Original Article

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Resin-based sealer penetration into dentinal tubules after the use of 2% chlorhexidine gel and 17% EDTA: *in vitro* study

Natalia Sabadin, Daiana Elisabeth Böttcher, Carolina Bender Hoppe, Régis Burmeister dos Santos, Fabiana Soares Grecca

Universidade Federal do Rio Grande do Sul – UFRGS, School of Dentistry, Department of Conservative Dentistry, Porto Alegre, RS, Brazil

Abstract

Aim: To evaluate, by confocal laser scanning microscopy (CLSM), the influence of 17% EDTA final irrigation on the penetration of an endodontic resin-based sealer into dentinal tubules after use of 2% chlorhexidine gel. **Methods:** Forty extracted bovine incisors were instrumented according to the groups: G1 – root canal preparation with 2% chlorhexidine gel (n=10); G2 – root canal preparation with 2% chlorhexidine gel and final irrigation with 17% EDTA (n=10); G3 – root canal preparation with saline and final irrigation with 17% EDTA (n=10); G4 – root canal preparation with saline (n=10). The samples were filled with gutta-percha using AH Plus sealer with rhodamine B fluorescent dye. After seven days, the teeth were sectioned at the coronal, middle, and apical thirds and viewed under confocal microscope. The most representative area of penetration depth was measured in each group. Statistical significance for the sealer penetration area was determined among groups using one-way ANOVA followed by Tuckey test. For thirds comparison, in each group, data were statistically analyzed using Friedman test ($p<0.05$). **Results:** The maximum penetration was provided by G2 - 2% chlorhexidine + EDTA ($p=0.000$). According to this criterion, no differences were found among the other groups and among thirds within the same group. **Conclusions:** Based on these results, the use of 17% EDTA should be indicated after root canal preparation with 2% chlorhexidine gel for smear layer removal, enhancing the AH Plus sealer penetration.

Keywords: endodontics; root canal filling; smear layer; *in vitro*.

Introduction

Cleaning and shaping are considered the most important steps for the management of an infected root canal space. Furthermore, a complete and three-dimensional sealing of the root canal system is critical to prevent oral pathogens from colonizing and re-infecting the endodontic space[1].

Endodontic sealers are used in conjunction with core filling materials in order to avoid gaps and voids. According to Mamootil and Messer[2] (2007) penetration of sealer into dentinal tubules will increase the interface between the filling material and dentin thus improving the sealing ability and the retention of material by mechanical locking. Root canal filling may also entomb any residual bacteria within the tubules and the chemical components of the sealer may exert an antibacterial effect[3]. Penetration of sealer into dentinal tubules is influenced by smear layer removal, by irrigation solutions and the filling technique[4-5].

During chemo-mechanical preparation, an amorphous layer of organic and

Correspondence to:
Daiana Elisabeth Böttcher
Av. Ramiro Barcelos, 2492
CEP: 90035-003 Porto Alegre - RS - Brazil
Phone: +55 51 33085191
E-mail: daibottcher@hotmail.com

inorganic materials, known as smear layer, is formed on the root canal walls[6-7]. This layer acts as a physical barrier and its removal is mandatory to facilitate the penetration of sealers into dentinal tubules[5,8]. Demineralizing agents such as tri-sodium salt of ethylenediaminetetraacetic (EDTA) have therefore been recommended as adjuvants in root canal therapy[8].

Chlorhexidine (CHX) has been recommended as an alternative to NaOCl during root canal preparation, especially in cases of open apex, root resorption, foramen enlargement and root perforation, due to its biocompatibility, or in cases of allergy related to bleaching solutions[9]. Clinical investigations have reported that CHX and NaOCl have comparable effects in eliminating bacteria[10-11]. However, only CHX has a property known as substantivity – capacity to adsorb to surfaces and maintain a prolonged antimicrobial activity[12]. In addition, CHX has the potential to inhibit matrix metalloproteinases - a group of enzymes that regulates the physiologic and pathologic metabolism of collagen-based tissues[13].

The use of irrigants during root canal preparation may alter the chemical composition of dentin surface, thereby influencing the interaction between dentin and filling materials. In this regard, CHX has been shown to be beneficial for the longevity of root canal obturations[14-15] and it does not affect the ability of root fillings to prevent fluid penetration into the root canal system through the apical foramen[9,16].

On account of this, the aim of this study was to evaluate by confocal laser scanning microscopy the influence of 17% tri-sodium salt of ethylenediaminetetraacetic acid on the penetration of AH Plus sealer into dentinal tubules after use of 2% CHX gel.

Material and methods

The present study was approved by the Ethics in Research Committee from the Federal University of Rio Grande do Sul (Protocol # 22234). Forty extracted central bovine incisors were selected for this study and stored in 0.2% thymol solution (Pharma&Cia, Porto Alegre, RS, Brazil).

The teeth were removed from storage and immersed in 5% NaOCl (Biodinâmica, Ibiporã, PR, Brazil) for 30 min. External debris were removed using a scalpel blade number 12 (Becton Dickinson Indústrias Cirúrgicas Ltda., Juiz de Fora, MG, Brazil). Then, they were cleaned with pumice (Maquira Indústria de Produtos Odontológicos Ltda., Maringá, PR, Brazil) and water and stored in distilled water at 4 °C (Pharma&Cia).

The crown surface of each tooth was sectioned below the cemento-enamel junction, perpendicular to the long axis of the tooth, with a slow speed saw (Isomet; Buehler, Lake Bluff, IL, USA) under water irrigation. Root length was standardized at 15 mm. The inclusion criterion for the roots was that canals should be up to 3 mm cervical diameter.

The selected roots were randomly assigned into four groups according to the irrigant (Figure 1). Irrigation was performed using a syringe and a 30G needle (Ultradent Products, South Jordan, UT, USA). A #20 K-Flexofile (Maillefer Instruments, Ballaigues, Switzerland) was used to remove pulp tissue and introduced further into the root canal until the tip was just visible at the apical foramen. Canals were kept flooded with 2.5% NaOCl (Biodinâmica). After complete pulp tissue removal, the teeth were dried with paper points, embedded in wet gauze and individually autoclaved at 121 °C and 1 atm for 15 min.

Before preparation, working length was determined by subtracting 1 mm from this length. The root canals were manually prepared with K-files (Maillefer Instruments) from size #70 until #110.

- G1: **Preparation with 2% CHX gel.** The root canal was filled with 0.3 mL 2% CHX gel (Pharma&Cia). The gel remained inside the canal during the entire instrumentation. Before each file was changed, CHX was removed with 3.0 mL sterile saline and renewed. A total amount of 1.5 mL of CHX gel was used during preparation. A final rinse with 4.0 mL sterile saline was performed.

- G2: **Preparation with 2% CHX gel and EDTA.** The root canal was filled with 0.3 mL 2% CHX gel. The gel remained inside the canal during the entire instrumentation. Before each file was changed, CHX gel was removed with 3.0 mL sterile saline and renewed. A total amount of 1.5 mL of CHX gel was used during preparation. A final rinse with 3.0 mL sterile saline was performed, followed by 1.0 mL 17% EDTA (Biodinâmica) for 3 min. EDTA was removed with 1.0 mL of sterile saline.

- G3: **Preparation with sterile saline and EDTA.** The root canal was filled with 3 mL of saline. Before each file was changed, the same amount of the solution was renewed. A total amount of 15 mL of saline was used during

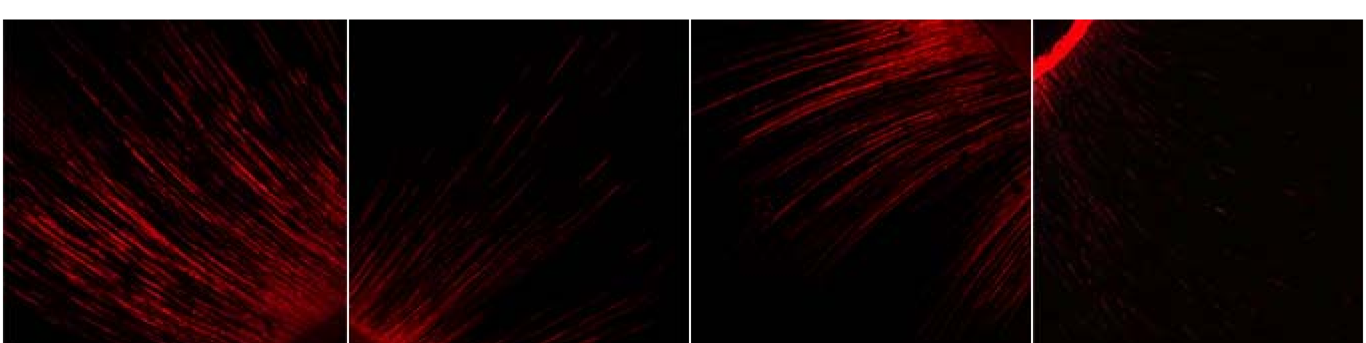


Fig. 1. Flowchart of preparation procedures.

preparation. A final rinse with 3.0 mL sterile saline was performed, followed by 1.0 mL 17% EDTA for 3 min. EDTA was removed with 1.0 mL of sterile saline.

- G4: **Preparation with sterile saline.** The root canal was filled with 3 mL saline. Before each file was changed, the same amount of the solution was renewed. A total amount of 15 mL of saline was used during preparation. A final rinse with 4.0 mL sterile saline was performed.

Root canals were then dried with paper points (Tanariman Industrial Ltda, Manacapuru, AM, Brazil). To allow analysis under the CLSM, AH Plus sealer (Dentsply/ De Trey, Konstanz, Germany) was labeled with rhodamine B (Vetec Química Fina Ltda, Duque de Caxias, RJ, Brazil) to an approximate concentration of 0.1%. The sealer was introduced into the root canals with a size #110 calibrated gutta-percha point (Dentsply Ind Com Ltda, Petrópolis, RJ Brazil). Canals were filled by the lateral compaction technique using a finger spreader size C (Maillefer Instruments). Accessory gutta-percha (Dentsply Ind Com Ltda) points were used until the entire length of the root canal was filled.

Radiographs were taken in the mesio-distal and bucco-lingual directions to evaluate the quality of root filling regarding homogeneity and apical extension. Canal filling was complemented if voids were detected in order to obtain well-compacted fillings. The excess of gutta-percha was removed using a heated plugger and vertical compaction was performed at the orifice level. Teeth were stored in an incubator for 7 days at 37 °C and 100% relative humidity.

After this, the teeth were sectioned using a 0.3-mm Isomet saw (Isomet; Buehler) at 200 rpm and continuous water-cooling to prevent frictional heat. Each specimen was horizontally sectioned at 3, 8 and 12 mm from the apex. In this manner, three, 2-mm-thick slices were obtained per root. Surfaces were polished with Arotec paste (Arotec, Cotia, SP, Brazil) in order to eliminate dentin debris generated during the cutting procedures.

The dentin segments were examined on Olympus Fluoview 1000 scanning confocal microscope (Olympus Corporation, Shinjuku-ku, Tokyo, Japan). The respective absorption and emission wavelengths for rhodamine B were 540 nm and 590 nm. A total of 120 sections were evaluated at the 3, 8 and 12 mm levels. The recorded images were 70 µm deep (800 x 800 pixels). Due to the wide diameter of the bovine root canal, the entire circumference was scanned at 10x and the most representative region (512 x 512 pixels) was selected for measuring the sealer's penetration area.

Each image was analyzed using Adobe Photoshop software v. 8.0 (Adobe Systems, San Jose, CA, USA). The Color Range tool was used to select the endodontic sealer penetration region into dentinal tubules. This tool marks all red pigments of the image and presents the values in pixels. The total area was set and values were converted into cm (Excel, Microsoft Corporation, Redmond, WA, USA). Normality was assessed using the Kolmogorov-Smirnov test. Statistical significance for the sealer penetration area was determined among groups, using one-way ANOVA followed by Tukey test. For thirds comparison in each group, data were statistically analyzed using Friedman test. Significance level was set at $p<0.05$.

Results

The results are presented in Table 1. The CHX/EDTA group (G2) presented the best results regarding the sealer's penetration depth ($p<0.05$; ANOVA – Tukey tests). According to this criterion, no differences were found among the other groups. There were also no statistical differences among the thirds in each group ($p>0.05$; Friedman test).

Figure 2 shows representative patterns of sealer penetration depth in the cervical third (12 mm). AH Plus displayed different amounts of penetration into dentinal tubules and the increased penetration area in the groups where EDTA was used is remarkable (Figures 2a and 2c).

Discussion

Confocal laser scanning microscopy has been used to verify the overlapping of sealers into dentinal tubules[17]. According to Ordinola-Zapata et al.[17] (2009), CLSM offers

Table 1. Comparative mean values and standard deviations (SD) from the total area (cm^2) of sealer penetration into dentinal tubules according to the groups and teeth thirds.

	Cervical Mean (SD)	Medium Mean (SD)	Apical Mean (SD)	p	TOTAL Mean (SD)
G1	78.37	92.11	102.57	0.122	91.02^{AB†}
CX	(±79.38)	(±28.84)	(±61.84)		(±59.17)
G2	133.05	159.28	124.07	0.122	138.80^{C}
CX + EDTA	(±30.54)	(±52.35)	(±42.91)		(±44.07)
G3	104.32	59.64	132.81	0.082	98.92^{A}
Saline + EDTA	(±45.72)	(±17.89)	(±70.87)		(±56.96)
G4	61.33	74.50	57.83	0.273	64.55^{B}
Saline	(±26.90)	(±31.92)	(±21.58)		(±27.18)
p	-	-	-	-	0.000

Different superscript capital letters indicate statistically significant differences among groups (p<0.05).

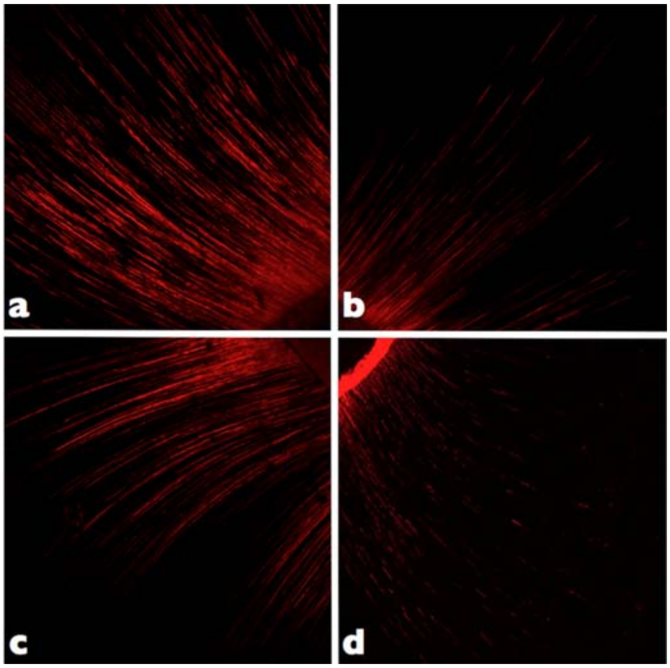
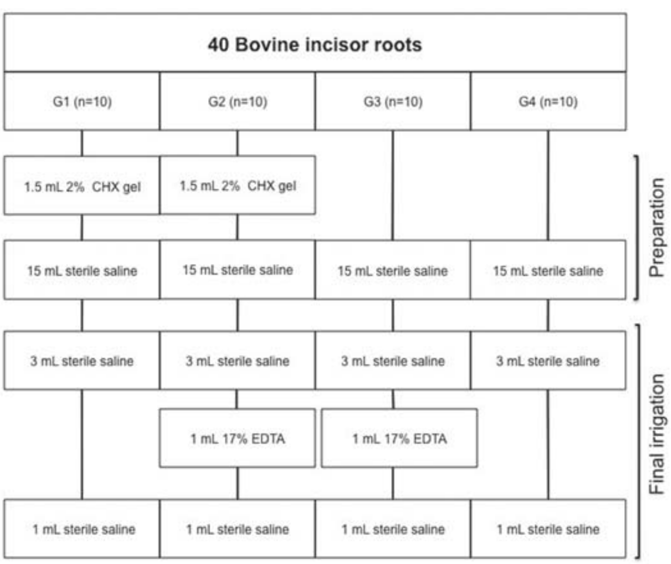


Fig. 2. Representative images of the sealer's penetration into the cervical dentinal tubules of each group: (a) 2% CHX gel and 17% EDTA; (b) 2% CHX gel; (c) Saline and 17% EDTA; (d) Saline. Confocal laser scanning microscopy images, 10x lens.



several advantages compared to scanning electron microscopy and other methodologies to assess penetration and the interface between dentin and endodontic filling materials. In the present study, smear layer removal by 17% EDTA promoted an increased penetration of the AH Plus sealer which could be observed by the addition of rhodamine B in the endodontic sealer. Due to its fluorescence, rhodamine B enables the overview of the filling adaptation in dentin cross-sectional slices.

Smear layer formation is a consequence of the biomechanical preparation and its remains may impair the penetration of root canal sealers into the dentinal tubules[5,18]. It has been advocated that it should be removed prior to the insertion of root filling[8]. Although CHX does not dissolve organic tissues, due to viscosity and rheological action, the gel formulation seems to promote a better mechanical cleansing of the root canal[19]. Additionally, it also decreases the smear layer formation[19]. Despite of the emphasized cleaning properties, the use of CHX gel did not favor the sealer's penetration without the use of a chelating agent in the present study.

Endodontic irrigants do not have all the desired physicochemical properties and need to act in association with other auxiliary chemicals. According to Hülsmann, Heckendorff and Lennon[20] (2003), EDTA has calcium ion chelating capacity. It is able to act on tooth mineral matrix, promoting the removal of smear layer formed during biomechanical preparation, and allows a better penetration of the sealer into the dentinal tubules[14]. In the present study, use of 17% EDTA after CHX or saline, improved the penetration of the filling material probably because of the greater cleanliness of the dentin walls.

Combination of NaOCl and CHX has been advocated to enhance their antimicrobial properties, and the advantage of using a final rinse with CHX would be the prolonged antimicrobial activity due to CHX substantivity[21]. However, it is already known that the interaction between NaOCl and CHX produces a reddish-brown precipitate containing the suspected carcinogen p-chloroaniline[22]. In addition, this precipitate appears to interfere with the penetration of the filling material into the dentinal tubules[23].

Rasimick et al.[24] (2008) evaluated the white precipitate formed by the interaction between CHX and EDTA. More than 90% of the precipitate's mass was found to be EDTA or CHX. Parachloroaniline, the potentially carcinogenic decomposition product of CHX, was not detected in this precipitate. Based on the results, CHX forms a salt with EDTA rather than undergoing a chemical reaction[24]. The clinical significance of the EDTA/CHX precipitate is largely unknown. Furthermore, it is unknown if any adhering precipitate interferes with the apical seal[24]. As a result, we chose to remove CHX with saline prior to the use of EDTA in order to avoid precipitate formation, which could impair the sealer penetration.

Ideally, endodontic sealers should seal the canal laterally and apically and have good adaptation to the root canal dentin[18]. According to Tay et al.[25] (2005), the filling critical zone is located at the sealer/dentin interface. The epoxy resin-based sealer, AH Plus appears to provide long-term dimensional stability, improved adhesion to root canal walls and presents adequate flow rate[26], when compared to other commonly employed endodontic sealers.

Mechanical adhesion occurs by entrapment of a material into another body, within natural or artificial cavities. For adhesion to occur, it is necessary that the materials to be adhered are sufficiently close to each other[14]. Nunes et al.[14] (2008) confirm that the presence of smear layer affects negatively the adhesion of root canal sealers because it forms an interface between the sealing material and dentin, hindering or impeding sealer penetration into the dentinal tubules. The authors also state that AH Plus has better penetration into the microirregularities because of its creep capacity and long setting time, which increases the mechanical inter-locking between sealer and root dentin[14]. This fact, together with the cohesion among sealer molecules, increases the resistance to removal and/or displacement from dentin[20], which can be translated as greater adhesion[14].

Kandaswamy et al.[27] (2011) and Ravikumar et al.[28] (2014) have evaluated the bond strength of resin-based sealers after treating with different final irrigants and concluded that EDTA improved the bond strength of endodontic sealers, which can be explained by the better removal of smear layer by 17% EDTA.

Do Prado, Simão and Gomes[29] (2013) found that the irrigation protocols influenced the bond strength of the resin sealers to dentin. In the gutta-percha/AH Plus groups, the bond strength was higher when NaOCl was combined with phosphoric acid or the CHX with EDTA. The use of CHX as a final irrigant did not affect negatively the bond strength.

Some studies evaluate the sealers' penetration linearly[17,19]. However, it is known that variations in dentinal tubules density occur along the root canal as well as the presence of dentinal sclerosis, which may eventually interfere with the sealer's penetration. Accordingly, Ordinola-Zapata et al.[17] claim that penetration is not uniform around the root canal walls. For this reason, we consider that sealer's penetration capability is better represented by the evaluation of the mean area instead of considering the points of greatest penetration, as done by Ordinola-Zapata et al.[17].

To minimize the limitations of an *in vitro* model, single-rooted bovine teeth were selected for this study. Camargo[30] (2007) reports the ethical aspects of using human teeth in dental research and suggests the use of bovine teeth. Despite presenting a higher number of dentinal tubules compared to human teeth[30], the use of bovine teeth may be accepted. After all, the purpose was not to compare the penetration of different sealers, but the 17% EDTA influence in the penetration of the same sealer.

Based on the present results, even with the AH Plus favorable properties, the maintenance of smear layer reduced the sealer penetration rate into dentinal tubules. For this reason, the use of 17% EDTA should be indicated for smear layer removal after root canal preparation with 2% CHX gel. Based on these results, use of 17% EDTA should be indicated after root canal preparation with 2% CHX gel for smear layer removal, enhancing the AH Plus sealer penetration.

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ERRATUM

In Article 13 "Resin-based sealer penetration into dentinal tubules after the use of 2% chlorhexidine gel and 17% EDTA *in vitro* study" published in the Brazilian Journal of Oral Sciences, Volume 13 Number 4, page 308-313, on page 309 where it is seen: Figure 1 is duplicated, but with the correct label. Therefore, Figure 1 has been replaced by Flowchart which was on page 309, maintaining the same label. After the publication was detected that error in the figures.

Where it reads:

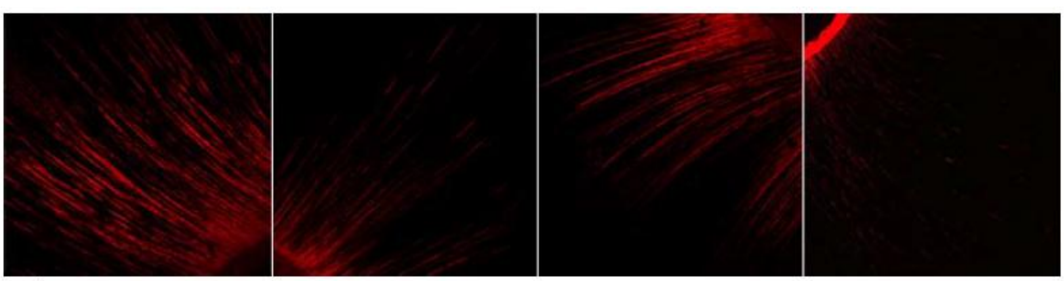

Fig. 1. Flowchart of preparation procedures.

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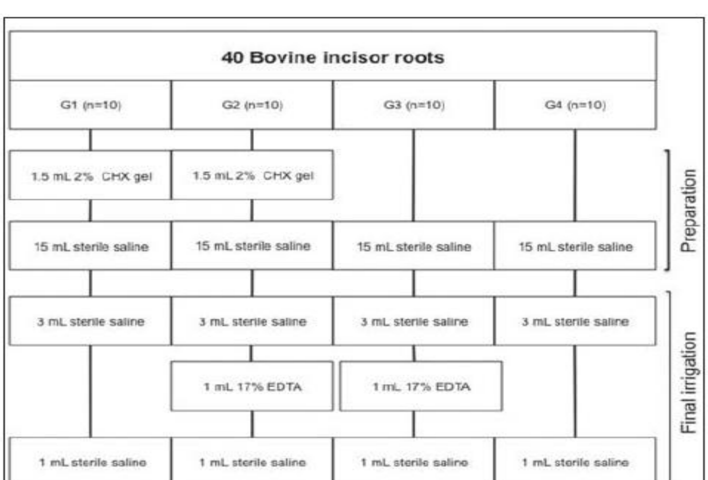

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