switzerland) so that each specimen weighed 0.15 g. Weight equality among each specimen was achieved by reducing the weight whenever necessary with silicon carbide paper of 600 grit (Ballerup, Denmark) from the central part of the specimen. Thereafter, the specimens were covered with 2 consecutive layers of nail varnish leaving the root canal surface exposed. Specimens were divided randomly into 3 groups (n = 10):

1. EDTA group: samples treated with 17% EDTA solution (Merck, Darmstadt, Germany).
2. MA group: samples treated with 7% MA solution (KMC Pharmacy, Manipal, Karnataka, India).
3. Saline group: samples treated with 0.9% saline solution (control).

In each group, the specimens were immersed in a magnetic stirrer bath containing 10 mL of the experimental solutions. The same specimens (n = 10/group) were used for 0, 1, 5, 10, and 15 minutes’ treatment. At each time interval, the specimens were removed from the stirrer bath, thoroughly rinsed with distilled water, dried on absorbent paper at room temperature and prepared for energy-dispersive spectrometric analysis. Levels of Ca2+, PO4<sup>3-</sup>, Mg2+, Na<sup>+</sup>, S, and O2<sup>-</sup> in the root dentin surface of each specimen were measured using a scanning electron microscope (SEM; LEO 440i; Carl Zeiss, Tokyo, Japan) and an energy-dispersive spectrometer (Oxford, Concord, MA, USA) with a minimum detectable range of 1%. Each specimen was irradiated at the center and at 2 other equidistant areas at a voltage of 20 kV for 60 seconds.

Changes in the mineral levels were recorded and differences between the groups were statistically analyzed using 1-way ANOVA. Tukey honest significant difference test was used for comparison between the groups and Bonferroni test was used for comparison between the time periods.

**RESULTS**

The mean values of Ca2+, PO4<sup>3-</sup>, Mg2+, Na<sup>+</sup>, S, and O2<sup>-</sup> release from the root dentin after different time interval treatments with the test solutions are presented in Table I. There was a significant decrease in the Ca2+ and PO4<sup>3-</sup> level after treatment with 7% MA when compared with 17% EDTA at all the time intervals tested. But, only up to 5 minutes time interval it was statistically significant (P < .001). Saline which was used as a control decreased the amount Ca<sup>2+</sup> and PO4<sup>3-</sup> to the least at all the time intervals tested which was statistically not significant (P > .05).

The O2<sup>-</sup>, S, and Mg2+ levels were decreased significantly more after the treatment with saline solution compared with MA and EDTA at all the time intervals tested (P < .001). MA reduced O2<sup>-</sup>, S, and Mg2+ content to the least.

The Na<sup>+</sup> level was decreased significantly more after the treatment with MA than EDTA and saline at all the time intervals tested (P < .001). EDTA had the least efficiency in terms of Na<sup>+</sup> removal. The graphical representation of the release of Ca<sup>2+</sup>, PO4<sup>3-</sup>, Mg2+, Na<sup>+</sup>, S, and O2<sup>-</sup> from the root dentin are presented in Figures 1, 2, and 3.

**DISCUSSION**

The efficacy of agents used to demineralize root dentin has been examined by various techniques, such as flame photometry, atomic absorption spectrometry, inductively coupled plasma–atomic emission spectrometry or energy dispersive spectrometer. In the present study, scanning electron microscope with energy-dispersive x-ray (EDX) analysis was used, because it is a microanalytical technique that is used to estimate quantitatively the amounts of mineral in a given tooth sample. This method allows a fast and nondestructive chemical analysis of the specimen with a spatial resolution in the micrometer regime. The principle is based on the energy emitted in the form of x-ray photons when the electrons from external sources hit the atoms in a material, thus generating characteristic x-rays of that element. When the sample is bombarded by the electron beam of the SEM, electrons are ejected from the atoms on the specimen’s surface (secondary electrons). A resulting electron vacancy is filled by an electron from a higher shell, and an x-ray is emitted (characteristic x-rays) to balance the energy difference between the 2 electrons. The EDX detector measures the number of emitted x-rays versus their energy. The energy of the x-ray is characteristic of the element from which the x-ray was emitted. A spectrum of the energy versus relative counts of the detected x-rays is obtained and evaluated for qualitative and quantitative determinations of the elements present in the specimen by using a computer-based program. The data generated by EDX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the specimen being ana-
lyzed. The technique can be qualitative, semiquantitative, and quantitative as well as provide spatial distribution of elements through mapping. The EDX technique is nondestructive and if required, specimens of interest can be examined in situ with little or no sample preparation. The EDX systems also have image analysis packages that can be applied to any images generated by the SEM/EDX technique, allowing for the identification of the critical characteristics of particles. It offers the ability to gather information about finer particles than by optical microscopes and can readily distinguish between clusters and agglomerates of particles in addition to the chemical analysis available by EDX. The strength of this analysis technique is its ability to gather statistically significant data on the size, morphology, and composition of the particles in a time-efficient manner beyond the capabilities of conventional optical microscopy.

Fig. 1. Comparison of mean calcium (A) and phosphorus (B) level among the three test solutions at different time periods.
In the present study, the root canals were not prepared before the analysis and therefore there was no smear present on the root dentin surface. The rationale behind not performing this step was to enable the measurement of Ca\(^{2+}\) loss that occurred solely on intact root dentin while avoiding any possible contamination of readings that could have been caused by the Ca\(^{2+}\) incorporated into the loosely bound smear layer. Furthermore, the adhesive restoration of endodontically treated teeth generally involves the pulp chamber, whose mineral content is adversely affected by exposure to endodontic irrigants, and this region generally does not contain smear layer, because of the conservative nature of endodontic access cavity preparation.

Fig. 2. Comparison of mean magnesium (A) and sulphur (B) level among the three test solutions at different time periods.
Previous studies have demonstrated that EDTA solution changed the Ca$^{2+}$/PO$_4^{3-}$ ratio significantly differently from the root canal dentin. In the present study, MA reduced the Ca$^{2+}$/PO$_4^{3-}$ ratio significantly more than EDTA up to 5 minutes. This may be attributed to the acidic pH of maleic acid (1.04) compared with that of EDTA. At 10 and 15 minutes, EDTA also removed significantly more Ca$^{2+}$ and PO$_4^{3-}$. This is in accordance with other studies that have shown that EDTA resulted in the maximum amount of Ca$^{2+}$ and PO$_4^{3-}$ removal from the root dentin at an extended period of time. Studies have shown that the optimal working time for EDTA is 15 minutes after which no chelating action can be expected, because of the self-
limiting decalcifying action of EDTA. Hence in the present study the decalcifying effect was observed for maximum of 15 minutes time interval.27

In addition to \( \text{Ca}^{2+} \) and \( \text{PO}_4^{3-} \), trace amount of \( \text{Mg}^{2+} \) is also detectable in root canal dentin, which has been considered to influence the mineralization process, especially crystal growth.28 Sulfur, which is a marker of proteoglycans, is also present in the matrix of hard tissue. Changes in the level of this mineral may cause damage to the organic component of the matrix. Trace amounts of \( \text{Na}^+ \) and \( \text{O}_2^{2-} \) are also detectable in the root dentin. Little is known about the role of these minerals which are usually present in the apatite crystals.29 In the present study, the level of \( \text{O}_2^{2-} \), \( \text{S} \), and \( \text{Mg}^{2+} \) was reduced more with saline than with MA and EDTA due to the increase in ionic concentration of saline solution which is a strong electrolyte. The insoluble material dentin consisting of hydroxyapatite when treated with saline, its solubility increases since saline has got ‘ionic strength.’30

Reduction in the level of sodium by MA more than by EDTA and saline solution may be due to its high acidic pH. Further studies have to be conducted to evaluate the demineralizing potential of maleic acid in combination with NaOCl since, previous studies have shown that sodium hypochlorite helps in removal of the organic matrix and thus increases the demineralizing effect of chelators when they are used along with it.7,31

Also, the demineralizing effect of MA on the adhesion of endodontic sealers and adhesive cements has to be evaluated.

### Table 1. Comparison of the mean values of the 6 elements from the root canal dentin after treatment with the test solutions at different time periods

<table>
<thead>
<tr>
<th>Element</th>
<th>Test agent</th>
<th>Time period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Calcium</td>
<td>Maleic acid</td>
<td>29.336</td>
</tr>
<tr>
<td></td>
<td>Saline sol.</td>
<td>29.654</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>Maleic acid</td>
<td>14.809</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td>14.782</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Maleic acid</td>
<td>0.828</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td>0.732</td>
</tr>
<tr>
<td></td>
<td>Saline sol.</td>
<td>0.858</td>
</tr>
<tr>
<td>Sodium</td>
<td>Maleic acid</td>
<td>0.540</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
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</tr>
<tr>
<td></td>
<td>Saline sol.</td>
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</tr>
<tr>
<td>Sulfur</td>
<td>Maleic acid</td>
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</tr>
<tr>
<td></td>
<td>EDTA</td>
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</tr>
<tr>
<td></td>
<td>Saline sol.</td>
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</tr>
<tr>
<td>Oxygen</td>
<td>Maleic acid</td>
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<tr>
<td></td>
<td>EDTA</td>
<td>54.457</td>
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<tr>
<td></td>
<td>Saline sol.</td>
<td>53.232</td>
</tr>
</tbody>
</table>

### REFERENCES

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