Effects of chelating agents on the mineral content of root canal dentin

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Objective. The objective of this in vitro study was to assess the effect of several chelating agents on the mineral content of root dentin.

Study design. Extracted human mandibular incisor roots were prepared and divided into groups according to the following irrigation protocols: 1) 17% ethylenediaminetetraacetic acid (EDTA); 2) 10% citric acid solution; 3) 18% etidronate; 4) 2.25% peracetic acid; 5) and deionized water (control). Dentin chips were obtained (Gates-Glidden nos. 3, 4, and 5). The levels of different minerals were analyzed with the use of inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Results. 1) Peracetic acid significantly decreased P, K, Mg, Na, and S levels compared with the other groups (P < .05). 2) S decreased by different levels in all of the chelating solutions (P < .05), and the greatest decrease was observed in peracetic acid. 3) Ca levels significantly decreased in peracetic acid, citric acid, and EDTA (P < .05). 4) Mn levels significantly decreased in the citric acid and peracetic acid groups (P < .05). 5) Na and Zn levels significantly decreased in the peracetic acid, citric acid, and etidronate groups (P < .05).

Conclusions. The chelation agents can create different effects on mineral contents of root dentin, so it is important to know what effects each solution will have on root dentin before their clinical use. In addition, according to the results of this in vitro study, it might be recommended that peracetic acid, in particular, should be used with caution. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:e149-e154)

Root canal treatment is a series of procedures in which the goals include the disinfection and sealing of the root canal system. After canal enlargement using reamers and files, the dentin surface is covered with a smear layer, which has an amorphous, irregular, and granular aspect and is composed of inorganic material (dentin chips containing hydroxyapatite) and organic material (necrotic or vital pulp tissue, odontoblastic remnants, coagulated proteins, blood cells, nerve fibers, collagen, tissular fluid, saliva, bacteria, and their by-products). Even though there is no consensus regarding smear layer removal, it is known that the removal of this layer reduces microflora and its toxins, which increases the sealing capacity and reduces the potential for bacterial survival and reproduction. It is deposited on the dentinal walls and obliterates the dentinal tubules, thus reducing dentinal permeability. As a result, the penetration of chemical substances and intracanal medications into the dentinal mass is hindered, thereby preventing adequate cleaning and disinfection of the root canal.

For smear layer removal, a chemical chelating solution together with sodium hypochlorite should be used. Most researchers have analyzed only the ability of chelating solutions to remove the smear layer. However, their effects on dentin have also been important. Some researchers reported that some chemical agents cause alterations in the chemical structure of human dentin. In particular, chelating solutions might play a part in influencing the physical and mechanical properties of dentin.

Dentin consists of both organic and inorganic components. The calcium present in hydroxyapatite (Ca₃(PO₄)₂OH) crystals is one of the main inorganic elements of dentin. The chelating agents are chosen due to their direct action on calcium ions. Any change in the calcium ratio can significantly alter the original proportion of organic and inorganic components, which can alter dentin permeability, microhardness, and solubility and can affect the bonding/sealing properties of dental materials to dental hard tissues.

Ethylenediaminetetraacetic acid (EDTA) and citric acid are probably the most frequently used chelating agents for that purpose today. However, some researchers have proposed that peracetic acid and etidronate are potential alternatives to EDTA or citric acid. Etidronate (also known as 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) or etidronic acid) is a biocom-
patible chelator that can be used in combination with sodium hypochlorite without a short-term loss of the desired properties of either compound. A sodium hypochlorite–etidronate combination could be advantageously used as a single irrigant during and after instrumentation so that a smear layer is never created. Etidronate is nontoxic and has been given orally to treat bone diseases. Furthermore, like EDTA, it is a chelator commonly used as an adjunct in household and personal care products, such as soaps. However, the chelating capacity of etidronate is relatively weak. Peracetic acid is also a strong candidate that can be used as a final irrigant. This peroxygen is sporicidal, bactericidal, virucidal, and fungicidal at concentrations <0.5%, even in the presence of protein. It decomposes to safe by-products acetic acid and oxygen. Peracetic acid actually does not exist in a pure form in aqueous solution, but occurs in equilibrium with hydrogen peroxide, acetic acid, and acetylhydroperoxide. The fact that acetic acid is liberated from or present in a peracetic acid solution poses the possibility that a peracetic acid solution could be used after instrumentation to dissolve the smear layer and provide a thorough disinfection of the root canal system pretreated with NaOCl. Lottanti et al. suggested that both etidronate and peracetic acid have the potential to replace the conventional treatment with EDTA.

The aim of the present in vitro study was, therefore, to assess the effect of several chelating agents (17% EDTA, 10% citric acid, 18% etidronate, and 2.25% peracetic acid) on the mineral content of root canal dentin using inductively coupled plasma-atomic emission spectrometry (ICP-AES) as the analytical method.

MATERIALS AND METHODS

Sixty human mandibular anterior teeth that had been recently extracted for periodontal reasons were used. The teeth were placed in saline solution, and the soft tissue covering the root surface was removed with curettes. The teeth were decoronated using a high-speed diamond disk with a cooling system. The pulp tissue was extirpated using a barbed broach, and the working length was established by inserting a #10 K-file (Mani, Tochigi Ken, Japan) into each root canal until it was just visible at the apical foramen and then subtracting 1 mm from that distance. The root canals were enlarged with K-files (MAF #35) and then Gates-Glidden burs (nos. 1, 2, and 3). During preparation, the root canals were irrigated with 2 mL deionized water at each file change. Root canals were rinsed with 5 mL deionized water to remove possible dentin chips. After cleaning and shaping, the apical foramen was sealed with composite resin to keep the test solutions inside the root canal, and specimens were randomly divided into 5 groups to test the following solutions (n = 12 per group):

- **Group 1**: 5 mL 17% EDTA (pH 7.7; Merck, Darmstadt, Germany)
- **Group 2**: 5 mL 10% citric acid (pH 2; Merck)
- **Group 3**: 5 mL 18% etidronate (pH 10.5; Zschimmer and Schwartz, Burgstadt, Germany)
- **Group 4**: 5 mL 2.25% peracetic acid (pH 2.5; Merck)
- **Group 5 (control)**: 5 mL of deionized water

All of the solutions were prepared in the research laboratory of the Faculty of the Dentistry, University of Selcuk, Konya, Turkey, and used within 24 hours. All of the irrigating solutions were introduced into the canal with the use of stainless steel 30-gauge needles (KerrHawe, Bioggio, Switzerland). The needle was placed within 1-2 mm of the working length in each canal. Each solution remained in the root canal for 5 minutes (1 mL/min). The root canals were finally irrigated with 2 mL of deionized water for 1 minute to remove any precipitate that might have formed. The canals were dried with sterile paper points (Dentsply-Maillefer, Ballaigues, Switzerland).

A test method similar to that previously reported by Secilmis et al. and Ari and Erdemir was used to evaluate the mineral content of root canal dentin with the use of ICP-AES. This method uses a sample that is passed through argon in a ray fluorescence field. When the sample is introduced into the plasma, the atoms are excited and emit very stable light of varying wavelengths that permit identification of the elements. In the present study, dentin chips obtained using Gates-Glidden burs (nos. 3, 4, and 5) were saved in plates and stored at 70°C in an incubator (Venticell, MMM, Planegg/Munchen, Germany) for 4 days until they reached a fixed weight. After their weights were recorded with an electronic balance (AX200; Shimadzu Corp., Kyoto, Japan), 9 mL nitric acid (HNO₃) and 3 mL hydrochloric acid (HCl) were added; next, the specimens were burned at 180 psi and 180°C in a microwave (CEM Corp., Matthews, NC, USA) until they dissolved. After calibration of the ICP-AES (Vista AX; Varian Inc., Melbourne, Australia), a 2-mL aliquot was removed. The solutions were carried to a nebulizer with the help of a peristaltic pump. The specimens were turned into aerosols and were carried by an argon spray. The aerosols were heated by conduction and radiation until they reached ~10,000°C, at which point they are completely atomized. Therefore, energy is released. The light was transferred to the detector, and every element was described according to its different wavelength. In this study, 3 measurements were performed on each element for each solution to increase measurement sensitivity. The means of the measurements were calculated as mg/L by a computer.
All decalcification procedures were carried out on the same day at the same room temperature, because an increase in temperature accelerates the demineralization process.

The levels of 8 elements—calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), sulfur (S), manganese (Mn), and zinc (Zn)—in each specimen were measured by ICP-AES. The differences in mineral content between the groups were analyzed with 1-way analysis of variance and the comparison of means with Duncan tests.

RESULTS

Table I shows the mean mg/L values of Ca, P, Mg, Na, Mn, K, Zn, and S in root canal dentin after treatment with the chelating solutions.

Peracetic acid significantly decreased P, K, Mg, Na, and S levels of root dentin compared with the other groups (P < .05). The S level of root dentin decreased by different levels in all chelating solutions compared with the control group (P < .05), and the greatest decrease was observed in the peracetic acid group. There were significant decreases in the Ca levels of root dentin after treatment with peracetic acid, citric acid, and EDTA compared with the other groups. The Mn level of root dentin significantly decreased in the citric acid and peracetic acid groups (P < .05). Na and Zn levels significantly decreased in the peracetic acid, citric acid, and etidronate groups (P < .05). The Ca/P ratios of the groups were not affected by the tested chelating solutions (P > .05). However, peracetic acid had a tendency to show a lower Ca/P ratio than the other solutions, and EDTA, etidronate, and citric acid had a tendency to show a higher Ca/P ratio. However, these differences were not significant at the 5% level compared with the control group.

DISCUSSION

It has been important to test the effect of irrigation solutions on all dentin tissues, because contact might occur during irrigation procedures. Earlier studies showed that irrigating solutions significantly change the mineral content of root dentin. In particular, chelating solutions might play a part in influencing the mineral content of dentin.

In the current study, we evaluated the mineral contents of root canal dentin that were treated with different chelating agents using ICP-AES. Conventional (EDTA and citric acid) and nonconventional solutions (etidronate and peracetic acid) were used.

Zehnder et al. found that a 10% citric acid solution significantly removes more calcium than a 15.5% EDTA solution or an 18% etidronate solution. Spano et al. evaluated the concentration of calcium ions by
suitable mineral acid. Among the analytical methods, some portion of enamel or dentin is digested in a determined by bulk techniques, in which whole teeth or

effect than other solutions on the level of K of dentin.

P acid (\textsuperscript{33}). Among the analytical methods, flame atomic absorption spectrometry, neutron activation analysis, and anodic flame atomic absorption spectroscopy, ICP-AES, mass spectrometry, neutron activation analysis, and anodic stripping voltammetry are used to measure trace elemental content in digested teeth. The ICP-AES method has become highly popular for element analysis.

In conclusion, the results obtained under the experimental conditions of this study provided evidence that chelation agents may create different effects on the mineral content of root dentin. Therefore, it is important to know how each solution affects the root dentin before clinically using them. In addition, we recommend that peracetic acid, in particular, should be used with caution. However, further in vivo and in vitro studies are required to confirm the findings obtained in this study.
REFERENCES


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