doi: 10.1111/j.1365-2591.2006.01094.x

Evaluation of the effect of EDTA, EDTAC and citric acid on the microhardness of root dentine

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Abstract

De-Deus G, Paciornik S, Mauricio MHP. Evaluation of the effect of EDTA, EDTAC and citric acid on the microhardness of root dentine. *International Endodontic Journal*, **39**, 401–407, 2006.

Aim To evaluate the effect of citric acid, ethylenediaminetetraacetic acid (EDTA) and ethylenediaminetetraacetic acid plus Cetavlon (EDTAC) solutions on the microhardness of human root canal dentine.

Methodology Sixteen maxillary human canines were sectioned transversely at the cemento-enamel junction and the crowns were discarded. Subsequently, each root was embedded in an epoxy resin cylinder and their middle third sectioned horizontally into 4 mm thick slices. The samples were randomly divided into three groups according to the chelating agent employed, as follows ($n = 6$): group 1: EDTA 17%, group 2: EDTAC 17% and group 3: citric acid 10%. Dentine microhardness was then measured with a load of 50 g for 15 s. At the beginning of the experiment, reference microhardness values were obtained for samples without any etching ($t = 0$ min). The same samples were then exposed to 50 μL of the chelator solution for 1, 3 and 5 min. The Student's t-test ($P < 0.05$) was used to compare results for different times for each chelator and different chelators for each time.

Results Microhardness decreased with increasing time of application of chelating solutions. There were no significant ($P > 0.05$) differences between initial microhardness for the three groups as well as after 1 min of application of the substances. After 3 min, EDTA produced a significantly greater reduction in microhardness. However, there was no significant difference between EDTA and EDTAC after 5 min. Citric acid caused significantly less reduction in microhardness.

Conclusions Overall, citric acid was least effective in reducing dentine hardness whilst EDTA had the strongest effect.

Keywords: chelating agents, dentine microhardness, endodontics, smear layer.

Received 25 May 2005; accepted 7 November 2005

Introduction

Chelating agents are used to improve chemo-mechanical debridement in root canal treatment by removing the smear layer (Østby 1957, Goldberd & Abramovich 1977). The smear layer can be removed with various substances but the most commonly used are based on different concentrations of EDTA (Hülsmann *et al.* 2003). Chelation is a physico-chemical process that prompts the uptake of multivalent positive ions by specific chemical substances. In the specific case of root dentine, the agent reacts with the calcium ions in the hydroxyapatite crystals. This process can cause changes in the microstructure of the dentine and changes in the Ca : P ratio. Initially, the use of EDTA solution was proposed by Østby (1957) 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).

The fact that the EDTA solution acts only through direct contact with the substrate led to a wetting agent being added, Cetavlon (Hill 1959). This combined substance is known as EDTAC and acts on the dentine

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walls to produce a clean surface, as well as open dentinal tubules (Goldberd & Abramovich 1977). The association of EDTA with a wetting agent enhances its bactericide effectiveness (Guerisoli *et al.* 2002) and produces solutions with reduced surface tension, that may lead to improved clinical performance.

Citric acid, a weak organic acid, has been applied previously on root surfaces altered by periodontal disease and instrumentation in order to increase cementogenesis and to accelerate healing and regeneration of a normal periodontal attachment after flap surgery (Hanes *et al.* 1991, Hennequin & Douillard 1995). In operative dentistry, citric acid has been proposed as a mild etchant for dental hard tissue, particularly for dentinal conditioning and enhanced smear layer and smear plug removal (Hennequin *et al.* 1994). In endodontic research, Loel (1975) used 50% citric acid alternately with 5% NaOCl during instrumentation and found that it was an effective agent for removing necrotic tissue and preparing the dentine for subsequent sealing with endodontic sealers. Tidmarsh (1978) also reported that 50% citric acid irrigation was effective in the removal of the superficial smear layer.

EDTA, EDTAC and citric acid are widely used in endodontic therapy (Hülsmann *et al.* 2003). The efficiency of such agents depends on many factors, such as the root canal length, penetration depth of the material, hardness of the dentine, duration of application, the pH and the concentration of the materials (Çalt & Serper 2002). For effective removal of both organic and inorganic components of the smear layer, it is generally recommended to use the endodontic chelator solution followed by NaOCl (Yamada *et al.* 1983).

It has been indicated that microhardness determination can provide indirect evidence of mineral loss or gain in dental hard tissues (Arends & ten Bosch 1992). As microhardness is sensitive to composition and surface changes of tooth structure (Panighi & G'Sell 1992), the effects of several solutions on dentine hardness were previously evaluated: sodium perborate (Chang *et al.* 2002), EDTA and a combination of hydrogen peroxide and sodium hypochlorite (Saleh & Ettman 1999), EDTAC, cyclohexane - 1,2- diaminetetraacetic acid (CDTA) and ethylene glycol-bis-(beta-amino-ethyl ether) N, N, N′, N′-tetraacetic acid (EGTA) (Cruz-Filho *et al.* 2001). Hülsmann *et al.* (2003) have pointed out that even after several investigations the real clinical relevance of the tests for evaluating the efficiency of the chelating solutions remains undefined. Effectively, the experimental conditions of the bench tests differ substantially from the clinical situation. In the tests, it is possible to apply a relatively large amount of the substance that remains in close contact with the dentine surface. This is not the case in clinical situations, e.g. narrow root canals.

However, the present paper aims to demonstrate that microhardness tests, being a simple and effective method to evaluate and compare the effect of different substances, can contribute to the comparison of their demineralization power, given that the tests are carefully calibrated. More specifically, in the present *in vitro* study the effect of 10% citric acid, EDTA and EDTAC solutions on the microhardness of human dentine was evaluated quantitatively.

Materials and methods

This study was revised and approved by the Ethics Committee, Nucleus of Collective Health Studies of the Rio de Janeiro State University, Rio de Janeiro, Brazil. Sixteen maxillary human canine teeth were selected from the tooth bank of Rio de Janeiro State University. The teeth were stored in 10% neutral formalin. Subsequently, each sample was embedded in an epoxy resin cylinder (Arazyn 1.0; Ara Química, São Paulo, SP, Brazil) to facilitate manipulation and improve the metallographic preparation. After this time, each sample was sectioned horizontally into 4 mm thick slices, in their middle third using a low-speed saw (Isomet; Buhler Ltd, Lake Bluff, NY, USA) with a diamond disc (Ø 125 mm × 0.35 mm × 12.7 mm – 330°C), under continuous water irrigation in order to prevent overheating. A standard metallographic procedure was employed, involving grinding and polishing, to prepare the surfaces for microhardness tests. The samples were randomly divided into three groups according to the chelating agent used, as follows: group 1: EDTA 17% ($n = 6$); group 2: EDTAC 17% ($n = 6$) and group 3: citric acid 10% ($n = 6$). All used solutions were freshly prepared by the manufacturer (Formula & Ação Ltda, São Paulo, SP, Brazil) and buffered to a pH of approximately 7.

A MicroMet® 5100 durimeter (Buehler Ltd, Lake Bluff, IL, USA) was used. All experiments were completed under the same conditions: 50 g load and 15 s dwell time, following the suggestions by Cruz-Filho *et al.* (2001). In each sample, three indentations were made along lines parallel to the edge of the root canal lumen, for each experimental time, taking care to avoid any overlap between them (Fig. 1). There was no blinding of the samples.

The diamond-shaped indentations were carefully observed in an optical microscope with a digital camera

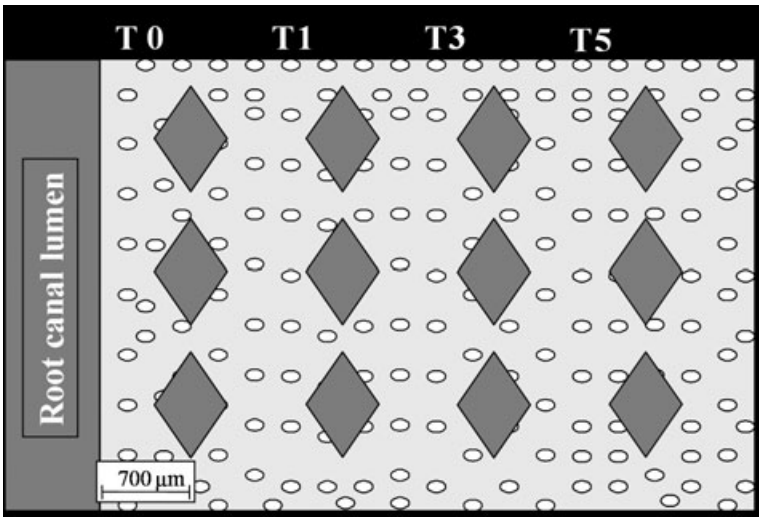

Figure 1 Sketch of the dentine location of indentation marks for different experimental times.

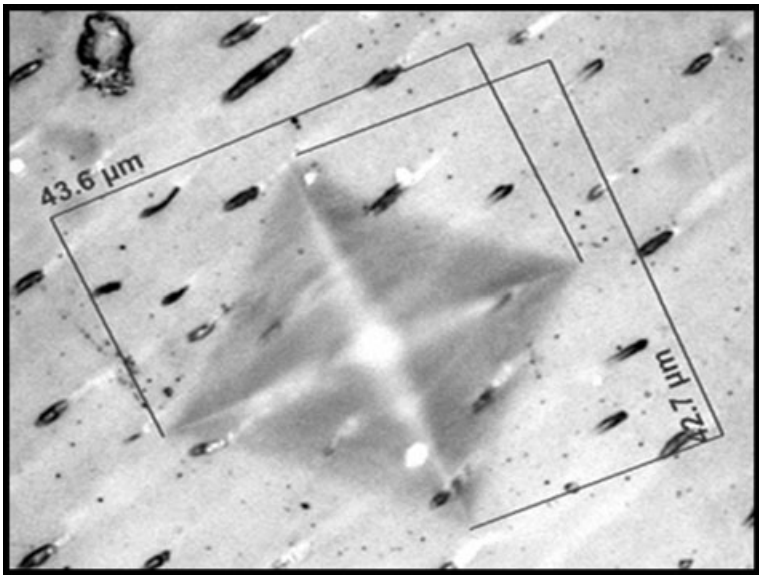

Figure 2 Illustration of a Vickers indentation and the digital measurement of the diagonals.

and image analysis software, allowing the accurate digital measurement of their diagonals (Fig. 2). The average length of the two diagonals was used to calculate the microhardness value (MHV). The representative hardness value for each sample was obtained as the average of the results for the three indentations.

At the beginning of the experiment, reference MHVs were obtained for samples prior to application of the solutions ($t = 0$ min). The samples were then subjected to the test solution for 1 min, with 50 µL of the chelating solution and a second set of measurements, adjacent to the previous ones, was obtained. The procedure was repeated and a third set of measurements was obtained after 2 more minutes of exposure ($t = 3$ min). Finally a fourth and last set was obtained after 2 more minutes ($t = 5$ min). Between each set, the solutions were neutralized with 1 mL of bi-distilled water. The Student's *t*-test ($P < 0.05$) was used to compare results for different times for each chelator and different chelators for each time.

Results

The MHVs are summarized in Table 1. The value in each table cell corresponds to the average of three measurements in six different samples for a total of 18

Table 1 Microhardness values (average ± SD) for all chelators and all times

Chelator	$t = 0$	$t = 1$ min	$t = 3$ min	$t = 5$ min
EDTA	47.6 ± 7.3	45.4 ± 7.9	34.2 ± 5.4	34.7 ± 6.3
EDTAC	49.9 ± 9.0	49.6 ± 6.9	40.5 ± 6.4	36.6 ± 3.8
CA	47.3 ± 7.0	47.5 ± 6.4	43.9 ± 4.9	41.8 ± 6.2

EDTA, ethylenediaminetetraacetic acid; EDTAC, ethylenediaminetetraacetic acid plus Cetavlon; CA, citric acid.

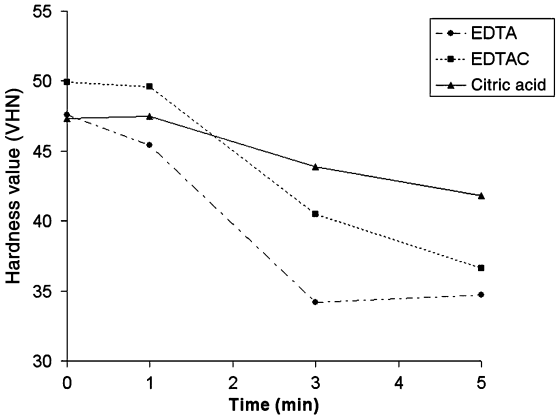

Figure 3 Microhardness value versus application time for three chelating agents.

Table 2 Statistical comparison between experimental times for each substance (*t*-test, $P < 0.05$)

Substance	Experimental times (*t*, min)	*P*-value $t = 1$ min	$t = 3$ min	$t = 5$ min
EDTA	0	NS	6.6E-7	1.6E-6
	1		5.2E-5	1.1E-4
	3			NS
	5			
EDTAC	0	NS	9.9E-4	4.8E-6
	1		4.7E-5	3.9E-7
	3			0.01
	5			
CA	0	NS	NS	0.02
	1		NS	0.02
	3			NS
	5			

NS, not significant.

measurements. These results can be better analysed with the plot in Fig. 3, and are summarized in Tables 2 and 3.

The graph in Fig. 3 shows that EDTA has the greatest overall effect, causing a sharp decrease in hardness from the reference state (0 min) to 3 min and then seems to saturate, as the hardness does not change further.

Table 3 Statistical comparison between substances for each experimental time (*t*-test, $P < 0.05$)

Experimental time (*t*, min)	Substance	*P*-value EDTAC	CA
0	EDTA	NS	NS
	EDTAC		NS
	CA		
1	EDTA	NS	NS
	EDTAC		NS
	CA		
3	EDTA	0.003	2.9E-6
	EDTAC		NS
	CA		
5	EDTA	NS	0.002
	EDTAC		0.005
	CA		

NS, not significant.

EDTAC had a slower effect on hardness, as shown by the value for 3 min when compared with EDTA. However, for 5 min, it reaches a similar decrease in hardness. These statements are confirmed by the data in Table 2.

Citric acid seems to be the least effective substance in terms of its effect on hardness. The results for 1 and 3 min are statistically similar to the reference state and it took 5 min to show a significant change.

Discussion

Results

In the present study, chelator solutions were applied on root canal dentine and the surface microhardness was used to determine their effect. EDTA had the strongest time–effect relationship whilst citric acid showed the weakest effect at the end of the experimental time. Patterson (1963) stated that the main effect of the chelator substances occurred after 5 min of application. Hülsmann *et al.* (2002) extended the experimental time to 10 min. EDTA has been reported to remove smear layer in 1 min if the fluid is able to reach the root canal wall surface (Yamada *et al.* 1983); however, it has also been suggested that the fluid should be kept in the root canal for at least 15 min to obtain optimal results (Goldberg & Spielberg 1982, Çalt & Serper 2002). It has also been reported that irrigation with 10% citric acid for 3 min had a similar pattern of Ca^{2+} extraction compared with EDTA-T used for a much longer time

interval (15 min) (Scelza *et al.* 2003). There is no consensus on the time a decalcifying agent must be in contact with the root canal wall to adequately remove smear layer; reports vary from 1 to 15 min. In the present study, etching was limited to 5 min, a more realistic time in terms of clinical practice (Yamada *et al.* 1983). As the goal of the present work was restricted to a direct comparison of the capacity of endodontic chelator solutions in reducing dentine hardness, the application of these results to the clinical situation is not straightforward.

The most significant difference between substances was obtained for an experimental time of 3 min. At this time EDTA had more effect than EDTAC ($P = 0.003$) and citric acid ($P = 2.9E\text{-}6$), whilst no significant difference between EDTAC and citric acid was found. At the end of the experimental time (5 min), the effect of EDTAC increased and there were no significant differences in relation to EDTA. At this time, only citric acid had a weaker effect on dentine hardness, significantly different from EDTA ($P = 0.002$) and EDTAC ($P = 0.005$).

Some of the present results differ from those found in the literature. In the present work citric acid was found to have the least effect on dentine microhardness. In contrast, Scelza *et al.* (2003), employing atomic absorption spectrometry, did not find differences between citric acid 10% and EDTA 17%, regarding their capacity to remove calcium ions. Moreover, the authors reported that EDTAC was less efficient, which again contradicts the present results. Another study (Scelza *et al.* 2004), in which demineralized dentine SEM images were digitally analysed, did not show any advantage in the use of an endodontic chelator solution associated with a wetting agent (EDTA-tergentol (EDTAT) solution). Zehnder *et al.* (2005) reported that the association of an endodontic chelator solution with a wetting agent that reduces surface tension did not improve the effectiveness of Ca ion removal. This conclusion is confirmed by the present work, as EDTAC was not more effective than EDTA.

Hülsmann *et al.* (2002) did not find significant microhardness differences between three chelator pastes within an experimental time of 10 min. This result may be due to the small dwell time (10 s) of the load during the hardness tests or due to the specific physical characteristics of the chelators in paste form. Eldeniz *et al.* (2005) compared 19% citric acid and EDTA 17% and reported a stronger decrease in dentine hardness because of the former, with a simultaneous increase in surface roughness. It should be noted that they used a 19% citric acid with pH = 1.3 against 10% citric acid with a buffered pH in the present study.

Methodology

The measurement of the hardness of a material is one of the simplest nondestructive mechanical characterization methods. Hardness is measured as the resistance to the penetration of an indenter that is necessarily harder than the sample to be analysed. Hardness tests provide a numerical value that allows a distinction between materials submitted to the penetration of a specific indenter. The values obtained depend on several factors such as: the Young's modulus of the material, the yield stress in compression, anisotropy, amongst others. Thus, the hardness value cannot be considered a basic property of the material, but rather an indication of its behaviour given the specific conditions of the penetration test. In a conventional Vickers hardness test a sample is indented with a pyramidal probe with load Q. The visualization of the indentation mark allows the measurement of its diagonals and, therefore, the determination of the Vickers hardness number (VHN).

In previous studies, the Vickers indenter method (Lewinstein *et al.* 1994, Cruz-Filho *et al.* 2001, Kuramato *et al.* 2001) was used for measuring the hardness of dentine 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 dentine, in particular, are far less homogenous and this may lead to deviations in the results because of differences in adjacent regions of the dentine tissue. This is clearly seen in the optical microscope image of Fig. 4 that reveals distinct density, spatial distribution and orientation of dentine tubules. Dentine hardness is related to location and its value decreases as the indentations tested are made closer to the pulp (Pashley *et al.* 1985). Pashley *et al.* (1985) reported that the microhardness of dentine declined when dentine was tested from superficial to deep regions. The increased number of widely opened dentine tubules free of peritubular dentine near the pulp offered little resistance to the testing indenter (Seaman & Shannon 1979, Burrow *et al.* 1984). Carrigan *et al.* (1984) showed that tubule density decreased from cervical to apical dentine and Pashley *et al.* (1985) reported an inverse correlation between dentine microhardness and tubular density. This histological pattern probably contributes to the hardness reduction at the cervical region of the root.

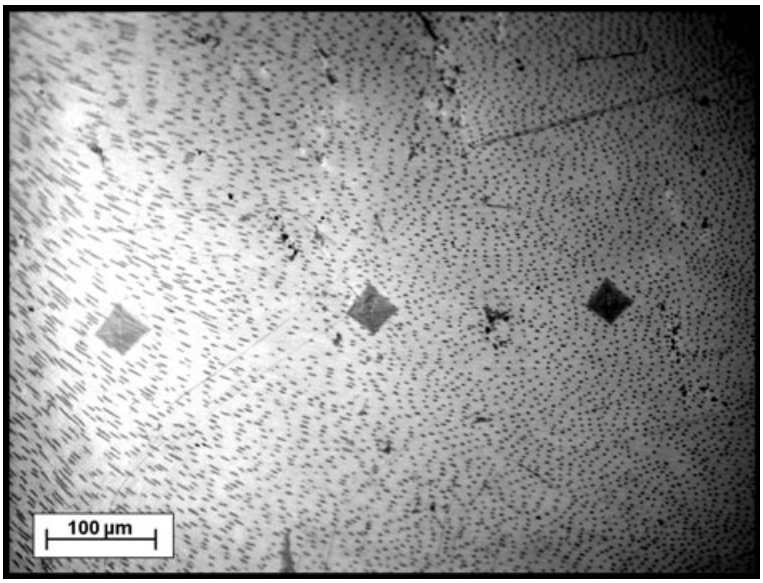

Figure 4 Microhardness indentations located on different regions of dentine. The root canal lumen is located to the left of the figure. Notice the strong morphological variations of the dentine surface.

The image in Fig. 4 corresponds to a pilot experiment in which the three microhardness measurements of a given sample were obtained at increasing distances from the root canal lumen, located on the left of the image. It is clear that the comparison between the MHV values would be biased by the underlying differences in dentine morphology. Thus, in the present work, the actual measurements were obtained from three indentations located in a central region, approximately halfway between the canal lumen and the root cement, where the dentine surface was more uniform. This methodological approach differs from the clinical situation in which the chelator substances affect more strongly the dentine walls. However, this approach allows a much better control of experimental variables, leading to readily comparable results that are fundamental for the present study.

In the present study the measurements of microhardness against increasing chelating time for a given acid were all performed on the same samples. Adjacent indentations were imprinted after the application of each chelator for a given time, including the measurement of the reference state, before application. Thus, the evolution of microhardness with chelating time can be analysed in a more robust fashion, improving the statistical value of the results.

Conclusions

Microhardness decreased with increasing time of application of chelating solutions. There were no significant differences between initial microhardness for the three groups as well as after 1 min of application of the substances. Overall, citric acid was least effective in reducing dentine hardness whilst EDTA had the strongest effect.

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