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Research Article

The Influence of Passive Ultrasonic Irrigation Associated with Different Irrigating Solutions on Radicular Dentin Microhardness

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Abstract

Introduction: This study's aim was to evaluate the effects of irrigating solutions with 2% chlorhexidine gluconate (CHX) 17% ethylenediaminetetraacetic acid (EDTA), and 2.5% sodium hypochlorite (NaOCl) on root dentin microhardness.

Methods: Eighty mesiobuccal canals from lower molars were irrigated with conventional irrigation

(CI) or passive ultrasonic irrigation (PUI). The roots were divided into eight groups: CHX+CI; CHX+PUI; EDTA+CI; EDTA+PUI; NaOCl+CI; NaOCl+PUI; distilled water (DW)+CI; DW+PUI. The Knoop microhardness test was used to evaluate indentations at 100 µm and 500 µm in the cervical, middle and apical thirds. The results were analyzed with the Kruskal-Wallis (Dunn) test.

Results: At 100 µm in the cervical third, the highest and lowest values were found in the CHX+PUI Group and EDTA+PUI groups, respectively (P<0.05). In the middle third, the highest and lowest values were found in the CHX+CI and EDTA+PUI groups, respectively (P<0.05). In the apical third, the highest and lowest values were found in the CHX+CI and EDTA+CI groups, respectively (P<0.05). At 500 µm in the cervical third, the highest and lowest values were found in the NaOCl+PUI and EDTA+PUI groups, respectively (P<0.05). In the apical third, the highest and lowest values were found in the NaOCl+PUI and EDTA+CI groups, respectively (P<0.05).

Conclusion: Dentin microhardness was affected by these irrigating solutions. There was a greater reduction of microhardness in the cervical and middle thirds with 17% EDTA plus PUI. When associated with PUI, 2% CHX and 2.5% NaOCl had no impact on microhardness.

Keywords: Passive ultrasonic irrigation; Irrigating solutions; Dentin hardness

1. Introduction

In infected root canals, intraradicular tissues and dentin debris provide niches for the growth of microorganisms and the dissemination of their byproducts, which are responsible for endodontic treatment failure [1]. Thus, auxiliary chemical agents are essential to disinfect this complex channel system [2, 3]. Sodium hypochlorite (NaOCl) is a chemical solution used worldwide for biomechanical preparation of the root canal system [4] due to its proven antimicrobial action and its properties as a solvent of organic matter and necrotic tissue and as a lubricant [5-7]. Ethylenediaminetetraacetic acid (EDTA) dissolves the inorganic portion of dentin in the smear layer through chelation and by increasing dentin permeability [7, 8]. A solution of 17% EDTA is used as a final irrigant for dentin cleansing [9]. It helps widen the dentinal tubule by dissolving peritubular dentin [10, 11]. Chlorhexidine gluconate (CHX) has bactericidal, antimicrobial and substantivity properties [9, 12, 13]. The literature demonstrates that some chemical agents cause changes in the chemical microstructure and mechanical properties of dentin, potentially altering the original proportion of organic and inorganic components by modifying microhardness [10, 11]. Dentin microhardness, defined as local resistance to deformation, is sensitive to composition and surface changes in the tooth structure [14]. Determining microhardness could provide indirect evidence of mineral loss or gain in dental hard tissues [11]. The Knoop Hardness Test has been used to assess superficial changes in dentin tissue [15, 16]. Ultrasonic agitation was introduced to increase the effectiveness of chemical-mechanical preparation by more effectively cleaning the canal system and disorganizing bacterial communities [17, 18]. Passive ultrasonic irrigation (PUI) involves the transmission of acoustic energy from an oscillating file or tip to an irrigant within the root canal. This *in vitro* study aimed to evaluate the effects of final irrigation with different solutions vs. conventional irrigation

(CI) or PUI on root dentin microhardness. The null hypothesis was that there would be no significant difference between the protocols.

2. Materials and Methods

The study was approved by the local Research Ethics Committee (register no. 2.604.320). A total of 80 human mandibular molars indicated for exodontia were obtained, donated by patients after providing written informed consent. The sample size was calculated using ANOVA: for a power of 0.80, 10 teeth per group were necessary for the 8 treatments. The inclusion criteria were fully formed roots and foramina, mesial canals with independent foramina (visual confirmation through the apical foramen with # 10 K file), bends of 10° and 20° according to Schneider [19], and an initial anatomical diameter compatible with a #10 K-file. After the root surfaces were cleaned with periodontal curettes (Gracey Neumar Instruments Surgical Ltda, São Paulo, Brazil), the samples were stored in 0.1% thymol (Formula and Action, Palmas, Brazil) [14] at 4 °C. The crowns were sectioned with a double-sided diamond disk (KG Sorensen, Barueri, Brazil) under air/water cooling. The root length was standardized at 16 mm in the apical-cervical direction with a digital caliper (Western PRO, São Paulo, Brazil). A #10 K-type file (Maillefer-Dentsply, Ballaigues, Switzerland) was used to determine root length, i.e. until it was observed exiting the apical foramen, and the working length was set at 1 mm beyond this point. Biomechanical preparation of the mesiobuccal canal was performed with an X-Smart Plus motor (Maillefer-Dentsply, Ballaigues, Switzerland) in “Reciproc All” mode, with an R25/0.08 file (VDW, Munich, Germany). Each file was used to prepare four mesiobuccal canals. In all specimens, a #10 K-type file was used at a length of 1 mm beyond the foramen to maintain foraminal patency. During root canal preparation, irrigation with distilled water (Asfer Ind. Química Ltda., SP, Brazil) was performed using a 5 mL syringe (Injex Ind. Cirurgicas Ltda., SP, Brazil) and a 30-gauge Endo-Eze intracanal needle (Ultradent Products Inc., South Jordan, UT, United States) for 30 seconds. The speed (rpm), torque (N.cm) and kinematics followed the endodontic motor manufacturer's parameters. The instrumentation and specimen irrigation procedures were always performed by the same operator.

2.1 Final irrigation protocol

The tested solutions were: 2% CHX (Biodynamics, Ibiporã, Brazil) and 17% EDTA (Asfer Ind. Química Ltda., SP, Brazil), followed by final irrigation of 2.5% NaOCl; 2.5% NaOCl (Asfer Ind. Química Ltda., SP, Brazil); and distilled water (DW) was used as a negative control (Asfer Ind. Química Ltda., SP, Brazil).

The final irrigation protocol was performed as follows:

1. CI: root canal irrigation was performed with a 30-gauge needle (Endo-Eze) positioned 1 mm below the working length. The volume of each test solution was 5 mL and the irrigation duration was 5 min. A final irrigation with distilled water was performed.
2. PUI: the ultrasonic irrigation tip was placed 1 mm below the working length, and the test solution was dispensed until it filled the root canal. The PUI procedure followed Van der Sluis et al. (18). An Irrisonic E1 tip (20/.01) (Helse Ultrasonic Dabi, Santa Rosa de Viterbo, Brazil) mounted on an ultrasound device (Profi Neo Dabi Atlante, SP, Brazil) and set at a frequency of 30 kHz was placed 1.0 mm short of the working

length and initially activated with 5 mL of the test solution for 3 cycles of 20 s (intermediate irrigation with distilled water), followed by irrigation with 5 mL of 2.5% NaOCl solution for 3 cycles of 20 s. Aspiration was performed
with capillary tips.

2.2 Experimental groups

The roots were randomly divided (www.random.org) into eight groups (n = 10): CHX+CI; CHX+PUI; EDTA+CI; EDTA+PUI; NaOCl+CI (Control Solution Group); NaOCl+PUI (Control Solution Group); DW+CI (Negative Control Group); DW+PUI (Negative Control Group). The experimental procedures are illustrated in Table 1. An intracanal rinse with distilled water was performed between applications of the test solutions and again at the end. The irrigation procedure was followed by sectioning the root perpendicularly along the axis with a high concentration diamond wafering blade (Extec Corp., Enfield, USA) in a precision sectioning saw (Buehler Ltd., Bluff, IL, USA) to obtain 1.0 mm thick slices of the cervical, middle and apical thirds. Both slice surfaces were polished with silicon carbide sandpaper (3M, Minnesota, USA) (400, 600 and 1,200 grit), followed by polishing with felt discs (Buehler, Lake Bluff, Illinois, USA).

	Conventional irrigation							**Passive ultrasonic irrigation**						
	Test solution			**DW**	**NaOCl**			**Test solution**			**DW**	**NaOCl**		
Group	**Conc. (%)**	**mL**	**Time (min)**	**mL/min**	**Conc. (%)**	**mL**	**Time (min)**	**Conc. (%)**	**mL**	**Time (s)**	**mL/min**	**Conc. (%)**	**mL**	**Time (s)**
CHX	2	5	5	5 / 5	2,5	5	5	2	5	3 x 20	5 / 5	2,5	5	3 x 20
EDTA	17	5	5	5 / 5	2,5	5	5	17	5	3x 20	5 / 5	2,5	5	3 x 20
NaOCl	2.5	5	5	5 / 5	-	-	-	2.5	5	3 x 20	5 / 5	-	-	-
DW	-	5	5	-	-	-	-	-	5	3 x 20	-	-	-	-

CHX = chlorhexidine gluconate; DW = distilled water, NaOCl = sodium hypochlorite.

Table 1: Experimental groups.

2.3 The knoop microhardness test

Microhardness was assessed with a digital microhardness tester (PanTec HVS1000, Panambra, São Paulo, SP, Brazil), with 40X magnification and a 10 gram load for 20 seconds. In each sample, three indentations were made at 100 µm and 500 µm from the canal lumen wall in the cervical, middle and apical thirds. The hardness value for each specimen at each recorded distance was obtained by averaging the values of the three indentations in each third.

2.4 Statistical analysis

The results were analyzed in Biostat 4.0. The
Kruskal-Wallis (Dunn) nonparametric test was applied, with a significance level of 5%.

3. Results

At 100 µm in the cervical third, the highest microhardness value was found in the CHX+PUI group (30.37), which was significantly different from the EDTA+PUI (11.11) and DW+PUI (16.43) groups (P<0.05). The lowest microhardness value in the cervical third was found in the EDTA+PUI group (11.11), which was significantly different from the NaOCl+CI (29.37), NaOCl+PUI (28.95) and DW+CI (28.45) groups (P<0.05). In the middle third, the highest microhardness value (100 µm) was obtained in the CHX+CI group (31.51), which was significantly different from the EDTA+PUI (13.47) and NaOCl+PUI (15.43) groups (P<0.05). In the apical third, the highest microhardness value (100 µm) was found in the CHX+CI group (18.36), which was significantly different from the EDTA+CI (8.92) group (P<0.05). The lowest microhardness value was found in the EDTA+CI group (8.92), which was significantly different from the CHX+CI (18.36), NaOCl+PUI (23.36) and DW+CI (17.53) groups (P<0.05) (Table 2). At 500 µm in the cervical third, the highest microhardness value was found in the NaOCl+PUI group (31.26), which was significantly different from the EDTA+PUI (14.23) group (P<0.05). In the middle third (500 µm), there was no significant difference in the microhardness values (P>0.05). In the apical third, the highest microhardness value (500 µm) was found in the NaOCl+PUI group (20.95), which was significantly different from the EDTA+CI (7.76) group (P <0.05) (Table 3).

4. Discussion

Aslantas et al. [13] evaluated the effect of 2% CHX and CHX-Plus (i.e. with surface modifier) irrigating solutions on dentin microhardness at 300 µm, finding that treatment with 5 mL of these solutions for 5 minutes did not affect microhardness. Considering that changes in the mechanical properties of dentin are dependent on contact time [20, 21], the short exposure time of 5 min could have contributed to this result. According to Ari et al. [15], irrigation with 0.2% CHX solution for 15 min did not affect root dentin microhardness, which might be explained by the fact that CHX preserves both the organic tissue and smear layer in dentinal tubules [7, 9], causing little disturbance to inorganic content and, thus, having no effect on microhardness [13, 21, 22]. However, Marcelino et al. [20] observed that irrigation with 2% CHX for 5 min significantly reduced dentin microhardness. Nevertheless, their preparation process involved the Protaper system, including previous irrigation with 2.5% NaOCl and 17% EDTA for 5 min. This method promotes removal of the smear layer and dissolution of organic tissue, which could have contributed to the 2% CHX results. At 500 µm, the NaOCl+PUI group presented the highest microhardness values, both in the cervical and apical thirds (P<0.05). According to reports by Vadhana et al. [23] and Generali et al. [24], the average penetration of 6% NaOCl (20 min, 45°C) in dentinal tubules is 300 µm, which could explain the relative lack of impact on microhardness found in the present study. The penetration of NaOCl is directly influenced by the concentration, contact time and temperature [25]. Given that NaOCl solutions have been associated with greater penetration at 20 min than at 10 min [26], shorter working times, such as the 5 min used in the present study, might limit its penetration and minimize its effects on microhardness. A number of studies corroborate the microhardness reduction results of EDTA, such as Aslantas et al. [13], Ghisi et al. [25], Kandil et al. [27], Saha et al. [14]. Baldasso et al. [16] found that 17% EDTA applied for 5 min reduced microhardness at 500 µm. According to Cruz-Filho et al. [4], this is because EDTA removes the smear layer, affecting the inorganic content of the root canal walls, which facilitates biomechanical preparation and increases the access of the irrigating solution to dentinal tubules, thus interfering with microhardness. The EDTA+CI group had the lowest microhardness values in the apical third, which might be explained by the finding of Van der Sluis et al. [28] and Generali et al. [24] that mechanical preparations with instruments whose tips are smaller than 30 gauge can reduce the effectiveness of PUI. Thus, the use of an R25/0.08 file in this study may have contributed to this result, since it did not create sufficient space for PUI to agitate the irrigant. Studies suggest that changes in the intrinsic structure of dentin can affect fracture resistance [22, 25, 29]. Uzunoglu et al. [30] found that the fracture resistance of endodontically treated roots was affected by different EDTA concentrations at different exposure times. This association reinforces the clinical importance of studying the impact of irrigation solutions on dentin microhardness.

Thirds	**Groups**	**Minimum**	**Maximum**	**Median (interquartile deviation)**	**(p-KW)**
Cervical	CHX + CI	11.32	42.97	18.61 (10.64)	
Cervical	CHX + PUI	16.80	45.60	30.37 (8.49)^{A}	
Cervical	EDTA + CI	16.37	44.33	23.03 (9.35)	
Cervical	EDTA + PUI	6.74	17.80	11.11(7.79)$^{B, a}$	0.0000
Cervical	NaOCl + CI	9.60	41.80	29.37 (8.89)^{b}	
Cervical	NaOCl + PUI	15.10	38.23	28.95 (6.86)^{b}	
Cervical	DW + CI	17.27	41.77	28.45 (13.20)^{b}	
Cervical	DW + PUI	10.57	22.80	16.43 (5.06)^{B}	
Middle	CHX + CI	12.93	86.40	31.51 (12.41)^{A}	
Middle	CHX + PUI	13.50	38.50	21.20 (6.83)	
Middle	EDTA + CI	4.23	30.00	20.01 (15.90)	
Middle	EDTA + PUI	5.56	29.73	13.47 (9.70)^{B}	0.0133
Middle	NaOCl + CI	10.84	50.77	17.18 (10.82)	
Middle	NaOCl + PUI	5.94	23.00	15.43 (6.09)^{B}	
Middle	DW + CI	8.71	43.30	16.96 (8.25)	
Middle	DW + PUI	5.19	25.27	28.20 (8.63)	
Apical	CHX + CI	7.16	36.83	18.36 (13.76)^{A}	
Apical	CHX + PUI	8.14	22.17	15.12 (3.56)	
Apical	EDTA + CI	4.55	12.43	8.92 (3.85)$^{B, a}$	
Apical	EDTA + PUI	5.47	24.30	16.63 (7.55)	0.0018
Apical	NaOCl + CI	6.51	18.83	11.14 (5.63)	
Apical	NaOCl + PUI	10.90	29.57	23.36 (10.31)^{b}	
Apical	DW + CI	6.73	34.33	17.53 (14.10)^{b}	
Apical	DW + PUI	6.67	24.27	16.20 (12.29)	

Table 2: Microhardness results at 100 µm.

CHX = chlorhexidine gluconate; CI = conventional irrigation; DW = distilled water; EDTA = ethylenediaminetetraacetic acid; NaOCl = sodium hypochlorite; PUI = passive ultrasonic irrigation. Different upper or lower case letters: statistically significant differences (P<0.05). No letters: no statistically significant differences (P>0.05).

Thirds	Groups	Minimum	Maximum	Median (interquartile deviation)	*P*
Cervical	CHX + CI	12.11	27.30	19.77 (3.21)	
Cervical	CHX + PUI	14.67	53.53	25.93 (19.05)	
Cervical	EDTA + CI	17.67	36.00	22.85 (10.63)	
Cervical	EDTA + PUI	5.73	21.90	14.23 (9.09)[A]	0.0000
Cervical	NaOCl + CI	23.23	46.80	29.50 (6.98)[B, a]	
Cervical	NaOCl + PUI	19.57	40.37	31.26 (10.18)[B, a]	
Cervical	DW + CI	18.57	40.43	25.95 (5.54)[B]	
Cervical	DW + PUI	7.45	40.30	15.91 (8.87)[b]	
Middle	CHX + CI	14.53	74.10	24.42 (7.78)	
Middle	CHX + PUI	10.77	32.10	21.20 (7.08)	
Middle	EDTA + CI	7.21	30.53	18.27 (16.23)	
Middle	EDTA + PUI	5.04	31.27	16.40 (8.76)	0.3165
Middle	NaOCl + CI	9.70	26.50	18.48 (5.74)	
Middle	NaOCl + PUI	9.81	27.93	17.75 (7.94)	
Middle	DW + CI	7.86	32.03	17.95 (12.37)	
Middle	DW + PUI	13.38	28.27	17.19 (4.61)	
Apical	CHX + CI	7.38	25.87	14.10 (5.93)	
Apical	CHX + PUI	10.26	24.40	17.30 (9.87)[B]	
Apical	EDTA + CI	6.23	12.64	7.76 (1.77)[A]	
Apical	EDTA + PUI	8.72	26.90	18.57 (6.39)[B]	0.0002
Apical	NaOCl + CI	7.74	18.40	12.77 (4.19)	
Apical	NaOCl + PUI	11.15	27.53	20.95 (8.30)[B]	
Apical	DW + CI	10.13	39.90	18.90 (13.58)[B]	
Apical	DW + PUI	8.17	26.27	16.08 (5.88)	

Table 3: Microhardness results at 500µm.

CHX = chlorhexidine gluconate; CI = conventional irrigation; DW = distilled water; EDTA = ethylenediaminetetraacetic acid; NaOCl = sodium hypochlorite; PUI = passive ultrasonic irrigation. Different upper or lower case letters: statistically significant differences (*P*<0.05). No letters: no statistically significant differences (*P*>0.05).

5. Conclusion

Dentin microhardness was affected by the tested irrigating solutions. The association of 17% EDTA and PUI led to greater microhardness reduction in the cervical and middle thirds. Associations of 2% CHX and 2.5% NaOCl with PUI had no impact on microhardness.

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