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ORIGINAL ARTICLE

Final irrigation protocols may affect intraradicular dentin ultrastructure

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Abstract
Objectives The aim of this study was to evaluate the effect of different irrigation protocols on the root dentin structure using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).
Materials and methods Thirty-nine lower bovine incisors were hemisected longitudinally and randomly divided into 13 groups ($n = 3$). After the root halves were reassembled, it was applied a specific irrigation protocol for each group, as following: G1, distilled water (control); G2, 0.9% saline; G3, saline + 17% EDTA; G4, saline + PUI; G5, saline + PUI + EDTA; G6 to G9 received the same protocol as above replacing 0.9% saline by 2.5% NaOCl; and G10 to G13 by 2% CHX. One-half of each sample was prepared and evaluated using SEM and the other one by TEM observations.
Results TEM descriptive analysis showed modifications in dentin organic ultrastructure, characterized by the thinning of dentin collagen fibrils, caused by NaOCl, enhanced by EDTA and/or PUI. SEM analysis showed that NaOCl with PUI caused significantly larger erosion of the peritubular dentin than in all the other groups ($P < 0.05$), followed by NaOCl + EDTA and NaOCl + EDTA + PUI.
Conclusions NaOCl caused ultrastructural alterations in the dentin collagen, and enhanced by EDTA and/or PUI, promoted peritubular and intertubular erosion.
Clinical relevance The effect of irrigating solutions on dentin ultrastructure was still unclear. The acknowledgment about the kind of solution, concentrations, application time, and sequence of use was important to achieve the right sanitization without jeopardizing the dentin ultrastructure quality.

Keywords Chlorhexidine · Dentine · EDTA · Endodontics · Sodium hypochlorite

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Introduction

Irrigation protocols play a key role in sanitization, shaping, and three-dimensional filling of the root canal system, and they are essential for successful endodontic treatment [1]. Many substances have been used as irrigating solutions of the root canals in order to remove the pulp tissue and debris, as well as to eliminate microorganisms [2–4]. Some studies have shown that many areas of the root canal remain untouched by the instruments, which emphasizes the importance of chemical substances for cleaning and disinfecting all areas of the root canal [5] and for releasing chemical solutions in the apical third and inaccessible areas [6–10].

The final irrigation protocol to clean and disinfect root canals is essential for successful endodontic treatment [2, 3, 5]. NaOCl is the most widely used irrigating solution to dissolve necrotic tissues due to its antimicrobial potential [10]. The use of a chelating, associated with NaOCl, capable of dissolving the inorganic component agent, is necessary to remove the smear layer [11]. CHX has been used as an irrigating solution due to its antimicrobial property and low toxicity [12], but does not act as an organic solvent [13]. EDTA is a

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specific chelating for calcium and has been used to remove the smear layer [14]. However, the effect of using these irrigating solutions on dentin ultrastructure, especially with regard to the organizational structure, is still not fully elucidated [15, 16].

Furthermore, the use of ultrasonic activation to spread these substances into the root canal system has been widespread in endodontic therapy [7, 17, 18]. An important aspect to be considered when selecting the irrigating solution is its effect on the dentin organic [15] and inorganic matrices [19–23]. This effect can lead to changes on dentin elastic modulus [16], fracture toughness [24], and bond strength of luting cements and restorative materials [13, 21, 22, 25–27]. The concentration [20], time [19, 21], and sequence of use [23, 24, 27] of different irrigating solutions have influence on mineral structure of dentin. However, the effect of using these irrigating solutions on dentin ultrastructure, especially with regard to the organizational structure, is still not fully elucidated [15, 16].

Based on the above information, the aim of this study was to evaluate the effect of different irrigation protocols on the organic and inorganic structures of root canal dentin, using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The null hypothesis of this study was that there would not be significant differences among the irrigation protocols tested.

Materials and methods

Thirty-nine health bovine incisors were selected and had their crowns removed at a distance of 16 mm from the apex. All teeth were observed with a microscope to confirm the absence of cracks or fractures. After that they were randomly divided (drawing lots) into 13 groups (n = 3), as following: G1—distilled water (control); G2—0.9% saline; G3—saline + 17% EDTA; G4—saline + PUI; G5—saline + PUI + EDTA; G6 to G9 received the same protocol as above replacing 0.9% saline by 2.5% NaOCl; and G10 to G13 by 2% CHX. (Table 1).

All teeth from the group 1 (control) received simultaneous irrigation/aspiration with 10 ml of distilled water. Then, two longitudinal grooves were prepared, with a double-sided diamond disk, on the buccal and lingual surfaces, without reaching the canal lumen, followed by subsequent cleavage into two halves. These hemisected roots were placed into an ultrasound bath (Cristófoli, Campo Mourão, PR, Brazil) containing distilled water, which was ultrasound-scanned twice for 5 min.

For all the remaining groups, the root canals were prepared following cervical-apical direction, using Gates-Glidden drills (Maillefer, Ballaigues/VD, Switzerland), #6, #5, #4, #3, and #2, until reaching the working length limit of 15 mm. Irrigation and aspiration were performed with 2 ml of physiological saline while changing the drills; the specimens were held in a metal bench vise. Then, these roots were hemisected longitudinally, as described for the group 1. These root hemisections were immersed into a bath of 17% EDTA for 5 min to remove the smear layer, and then immersed into distilled water for another 5 min to remove residual EDTA.

Table 1 Group allocation according to irrigation protocols

Groups
G1—distilled water (control) = 10 ml
G2—0.9% saline = 10 ml
G3—0.9% saline (8 ml) + 17% EDTA (2 ml) = 10 ml
G4—0.9% saline (8 ml) + PUI (2 ml) = 10 ml
G5—0.9% saline + PUI (8 ml) + 17% EDTA (2 ml) = 10 ml
G6—2.5% NaOCl = 10 ml
G7—2.5% NaOCl (8 ml) + 17% EDTA (2 ml) = 10 ml
G8—2.5% NaOCl (8 ml) + PUI (2 ml) = 10 ml
G9—2.5% NaOCl + PUI (8 ml) + 17% EDTA (2 ml) = 10 ml
G10—2% CHX = 10 ml
G11—2% CHX (8 ml) + 17% EDTA (2 ml) = 10 ml
G12—2% CHX (8 ml) + PUI (2 ml) = 10 ml
G13—2% CHX + PUI (8 ml) + 17% EDTA (2 ml) = 10 ml

The 78 pairs of root hemisections were sealed with utility wax (Wilson, Polidental Indústria e Comércio Ltda, Cotia, SP, Brazil) from the outer surface, including the root apexes, and were wrapped in a necklace-like thermoplastic adhesive (Rendicolla Comércio, Exportação e Importação de Produtos Químicos Ltda, Palmeira, PR, Brazil). The roots were placed back on the bench vise, where they received irrigation protocols.

The root canals (except from the PUI groups) received 10 ml of the corresponding irrigating solution, using a disposable 10-ml syringe (BD®, Curitiba, PR, Brazil) with a 30-gauge needle (Navitip, Ultradent, South Jordan, UT, USA) placed 2 mm from the working length. Simultaneously, complete aspiration of the irrigating solution was performed using a metal cannula (Konnen, São Paulo, SP, Brazil) coupled to a suction pump. For the groups that received 2 ml of 17% EDTA, the same protocol as described above was used for its application during 3 min.

The root canals in the PUI groups received 8 ml of the corresponding irrigating solution as mentioned above and then were ultrasonically activated three times for 20 s using 1 ml of irrigating solution renewed through the irrigation/aspiration process at each interval, totalizing 10 ml. The PUI was performed using MultiSonic S (Satelec System, Gnatus, Ribeirão Preto, SP, Brazil), in endo function, power 5, with smooth insert (E1 Irrisonic; Helse, Capelli and Fabris, São Paulo, SP, Brazil) equivalent to a # 20-K file, positioned 1 mm short of the working length and centered in the root canal.

After the protocols were completed, the root canals were irrigated with 3 ml of distilled water and dried with absorbent paper points.

The root hemisections were split and placed individually in plastic tubes (Eppendorf Brazil Ltda, São Paulo, SP, Brazil) containing 2.5% glutaraldehyde for those intended for SEM and a specific fixative solution for those intended for TEM.

A representative sample of each experimental group was prepared for TEM analysis, corresponding to the middle third of the root hemisection [21, 22]. Firstly, semi-thin sections of 7 μm allowed the visualization under optical microscope at 100× of the area, which dentinal tubules would be displayed longitudinally. The samples were partially decalcified and then prepared for ultramicrotomy. Sections of 100-nm thick were made towards the long axis of the dentinal tubules. These sections were examined under a transmission electron microscope, JEM 1200 EXII model (JEOL, Tokyo, Japan) at 120 kV, and images were captured at 10,000×, 100,000×, and 200,000× magnifications. At least three images were obtained at each magnification and made available for the blinded examiner. Collagen analysis considered the following status classifications: intact (INT), dispersed (DIS), and altered (ALT).

The root hemisections for SEM analysis were washed under tap water, dehydrated, mounted on stubs, and coated with gold-palladium (Bal-tec SCD 050, Tokyo, Japan). The specimens were analyzed through SEM using a JEOL JSM 6060 microscope (JEOL, Tokyo, Japan) at 2000× and 7500× magnifications. The images were captured in the neighboring portions between the apical and middle thirds and between the middle and cervical thirds.

An experienced examiner on ultrastructure of dentin performed the analysis of electron micrographs. Particular attention was paid to the appearance of the dentin collagen in the TEM images and the possible erosion of peritubular and intertubular dentin. The cleaning of dentinal walls was evaluated by observing the presence of debris and smear layer in the SEM images. These factors were also evaluated quantitatively using a 4-point numerical scale: score 1—no debris, smear layer, or peritubular and intertubular dentinal erosion (0%); score 2—less than 50%; score 3—more than 50%; and score 4—dentinal wall completely covered by smear layer [9]. Two blinded calibrated examiners performed the quantitative evaluation.

Kruskal-Wallis and post hoc Dunn multiple comparison tests were used for data analysis, with a significance level of 5%.

Results

TEM qualitative analysis

The surface of the tubules presented smooth and intact collagen, dispersed, with characteristics of detachment from the surface, and altered, demonstrating loss of substance and tapered ends of collagen fiber ribbons, as shown in Table 2 and Figs. 1 and 2.

In all the three analyses, the change in dentin ultrastructure was evidenced in the G8 group (NaOCl + PUI) and G9 group (NaOCl + PUI + EDTA), with displacement of the collagen surface and thinning of the fibrils, as well as extensive erosion of peritubular and intertubular dentin. This collagen degeneration was also evident when NaOCl was used alone (G6).

Table 2 Quantities of debris, smear, erosion of peritubular dentin, erosion of intertubular dentin among the groups, and characteristics of collagen on surface of the dentinal tubules

	Debris		Smear			Erosion peritubular				Erosion intertubular			Collagen		
%	0	−50	0	−50	+50	0	−50	+50	100	0	−50	+50	Int	Dis	Alt
G1		X		X		X				X			X		
G2		X		X		X				X			X		
G3		X			X	X				X			X		
G4		X		X			X			X			X		
G5		X			X		X			X				X	
G6	X			X			X			X					X
G7	X		X					X			X				X
G8	X		X						X			X			X
G9	X		X					X			X				X
G10		X		X		X				X			X		
G11		X		X		X				X				X	
G12		X		X		X				X				X	
G13		X		X		X				X				X	

G1 (distilled water), *G2* (saline), *G3* (saline + EDTA), *G4* (saline + PUI), G5 (saline + PUI + EDTA), G6 (NaOCl), G7 (NaOCl + EDTA), G8 (NaOCl + PUI), *G9* (NaOCl + PUI + EDTA), *G10* (CHX), *G11* (CHX + EDTA), *G12* (CHX + PUI), and *G13* (CHX + PUI + EDTA)

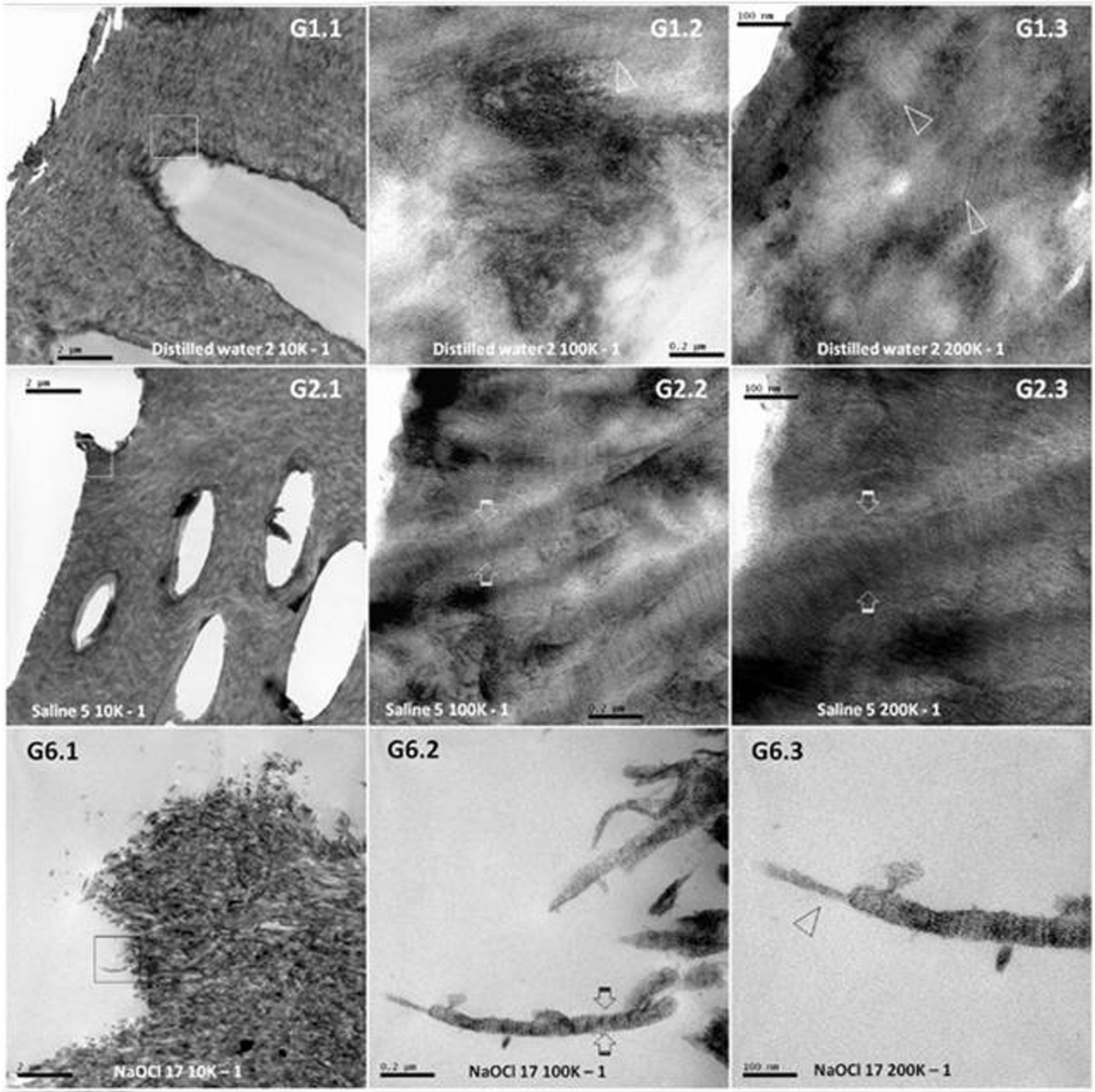


Fig. 1 The letter *G* represents the group assignment and the numbers *.1*, *.2*, and *.3* the magnification of 10,000, 100,000, and 200,000×, respectively. *Squares* are denoting the amplified areas. G1.1 to G2.3: smooth surface and intact collagen structure. Long and intact fibers are found between *arrows* (⇧) and *arrow tips* (◁). G6.1, .2, and .3: disperse collagen along surface (⇧); collagen structure displaying partially dissolved tip. Loss of substance is denoted thinning the collagen tip (◁)

SEM qualitative analysis

The dentin walls showed less than 50% or none of debris, as shown in Table 3 and Fig. 3.

The dentinal tubules showed less than 50%, more than 50%, or 0% of smear, as shown in Table 3 and Fig. 3.

The peritubular dentin was intact (0% erosion), had less than 50% erosion, over 50% erosion, or 100% erosion, as shown in Fig. 3.

The lack of erosion in the intertubular dentine, less than 50%, and more than 50% erosion are shown in Fig. 3.

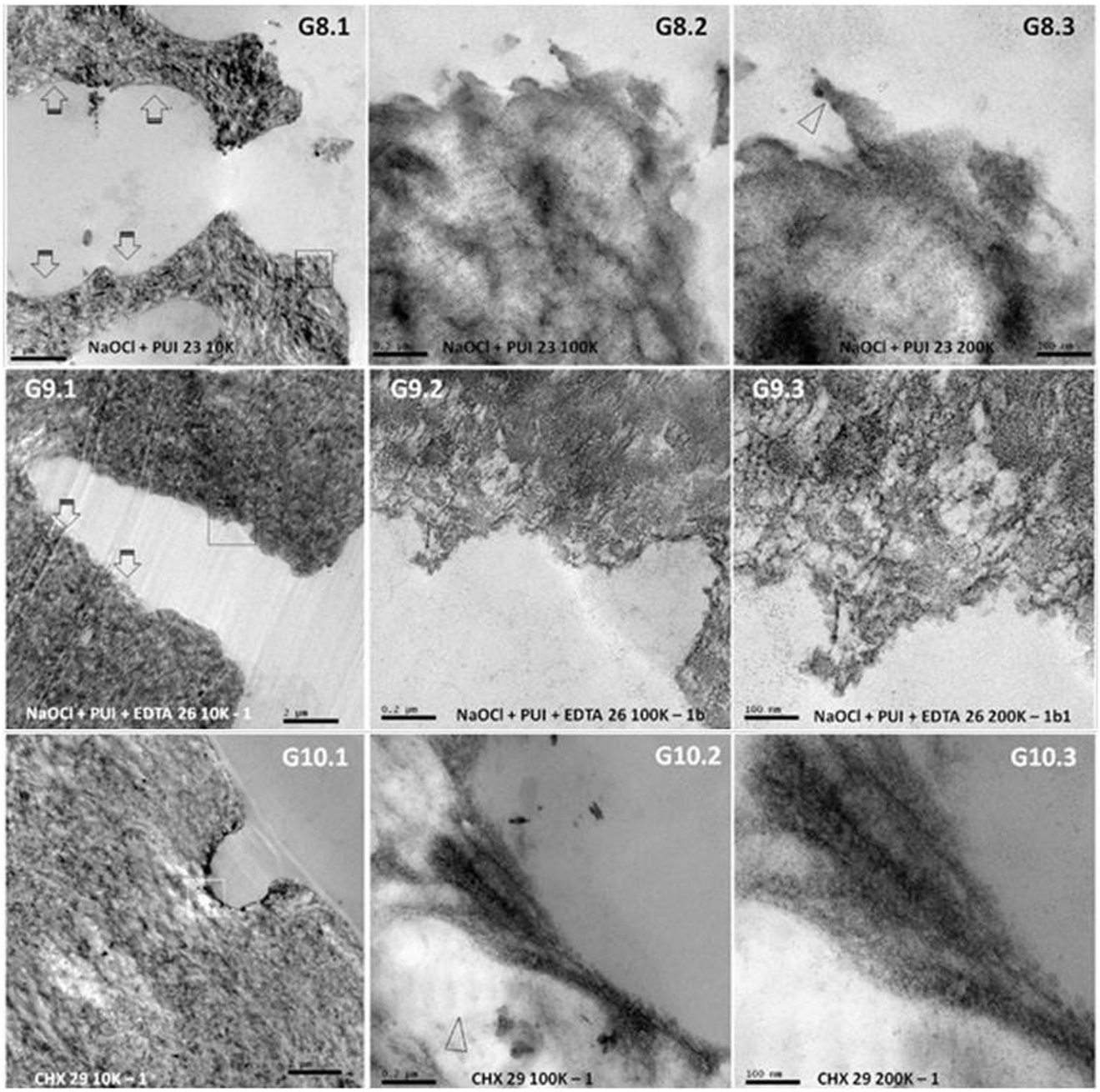


Fig. 2 The letter *G* represents the group assignment and the numbers *.1*, *.2*, and *.3* the magnification of 10,000, 100,000, and 200,000×, respectively. *Squares* are denoting the amplified areas. G8.1, .2, and .3; and G9.1, .2, and .3: disperse collagen along surface (⇧); collagen structure displaying partially dissolved tip. Loss of substance is denoted thinning the collagen tip (◁). This feature can be seen also at the tubule end. G8.1: globular enlargement of the tubules (⇧). G10.1, .2, and .3: smooth surface and intact collagen structure

Change in dentin ultrastructure was evidenced in the G8 group (NaOCl + PUI) and G9 (NaOCl + PUI + EDTA), with extensive erosion of peritubular and intertubular dentin.

SEM quantitative analysis

Peritubular dentin erosion in the G8 (NaOCl + PUI) was significantly higher than that in all the groups ($p < 0.05$). The G7 (NaOCl + EDTA) and G9 (NaOCl + PUI + EDTA) showed peritubular dentin erosion, but less accentuated than the G8 ($p < 0.05$).

Intertubular dentin erosion was higher in the G8 (NaOCl + PUI) than that in the other groups, but not much different from the G9 (NaOCl + PUI + EDTA) and G7 (NaOCl + EDTA) ($p > 0.05$). The other groups showed no intertubular dentin erosion.

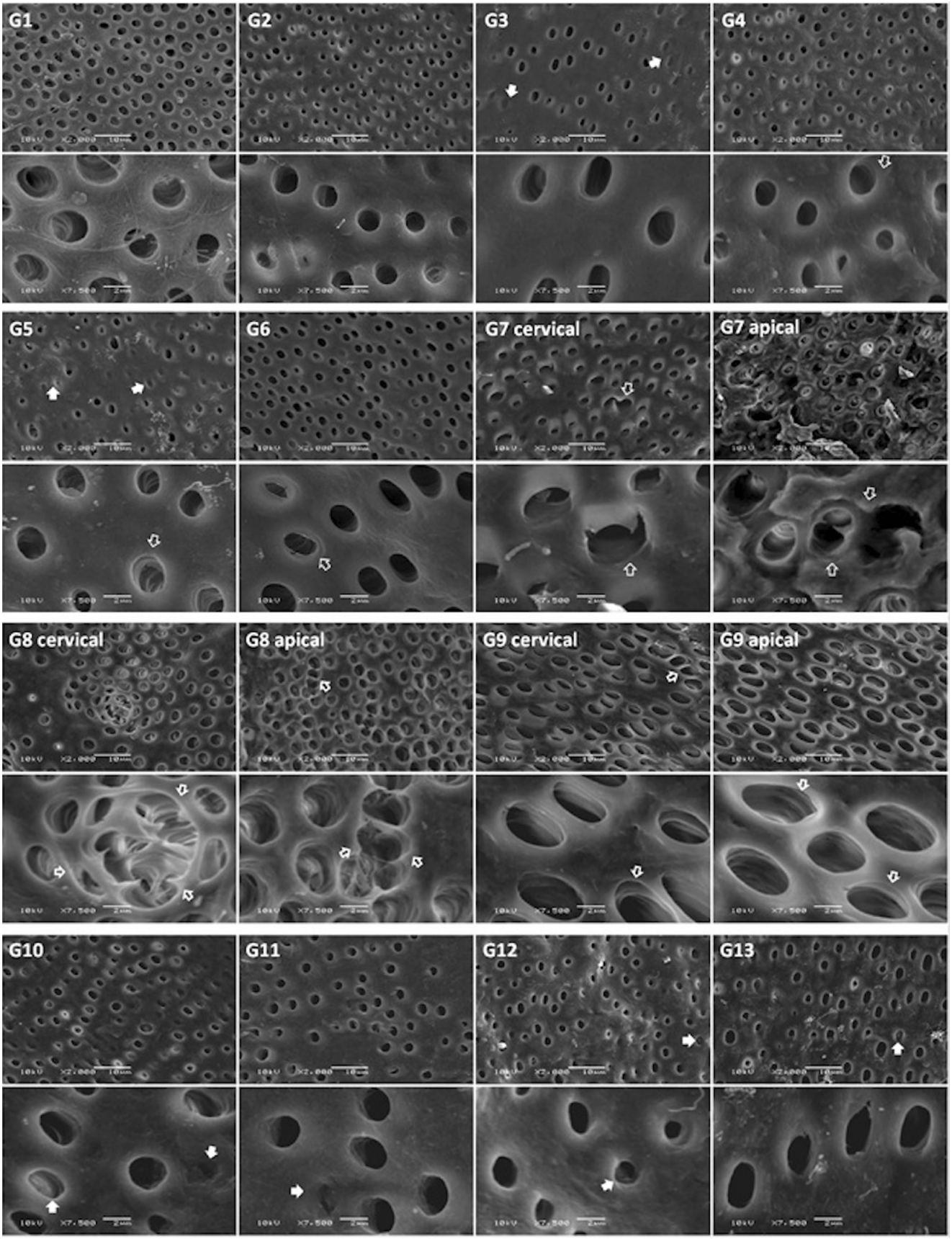
Table 3 Debris, smear layer and erosion scores of peritubular and intertubular dentin, and SEM quantitative analysis (median and range)

Groups	Irrigation protocol	Debris	Smear layer	Peritubular dentin erosion	Intratubular dentin erosion
G1	Distilled water				
	Median	2 ^{cde}	2 ^{a}	1 ^{a}	1 ^{a}
	Range	2–2	2–3	1–1	1–1
G2	Saline				
	Median	2 ^{abcde}	3 ^{a}	1 ^{a}	1 ^{a}
	Range	1–2	2–3	1–1	1–1
G3	Saline + EDTA				
	Median	2 ^{de}	3 ^{a}	1 ^{a}	1 ^{a}
	Range	2–2	3–3	1–1	1–1
G4	Saline + PUI				
	Median	2 ^{cde}	2 ^{a}	2 ^{a}	1 ^{a}
	Range	2–2	1–3	1–2	1–1
G5	Saline + PUI + EDTA				
	Median	2 ^{e}	3 ^{a}	2 ^{b}	1 ^{a}
	Range	1–3	1–3	2–2	1–1
G6	NaOCl				
	Median	1 ^{abcde}	2 ^{ac}	2 ^{b}	1 ^{a}
	Range	1–2	1–3	2–2	1–1
G7	NaOCl + EDTA				
	Median	1 ^{ab}	1 ^{c}	3 ^{b}	2 ^{ab}
	Range	1–1	1–1	2–3	1–3
G8	NaOCl + PUI				
	Median	1 ^{a}	1 ^{c}	4 ^{c}	3 ^{b}
	Range	1–1	1–1	3–4	3–3
G9	NaOCl + PUI + EDTA				
	Median	1 ^{a}	1 ^{c}	2 ^{b}	2 ^{ab}
	Range	1–1	1–1	2–4	1–3
G10	CHX				
	Median	1 ^{abc}	2 ^{a}	1 ^{a}	1 ^{a}
	Range	1–2	2–3	1–1	1–1
G11	CHX + EDTA				
	Median	2 ^{bc}	2 ^{ac}	1 ^{a}	1 ^{a}
	Range	1–2	1–2	1–1	1–1
G12	CHX + PUI				
	Median	2 ^{abc}	2 ^{a}	1 ^{a}	1 ^{a}
	Range	1–2	1–4	1–1	1–1
G13	CHX + PUI + EDTA				
	Median	2 ^{e}	2 ^{a}	1 ^{a}	1 ^{a}
	Range	2–3	2–3	1–1	1–1

Different superscript letters in the same column indicate statistically significant difference with Kruskal-Wallis and post hoc Dunn tests ($p < 0.05$)

The G7 (NaOCl + EDTA), G8 (NaOCl + PUI), and G9 (NaOCl + PUI + EDTA) presented cleaner walls, with less debris and more open dentinal tubules than the other groups.

The NaOCl + EDTA, NaOCl + PUI, and NaOCl + PUI + EDTA groups presented cleaner walls, i.e., without debris and open dentinal tubules than the other groups. The use of EDTA and PUI neither reduced the amount of debris, nor the smear layer, when combined with saline and CHX. However, in the NaOCl groups, EDTA and/or PUI decreased the presence of smear layer.


G1
G2
G3
G4
G5
G6
G7 cervical
G7 apical
G8 cervical
G8 apical
G9 cervical
G9 apical
G10
G11
G12
G13

◀ **Fig. 3** Erosion profile under SEM with magnifications of 2000× (*upper images*) and 7500× (*lower images*). G1 (distilled water) and G2 (saline): partially open tubules, intact peri and intertubular dentin. G3 (saline + EDTA): partially closed tubules, presence of smear plugs, intact peri and intertubular dentin. G4 (saline + PUI): open tubules, partially open peritubular dentin and intact intertubular dentin. G5 (saline + PUI + EDTA): partially closed tubules, presence of smear plugs, partially open peritubular dentin and intact intertubular dentin. G6 (NaOCl): open tubules, slightly modified peritubular dentin and intact intertubular dentin. G7 (NaOCl + EDTA) cervical: open tubules, modified peritubular dentin with erosion areas, slightly modified intertubular dentin. G7 (NaOCl + EDTA) apical: open tubules, modified peritubular dentin with extensive erosion areas and intertubular dentin modified by erosive process. G8 (NaOCl + PUI) cervical and apical: open tubules, modified peritubular dentin with extensive erosion areas and intertubular dentin modified by erosive process. G9 (NaOCl + PUI + EDTA) cervical and apical: open tubules, modified peritubular dentin, with small erosion areas and intertubular dentin slightly modified by erosive process. G10 (CHX), G11 (CHX + EDTA), G12 (CHX + PUI), and G13 (CHX + PUI + EDTA): partially open tubules, small smear plug areas, intact peri and intertubular dentin

Discussion

The samples selected for the TEM analysis consisted of fragments of the middle third of root hemisections [21, 22]. The visualization in the apical third was avoided due to the effect of vapor lock [9], due to this region could not receive the same amount of irrigating solution than the other thirds [22]. The root canal central areas tend to have more regular and smooth dentin walls [14, 23]. The same aspects were considered to capture images for the SEM analysis.

The purpose of subjecting the root canal surface to EDTA was to remove smear layer prior to exposure to the irrigating solutions, allowing direct contact with dentinal surface. Smear layer could become a physical barrier to contact with the solution. The main objective was not to evaluate the cleaning ability, as there is a lot of this in the dental literature. The major concerns were the influence of the irrigating solution on dentin structure, especially its collagen content, as well as the potential to cause erosion of peritubular and intertubular dentin.

Depth of penetration of the solution into dentinal matrix would be jeopardized by smear layer. Even if this does not mimic the clinical scenario, some inference could be taken as to the potential risk of some substances to degrade collagen. This is why bovine teeth were used, as they reduce variables present in human teeth, such as age, influence of caries, periodontal disease, etc. Additionally, they allowed a more standardized collagen to be subjected to the test substances and the control.

The four scores that were set to evaluate the removal of debris, smear layer, and erosion were based on quantitative criteria (<50 or >50%), according to van der Sluis et al. (2010) [7]. The use of subjective criteria [17] that could contribute to lower intra- and inter-observer agreement was avoided.

The results of SEM quantitative and qualitative analyses were similar and comparable to those of TEM. This fact demonstrates the importance of quantitative SEM analysis that reinforces the qualitative findings, and confirms the evidence from this study. In all the three analyses, the change in dentin ultrastructure was evidenced in the G8 group (NaOCl + PUI) and G9 (NaOCl + PUI + EDTA), with displacement of the collagen surface and thinning of the fibrils, as well as extensive erosion of peritubular and intertubular dentin. This collagen degeneration was also evident when NaOCl was used alone (G6).

This confirms the NaOCl capability to dissolve organic components [2–4, 8]. No other studies showing collagen degenerations through TEM analysis were found after the use of irrigating solutions. However, changes in the dentin collagen were demonstrated by using immunohistochemical evaluation. It was found that 5% NaOCl induced major changes in demineralized intertubular collagen and intratubular glycosaminoglycans [15]. This study has also demonstrated the protective role of hydroxyapatite on the stability of the organic matrix. The null hypothesis stating that 2.5% NaOCl does not cause structural and ultrastructural changes in dentin was rejected.

In the descriptive TEM analysis, the fact that the collagen structure was intact in the saline, CHX, and distilled water groups confirmed the null hypothesis, since these substances were not capable of dissolving organic matter. Previous studies have shown that water is less effective than NaOCl during PUI in the removal of inorganic matter from the root canals [6]. Only the samples in which saline + PUI + EDTA and CHX + PUI, and/or EDTA were used, the collagen was dispersed, but without degradation. This was probably due to the flow of the irrigating solution that was driven against the dentinal walls by ultrasonic acoustic transmission, which generated the shock wave and made the collagen fibrils cut by the action of Gates-Glidden drills be partially displaced from the dentin wall.

The quantitative analysis revealed that the NaOCl + EDTA, NaOCl + PUI, and NaOCl + PUI + EDTA groups presented cleaner walls, i.e., without debris and open dentinal tubules than the other groups. Unlike the results found in previous studies [7, 17, 31, 32], the use of EDTA and PUI neither reduced the amount of debris, nor the smear layer, when combined with saline and CHX. However, in the NaOCl groups, EDTA and/or PUI decreased the presence of smear layer. This fact shows that EDTA and PUI, without the dissolution of organic matter performed by NaOCl, did not remove the smear layer effectively. This NaOCl chemical effect was already advocated by van der Sluis et al. (2010) [7].

The NaOCl + PUI group showed significantly greater peritubular dentin erosion than all the other groups ($p < 0.05$). The NaOCl + EDTA and NaOCl + PUI + EDTA groups had a less pronounced peritubular dentin erosion than

the NaOCl + PUI group ($p < 0.05$), though higher than the NaOCl group alone. Consequently, it can be inferred that ultrasound enhanced the erosion effect of NaOCl. Similar results were found in other studies after using NaOCl and EDTA [11, 19, 20].

Similarly, the NaOCl + PUI group showed greater intertubular dentin erosion than the other groups, but did not differ significantly from the NaOCl + PUI + EDTA and NaOCl + EDTA groups ($p > 0.05$). This fact supports the findings from other studies that showed that EDTA used as a final irrigating agent causes erosion of the dentinal walls only when NaOCl was used previously [21, 22].

When EDTA is used alone, it removes the calcium ions from the mineral tissue, the organic matter being the limiting factor for the continuation of inorganic dissolution [15]. The subsequent application of NaOCl removes the organic matter and facilitates the exposure of the inorganic matrix, thus enhancing the demineralization effect [33]. It can be considered that EDTA accelerates the destructive action of NaOCl [27]. The supposed aggressiveness of EDTA in causing erosion of the root canal walls is attributed to and associated with the prolonged use of NaOCl. Interestingly, these irregularities occurred more markedly in the G8 (NaOCl + PUI), which demonstrates that regardless of the absence of EDTA, there was an even more severe peritubular and intertubular dentin erosion.

The clinical relevance of this study has been demonstrated by alerting the dentists who will restore the endodontically treated tooth on the alterations that occur in dentin ultrastructure when different irrigation protocols are used, which may interfere with both its mechanical resistance and bond strength in adherence to restorative materials. Some studies have assessed the dentine/fiberglass-bonded interface by scanning electron microscopy and the push-out bond strength of different luting cements, and concluded that glass ionomer cements have the highest push-out bond strength values when compared with the composite resin cements [28, 29]. When dentin collagen is dissolved by NaOCl, the release of oxygen occurs and can interfere with the polymerization of resins and adhesive cements [25, 26]; this could be one more explanation about the lower results of composite resin cements and why the main failure of prefabricated posts is decementation when these cements are used [30]. Fine integration among professionals involved in endodontic treatment and restorative protocols allows for adequate irrigation protocols, according to the restorative procedure to be used.

The results obtained from this study suggest that PUI enhances the NaOCl effect because of acoustic cavitation [7]. However, the NaOCl effect was also enhanced by EDTA, and this caused great impact upon the erosion degree of dentinal walls. These results indicate the importance of careful irrigation, either conventional or activated. The NaOCl concentration and length of use should be carefully considered. Some authors have suggested that the use of both NaOCl [34] and EDTA [20] should be used in low concentrations, and the exposure of dentin to EDTA should be short [19, 24]. Attention should be paid to the sequence of irrigating agents used as well [23, 27].

Based on the results from this study, and considering that PUI is an important aid in the irrigation of the root canals, new questions are inevitable. Thus, further research is needed to determine the depth of erosion that occurs in the dentin walls when using the combination of NaOCl and PUI and/or EDTA, and how this erosion affects the tooth structure and longevity. Moreover, it is necessary to devise irrigation protocols with well-defined time, concentration, and sequence of irrigating solutions to provide cleaning and disinfection of the dentin with minimum adverse effects to the tooth. Another point could be the direct influence of these structures and collagen alterations on the choice of different cements used to cement intraradicular posts.

Conclusions

Given the experimental conditions and the results obtained, this study concluded the NaOCl provided better cleaning of the root canal walls than the other irrigating solutions. However, it also caused alterations in the collagen ultrastructure and erosion of peritubular and intertubular dentin. The synergistic action of PUI and/or EDTA enhanced the effects of NaOCl on dentin structures.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Project n. 23051 was approved by the Committee of Research and Ethics of the Federal University of Rio Grande do Sul.

Informed consent For this type of study, formal consent is not required.

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