

ORIGINAL ARTICLE

# Effect of different irrigation protocols and calcium hydroxide dressing on the microhardness of root canal dentin

## ABSTRACT

**Aim:** This study aimed to evaluate the effect of different irrigation protocols and calcium hydroxide dressing on the microhardness of root canal dentin.

**Methodology:** Thirty human teeth were decoronated and root segments were obtained from pre-established cuts made below the cemento-enamel junction. After specimen's preparation, the initial dentin microhardness (H0) was measured with a Vickers indenter, on the coronal surface, at 150  $\mu\text{m}$  and 500  $\mu\text{m}$  from the root canal lumen. Next, the specimens were randomly distributed into three groups ( $n=10$ ), according to the different irrigation protocols: 1% NaOCl; 1% NaOCl + 17% EDTA; and 5% NaOCl. After irrigation, the hardness measurement was repeated (H1), in another quadrant. Then, Ca(OH)<sub>2</sub> dressing was applied and left for 30 days, until it was removed and a new microhardness measurement (H2) was made, in a third quadrant. The data were statistically analyzed using the three-way analysis of variance (ANOVA) and Tukey tests, set at  $\alpha=0.05$ .

**Results:** The factors analyzed (moment of VH, distance from the canal lumen and irrigation protocol) were statistically significant ( $p<0.05$ ). There was a significant decrease of dentin microhardness after the irrigation protocols ( $H1<H0$ ,  $p<0.05$ ), however, with no significant difference after Ca(OH)<sub>2</sub> dressing ( $H2\sim H1$ ,  $p>0.05$ ). The 5% NaOCl group shown the greatest difference between H0 and H1 measurements ( $p<0.05$ ).

**Conclusions:** All irrigation protocols promoted significant decrease of the dentin microhardness. The Ca(OH)<sub>2</sub> dressing for 30 days did not significantly affect the microhardness of the root canal dentin.

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## Introduction

The main objective of the chemomechanical preparation is to promote cleaning, shaping and disinfection of the root canal system (1). However, the complex anatomy of the root canal, which includes accessory canals, isthmuses, and apical ramifications, may hinder a proper disinfection (2). Therefore, the success of the endodontic treatment also depends on the chemical action of irrigating solutions (3) and an intracanal medication, especially in treatment of teeth with pulp necrosis (4).

Sodium hypochlorite (NaOCl) is the irrigating solution widely used in the root canal chemical preparation due to its antimicrobial properties (5) and the ability to dissolve organic tissues (3). This solution is used as a canal irrigant in several concentrations, which may range from 0.5% to 6% (6). At 1% concentration, NaOCl has shown to be efficient in dissolving organic matter and promote disinfection (7). Conversely, higher concentrations, such as 5.25%, may cause change in the dentin mechanical properties (8), as microhardness (9-11), because of the proteolytic action of NaOCl solution in the dentin collagen matrix (12).

However, NaOCl is not able to dissolve inorganic particles from the smear layer created from the instrumentation process (13), requiring the use of a chelating agent, as the ethylenediaminetetraacetic acid (EDTA) (14). The use of the EDTA increases the dentin permeability (15), which contributes to the intracanal medication diffusion (16), as well as the sealer penetration into dentinal tubules (17).

Even after an adequate chemomechanical preparation, studies have shown the permanence of opportunistic microorganisms inside of the root canal system (18, 19), which makes prudent the use of an intracanal medication (4). Due to its biological properties, calcium hydroxide [Ca(OH)<sub>2</sub>] is widely used as intracanal medication (20, 21). Once introduced into the canal, Ca(OH)<sub>2</sub> dressing dissociates and releases

calcium and hydroxyl ions, which are responsible for the dentin alkalization and, consequently, an antimicrobial effect (22). This ionic dissociation is also responsible for other properties of Ca(OH)<sub>2</sub>, as the inhibition of phospholipase enzyme and stimulates the formation of mineralized tissue, which favors bone repair (23, 24). However, the alkalinity may reduce the organic matrix maintenance and interrupt the interaction between collagen fibrils and hydroxyapatite crystals (25), which negatively affects the dentin mechanical properties (26, 27).

Therefore, the present study aimed to evaluate the effect of different endodontic irrigation protocols and Ca(OH)<sub>2</sub> dressing on the microhardness of root canal dentin. The null hypothesis tested was that the different irrigating solutions and the subsequent use of intracanal medication would have no effect on dentin microhardness.

## Materials and Methods

### *Sample Size Calculation*

The sample size was estimated based on a previous study which assessed the effect of different irrigation protocols on the microhardness of the root dentin (28). Accordingly, for analysis with  $\alpha=0.05$  and 80% power, at least 10 teeth were allocated for each experimental group.

### *Sample Selection and Preparation*

This study was previously approved by the local Human Research Ethics Committee (Protocol number 2083FR440212). Thirty freshly extracted human mandibular premolars teeth, with single root, straight, and fully formed roots were selected for the study. The teeth were extracted from young adult patients for orthodontic reasons. Initially, the teeth were cleaned with periodontal curettes (SM 17/18, Hu-Friedy, Rio de Janeiro, RJ, Brazil), followed by disinfection in 0.5% chloramine T solution at a temperature of 4 °C for 48 hours, and washing under running water for 24 hours. Next, a radiographic examination was performed, in both mesio-distal and buccal-lingual directions, in order to verify

the existence of a single and straight root canal; and fully formed roots. Also, the teeth were examined with stereoscopic lens under  $\times 4$  magnification (Illuminated Magnifying Glass, Tokyo, Japan) to exclude from the final sample teeth with caries, restorations and any signs of cracks.

A double-sided diamond disc (Brasseler Dental Products, Savannah, GA, USA), in low rpm and under air/water spray, was used to create perpendicular cuts along the teeth axis, 2 mm and 8 mm below the cemento-enamel junction, in order to obtain root segments with 6 mm length long. The remaining pulp tissue was removed with the aid of a size 30 K-type file (Dentsply-Maillefer, Tulsa, OK, USA). Next, the root segments were placed with the coronal surface down on a glass surface containing a double-sided adhesive tape (3M ESPE, St. Paul, MN, USA). Then, the specimens were individually mounted in a customized aluminum cylinder (6.0 mm height  $\times$  12.0 mm in diameter) and embedded in self-curing acrylic resin.

After the creation of the root segments, the root canals were initially scouted with a size 15 K-file (Dentsply-Maillefer). To standardize the size and taper, the root canals, they were prepared by using Gates-Glidden drills (no 1 to 4) (Dentsply-Maillefer), and with a no 2 drill from the DT Light-Post System (BISCO, Schaumburg, IL, USA). At each use of the drills, the root canals were rinsed with 2 mL of distilled water using a syringe with Navitip 30-gauge needle (Ultradent, South Jordan, UT, USA) inserted up the canal length. The root canal preparation was performed in order to standardized the coronal diameter (1.3 mm) and the apical size (1.1 mm).

Afterwards, the specimens were sequentially polished with silicon carbide sandpapers (3M ESPE) with progressively increasing grit sizes (#400, 600, 800 and 1200), under constant irrigation. The dentin and cement surface exposition, coronal and apical, were verified through a stereomicroscope (SteREO Discovery V12, Carl Zeiss, Jena, Germany) under  $\times 100$  magnification. A final polishing procedure was performed with alumina-based pastes in decreasing order of granulation (1.0, 0.5

and 0.03  $\mu\text{m}$ ) (Buehler, Ltda, Lake Bluff, IL, USA). At each change of abrasive paper or paste, the specimens were extensively rinsed in tap water, and at the end, they were washed in an ultrasonic tank (Cristófoli, Campo Mourão, PR, Brazil) for 10 minutes.

#### *Irrigation Protocols*

Before starting the irrigation protocols, an initial evaluation of the dentin microhardness of the specimens was performed (H0: hardness control). For this, the specimens were submitted to a microhardness test in a Shimadzu HMV2 hardness tester (Newage Testing Instruments, Inc., Southampton, PA, USA) with a Vicker's diamond indenter. In each specimen, the representative hardness value was obtained at two different lateral areas of the dentin, at the coronal surface, determined at distances of 150  $\mu\text{m}$  and 500  $\mu\text{m}$  in relation to the root canal lumen. At each lateral distance (150  $\mu\text{m}$  and 500  $\mu\text{m}$ ), the microhardness was calculated as the average of three measurements, totaling six indentations in each specimen (Figure 1). The minimum distance between each indentation in the same line was established at 150  $\mu\text{m}$ . Each indentation was made using a 50 g static load and dwell time of 10 seconds. Vickers microhardness number (VHN) was calculated based on the average of the diagonals measured under examination with an optical microscope ( $\times 400$ , Shimadzu HMV2) (Figure 2), with the aid of the Newage C.A.M.S software (Newage Testing Instruments, Inc., Southampton, PA, USA). Then, the specimens were randomly assigned to the following three experimental groups, according to the irrigation protocols performed (n=10): 1% NaOCl; 1% NaOCl + 17% EDTA; and 5% NaOCl.

The specimens had the coronal surface protected with a non-porous tape (3M ESPE) to allow exposition only of the root canal to the irrigating solutions. The irrigation was performed using an endodontic syringe with Navitip 30-gauge needle (Ultradent) containing the solution of each group. Simultaneously to the irrigation, the aspiration of the solution reflux was performed by the capillary tip (.014) (Ul-



Table 1

Analysis of variance based on the following factors: distance from the canal, irrigating solution and moment of VHN† evaluation (3-way ANOVA, F test,  $\alpha=0.05$ )

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Distance from canal	1	2338	2338	25.37	0.0000
Irrigating Solution	2	1222	611	6.63	0.0017
Moment of VHN	2	2664	1332	14.45	0.0000
Distance from canal*Irrigating Solution	2	10	5	0.05	>.05
Distance from canal*Moment of VH	2	252	126	1.37	>.05
Irrigating Solution*Moment of VH	4	1104	276	2.99	0.0203
Distance from canal*Irrigating Solution*Moment of VH	4	72	18	0.19	>.05
Residue	162	14930	92		

†VHN: Vickers Hardness Number

tradent), coupled in a Luer cannula (Ultradent). The root canals were irrigated with 15 mL of the solution intended to each group, during 20 minutes, except for the group 1% NaOCl + 17% EDTA, which received initially 10 mL of 1% NaOCl for 15 minutes and, in the sequence, 3 mL of 17% EDTA for 3 minutes and 2 mL of 1% NaOCl for 2 minutes, also totalizing 15 mL of solution and 20 minutes of irrigating regimen. All the specimens were rinsed with 5 mL of distilled water to remove any residue of the chemical solutions.

Therefore, the specimens were dried with absorbent paper points (size 40, Dentsply-Maillefer) and submitted to a second microhardness evaluation (H1), in another quadrant of the coronal surface, in the same above-mentioned distances, as previously described.

#### *Ca(OH)<sub>2</sub> Dressing*

Next, the specimens received an intracanal medication with Ca(OH)<sub>2</sub> paste (Ultracal; Ultradent Products). In order to fill the root canal with the paste, each specimen was protected again with the non-porous tape on their coronal portion, exposing only the root canal entrance for the insertion of the needle with the medicament. The ready-to-use paste was introduced into the canal using the needle provided by manufacturer (Ultradent Products).

As soon as the quality of the root canal filling was confirmed, by radiographic examination, in both mesio-distal and

buccal-lingual directions, the specimens had the root canal entrance totally sealed, with the same non-porous tape, and were stored in oven at 37 °C with 100% relative humidity, during 30 days.

Over the course of this period, the tape was removed, the specimens had their root canals irrigated with 2 mL of distilled water and they were also washed in an ultrasonic tank (Cristófoli). The final microhardness evaluation (H2) was performed in another quadrant of the coronal surface, in the same above-mentioned distances, as previously described.

#### *Statistical Analysis*

Data were analyzed using the SAEG statistical software (UFV, Viçosa, MG, Brazil). The mean values and standard deviations were calculated, and data homogeneity and normality were tested by the Levene's and the Kolmogorov Smirnov's tests, respectively. Three-way analysis of variance (ANOVA) was used to compare the following variables: A) moment of VH evaluation (H0: control of the initial dentin microhardness; H1, after irrigation protocol and H2, after Ca(OH)<sub>2</sub> dressing; B) distance from the canal lumen (at 150 µm or at 500 µm); and C) irrigating solution, as well as its interactions. The significance level was set at 5%.

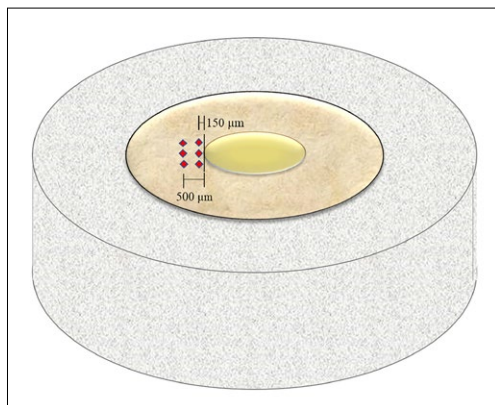
#### **Results**

The results showed that all factors analyzed (moment of VH, distance from the canal



**Figure 1.**

Schematic diagram showing a specimen with three indentations made for each lateral distance from the root canal lumen (150  $\mu$ m and 500  $\mu$ m). Six indentations were made at each moment of VH evaluation.



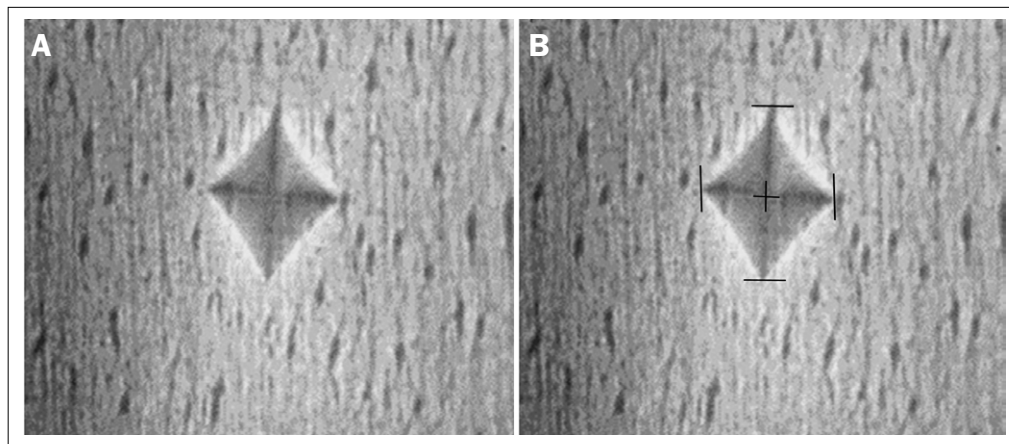
lumen and irrigation protocol) were statistically significant ( $p < 0.05$ ). However, the interaction of the factors showed significance only to the interaction between the irrigation protocol and moment of VH ( $p < 0.05$ ). The data are available in Table 1.

Overall, the dentin microhardness mean

values at the distances of 150  $\mu$ m and 500  $\mu$ m from the canal lumen were 19.2 ( $\pm 9.7$ ) and 25.7 ( $\pm 10.1$ ), respectively, which were significantly different from each other ( $p < 0.05$ ). When analyzing the interaction between irrigation protocol and the moment of VH, it was noticed a significant decrease ( $p < 0.05$ ) in dentin microhardness after irrigation (H1), in relation to the initial hardness (H0) previously assessed in all groups (Table 2). When compared to the other irrigation protocols, the 5% NaOCl group had greater decrease of VH between H0 and H1. After  $\text{Ca}(\text{OH})_2$  dressing (H2), it was not noticed any significant change in the VH results ( $p > 0.05$ ). The VH values for the 1% NaOCl and 1% NaOCl + 17% EDTA groups did not range significantly between them, regarding the moment of evaluation (H0, H1 and H2) (Table 2). Differences in dentin microhardness

**Figure 2.**

Representative optical microscopy image (400x) of the indentation performed during the hardness test in each experimental group (A), and at the moment at which the diagonals used to calculate Vickers hardness were measured (B).



**Table 2**

Comparison of the mean  $\pm$  SD values of the VH into the factor's interaction: group/irrigation protocol  $\times$  moment of VH evaluation\*

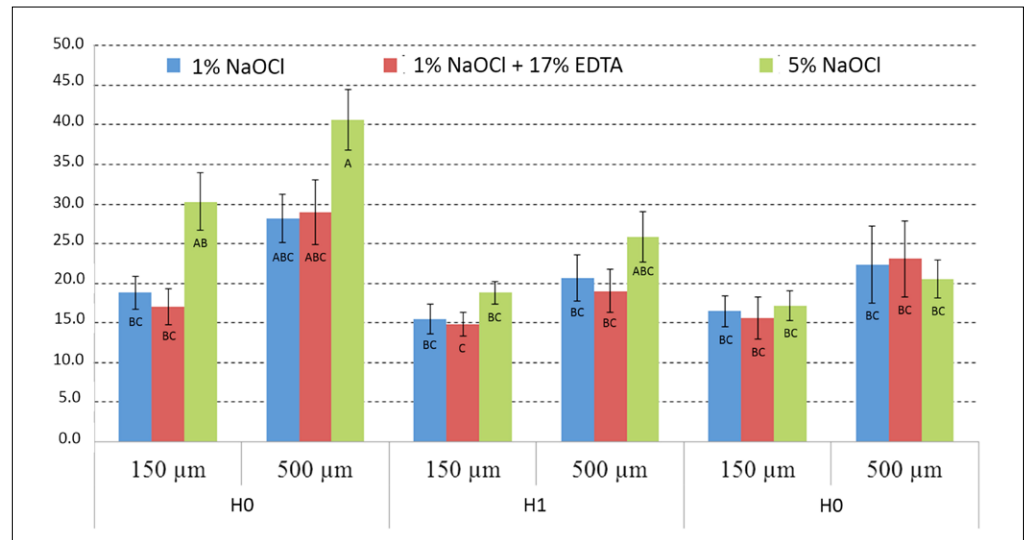
Moment of VHN†	Group/Irrigation Protocol		
	1% NaOCl	1% NaOCl +17% EDTA	5% NaOCl
H0	23,5 $\pm$ 9,5 <sup>Ba</sup>	23,0 $\pm$ 9,8 <sup>Ba</sup>	35,5 $\pm$ 9,9 <sup>Aa</sup>
H1	18,1 $\pm$ 8,0 <sup>Bb</sup>	16,9 $\pm$ 7,1 <sup>Bb</sup>	22,3 $\pm$ 8,4 <sup>Ab</sup>
H2	19,5 $\pm$ 9,8 <sup>Ab</sup>	19,4 $\pm$ 9,9 <sup>Aab</sup>	18,8 $\pm$ 6,9 <sup>Ab</sup>

\*Different uppercase letters in the lines indicate statistical difference among the irrigation protocols (Tukey's test,  $p < 0.05$ ).

\*Different lowercase letters in the columns indicate statistical difference among the VH values obtained at different moments (Tukey's test,  $p < 0.05$ ).

**Figure 3.**

Results for Tukey's multiple-comparison post hoc test according to the distance from the canal lumen (150 or 500  $\mu\text{m}$ ), moment of VH (H0, H1 and H2) and Group (irrigating solution used before H1). The same letters in the bars do not differ significantly Tukey's test ( $p > 0,05$ ). H0: control, initial dentin microhardness. H1: after irrigation protocol. H2: after  $\text{Ca}(\text{OH})_2$  dressing.



before and after specimen's treatment and among groups, in both distances evaluated (150  $\mu\text{m}$  and 500  $\mu\text{m}$ ), are showed in Figure 3.

## Discussion

The results of the present study demonstrated that, irrespective of the distance evaluated, a significant decrease in dentin microhardness for all groups was noticed after the irrigating protocols. Other studies have also noticed significant decrease on dentin microhardness when the NaOCl was used as irrigating solution in an isolated manner (9, 10, 28, 29) or even when combined with EDTA (30, 31). However, in the present study, the  $\text{Ca}(\text{OH})_2$  dressing for 30 days did not promote any significant effect to the dentin microhardness, in none of the groups. These findings may be explained by the following reasons: applied methodology in the study, dentin structural diversity, concentration of the irrigating solutions used and action time of  $\text{Ca}(\text{OH})_2$  dressing.

It is important to emphasize that, even with the specimen's standardization, it is possible to exist structural differences in the dentin evaluated (32). Considerable variations of the dentin microhardness values may occur in the same tooth (32). These structural variations usually occur in dentine, and may be related to genetic in-

fluence, atmosphere conditions, age, number of dentinal tubules, and root region evaluated (29, 30, 32). Closest areas from the canal lumen have lowest microhardness values (29), which may be justified by the higher quantity and bigger diameter of the dentinal tubules in this region (32). The results of the present study showed that the microhardness evaluated at 150  $\mu\text{m}$  from the root canal lumen was significantly lower than the one evaluated at the distance of 500  $\mu\text{m}$ . This may also be explained by the mineralization degree variation and the amount of hydroxyapatite in intertubular dentin (33).

In addition, in the present study, all irrigation protocols promoted a significant decrease in dentin microhardness, which suggests direct and powerful effect of these solutions on the structural dentin components (28-30). The 5% NaOCl group presented the greatest decrease in the microhardness values after irrigation. Such results were also found by Ari et al. (9) and Slutzky-Goldberg et al. (10), which corroborates with this study.

Keine et al. (34) have reported greater reduction in dentin microhardness when the association between NaOCl and EDTA occurs. Conversely, in the present study, the additional irrigation with 17% EDTA did not intensified the decrease of dentin microhardness, since similar results were observed for 1% NaOCl and 1% NaOCl +17% EDTA groups. However, these results

corroborate with a previously study which demonstrated that the association between EDTA and NaOCl may reduce the NaOH/HClO release, inactivating the action of the NaOCl (35). NaOCl is a non-specific proteolytic agent that acts dissolving dentin organic components (11, 29). Dentin has a high percentage of organic content, mainly type I collagen, which sustain dentinal mineral content (32). The loss of organic support may cause collapse of the inorganic structures leading to the dentin weakening and lower hardness (8, 11). The use of EDTA associated to NaOCl did not significantly affect the dentin structure. It is possible that the amount of EDTA used (3 mL) was not enough to cause harmful effects to dentin, especially due to the small volume of NaOCl used in the sequence (only 2 mL).

According to Zou et al. (36), the temperature, concentration and time are factors that may potentially affect the NaOCl penetration into the dentin. These authors also reported the increase of 30 to 50% in the penetration of NaOCl when its concentration ranged from 1% to 6%. Some other researches showed that higher concentrations of NaOCl solution promote higher penetration capacity and, consequently, higher mechanical changes in the dentin, as the microhardness (11, 37). These results support the evidences found in this research, which the 5% NaOCl group had the highest dentine microhardness decrease, in both distances evaluated.

The use of 20 min in the irrigating protocol was based on the study of Zou et al. (36), in which was observed a higher penetration of the NaOCl into the dentinal tubules using the same time. Also, this time was selected in order to standardize the period in which the solutions kept in contact with the root canal walls, simulating what usually happens in a clinical situation during chemo-mechanical preparation. Likewise, 15 mL of irrigating solution was chosen taking into account the amount used during the chemo-mechanical (including final irrigation). During a conventional preparation, we usually use 2 to 3 mL of irrigating solution at each instrument change. At the end, a final irrigation is performed with

amounts that may range from 5 mL to 10 mL, depending on the EDTA and NaOCl solutions used (2). The frequent NaOCl solution renewal inside the root canals is extremely important, because its solubilization capability is reduced in the presence of organic matter and most of its activity is lost 2 minutes after contact (38).

The volume of irrigating solution used in experimental studies varies widely from one study to another. We observed variations ranging from 0.1 mL (29, 34) to more than 40 mL (1, 37). In the study of Akay et al. (2012), for example, the specimens of each experimental group were soaked in 50 mL of the tested solutions. In other study (36), the authors used 5% NaOCl during root canal preparation, followed by a final irrigation with 6% NaOCl. Then, the authors have immersed the teeth for 20 minutes into 10 mL of NaOCl (at different concentrations), which resulted in an amount greater than 15 mL of irrigating solution delivered in root canal dentin. A recent study suggests that clinical studies must be performed using 15 mL to 20 mL during root canal preparation (39). According to this, the choice of using 15 mL of irrigating solution during root canal preparation appears to be a feasible option.

The results of the present study showed that the Ca(OH)<sub>2</sub> dressing for 30 days did not promote significant change in the dentin microhardness, in any of the distances evaluated, irrespective of the previous use of the irrigating solution. However, previous studies have reported that the use of intracanal dressing for 4 weeks reduced the dentin microhardness and promoted changes in the surface chemical structure (40, 41). Also, Yoldas et al. (41) reported a significant reduction in the dentin hardness after direct contact of specimens with Ca(OH)<sub>2</sub> for 3 and 7 days in 1 mm from the root lumen.

The moment of VH, in this research, performed in the mentioned distances, and considering a no direct dentine exposure and contact with the medicament, may partially explain the microhardness lack of changes. Thus, further studies are necessary to understand the exact mechanism of microhardness decrease after being ex-



posed to the Ca(OH)<sub>2</sub> dressing. It is important to emphasize that the results of the present study must be analysed with caution. Currently, due to the faster root canal instrumentation achieved with rotating and reciprocating systems, the contact time and the amount of irrigation solution used are becoming smaller (42). Therefore, new studies must be carried out to verify the performance of irrigation solutions in these scenarios.

The results found in this study suggest that, in clinical practice, irrigating substances such as NaOCl and EDTA would be used sparingly and safely, in order to achieve cleaning and disinfection objectives without, however, affecting dentin properties (3). Although, our study shown a reduction in the dentin microhardness with all irrigating protocols used, these findings may not be clinically relevant regarding to the longevity of the endodontically treated tooth. It is premature to state that the use of a smaller amount of irrigating solution may be beneficial for the treatment, as this might negatively interfere with other important outcomes, such as cleaning and disinfecting the root canal system.

### Conclusions

The different irrigation protocols promoted significant decrease in the dentin microhardness. The subsequent Ca(OH)<sub>2</sub> dressing for 30 days did not significantly affect the microhardness of the root canal dentin, in none of the experimental groups.

### Clinical Relevance

In clinical practice, irrigating substances, such as NaOCl and EDTA, would be sparingly and safely used, in order to achieve cleaning and disinfection of the root canal system, without affecting dentin properties.

### Conflict of Interest

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

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### References

1. Akcay I, Sen BH. The effect of surfactant addition to EDTA on microhardness of root dentin. *J Endod* 2012;38:704-7.
2. Tuncer AK, Tuncer S. Effect of different final irrigation solutions on dentinal tubule penetration depth and percentage of root canal sealer. *J Endod* 2012;38:860-3.
3. Haapasalo M, Shen Y, Gian W, Gao Y. Irrigation in endodontics. *Dent Clin North Am* 2010;54:291-312.
4. Sirén EK, Kerosuo E, Lavonius E et al. CaOH<sub>2</sub> application modes: in vitro alkalinity and clinical effect on bacteria. *Int Endod J* 2014;47:628-38.
5. Perdigão J, Lopes M, Geraldini S et al. Effect of a sodium hypochlorite gel on dentin bonding. *Dent Mater* 2000;16:311-23.
6. Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. *J Endod* 1992;18:605-12.
7. Sirtes G, Waltimo T, Schaetzle M, Zehnder M. The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy. *J Endod* 2005;31:669-71.
8. Sim TP, Knowles JC, Ng YI et al. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *Int Endod J* 2001;34:120-32.
9. Ari H, Erdemir A, Belli S. Evaluation of the effect endodontic irrigation solutions on the microhardness and the roughness of root canal dentin. *J Endod* 2004;30:792-5.
10. Slutzky-Goldberg I, Liberman R, Heling I. Effect of sodium hypochlorite on dentin microhardness. *J Endod* 2004;30:880-2.
11. Oliveira ID, Carvalho CA, Nunes W et al. Effects of chlorhexidine and sodium hypochlorite on the microhardness of root canal dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;104:e125-8.
12. Grigoratos D, Knowles J, Ng YI, Gulabivala K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. *Int Endod J* 2001;34:113-9.
13. Ulusoy ÖI, Görgül G. Effects of different irrigation solutions on root dentine microhardness smear layer removal and erosion. *Aust Endod J* 2013;39:66-72.
14. Serper A, Calt S, Dogan AL et al. Comparison of the cytotoxic effects and smear layer removing capacity of oxidative potential water, NaOCl and EDTA. *J Oral Sci* 2001;43:233-8.
15. Soares JA, De Carvalho MAR, Santos SMC et al. Effectiveness of chemomechanical preparation with alternating use of sodium hypochlorite and EDTA in eliminating intracanal enterococcus faecalis biofilm. *J Endod* 2010;36:894-8.
16. Uzunoglu E, Aktemur S, Uyanik MO et al. Effect of ethylenediaminetetraacetic acid on root fracture with respect to concentration at different time exposures. *J Endod* 2012;38:1110-3.



17. Machado R, Garcia LDFR, da Silva Neto UX et al. Evaluation of 17% EDTA and 10% citric acid in smear layer removal and tubular dentin sealer penetration. *Microsc Res Tech* 2018;81:275-82.
18. Siqueira Júnior J, Rôças IN, Oliveira JC. Prevalence of *Prevotella intermedia* and *Prevotella nigrescens* in infected root canals from a Brazilian population. *Aust Endod J* 2000;26:110-2.
19. Nair PN. On the causes of persistent apical periodontitis: a review. *Int Endod J* 2006;39:249-81.
20. Siqueira Jr JF, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *Int Endod J* 1999;32:361-9.
21. Rahimi S, Janani M, Lotfi M et al. A review of antibacterial agents in endodontic treatment. *Iran Endod J* 2014;9:161-8.
22. Sjogren U, Figdor D, Spangberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. *Int Endod J* 1991;24:119-25.
23. Lima RK, Guerreiro-Tanomaru JM, Faria-Junior NB, Tanomaru-Filho M. Effectiveness of calcium hydroxide-based intracanal medicaments against *enterococcus faecalis*. *Int Endod J* 2012;45:311-6.
24. Slutzky-Goldberg I, Hanut A, Matalon S et al. The effect of dentin on the pulp tissue dissolution capacity of sodium hypochlorite and calcium hydroxide. *J Endod* 2013;39:980-3.
25. Andreasen JO, Munksgaard EC, Bakland IK. Comparison of fracture resistance in root canals of immature sheep teeth after filling with calcium hydroxide or MTA. *Dent Traumatol* 2006;22:154-6.
26. Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. *Dent Traumatol* 2002;18:134-7.
27. Doyon GE, Dumsha T, VonFraunhofer JA. Fracture resistance of human root dentine exposed to intracanal calcium hydroxide. *J Endod* 2005;31:895-7.
28. Baldasso FER, Roletto L, Silva VDD et al. Effect of final irrigation protocols on microhardness reduction and erosion of root canal dentin. *Braz Oral Res* 2017;31:e40.
29. Garcia AJ, Kuga MC, Palma-Dibb RG et al. Effect of sodium hypochlorite under several formulations on root canal dentin microhardness. *J Investig Clin Dent* 2013;4:229-32.
30. Pedersen ND, Uzunoglu-Özyürek E, Dogan Buzoglu H. Influence of different irrigation protocols on microhardness and flexural strength values of young and aged crown dentin. *Gerodontology* 2020;37:53-8.
31. Saha SG, Sharma V, Bharadwaj A et al. Effectiveness of Various Endodontic Irrigants on the Micro-Hardness of the Root Canal Dentin: An in vitro Study. *J Clin Diagn Res* 2017;11:ZC01-ZC04.
32. Lopes MB, Sinhoreti MA, Gonine Júnior A et al. Comparative study of tubular diameter and quantity for human and bovine dentin at different depths. *Braz Dent J* 2009;20:279-83.
33. Panighi M, G'Sell C. Influence of calcium concentration on the dentin wettability by an adhesive. *J Biomed Mater Res* 1992;26:1081-9.
34. Keine KC, Kuga MC, Coaguila-Llerena H et al. Peracetic acid as a single endodontic irrigant: effects on microhardness, roughness and erosion of root canal dentin. *Microsc Res Tech* 2020;834:375-80.
35. Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. *J Endod* 2005;31:817-20.
36. Zou L, Shen Y, Li W, Haapasalo M. Penetration of sodium hypochlorite into dentin. *J Endod* 2010;36:793-6.
37. Tartari T, de Almeida Rodrigues Silva E Souza P, Vila Nova de Almeida B et al. A new weak chelator in endodontics: effects of different irrigation regimens with etidronate on root dentin microhardness. *Int J Dent* 2013;2013:743018.
38. Clarkson RM, Moule AJ, Podlich H et al. Dissolution of porcine incisor pulps in sodium hypochlorite solutions of varying compositions and concentrations. *Aust Dent J* 2006;51:245-51.
39. Arruda, JAA, Schuch, LF, Pereira, A et al. Investigation of different sodium hypochlorite volumes, concentrations and times of irrigation in endodontic therapy: a systematic review. *Arch Health Invest* 2019;8:185-191.
40. Naseri M, Eftekhari L, Gholami F et al. The Effect of Calcium Hydroxide and Nano-calcium Hydroxide on Microhardness and Superficial Chemical Structure of Root Canal Dentin: An Ex Vivo Study. *J Endod* 2019;45:1148-54.
41. Yoldaş O, Doğan C, Seydaoğlu G. The effect of two different calcium hydroxide combinations on root dentine microhardness. *Int Endod J* 2004;37:828-31.
42. Gazzaneo I, Vieira GCS, Pérez AR, et al. Root Canal Disinfection by Single- and Multiple-instrument Systems: Effects of Sodium Hypochlorite Volume, Concentration, and Retention Time. *J Endod* 2019;45:736-41.