

# Microhardness Test to evaluate the Effect of Chelating Agents on the Superficial Layer of Root Canal Lumen Dentin

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## ABSTRACT

**Aim:** The aim of the present study was to compare the effects of different chelating agents on the microhardness of the most superficial layer of root canal lumen dentin.

**Materials and methods:** Forty-two extracted single-rooted teeth were instrumented, and the roots were longitudinally sectioned in a buccolingual direction to expose the entire root canal extension. The specimens were distributed into five groups according to the different chelating agents used: 15% ethylenediaminetetraacetic acid (EDTA) solution, 15% EDTA gel, 10% citric acid, 5% malic acid, and control [no irrigation (n=2)]. A standard volume of 50  $\mu$ L of each chelating agent was used for 5 minutes. Dentin microhardness was measured with a Vickers indenter under a 50 gm load and a 15 second dwell time. Data were analyzed statistically by one-way analysis of variance and *post hoc* multiple comparison test at 5% significance level.

**Results:** Ethylenediaminetetraacetic acid solution, EDTA gel, and citric acid had the greatest overall effect causing decrease in dentin microhardness without a significant difference ( $p>0.05$ ) from each other. However, these chelating agents differed significantly with malic acid ( $p<0.05$ ).

**Conclusion:** All tested chelating solutions reduced microhardness of the most superficial root canal dentin layer. Ethylenediaminetetraacetic acid and citric acid were the most efficient.

**Keywords:** Chelators, Dentin microhardness, Ethylenediaminetetraacetic acid, Irrigants.

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**Conflict of interest:** None

## INTRODUCTION

The complexity of root canal morphology presents a challenging objective to the endodontic community. These

complexities hamper the ability to thoroughly achieve pulp cavity disinfection. The objective therefore, is to remove all of the pulpal and dentinal debris from the root canal system. Success in endodontic therapy depends on chemomechanical debridement of the root canal system through the use of instruments and effective irrigating solutions.<sup>1</sup> Root canal instrumentation consists in the combined action of endodontic instruments and irrigation solution, aiming at the elimination of preexisting organic and inorganic remnants or debris resulting from the operative procedure as well as the reduction of the microbial content and its by-products.<sup>2</sup> An irrigation solution should present a number of physicochemical properties in order to be effective in endodontics.<sup>3</sup> It is known, however, that no endodontic irrigant present all ideal properties, and thus, the combination of auxiliary solutions is necessary to achieve the desired effects.

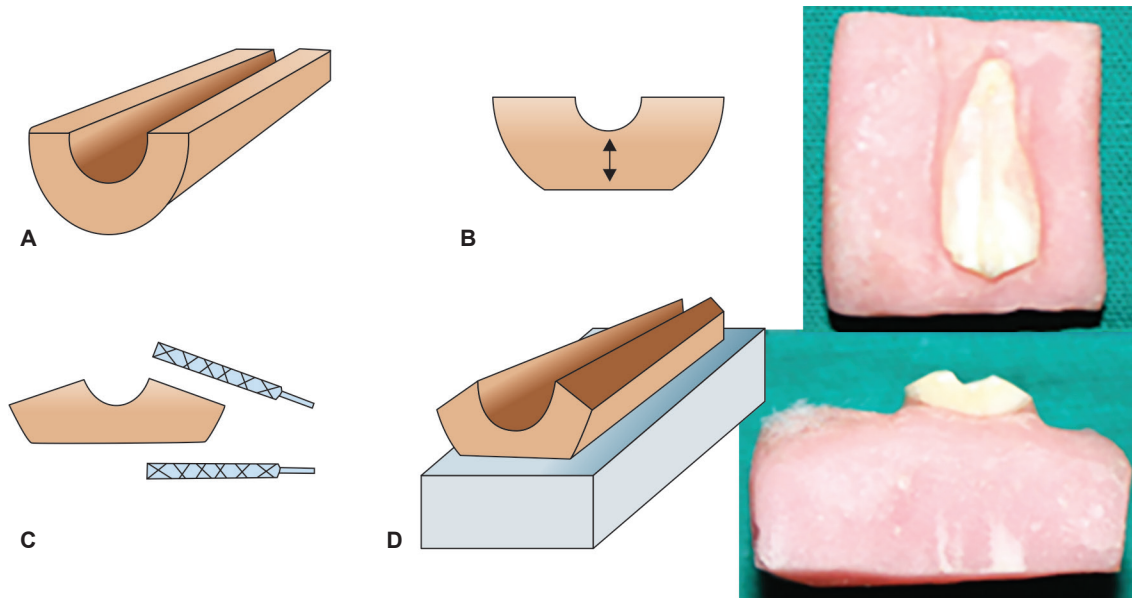
Sodium hypochlorite (NaOCl) is the most widely used chemical solution in the biomechanical preparation of the root canal system, and it has been systematically used in endodontics in concentrations ranging from 0.5 to 5.2%.<sup>4</sup> Chelating agents were introduced to endodontics by Nygaard-Ostby in 1957 as an aid for the preparation of narrow and calcified canals. The demineralizing effect of chelators acts indistinguishably on the smear layer and the root dentin, with consequent exposure of collagen and decrease in dentin microhardness.<sup>5</sup> Ethylenediaminetetraacetic acid (EDTA) is available in aqueous and gel form. An aqueous form 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.<sup>6</sup> Citric acid is very acidic (pH=1.28) and used as chelating agent in various percentages. It removed smear layer from both middle and apical thirds of the canal. It is equally effective for smear layer removal as EDTA.<sup>7</sup> Malic acid is a mild organic acid used for dentin and enamel etching and as an acid conditioner in adhesive dentistry. Malic acid can decalcify and chemically adhere to hydroxyapatite.

It has been studied that evidence of mineral loss or gain of dentin can be estimated by microhardness determination.<sup>8</sup> To simulate the clinical situation, this study evaluated the action of chelating agents by irrigating the main canal with the test agents and then measuring the

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**Figs 1A to D:** A schematic illustration of specimen preparation: (A) Hemisection 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; and (D) specimen (root/acrylic block set)<sup>2</sup>

microhardness of the most superficial layer of dentin of the root canal lumen using a methodology in which the roots were split longitudinally.<sup>9</sup>

**MATERIALS AND METHODS**

Forty-two extracted single-rooted teeth were selected. The teeth were decoronated at the cemento-enamel junction with a water-cooled diamond grid, and cervical preflaring was done with Gates Glidden drills no. 3,2,1 (Mani, Japan). Working length 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. Nickel-titanium rotary system (Protaper universal, Dentsply Maillefer) was employed for cleaning and shaping according to a crown-down technique. During preparation, the root canals were irrigated with 2 mL, 2.5% NaOCl at each change of file with an irrigation time of 30 seconds for each flush. A final irrigation with 10 mL 2.5% NaOCl was performed for removal of possible dentin shavings.

**Specimen Preparation**

Grooves were prepared along the long axis of the roots with a water-cooled diamond grid mounted on a high-speed handpiece, and the same was used to cleave the root longitudinally sectioned in a buccolingual direction to expose the entire root canal extension (Fig. 1A).

The convex surface of the half covered with cementum was flattened with a diamond cylindrical abrasive point (Mani, Japan) mounted on a high-speed handpiece to maintain a minimal thickness of 2 mm (Fig. 1B) 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. 1C) to facilitate the polishing of the root canal lumen dentin and its visualization in the microhardness tester. Each specimen was attached to an autopolymerized acrylic resin block with cyanoacrylate adhesive, and root/acrylic block sets (Fig. 1D) were randomly distributed in five groups according to the final chelating agents: 15% EDTA gel, 15% EDTA solution, 10% citric acid, 5% malic acid, and control (no irrigation).

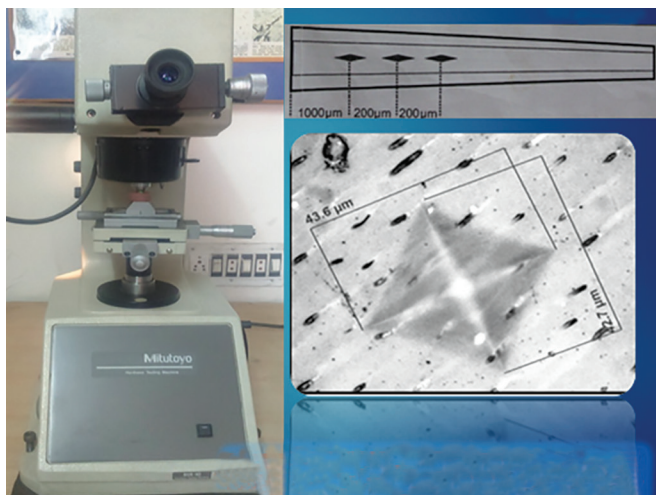
Before irrigating the root canal lumen with the test substance, the dentin surface was polished with felt disk embedded in aluminum oxide paste at a low speed. This procedure is necessary because the measurement of microhardness is only possible on polished dentin surface. The indentations are not visible on nonpolished surfaces.

**Treatment of the Specimens**

A standardized volume of 50 µL of each chelating agent was delivered directly on root canal dentin using an automated micropipette filling the whole canal extension. After 5 minutes, the specimens were rinsed with 10 mL 2.5% NaOCl to remove any residues of the test solution.

**Microhardness Measurements**

Dentin microhardness was measured with a Vickers indenter at 40× magnification (Shimadzu HMV-2000; Shimadzu Corporation, Kyoto, Japan) under a 50-gm



**Fig. 2:** A pictorial representation showing the distance between orientations of the indentations (upper view of the specimen). Illustration of a Vickers indentation and the digital measurement of the diagonals

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.

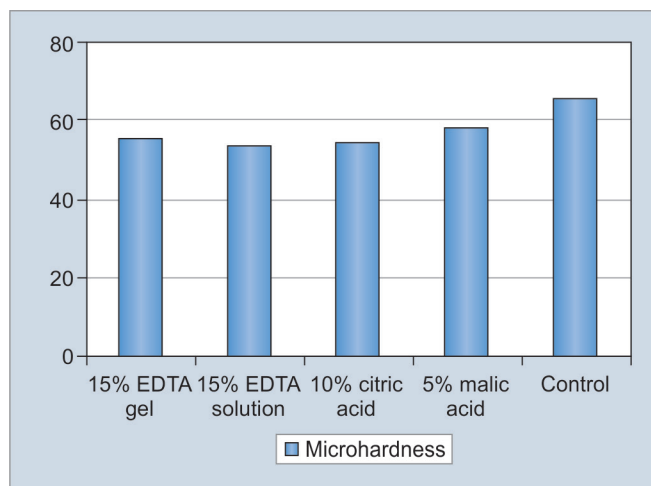
The first indentation was made 1,000 µm from the root canal entrance, and two other indentations were made at a distance of 200 µm from each other (Fig. 2). The average length of the two diagonals was used to calculate the microhardness value (Vickers hardness). The representative hardness value for 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 *post hoc* multiple-comparison test. A significance level of 5% was set for all analyses.

**RESULTS**

The Vickers microhardness values (mean standard deviation) for the chelating agents are summarized in Table 1 and pictorially represented in Graph 1. The value in each table row corresponds to the average of three measurements in 10 different specimens for a total of 30 measurements except for control group. In the control group, average of three measurements in two different specimens for total of six measurements was taken. Statistically significant difference was detected among

**Table 1:** Vickers microhardness values (mean, standard deviation) of root canal dentin after the use of the tested chelating agents

Group	Microhardness		
	Mean	n	Std. deviation
1.00	55.7610	10	1.14870
2.00	53.8580	10	3.92716
3.00	54.1520	10	3.36711
4.00	58.2950	10	2.21197
5.00	65.7010	02	1.48093
Total	57.5534	42	5.09142



**Graph 1:** Graphical representation of Vickers microhardness values of root canal dentin after the use of the tested chelating agents

the chelators by one-way analysis of variance ( $p < 0.0001$ ). Ethylenediaminetetraacetic acid and citric acid had the greatest overall effect, causing a decrease in dentin microhardness without statistically significant difference between them ( $p > 0.05$ ).

**DISCUSSION**

Success in root canal therapy depends on chemomechanical debridement and effective irrigating solutions, including chelating agents. Chelating agents were introduced into endodontics as an aid for the preparation of narrow and calcified root canal. These chelating agents were thought to chemically soften the root canal dentin and dissolve the smear layer, as well as increase dentin permeability. The aim of instrumentation and irrigation is to prepare a clean, debris-free canal for subsequent obturation.<sup>1,10</sup>

*In vitro* studies investigating the effect of chelating agents on dentin microhardness have traditionally used dentin discs cut transversally from roots of bovine or human teeth. Accordingly, 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 agents and then to measure the microhardness of the most superficial layer of dentin of the root canal lumen.<sup>2</sup> Microhardness of superficial dentin can be evaluated by means of two indentation methods (Knoop and Vickers) under two different applied loads. The measurement of the hardness of a material is one of the simplest nondestructive mechanical characterization methods. In previous studies, the Vickers indenter method was used for measuring the hardness of dentin and it is also important to mention that hardness tests have been traditionally employed to evaluate materials presenting a certain morphological homogeneity, e.g., metals. Biological materials in general and dentin, in particular, are far less homogeneous, and this may lead to deviations

in the results because of differences in adjacent regions of the dentin tissue.<sup>5</sup> For this reason, a Vickers hardness indenter was used in the present study to evaluate the most superficial layer of dentin of the root canal lumen.

The 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. Chelators, such as EDTA form a stable complex with the calcium ions in dentin. In this particular moment, carboxyl groups of the EDTA molecule are ionized, releasing hydrogen atoms that compete with the calcium ions.<sup>10</sup>

Ethylenediaminetetraacetic acid solution has a better action than EDTA gel because in small volume root canal contributes to rapid saturation of the chemical and thereby loss of effectiveness. Gels attain saturation level faster than solution form.<sup>11</sup>

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.<sup>12</sup> 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. This phenomenon can be explained using molar concentration, also called molarity, amount concentration, or substance concentration, which is a measure of the concentration of a solute in a solution. In the present study, EDTA was used at a concentration of 15% in mass, which corresponds to  $2.7 \times 10^{-5}$  mol, whereas citric acid was used at a concentration of 10% in mass, which corresponds to  $2.6 \times 10^{-5}$  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.<sup>2</sup>

In the present study, 5% malic acid shows less reduction in microhardness. This finding of the present study corroborates those of Spanó et al,<sup>13</sup> who used atomic absorption spectrophotometry to show that 5% malic acid removed similar amounts of calcium ions from the root canal but were less effective than 10% citric acid; 5% of malic acid that we used in this study showed significant reduction in microhardness when comparing with other chelators. It may be probably because of its lower concentration.

The findings of the present study showed that 15% EDTA in solution and gel form 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. However, we like to call attention to the fact that, in addition to reducing microhardness, repeated uses of chelating solutions cause erosion of root canal lumen dentin.

## CONCLUSION

In conclusion, all tested chelators reduced the microhardness of the most superficial layer of dentin of the root canal lumen. Ethylenediaminetetraacetic acid and citric acid were the most efficient. Also aqueous form of EDTA chelator has more action than gel form.

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