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A microleakage study of gutta-percha/AH Plus and Resilon/Real self-etch systems after different irrigation protocols

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ABSTRACT

The development and maintenance of the sealing of the root canal system is the key to the success of root canal treatment. The resin-based adhesive material has the potential to reduce the microleakage of the root canal because of its adhesive properties and penetration into dentinal walls. Moreover, the irrigation protocols may have an influence on the adhesiveness of resin-based sealers to root dentin. Objective: The objective of the present study was to evaluate the effect of different irrigant protocols on coronal bacterial microleakage of gutta-percha/AH Plus and Resilon/Real Seal Self-Etch systems. Material and Methods: One hundred ninety pre-molars were used. The teeth were divided into 18 experimental groups according to the irrigation protocols and filling materials used. The protocols used were: distilled water; sodium hypochlorite (NaOCl)+EDTA; NaOCl+H_3PO_4; NaOCl+EDTA+chlorhexidine (CHX); NaOCl+H_3PO_4+CHX; CHX+EDTA; CHX+ H_3PO_4; CHX+EDTA+CHX and CHX+H_3PO_4+CHX. Gutta-percha/AH Plus or Resilon/Real Seal SE were used as root-filling materials. The coronal microleakage was evaluated for 90 days against *Enterococcus faecalis*. Data were statistically analyzed using Kaplan-Meier survival test, Kruskal-Wallis and Mann-Whitney tests. Results: No significant difference was verified in the groups using chlorhexidine or sodium hypochlorite during the chemo-mechanical preparation followed by EDTA or phosphoric acid for smear layer removal. The same results were found for filling materials. However, the statistical analyses revealed that a final flush with 2% chlorhexidine reduced significantly the coronal microleakage. Conclusion: A final flush with 2% chlorhexidine after smear layer removal reduces coronal microleakage of teeth filled with gutta-percha/AH Plus or Resilon/Real Seal SE.

Keywords: Chlorhexidine. Dental leakage. Root canal irrigants. Root canal obturation.

INTRODUCTION

The major aim of root canal therapy is to prevent and treat periradicular inflammation by eliminating microorganisms from the root canal system. The methods commonly used for this purpose include root canal preparation using different instruments and irrigants, adequate filling, and coronal restoration[1,14].

Chemical irrigants are essential for successful debridement of root canals during cleaning and shaping procedures[11]. They are used during chemo-mechanical procedures not only as antimicrobial agents, but also to lubricate the dentinal walls, flush out debris and dissolve organic and inorganic components of the smear layer, thus cleaning the dentin surface[2,17]. Different irrigants have been proposed and used, including: 5.25% sodium hypochlorite, 2% chlorhexidine, 17% EDTA, 10% citric acid and 37% phosphoric acid solution[17,18,20].

Chlorhexidine has been used as irrigant during root canal therapy because of its antibacterial effects, substantivity, and relative absence of cytotoxicity, even though this solution is unable

to dissolve the tissue. Additionally, chlorhexidine has been suggested as a final irrigant[30]. Regarding its use as final irrigant, a final flush with 2% chlorhexidine favors the wettability of AH Plus and Real Seal SE sealers on the dentin surface. Furthermore, it was verified that the bond strength of ActiV GP, a glass ionomer based system, was improved by using 2% chlorhexidine in the final irrigation after 17% EDTA.

The development and maintenance of the sealing of the root canal system is the key to the success of root-canal treatment. The resin-based adhesive material has the potential to reduce the microleakage of the root canal because of its adhesive properties and penetration into dentinal walls[25]. Moreover, the irrigation protocols may have an influence on the adhesiveness of resin-based sealers to root dentin[8].

A variety of experimental models are used to detect and measure any leakage along endodontic fillings, such as dye penetration, clearing of the teeth, radioisotope tests, bacterial penetration, electrochemical tests, fluid filtration, and glucose penetration model[12,24].

The aim of the present study was to evaluate the effect of different irrigant protocols on coronal bacterial microleakage of gutta-percha/AH Plus and Resilon/Real Seal Self-Etch systems.

MATERIAL AND METHODS

Sample preparation

One hundred ninety single-rooted pre-molars with straight roots, mature root apices and similar anatomical characteristics were used in this study. All instruments used in the root canal preparation were sterilized previously to the procedure. The teeth were positioned on a metallic apparatus that allowed for all procedures to be carried out without manual contact with the roots. Conventional access was performed using high-speed diamond burs. A size 10 K-file (Dentsply Maillefer, Petrópolis, Rio de Janeiro, Brazil) was used to verify the patency of the canals and to determine the total length of the root canal, i.e. the work length. This was observed when the instrument reached the apical foramen. Next, the foramina were standardized by using a size 20 K-file and root canals were shaped by using MTwo NiTi rotary system (VDW, Münich, Bavaria, Germany). The sequence employed was the following: 10/.04, 15/.05, 20/.06, 25/.06, 30/.05, 35/.04, 40/.04, and 25/.07. The teeth were divided into groups of ten according to the irrigation regimen (Figure 1) and root canal filling.

Before the insertion of each file, 2% chlorhexidine (CHX) gel (Drogal, Piracicaba, São Paulo, Brazil) or 5.25% sodium hypochlorite (NaOCl) (Drogal, Piracicaba, São Paulo, Brazil) were used as chemical-auxiliary substance. Once the preparation was finished, 10 mL of distilled water (DW) was used to remove the chemical-auxiliary substance.

Groups	Chemical-auxiliary substance	Intermediate flush	Smear layer removal	Final flush
Distilled water (DW)	6 mL DW	10 mL DW	3 mL DW	10 mL DW
NaOCl/EDTA	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 17% EDTA	10 mL DW
$NaOCl/H_3PO_4$	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	10 mL DW
NaOCl/EDTA/CHX	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 17% EDTA	5 mL DW + 5 mL 2% CHX solution
$NaOCl/H_3PO_4$/CHX	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	5 mL DW + 5 mL 2% CHX solution
CHX/EDTA	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 17% EDTA	10 mL DW
CHX/H_3PO_4	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	10 mL DW
CHX/EDTA/CHX	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 17% EDTA	5 mL DW + 5 mL 2% CHX solution
CHX/H_3PO_4/CHX	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	5 mL DW + 5 mL 2% CHX solution

*CHX - chlorhexidine

Figure 1- Protocols for irrigation

Next, 17% EDTA or 37% phosphoric acid solution (Drogal, Piracicaba, São Paulo, Brazil) was used for 3 minutes to remove the smear layer, with changes every 1 minute (1 mL *per* minute). Again, DW was used to remove the remaining solution. Finally, 2% chlorhexidine solution (Drogal, Piracicaba, São Paulo, Brazil) was used for final flush. During the chemo-mechanical preparation, all teeth had their apices sealed with utility wax (Technew, Rio de Janeiro, Rio de Janeiro, Brazil) to prevent flow through them.

The root canals were dried with sterilized medium-sized paper points (Endopoints, Paraíba do Sul, Rio de Janeiro, Brazil). Groups 1 to 9 had the canals filled with gutta-percha cones (Odous, Belo Horizonte, Minas Gerais, Brazil) associated with AH Plus sealer (Dentsply, Petrópolis, Rio de Janeiro, Brazil), whereas Groups 10 to 18 had the canals filled with Resilon associated with Real Seal SE (SybronEndo, Orange, California, USA). The cones packages were opened and they were used immediately, and the sealers were manipulated in sterile plates according to the manufacturer's recommendations.

A System-B endodontic heat source unit (SybronEndo, Orange, California, USA) was used to down-pack and Obtura System (J Morita, São Paulo, São Paulo, Brazil) to backfill. All procedures were conducted at the laminar flow cabinet. Subsequently, teeth were radiographed mesiodistally and buccolingually to assess the quality of the filling.

All roots were kept on gauzes at 37°C and 100% humidity for 2 weeks before leakage measurement in order to allow the materials to set properly.

Next, the external root surface of all specimens was sealed with two layers of red nail varnish (Revlon, New York, New York, USA), except the last 1 mm of the apex.

Analysis of coronal bacterial microleakage

Figure 2a illustrates the apparatus used to evaluate coronal leakage[10]. Glass vials with rubber stoppers were adjusted for use. By using a shear, a hole was made at the center of each rubber stopper (Figure 2b), through which each tooth was inserted under pressure up to the cementoenamel junction, so that its crown was outside the vial and its root inside (Figure 2c). Cyanoacrylate glue (CG) was applied at the interface between tooth and stopper for sealing[15]. Cylinders prepared from 10 mL plastic syringes were adapted to the outer surface of the stoppers to create a chamber around the crown of the tooth (Figure 2d). Again, CG was used at the interface between syringes and stoppers, followed by a Parafilm layer (American National CanTM, Menasha, Wisconsin, USA) to help in the sealing. The syringe/stopper/tooth sets were submitted to sterilization by gamma-rays (Embrarad, São Paulo, São Paulo, Brazil). The glass flasks were autoclaved at 121°C for 15 minutes.

The sterilized glass flasks were then filled with sterile Brain Heart Infusion broth (BHI; Oxoid, São Paulo, São Paulo, Brazil) so that a 2-mm length of root apex was immersed in the broth. CG and Parafilm were used to seal the interface between stopper and flask (Figure 2e). In all samples, in order to ensure the efficiency of the seal, 2 mL of 1% sterile methylene blue dye was placed into the tube until the coronal portion of the sample was reached[15]. The flasks were then incubated at 37°C for 3 days to ensure sterilization. After the third day, the methylene blue was removed with sterile distilled water by using a pipette. When a green medium was observed the specimen was discarded. The green color was due to the blue dye association

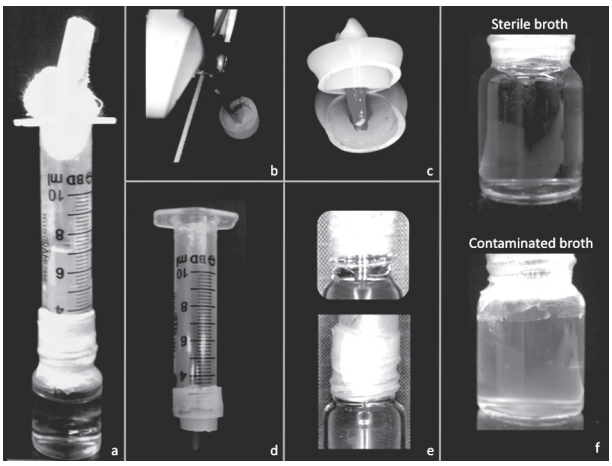
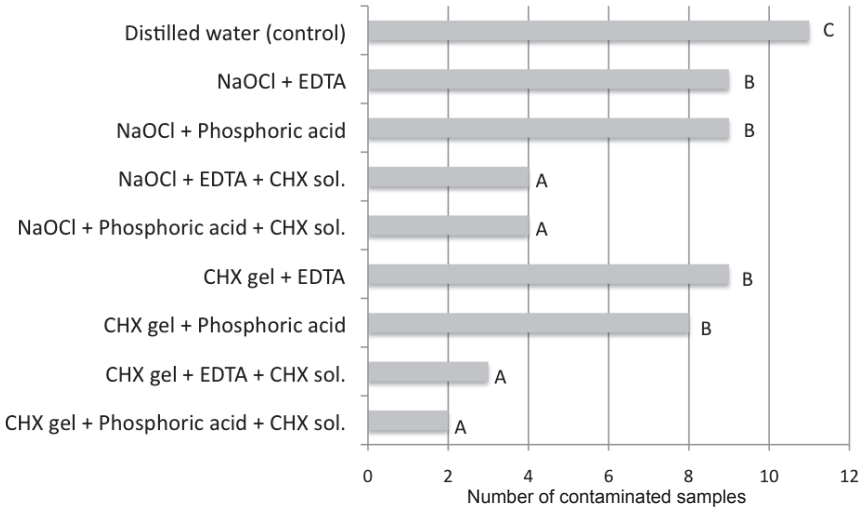


Figure 2- The apparatus used to evaluate coronal leakage (a). By using a shear, a hole was made at the center of the rubber stopper (b), through which the tooth was inserted (c). A cylinder prepared from a 10 mL plastic syringe was adapted to the outer surface of the stopper to create a chamber around the crown of the tooth (d). The glass flask filled with BHI broth was connected to the syringe/stopper/tooth set (e). Turbidity in the BHI broth was evaluated (f)



*Different letters indicate statistically significant values (P<0.05)

Figure 3- Graph showing the number of contaminated samples according to the irrigation protocol

with the yellow medium.

For preparation of microbial medium[28], *Enterococcus faecalis* (ATCC 29212) was grown on BHI agar plates (Brain Heart Infusion agar; Oxoid, São Paulo, São Paulo, Brazil) and supplemented with 5% sheep blood for 24 hours at 37°C in CO_2. Then, the *Enterococcus faecalis* was inoculated into tubes containing 5 mL sterile BHI suspension, which were adjusted spectrophotometrically at 800 nm (OD800) to a turbidity of $1.5x10^8$ colony-forming units (CFU)/mL. With the aid of a pipette, 5 mL of the suspensions were placed into the syringe apparatuses (in the upper region, removing the gauze stop), which were left at 37°C for 90 days in CO_2 and checked daily for turbidity in the BHI broth. When turbidity (Figure 2f) was observed, the day was recorded.

Every 2 days, 3 mL of the suspension (BHI+microorganisms) were removed from the chamber and replaced by 3 mL of BHI to avoid saturation and to confirm the growth of *Enterococcus faecalis*[5].

After this period, all apparatuses were opened to evaluate the sterile hood. Positive cultures were confirmed by using Gram staining (gram-positive), colony morphology on blood agar plates (cocci) and biochemical identification kits (Rapid ID 32 Strep, BioMérieux SA, Marcyl'Etoile, Charbonnieres-les-Bains, France).

Ten samples were used as positive (n=5) and negative (n=5) controls. The positive controls consisted of instrumented teeth without obturation, while negative controls consisted of sound teeth, both with no contamination.

The results were analyzed with Kaplan-Meier survival test, Kruskal-Wallis and Mann-Whitney tests ($p<0.05$).

RESULTS

No differences were found following the use of chlorhexidine or NaOCl associated with EDTA or phosphoric acid for smear layer removal. However, it was clearly observed that groups receiving a final flush with chlorhexidine showed a lower number of contaminated samples.

Figure 3 shows the number of contaminated samples according to the irrigation protocol.

Regarding the root canal filling system (gutta-percha/AH Plus and Resilon/Real Seal SE), there was no statistically significant differences in relation to the coronal microleakage.

Figure 4 shows the number of contaminated samples in relation to time. Statistic analysis of the contamination days revealed difference in the groups receiving the final flush with chlorhexidine. Chlorhexidine groups started to contaminate only in the 6th week, while in the others the microbial growth was verified in the 1st or 2nd week. The control apparatuses showed broth turbidity within 1 day of incubation in all samples, whereas no microbial growth was found in the negative control throughout the experiment.

DISCUSSION

Leakage of the root canal has been defined as the passage of bacteria, fluids, and chemical substances between the dentinal wall and the root canal filling material, and results from the presence of space at the interface of the filling material and the root canal wall. This space can result from deficient adaptation of the filling material to the root dentin, solubility of the sealer, or sealer expansion or shrinkage. There are 2 possibilities of leakage: at the interface between the main filling material and sealer, or between the sealer and root canal wall[26].

GROUPS	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	WEEK 7	WEEK 8	WEEK 9	WEEK 10	WEEK 11	WEEK 12	WEEK 13
DW/GPAH	0/10	2/10	0/10	2/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
NaOCl/EDTA/GPAH	0/10	1/10	1/10	0/10	0/10	1/10	0/10	0/10	1/10	0/10	0/10	0/10	1/10
NaOCl/H_3PO_4/GPAH	0/10	2/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
NaOCl/EDTA/CHX/GPAH	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	1/10
NaOCl/H_3PO_4/CHX/GPAH	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
CHX/EDTA/GPAH	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10	2/10
CHX/H_3PO_4/GPAH	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	1/10
CHX/EDTA/CHX/GPAH	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10
CHX/H_3PO_4/CHX/GPAH	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
DW/RRS	2/10	2/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	1/10
NaOCl/EDTA/RRS	1/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10
NaOCl/H_3PO_4/RRS	1/10	1/10	0/10	0/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
NaOCl/EDTA/CHX/RRS	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
NaOCl/H_3PO_4/CHX/RRS	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	1/10
CHX/EDTA/RRS	0/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10
CHX/H_3PO_4/RRS	0/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10	0/10	1/10
CHX/EDTA/CHX/RRS	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
CHX/H_3PO_4/CHX/RRS	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10

DW- Distilled water; CHX- chlorhexidine; GPAH- gutta-percha/AH Plus; RRS- Resilon/Real Seal SE

Figure 4- Number of contaminated samples *per* week

In the present study, the coronal bacterial microleakage was used. This methodology is reproducible and has clinical relevance, presenting reliable data and simulating clinical conditions[7,13,16]. This methodology allows the observation of the exact day of the sample contamination, showed by the broth turbidity.

The 37% phosphoric acid was evaluated in comparison with EDTA because this solution is effective for smear layer removal, showing better results than EDTA in the apical third during 3 minutes[19]. Additionally, the protocols associating NaOCl with phosphoric acid showed higher bond strength values when compared with NaOCl associated with EDTA[20]. Although phosphoric acid had showed better performance in removing the smear layer from the apical third[19], it did not influence the results of coronal leakage.

The 2% chlorhexidine, a cationic bisbiguanide, was used here for the final flush after smear layer removal. This substance has a broad-spectrum MMP-inhibitory effect that improves the integrity of the hybrid layer[6] and the resin-dentin bond stability[23]. Additionally, the use of CHX increases the wettability of endodontic sealers on dentin[3], which can be explained by the presence of surface surfactant in CHX, increasing the surface energy and promoting higher wetting ability to dentin. Our results showed that a final flush with chlorhexidine significantly reduced the coronal microleakage, when compared to the other experimental groups. It might be explained by the fact that chlorhexidine is adsorbed onto dentin and prevent microbial colonization[21], increasing the time required for recontamination of filled root[23] up to 12 weeks due to its substantivity[21].

Regarding the effect of a final flush with chlorhexidine on adhesion, previous studies showed that this solution did not affect the bond strength of resin-based sealers[17,20] and improved the adhesion of hydrophilic bonded materials such as ActiV GP and Epiphany[23].

NaOCl was not used as a final irrigant because a previous study showed that this solution decreased the bond strength between epoxy resin and dentin and increased the leakage[17].

Regarding the root canal filling system (gutta-percha/AH Plus and Resilon/ Real Seal SE), there was no statistically significant differences in relation to the coronal microleakage, agreeing with previous findings[4,9,26]. However, some studies reported that Resilon/Epiphany sealer was more efficient than gutta-percha/AH Plus[12,29], whereas others found the opposite[22,27].

CONCLUSION

In conclusion, a final flush with 2% chlorhexidine after smear layer removal reduces coronal microleakage of teeth filled with gutta-percha/AH Plus or Resilon/Real Seal Self-Etch. The bacterial leakage methodology used made possible the verification of this behavior.

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