
The effect of EDTA, EGTA, EDTAC, and tetracycline-HCl with and without subsequent NaOCl treatment on the microhardness of root canal dentin

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Objective. The purpose of this study was to evaluate the effect of single and combined use of ethylenediamine tetra acetic acid (EDTA), ethylene glycol bis [b-aminoethylether] N,N,N',N'-tetraacetic acid (EGTA), EDTA plus Cetavlon (EDTAC), tetracycline-HCl, and NaOCl on the microhardness of root canal dentin.

Study design. The crowns of 30 single-rooted human teeth were discarded at the cemento-enamel junction and the roots were bisected longitudinally to obtain root halves (N = 60). The specimens were embedded in autopolymerizing acrylic resin, leaving the root canal dentin exposed. Dentin surfaces were prepared for microhardness test by grinding and polishing. The reference microhardness values of untreated specimens were recorded using a Vicker's microhardness tester at the apical, midroot, and cervical levels of the root canal. Thereafter, the specimens treated with single (test solution only) or combined (test solution, followed by 2.5% NaOCl) versions of the irrigants for 5 minutes. Posttreatment microhardness values were obtained as with initial ones. Statistical comparisons between the test groups and among single and combined treatments were carried out using 2-way ANOVA with repeated measures ($P = .05$). Comparisons within each group with respect to application regions were made with Friedman's nonparametric 2-way analysis of variance at the same level of significance.

Results. All treatment regimens except distilled water significantly decreased the microhardness of the root canal dentin ($P < .05$). The single and combined use of EDTA decreased the microhardness of the root canal dentin significantly more than all other treatment regimens ($P < .05$). Compared with their single-treatment versions, all combined treatment regimens decreased the mean microhardness values significantly ($P < .05$). A comparison of single and combined treatment regimens revealed significant decreases only for EDTA and EDTA + NaOCl in the coronal region and for EDTAC and EDTAC + NaOCl in the apical and middle regions of the root canal ($P < .05$).

Conclusions. The use of EDTA alone or prior to NaOCl resulted in the maximum decrease in dentin microhardness. The softening effect of subsequent NaOCl treatment was both material and region dependent. However, for combined treatment regimens, subsequent use of NaOCl levels the statistical differences between the regional microhardness values obtained after treatment with EGTA, EDTAC, and tetracycline-HCl. (*Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;104:418-24)

The success of root canal treatment depends on the root canal system being thoroughly cleansed and disinfected, followed by obturation of this space. Since the first description of the smear layer in instrumented root canals by McComb and Smith,¹ there is an ongoing debate regarding the influence of this layer on the success rate of endodontic treat-

ment. However, accumulating evidence suggests the importance of removing the smear layer because it can result in a more thorough disinfection of the root canal system and the dentinal tubules, which would ensure a better adaptation between the obturation materials and the root canal walls.²⁻⁴ Smear layer created during root canal instrumentation is composed of dentin structure and some nonspecific inorganic contaminants.⁵ The organic components may consist of reacted coagulated proteins, necrotic or viable pulp tissue, odontoblastic processes, and microorganisms.^{5,6}

Different solutions have been used to remove the smear layer. Sodium hypochlorite (NaOCl) in a 1% to 5.25% concentration is an irrigant solution widely used in root canal treatment because of its bactericidal properties and ability to dissolve organic tissues.^{7,8} However, it has been shown to be ineffective in removing the entire smear layer when used

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Received for publication Oct 12, 2006; returned for revision Mar 7, 2007; accepted for publication Mar 19, 2007.

1079-2104/\$ - see front matter

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doi:10.1016/j.tripleo.2007.03.021

alone.⁹⁻¹¹ Thus, the use of chelating agents and acids have been suggested to remove the smear layer from the root canal, because the components of this loosely bound structure are very small particles with a large surface-mass ratio that makes them very soluble in acids.^{5,12,13} The most commonly used chelating agents are based on different concentrations of ethylenediamine tetra-acetic acid (EDTA).^{14,15}

Hill¹⁶ and Goldberg and Abramovich¹⁷ reported that addition of a quaternary ammonium bromide (Cetavlon) increased the action of EDTA by reducing its surface tension, because EDTA solutions act only through direct contact with the substrate.¹⁶ This combination, known as EDTA plus Cetavlon (EDTAC), was shown to be very effective in smear layer removal and increasing the diameter of the opened dentinal tubules.¹⁸ Recently, Çalt and Serper¹⁹ reported that ethylene glycol-bis [b-aminoethyl-ether]-N,N,N', N'-tetraacetic acid (EGTA) was also effective in removing the smear layer, without inducing dentinal erosion commonly caused by EDTA. Tetracycline-hydrochloride (HCl) has also been proposed as a root canal irrigant. In addition to its antimicrobial effect, tetracycline-HCl acts as a calcium chelating agent due to its low pH.^{20,21} One percent tetracycline HCl has been shown to be as effective as 50% citric acid in the removal of smear layer, while causing less demineralization in peritubular dentin.²¹

It has been reported that some chemicals used for endodontic irrigation are capable of causing alterations in the chemical composition of dentin.^{14,22-24} Any change in the Ca/P ratio may alter the original proportion of organic and inorganic components, which in turn change the microhardness, permeability, and solubility characteristics of dentin.²³ Indeed, studies have shown that different concentrations of EDTA, EDTAC, and EGTA are capable of decreasing the microhardness of root canal dentin,^{13,25} and that this effect can increase by extended application times.¹³ Changes in the mineral content of superficial dentin may also adversely affect the sealing ability and adhesion of dental materials such as resin-based cements and root canal sealers to dentin.²⁶⁻²⁹

For effective removal of both organic and inorganic components of the smear layer, it is generally recommended to use endodontic chelator solutions followed by NaOCl.⁷ Although NaOCl is not a chelating agent, it can significantly decrease the Ca/P ratio of superficial root dentin^{14,24} and its microhardness,^{30,31} depending on the concentration of the solution. To date, the effect of NaOCl on dentin microhardness following initial irrigation with chelating solutions has not been investigated. Consequently, the aim of this study was to evaluate the effect of single or combined use of NaOCl, EDTA, EGTA, EDTAC, and tetracycline-HCl on the microhardness of human root canal dentin.

MATERIAL AND METHODS

Thirty periodontally involved, human maxillary incisor and mandibular premolar teeth were extracted and stored in distilled water at 4°C for a maximum of 2 months. Before experiments, soft tissues covering the root surfaces were removed with gauze and a fine brush. The crowns were removed at the cementoenamel junction by using a high-speed bur under water cooling. Thereafter, the roots were bisected longitudinally in the buccolingual direction to obtain root halves (N = 60), after which the pulp tissue was removed with a toothbrush. The root halves were embedded in autopolymerizing acrylic resin, leaving the dentin surface exposed. Then, the specimens were ground flat on a circular grinding machine with ascending grades of SiC abrasive papers (500, 800, 1000, and 1200 grit) under constant water irrigation, and further polished with fine alumina suspension (0.1 µm) on rotary felt disk.

The following irrigation solutions were tested in the present study: 2.5% NaOCl, 17% EDTA (ethylenediaminetetraacetic acid), 15% EDTAC (EDTA + 0.1% cationic surfactant, Cetavlon [cetyltrimethylammonium bromide]), 17% EGTA (ethylene glycol bis[2-aminoethylether]-N,N,N'N'-tetraacetic acid), and 1% tetracycline hydrochloride. All chemicals except NaOCl were obtained from Sigma Chemical Co. (St. Louis, MO). The test solutions were freshly prepared in laboratory conditions. The pH of EDTA, EDTAC, EGTA, and tetracycline-HCl solutions was adjusted to 7.5 by addition of 0.1 N NaOH.

Prior to application of test solutions, the Vicker's hardness values of the specimens were measured on a Zwick-type 3212002 microhardness tester (Zwick GMBH, Ulm, Germany) and recorded. Accordingly, 3 separate indentations, each using 200 gram load and 20 second dwell time were made along the central axis of the root canal at the apical, midroot, and cervical levels.

The samples were then randomly distributed into the following treatment groups (n = 10/group): group 1, 2.5% NaOCl; group 2, 17% EDTA; group 3, 17% EGTA; group 4, 15% EDTAC; group 5, 1% tetracycline-HCl; and group 6, distilled water (negative control).

The specimens were immersed for 5 minutes in a magnetic stirrer bath that contained 10 mL of each test solution. Following treatment with the chelating agents (groups 2 to 5), the same specimens were treated with NaOCl (combined treatment). Thus, each specimen served as its own control. In groups 2 to 5, the specimens received a final flush of 10-mL distilled water immediately after treatment, to avoid the prolonged effect of chelating solutions. The same procedure was carried out after treatment with

Table I. Mean of changes (apical + middle + coronal) in the microhardness values of the root canal dentin following treatment with the test solutions

Treatment	Mean %	SD %	Min %	Max %
NaOCl	8.43	2.58	5.27	13.05
EDTA	21.59	4.47	14.84	28.57
EGTA	10.56	3.34	5.75	15.53
EDTAC	7.91	1.34	5.58	9.75
Tetracycline-HCl	8.53	3.39	4.22	13.59
EDTA + NaOCl	27.54	5.05	18.74	32.90
EGTA + NaOCl	13.19	5.08	6.54	20.73
EDTAC + NaOCl	11.81	4.45	5.96	18.57
Tetracycline-HCl + NaOCl	11.06	3.76	6.33	16.46
Distilled water	3.42	1.91	1.29	7.95

Min, minimum; Max, maximum; EDTA, ethylenediamine tetra-acetic acid; EGTA, ethylene glycol-bis [b-aminoethylether]-N,N,N', N'-tetraacetic acid; EDTAC, EDTA plus Cetavlon.

NaOCl. Posttreatment indentations were made on each specimen adjacent to the initial specimens in the same manner, and the microhardness values were recorded. For each specimen, the change (percentage) in microhardness values was calculated as follows:

$$\frac{|M_i - M_p|}{M_i} \times 100$$

where M_i = initial microhardness and M_p = posttreatment microhardness.

Statistical comparisons between the test groups and among single and combined treatments were carried out using 2-way ANOVA with repeated measures ($P = .05$). Comparisons within each group with respect to application regions were made with Friedman's non-parametric 2-way analysis of variance at the same level of significance.

RESULTS

Posttreatment changes in the microhardness values (percentage) of the entire root canal dentin (mean of apical, middle, and coronal regions) are presented in Table I. Changes in the microhardness values (percentage) with respect to single and combined treatment regimens are shown in Fig. 1. Changes in microhardness values with respect to the apical, middle, and coronal regions are shown in Tables II, III, and IV, respectively. All treatment regimens except distilled water significantly decreased the microhardness of the root canal dentin ($P < .05$). Ethylenediamine tetra-acetic acid decreased the overall microhardness of the root canal dentin significantly more than the other single-solution treatments (groups 1,

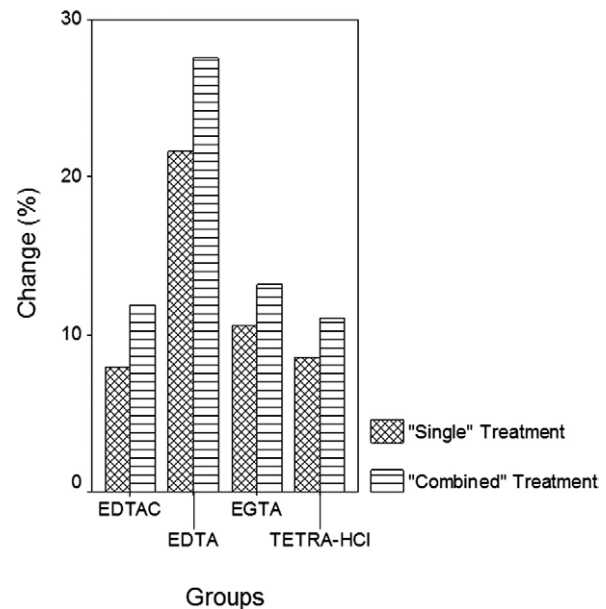


Fig 1. Changes in the microhardness values (percentage) with respect to single and combined treatment regimens. Tetra-HCl, tetracycline-HCl.

Table II. Changes in microhardness values with respect to apical root canal dentin following treatment with the test solutions

Treatment	Mean %	SD %	Min %	Max %
NaOCl	6.96	3.99	1.89	12.23
EDTA	17.98	6.14	10.55	27.87
EGTA	12.30	5.75	6.53	26.30
EDTAC	8.56	2.71	5.28	12.84
Tetracycline-HCl	7.28	3.03	2.71	12.64
EDTA + NaOCl	26.46	8.45	9.61	36.78
EGTA + NaOCl	13.73	4.38	3.98	20.22
EDTAC + NaOCl	12.00	4.47	6.84	20.42
Tetracycline-HCl + NaOCl	11.42	5.11	5.06	20.25
Distilled water	3.23	2.69	0.00	7.86

Min, minimum; Max, maximum; EDTA, ethylenediamine tetra-acetic acid; EGTA, ethylene glycol-bis [b-aminoethylether]-N,N,N', N'-tetraacetic acid; EDTAC, EDTA plus Cetavlon.

3, 4, and 5; $P < .05$). However, there was no significant difference between the microhardness values of EGTA, EDTAC, tetracycline-HCl, and NaOCl ($P > .05$). For single-solution treatments, a statistical ranking for the change in microhardness was obtained as follows (Table I):

$$\text{EDTA} > \text{EGTA} = \text{EDTAC} = \text{tetracycline-HCl}$$

A comparison of combined treatment regimens showed that EDTA + NaOCl induced significantly

Table III. Changes in microhardness values with respect to middle root canal dentin following treatment with the test solutions

Treatment	Mean %	SD %	Min %	Max %
NaOCl	7.72	4.50	2.77	17.21
EDTA	29.48	11.30	10.77	41.38
EGTA	9.08	4.95	4.51	20.00
EDTAC	5.61	1.86	3.91	8.82
Tetracycline-HCl	9.52	9.07	0.82	25.10
EDTA + NaOCl	28.32	17.20	10.96	67.97
EGTA + NaOCl	10.67	4.17	2.83	16.40
EDTAC + NaOCl	9.80	3.88	4.01	15.76
Tetracycline-HCl + NaOCl	10.69	10.01	2.38	33.92
Distilled water	3.30	1.80	0.00	6.39

Min, minimum; Max, maximum; EDTA, ethylenediamine tetra-acetic acid; EGTA, ethylene glycol-bis [b-aminoethylether]-N,N,N', N'-tetraacetic acid; EDTAC, EDTA plus Cetavlon.

Table IV. Changes in microhardness values with respect to coronal root canal dentin following treatment with the test solutions

Treatment	Mean %	SD %	Min %	Max %
NaOCl	10.60	6.94	1.39	25.61
EDTA	17.33	7.64	8.05	35.83
EGTA	10.30	4.43	2.02	17.73
EDTAC	9.55	2.93	3.69	14.56
Tetracycline-HCl	8.80	6.15	2.62	21.68
EDTA + NaOCl	27.85	10.84	10.93	49.31
EGTA + NaOCl	15.16	12.20	0.84	37.24
EDTAC + NaOCl	13.63	10.06	2.46	32.13
Tetracycline-HCl + NaOCl	11.08	2.71	8.23	17.77
Distilled water	3.73	3.90	0.37	12.77

Min, minimum; Max, maximum; EDTA, ethylenediamine tetra-acetic acid; EGTA, ethylene glycol-bis [b-aminoethylether]-N,N,N', N'-tetraacetic acid; EDTAC, EDTA plus Cetavlon.

more reduction in microhardness than EGTA + NaOCl, EDTAC + NaOCl, and tetracycline + NaOCl ($P < .05$). When compared with their single-treatment versions, all combined treatment regimens decreased the mean microhardness values significantly ($P < .05$). For changes in microhardness values achieved with combined treatment regimens, the following statistical ranking was obtained (Table I):

$$EDTA + NaOCl > EGTA + NaOCl \\ = EDTAC + NaOCl = tetracycline - HCl + NaOCl$$

With respect to the region being compared (apical, middle, or coronal), treatment with EDTA resulted in a significantly higher decrease in dentin microhardness compared with EGTA, EDTAC, tetracycline-HCl, and

NaOCl (Tables II, III, and IV; $P < .05$). For all single-solution treatments, a statistical ranking for the change in regional microhardness values was obtained as follows:

$$\text{apical region: } EDTA > EGTA > EDTAC \\ = tetracycline-HCl = NaOCl$$

$$\text{middle region: } EDTA > EGTA = NaOCl \\ = tetracycline-HCl = EDTAC$$

$$\text{coronal region: } EDTA > EGTA = NaOCl \\ = EDTAC = Tetracycline-HCl$$

In all regions, combined use of EDTA and NaOCl decreased the microhardness of the root canal dentin significantly more than EGTA + NaOCl, EDTAC + NaOCl, and tetracycline + NaOCl ($P < .05$). A comparison of single and combined treatment regimens revealed significant decreases only for EDTA and EDTA + NaOCl in the coronal region and for EDTAC and EDTAC + NaOCl in the apical and middle regions of the root canal ($P < .05$). In all regions, the same statistical ranking was obtained for changes in microhardness values achieved with combined treatment regimens:

$$EDTA + NaOCl > EGTA + NaOCl \\ = EDTAC + NaOCl = tetracycline-HCl + NaOCl$$

DISCUSSION

Current concepts of chemomechanical preparation imply that chemicals should be applied on instrumented root canal surfaces in order to remove the smear layer.^{7,12} Such procedures may induce considerable changes in the surface morphology of dentin, which may also exert changes in its mechanical and physical properties.^{14,19,24,25,32,33} Moreover, alteration of the inorganic phase of dentin surfaces by acidic pretreatments modifies their surface properties, and undoubtedly, their hardness.³⁴ Panighi and G'Sell³⁵ reported a positive correlation between hardness and the mineral content of the tooth. The determination of microhardness can thus provide valuable evidence of mineral loss (or gain) in dental hard tissues,³⁶ with special regard to the effects of irrigating solutions on dentin hardness.^{13,25,30,31}

In the present study, all specimens were subjected to a 5-minute contact with the test solutions. Currently, there is a lack of consensus on the duration a decalcifying agent must be in contact with the root canal to adequately remove the smear layer.¹³ As performed herein, De-Deus et al.¹³ limited the contact time of 3 chelator solutions (EDTA, EDTAC, and citric acid) to 5 minutes, stating that this duration is more realistic in

terms of clinical practice.⁷ Other researchers have suggested extending the application time to 10 to 15 minutes to obtain optimal results.^{18,37} It has also been reported that EDTA can remove the smear layer in 1 minute.³⁷ In addition to contact time, the concentration of the irrigation solution needs to be considered as another determinant in the posttreatment microhardness values of dentin. On the basis of the results obtained, EDTA decreased the microhardness of dentin by 17.33% to 29.48%, and this effect was significantly greater than that achieved with both the test and control solutions. Although EDTA and EDTAC had similar concentrations (17% vs. 15%), the efficacy of EDTAC was significantly lower than that of EDTA. This finding corroborates previous work,^{13,38,39} showing that reducing its surface tension does not improve the effectiveness of EDTA. According to De-Deus et al.,¹³ the lesser efficiency of EDTAC to remove calcium ions from dentin could be responsible for this finding. Although this explanation could be reasonably extended to the findings obtained with EGTA and tetracycline-HCl, there is currently no published study to support this assumption, especially when all solutions are adjusted to the same concentration and/or pH.

The relative softening effect on dentinal walls exerted by chemical irrigants could be of clinical benefit since it permits rapid preparation and facilitates negotiation of small tight canals,^{40,41} but these alterations also affect the sealing ability and adhesion of sealers to treated dentin surfaces.²⁶⁻²⁹ Thus, provided that the solution removes the entire smear within the same period of time, lower concentrations of EDTA should be preferred to reduce its adverse (softening) effect on root dentin. In this regard, the tested concentrations of EGTA and EDTAC can be considered less detrimental to dentin. However, the efficacy of lower concentrations of EGTA and EDTAC merits further evaluation, because these 2 solutions also significantly decreased the microhardness (Tables I-IV). The pH of the irrigating solutions also needs to be considered as another important factor. However, since the pH of all test solutions were adjusted to 7.5, comparisons cannot be made herein.

Results obtained within the experimental conditions of the present study indicate that the single use of NaOCl significantly reduces the microhardness of root canal dentin compared with control. Further, despite the lack of significant differences, comparison of numerical data has shown that the use of NaOCl alone can also induce more reduction in microhardness in comparison with EDTAC and tetracycline-HCl in the middle and coronal root canal dentin. NaOCl was not as effective in the apical region as it was in the coronal and middle thirds, probably because it has been shown to be less

effective in reducing the surface tension at the apical region than in the middle and coronal thirds.⁴² It could be expected that the removal of the inorganic content of dentin would reduce more its microhardness than remove the organic portion. Unlike what is commonly accepted, the treatment of dentin with NaOCl may not only remove the organic matrix but also some of the inorganic content that ultimately renders dentin much weaker than normal.^{43,44} The precise mechanism of this phenomenon is unknown, leaving room for speculation. With special regard to the combined treatment regimens tested, subsequent application of NaOCl may facilitate further exposure of the inorganic material on decalcified dentin substrate through removal of the organic matrix^{14,28,45} and thus increase the demineralizing effect that would eventually decrease the dentin microhardness. Nevertheless, this effect can be material and/or region dependent. For instance, compared with their respective single-treatment versions, significant microhardness reductions in the combined treatment groups were observed only when NaOCl was used after EDTA in the coronal third and after EDTAC in the apical and middle thirds of the root canal.

Microhardness tests have been traditionally employed to evaluate materials, presenting a certain homogeneity.¹³ Biological materials such as dentin are far less homogenous, with dentin tubule density increasing from cervical to apical dentin,⁴⁶ resulting in an inverse correlation between dentin microhardness and tubule density.⁴⁷ This may lead to deviations in the results because of differences in adjacent regions of the dentin tissue.¹³ This is clearly confirmed in the present study by the differences in the statistical ranking of single-solution treatments with EGTA, EDTAC, NaOCl, and tetracycline-HCl at the apical, middle, and coronal regions of root canal dentin. However, following subsequent treatment with NaOCl, the statistical ranking for all three regions was the same. This indicates that regional differences in microhardness are leveled in the combined treatment groups in a similar pattern observed in the general mean ranking of all 3 regions (Table I). Thus, a comparison of numerical values suggests that the combination of tetracycline-HCl and NaOCl appears to yield the least softening (adverse) effect on radicular dentin.

CONCLUSION

On the basis of the results obtained and experimental conditions of the present study, the use of EDTA alone or prior to NaOCl resulted in the maximum decrease in dentin microhardness. The softening effect of subsequent NaOCl treatment was both material and region dependent. However, for combined treatment regimens, subsequent use of NaOCl levels the differences be-

tween the microhardness values obtained after treatment with EGTA, EDTAC, and tetracycline-HCl.

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