Effect of Chelating Solutions on the Microhardness of Root Canal Lumen Dentin

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Abstract

Introduction: The greatest reduction in microhardness of the most superficial layer of dentin of the root canal lumen is desired. The use of chelating agents during biomechanical preparation of root canals removes smear layer, increasing the access of the irrigant into the dentin tubules to allow adequate disinfection, and also reduces dentin microhardness, facilitating the action of endodontic instruments. This study evaluated the effect of different chelating solutions on the microhardness of the most superficial dentin layer from the root canal lumen. **Methods:** Thirty-five recently extracted single-rooted maxillary central incisors were instrumented, and the roots were longitudinally sectioned in a mesiodistal direction to expose the entire canal extension. The specimens were distributed in seven groups according to the final irrigation: 15% EDTA, 10% citric acid, 5% malic acid, 5% acetic acid, apple vinegar, 10% sodium citrate, and control (no irrigation). A standardized volume of 50 μL of each chelating solution was used for 5 minutes. Dentin microhardness was measured with a Knoop indenter under a 10-g load and a 15-second dwell time. Data were analyzed statistically by one-way analysis of variance and Tukey-Kramer multiple-comparison test at 5% significance level. **Results:** EDTA and citric acid had the greatest overall effect, causing a sharp decrease in dentin microhardness without a significant difference ($p > .05$) from each other. However, both chelators differed significantly from the other solutions ($p < .001$). Sodium citrate and deionized water were similar to each other ($p > .05$) and did not affect dentin microhardness. Apple vinegar, acetic acid, and malic acid were similar to each other ($p > .05$) and presented intermediate results. **Conclusion:** Except for sodium citrate, all tested chelating solutions reduced microhardness of the most superficial root canal dentin layer. EDTA and citric acid were the most efficient. *(J Endod 2011;37:358–362)*

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0099-2399/$ - see front matter

doi:10.1016/j.joen.2010.12.001

Key Words

Apple vinegar, chelators, dentin microhardness, irrigants

Root canal instrumentation consists in the combined action of endodontic instruments and irrigating solutions, aiming at the elimination of preexistent organic and inorganic remnants or debris resulting from the operative procedures as well as the reduction of the microbial content and its byproducts (1). An irrigating solution should present a number of physicochemical properties in order to be effective in endodontics (2). It is known, however, that no endodontic irrigant presents all ideal properties, and, thus, the combination of auxiliary solutions is necessary to achieve the desired effects. As far as cleaning is concerned, the chances of success in the endodontic therapy increase as more debris and smear layer are removed. It is believed that removing this layer could dissolve attached microbiota and their toxins from root canal walls, improve the seal of root fillings, and reduce the potential of bacterial survival and reproduction (3, 4).

Sodium hypochlorite (NaOCl) is the most widely used chemical solution in the biomechanical preparation of the root canal system, and it has been systematically used in endodontics in concentrations ranging from 0.5% to 5.25%. However, despite its excellent antimicrobial action and capacity of dissolving organic materials, this solution alone does not effectively remove the smear layer, and its association with chelating agents that act on organic matter is necessary (1, 5, 6). The demineralizing effect of chelators acts indistinguishably on the smear layer and the root dentin, with consequent exposure of collagen and decrease of dentin microhardness (7–9). The greatest reduction in microhardness of the most superficial layer of dentin of the root canal lumen is desired. The use of chelating agents during biomechanical preparation of root canals removes smear layer, increasing the access of the irrigant into the dentin tubules to allow adequate disinfection, and reduces dentin microhardness, facilitating the access and action of endodontic instruments in narrow, calcified root canals.

Chelating agents were introduced to endodontics by Nygaard-Østby in 1957 (10) as an aid for the preparation of narrow and calcified root canals. A liquid solution of EDTA was the first chelator used in dentistry as an agent capable of chemically softening the root canal dentin, dissolving the smear layer, and increasing dentin permeability. In addition to EDTA (8, 11–16), the following chelating solutions have been investigated to assess their demineralizing capacity and reduction on dentin microhardness: EDTAC (EDTA + cetavlon); EDTA-T (EDTA + anionic detergent) (12, 14, 17); EGTA (ethylene glycol-bis[β-aminoethyl ether]-N,N,N′,N′-tetraacetic acid) (14, 18–21); CDTA (CDTA, trans 1,2diaminocyclohexaneNNN',N'tetraacetic acid (14, 19, 20); citric acid (8, 21–24); hydrogen peroxide (15); 6% NaOCl (7); sodium citrate (22); phosphoric acid (25, 26); MTAD (tetracycline isomer + acid + detergent) (27); chlorhexidine digluconate (28); etidronic acid (9, 29); and Smear Clear (SybronEndo, Orange, CA), which is a commercial product containing 17% EDTA along with centrimide and additional proprietary surfactants (17).

In vitro studies investigating the effect of chelating agents on dentin microhardness have traditionally used dentin discs cut transversally from roots of bovine (7) or human teeth (8,11–16, 18, 21, 23, 26). According to this methodology, the

chelating solution is applied to the surface of the dentin discs, in the region between the main canal and the cementum layer, and then the microhardness of this region is measured. In fact, some authors have shown that the irrigating solution diffuses through the dentin tubules from the main root canal to a distance of up to 1,500 μm toward the root cementum (7, 13, 28). However, under clinical conditions, it is evident that during canal irrigation the solution initially enters in direct contact with the most superficial dentin layer of the root canal lumen and then diffuses through the tubular root dentin structure. Therefore, it seems more accurate and closer to a clinical situation to evaluate the action of chelating agents by irrigating the main canal with the test solution and then measure the microhardness of the most superficial layer of dentin of the root canal lumen using a methodology in which the roots were split longitudinally instead of cut transversally into discs. The aim of this study was to evaluate the effect of different chelating solutions on the microhardness of the most superficial layer of dentin of the root canal lumen.

Materials and Methods

Biomechanical Preparation of the Root Canals

Thirty-five recently extracted single-rooted maxillary central incisors were selected and stored in 0.1% thymol solution under refrigeration until the moment of use. The teeth were decoronated at the cementoenamel junction with a water-cooled diamond saw and cervical preflaring was done with stainless steel LA Axxess burs (SybronEndo Corporation, Orange, CA). The working length (WL) was established with a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) introduced into each root canal until its tip was visualized at the apex and then pulled back 1 mm. The anatomic diameter of the canal at the WL was also checked by introducing first series K-type instruments of successively larger diameters, and the instrument that showed resistance to be removed from WL was recorded. A nickel-titanium rotary system (Quantec, SybronEndo Corporation) was employed according to a crown-down technique, using a sequence of instruments three sizes larger the diameter of the first instrument. During preparation, the root canals were irrigated with 2 mL 1% NaOCl at each change of file with an irrigation time of 30 seconds for each flush. A final irrigation with 20 mL 1% sodium hypochlorite was performed for the removal of possible dentin chips.

Specimen Preparation

Grooves were prepared along the long axis of the roots with a water-cooled diamond disk (KG Sorensen Ind Com, São Paulo, SP, Brazil) mounted on a high-speed handpiece, and a surgical chisel was used to cleave the roots longitudinally in a buccolingual direction to expose the entire canal extension (Fig. 1*a*). The convex surface of the half covered with cementum was flattened with a diamond cylindrical bur mounted on a high-speed handpiece to maintain a minimal thickness of 2 mm (Fig. 1*b*) between the abraded surface and the root canal lumen. The dentin layer between the canal lumen and the cementum was also abraded with angulation of approximately 45° (Fig. 1*c*) to facilitate the polishing of root canal lumen dentin and its visualization in the microhardness tester. During the whole preparation phase, abrasion of the root segment was performed under an operative microscope at ×24

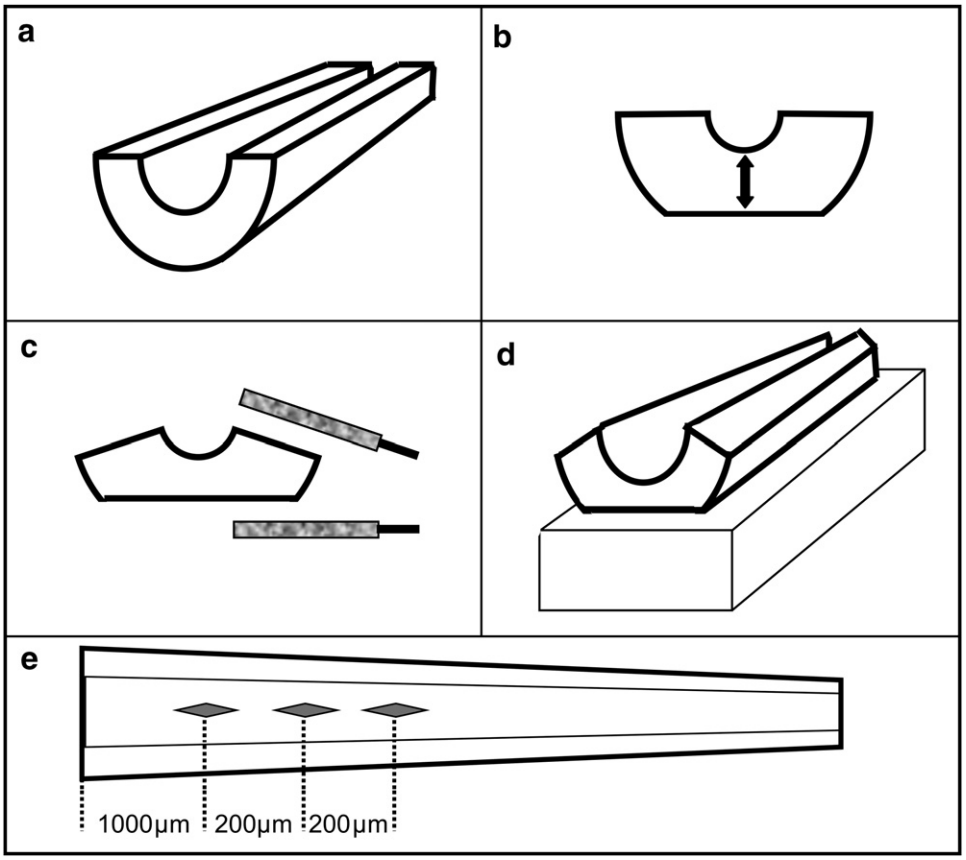

Figure 1. A schematic illustration of specimen preparation. (*a*) Hemi-section of the root after cleavage, (*b*) flattening of the root surface up to a thickness of 2 mm, (*c*) abrasion of the dentin layer between the canal lumen and the cementum at 45° and flattening of root surface, (*d*) specimen (root/acrylic block set), and (*e*) a pictorial representation showing the orientation of the indentations and the distance between them (upper view of the specimen).

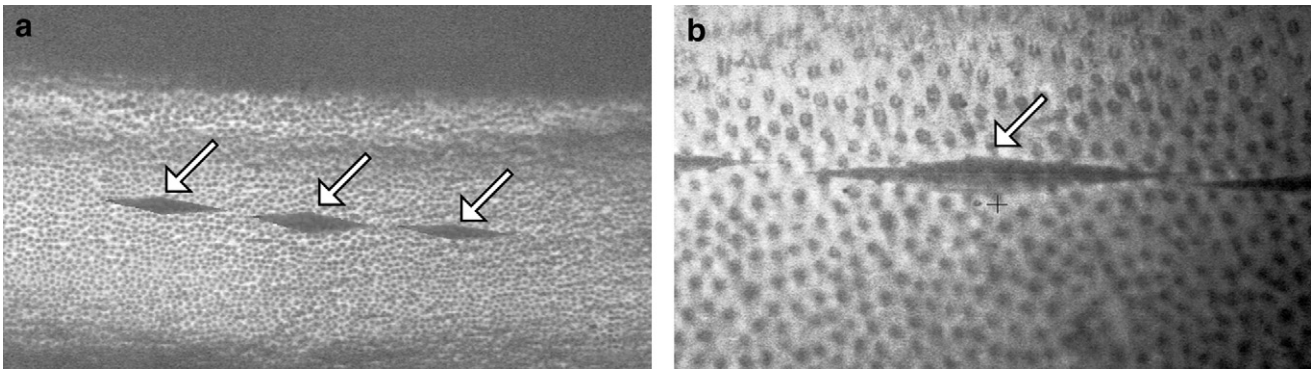

Figure 2. A photograph of the indentations in the root canal lumen dentin. (*a*) A row of three indentations (*arrows*) made along lines parallel to the edge of the root canal lumen (×10 magnification). (*b*) A close-up view of the indentation (×40 magnification).

magnification (Opto Dental, São Carlos, SP, Brazil) to increase the accuracy of preparation. Each specimen was attached to an autopolymerized acrylic resin block with cyanoacrylate adhesive (SuperBonder Instant Adhesive; Loctite Corp, Rocky Hill, CT) and the root/acrylic block sets (Fig. 1*d*) were randomly assigned to seven groups of five teeth each according to final irrigating solution: 15% EDTA, 10% citric acid, 5% malic acid, 5% acetic acid, apple vinegar, 10% sodium citrate, and control (no irrigation). Apple vinegar was obtained from Castelo Alimentos SA (Jundiaí, SP, Brazil). The other solutions were prepared at the Research Laboratory of the Department of Restorative Dentistry (University of São Paulo, Ribeirão Preto, SP, Brazil) using proanalysis reagents purchased from Sigma Aldrich Corp. (St Louis, MO, USA). Before irrigating the root canal lumen with the test substances, dentin surface was polished with felt discs embedded in aluminum oxide paste (Diamond, FGM, Joinvile, SC, Brazil) at a low speed. This procedure is necessary because the measurement of microhardness is only possible on polished dentin surfaces. The indentations are not visible on nonpolished surfaces.

Treatment of the Specimens

A standardized volume of 50 μL of each chelating solution was delivered directly on root canal dentin using an automated micropipette filling the whole canal extension. After 5 minutes, the specimens were rinsed with 20 mL 1% sodium hypochlorite to remove any residues of the test solution.

Microhardness Measurements

Dentin microhardness was measured with a Knoop indenter at ×40 magnification (Shimadzu HMV-2000; Shimadzu Corporation, Kyoto, Japan) under a 10-g load and a 15-second dwell time. In each sample, three indentations were made along lines parallel to the edge of the root canal lumen (Fig. 2). The first indentation was made 1,000 μm from the root canal entrance, and two other indentations were made at a distance of 200 μm from each other (Fig. 1*e*). The average length of the two diagonals was used to calculate the microhardness value (Knoop number hardness [KHN]). The representative hardness value for each specimen was obtained as the average of the results for the three indentations. Data were analyzed statistically by one-way analysis of variance and the Tukey-Kramer multiple-comparison test. A significance level of 5% was set for all analyses.

Results

The Knoop microhardness values (mean ± standard deviation) for the chelating agents are summarized in Table 1. The value in each table row corresponds to the average of three measurements in five different specimens for a total of 15 measurements. Statistically significant difference was detected among the chelators by one-way analysis of variance ($p < .0001$). EDTA and citric acid had the greatest overall effect, causing a sharp decrease in dentin microhardness without statistically significant difference between them ($p > .05$). However, both solutions differed significantly from the other solutions ($p < .001$). Apple vinegar, acetic acid, and malic acid were similar to each other ($p > .05$) and presented intermediate results, differing significantly from the other substances. Sodium citrate and deionized water were similar to each other ($p > .05$) and were the least effective in terms of their effect on dentin microhardness.

Discussion

A previous study evaluating the microhardness of superficial and deep dentin by means of two indentation methods (Knoop and Vickers) under two different applied loads revealed that only Knoop hardness was significantly higher for superficial than for deep dentin (30). Considering the topographic anatomy of human dentin, it has been well shown that the greater dentin tubule density near the canal lumen in root dentin (31) contributes to lower resistance in this region (32). The chief characteristic of the Knoop hardness test is its sensitivity to surface effects and textures. For a given load, the Vickers indenter penetrates about twice as far into the specimen as the shallower Knoop indenter, and the diagonal is about one third the length of the longest diagonal of the Knoop indentation. Thus, the Vickers test is less sensitive to surface conditions and, because of its shorter diagonals, more sensitive to measurement errors when equal loads are applied (33). For this reason, a Knoop hardness indenter was used in the present study to evaluate the most superficial layer of dentin of the root canal lumen.

According to the literature, a load of 100 g for 15 seconds should be used for the determination of root dentin Knoop hardness (13, 31). However, pilot studies that preceded the present experiment showed that the application of this load on the root canal lumen dentin

TABLE 1. Knoop Microhardness Values (Mean ± Standard Deviation) of Root Canal Dentin after the Use of the Tested Chelating Solutions

Groups	Mean ± standard deviation*
EDTA 15%	25.78 ± 0.43[a]
10% citric acid	26.66 ± 0.50[a]
5% malic acid	33.92bc ± 0.59bc
5% acetic acid	33.94bd ± 0.68bd
Vinegar	34.72cd ± 0.77cd
10% sodium citrate	36.30e ± 0.60e
Control	37.44e ± 0.42e

*Different letters indicate statistically significant difference ($p > .05$).

produced excessively large indentations that sometimes resulted in completely deformed images. A load of 10 g for 15 seconds was sufficient to promoted good visualization of the pre- and posttreatment indentations.

EDTA and citric acid solutions had the strongest effect on reducing dentin microhardness compared with the other solutions. The fact that EDTA acts efficiently in the reduction of dentin microhardness is because of its chelating property. Several theories have tried to explain this chemical reaction. According to the crystalline field theory, the attraction force between the central metal and the ligands is purely electrostatic. Therefore, the attraction force exerted by the metallic ion is greater than the repulsive force offered by the atoms of the EDTA molecule (1). Chelators such as EDTA form a stable complex with the calcium ions in dentin. In this moment, carboxyl groups of the EDTA molecule are ionized, releasing hydrogen atoms that compete with the calcium ions (1).

The reducing effect of EDTA on dentin microhardness has been reported by Sayin et al (21). Those authors verified that EDTA alone or followed by 2.5% NaOCl promoted a significantly greater decrease in dentin microhardness when compared with EGTA, a calcium-ion–specific chelator, and a combination of EDTA with a tensoactive agent (EDTAC). It is interesting to mention that when the root canal is irrigated with NaOCl followed by EDTA, the collagen degradation with a consequent decrease of flexural strength is caused by a hypochlorite action and has no association with the demineralization promoted by the final rinse with EDTA (34).

The effect of EDTA was statistically similar to that of citric acid. This acid, also known as hydrogen citrate, is capable of reacting rapidly with calcium, thus forming calcium citrate (35). Under normal reactive conditions, the salt resulting from the reaction of citrate with calcium is formed at a 1:1.5 ratio, whereas the chelate formed by the union of the EDTA ion with a bivalent metallic cation (eg, Ca^{2+}, Mg^{2+}, and Zn^{2+}) occurs at a 1:1 ratio (ie, 1 mol of EDTA chelates 1 mol of metallic ions) (36). If both solutions were used at the same concentration, citric acid would theoretically remove more calcium ions, thus contributing to a greater reduction in dentin microhardness. In the present study, EDTA was used at a concentration of 15% in mass, which corresponds to 2.7×10^{-5} mol, whereas citric acid was used at a concentration of 10% in mass, which corresponds to 2.6×10^{-5} mol. Because the molarity of the solutions is almost the same, the citric acid was expected to remove more calcium ions from dentin than EDTA. The similar result for both substances might be explained by calcium bioavailability. In dentin, calcium is not available in the form of ion but rather as a complex within the hydroxyapatite crystals, which impedes its complete reaction with the acid.

The results of the present study diverge from those of previous investigations. Eldeniz et al (23) observed that citric acid was much more efficient than EDTA in reducing dentin microhardness, whereas De Deus et al (8) had opposite results. Eldeniz et al used 19% citric acid (ie, a higher concentration than that used in the present experiment). It has been shown that the higher the concentration of a solution, the stronger the chelating effect is (24). In the study by De Deus et al (8), although citric acid had the same concentration as that of the EDTA used in the present study (10%), the pHs were different. In addition, those authors (8) used citric acid with a pH close to neutral. The more acid pH of a solution might favor the removal of calcium ions from dentin. Sousa and Silva (19) showed that 1% citric acid (pH = 1.0) removed significantly more calcium ions from dentin than 1% citric acid (pH = 7.4).

Apple vinegar, acetic acid, and malic acid had a similar reducing effect on microhardness to each other and smaller than that of EDTA and citric acid. The lower concentration used for malic and acetic acids may be an explanation for such a result. Ballal et al (37) observed that 7% malic acid reduced dentin microhardness in a similar manner as 17% EDTA. The findings of the present study corroborate those of Spanó et al (38), who used atomic absorption spectrophotometry to show that apple vinegar, 5% malic acid, and 5% acetic acid removed similar amounts of calcium ions from the root canal but were less effective than 10% citric acid.

The results of the present study showed that sodium citrate was not capable of reducing root canal lumen dentin microhardness. Machado-Silveiro et al (22) observed that 10% sodium citrate has a limited capacity of demineralizing dentin, mainly when compared with 1% and 10% citric acid and 17% EDTA. The authors explain that the physicochemical characteristics of the original acid are not preserved in the salt, and so it is possible that citrate maintained only the chelating effect of the acid, which is limited. The authors of a recent study (38) verified that 10% sodium citrate does not have the capacity of removing calcium ions from the smear layer, justifying that this is a stable compound that does not react with the calcium present in this layer. The same situation may explain why the citrate did not affect microhardness; if sodium citrate does not react with the calcium from the smear layer, it probably does not react with the calcium from dentin either.

The findings of the present study showed that 15% EDTA and 10% citric acid are effective in reducing the microhardness of the most superficial dentin layer of the root canal lumen, which facilitates the biomechanical preparation considerably under clinical conditions. Some authors, however, call the attention to the fact that, in addition to reducing microhardness, some chelating solutions cause erosion of root dentin (15, 39). There has been concern about this erosion because the alteration of dentin and enamel surface may affect the interaction of these tissues with root canal filling materials and restorative materials used for coronal seal and may decrease the resistance to penetration of bacteria and coronal leakage (40). Erosion can be avoided by using chelators at low concentrations, such as 1% EDTA (41), or shorter chelating times (42).

Conclusion

In conclusion, except for sodium citrate, all tested chelating solutions reduced the microhardness of the most superficial layer of dentin of the root canal lumen. EDTA and citric acid were the most efficient.

Acknowledgments

The authors deny any conflicts of interest related to this study.

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