Influence of antimicrobial solutions in the decontamination and adhesion of glass-fiber posts to root canals

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ABSTRACT

Objective: This study evaluated the effect of root canal disinfectants on the elimination of bacteria from the root canals, as well as their effect on glass-fiber posts bond strength. Material and Methods: Fifty-three endodontically treated root canals had post spaces of 11 mm in length prepared and contaminated with E. faecalis. For CFU/ml analysis, eight teeth were contaminated for 1 h or 30 days (n=4). Teeth were decontaminated with 5% NaOCI, 2% CHX, or distilled water. As control, no decontamination was conducted. After decontamination, sterile paper points were used to collect samples, and CFU/ml were counted. For push-out, three groups were evaluated (n=15): irrigation with 2.5% NaOCI, 2% CHX, or sterile distilled water. A bonding agent was applied to root canal dentin, and a glass-fiber post was cemented with a dual-cured cement. After 24 h, 1-mm-thick slices of the middle portion of root canals were obtained and submitted to the push-out evaluation. Three specimens of each group were evaluated in scanning electron microscopy (SEM). Data were analyzed with one-way ANOVA and Dunnett's T3 test (α =0.05). Results: The number of CFU/ml increased from 1 h to 30 days of contamination in control and sterile distilled water groups. Decontamination with NaOCI was effective only when teeth were contaminated for 1 h. CHX was effective at both contamination times. NaOCl did not influence the bond strength (p>0.05). Higher values were observed with CHX (p<0.05). SEM showed formation of resin tags in all groups. Conclusion: CHX showed better results for the irrigation of contaminated root canals both in reducing the bacterial contamination and in improving the glass-fiber post bonding.

Keywords: Shear strength. Post and core technique. Sodium hypochlorite. Chlorhexidine.

INTRODUCTION

Endodontically treated teeth with great coronal destruction generally need glass-fiber posts for better retention of crowns or resin composite restorations. Although there is evidence to support the idea that endodontically treated teeth are not reinforced by posts²¹, it is recognized that fiber posts better distribute the stress generated on teeth under function²². On the other hand, most failures involving the reconstruction of these teeth are due to bond strength failure in luting the post with resin-based cements²⁰. The luting relies on the formation of a hybrid layer into dentin, which depends on the treatment of dentinal surface, the flow of the resin cement into the root canal, as well as the interaction between the adhesive material and intertubular dentin⁹. However, the treatment of the post space is an aspect that is normally overlooked when considering an oral infected environment and the exposure of dentin to saliva.

The contamination of the root canal system with saliva has been identified as a potential cause of endodontic failure, especially when the guttapercha is removed from the coronal and middle thirds, leaving only a remnant of 3-5 mm^{10,17}. For

this reason, root canal cleanliness before adhesive luting is an indispensable step for preventing leakage and endodontic treatment failure. Sodium hypochlorite (NaOCI) and chlorhexidine (CHX) are widely used with the aim of biofilm removal⁵, solvency of organic tissues, and neutralization of toxic products³⁰. However, the long exposure to NaOCI could negatively affect adhesive techniques due to the superficial oxidation effects on the polymerization of resin monomers, decreasing the bond strength4. Some concerns regarding the influence of irrigating solutions on the bond strength of endodontic sealers, as well as of materials used to repair furcation perforations, have been raised^{11,19}. However, the effect of these solutions when there is contamination of the post space before the luting of fiber-reinforced posts still needs to be addressed.

The aim of this study was to evaluate the influence of these solutions on bond strength of cemented glass-fiber posts, including bonding failures. The formation of resin tags into the dentinal tubules, and the effectiveness of NaOCI and CHX to decontaminate root canals exposed to Enterococcus faecalis were also evaluated. The null hypotheses were: there would be no influence of the irrigating solutions on the shear bond strength of glass-fiber posts luted to contaminated root canals; and there would be no difference between the solutions employed to decontaminate root canals.

MATERIAL AND METHODS

Root canal preparation and post space preparation

Fifty-three sound human maxillary canines had their crowns sectioned to obtain roots of 16 mm in length. Teeth were sterilized in a steam autoclave at 121°C for 20 min and then maintained in 0.9% sterile saline solution at 9°C. The Research Ethics Committee of Positivo University, Curitiba, PR, Brazil approved this study under the protocol #77947.

The working length was established at 15 mm and final diameter at 0.60 mm. A ProFile.04 rotary system (Dentsply Maillefer, Balaigues, Switzerland) for the crown-down technique was used with an electric motor (X Smart, Dentsply Maillefer, Balaigues, Switzerland) at 250 rpm and torque of 1.5 N. The specimens were filled with lateral condensation employing #60 gutta-percha points (Dentsply Maillefer, Petrópolis, RJ, Brazil), accessory MF cones (Dentsply Maillefer, Petrópolis, RJ, Brazil), and AH Plus sealer (Dentsply DeTrey, Konstanz, Germany).

Post spaces were prepared by removing the filling material with #3 and #4 Largo drills (Dentsply-Maillefer, Petrópolis, RJ, Brazil), at an 11-mm-length, leaving 4 mm of apical seal. The preparation was completed with the specific drill for the glass-fiber post (WhitePost DC2, FGM, Joinville, SC, Brazil).

For the evaluation of the shear bond strength, forty-five teeth were contaminated for 1 h with Enterococcus faecalis (ATCC 19433). Before contamination, teeth's external surfaces were protected with nail polish varnish (Colorama, Maybelline, New York, NY, USA).

E. faecalis were cultivated in 5 ml of sterilized brain-heart infusion (BHI) broth and incubated for 24 h at 37°C in suitable respiratory conditions. The test tubes containing the BHI broth with the cultivated bacteria were adjusted to the 0.5 tube of the McFarland scale at a concentration of approximately 1.5×108 cells/ml with the aid of the same diluent. Each tooth was individually placed in a sterile 1.5 ml Eppendorf tube filled with bacterial inoculum of *E. faecalis*. To ensure proper contact to the root canal walls, the inoculum was firstly injected with a 20-ml-sterile-syringe (BD, São Paulo, SP, Brazil) and a 17-mm 30-gauge cannula (NaviTip, Ultradent, South Jordan, UT, USA). Samples were incubated at 37°C for 1 h.

The contaminated specimens were divided into three groups (n=15): G-I - distilled water (control), G-II - 2.5% NaOCl, and G-III - 2% CHX (Clorhexidina S, FGM, Joinville, SC, Brazil). Each specimen had its canal irrigated with 10 ml of each solution for approximately 1 min. Glass-fiber posts (WhitePost DC2, FGM, Joinville, SC, Brazil) were cemented with a dual-cured resin-based cement (AllCem, FGM, Joinville, SC, Brazil) following the manufacturer's recommendations: 15-s-etching with 37% phosphoric acid, 20-s-washing with sterile water and a syringe, and drying with paper cones. A two-step etch-and-rinse adhesive (Ambar, FGM, Joinville, SC, Brazil) was applied using a long applicator tip, followed by 15-s-air-drying and 20-s-light-curing with an LED device (LD Max, Gnatus, São Paulo, SP, Brazil; 400 mW/cm²; 470 nm). The silane (Prosil, FGM, Joinville, SC, Brazil) was applied at the glass-fiber post followed by the application of the same adhesive. The resin cement was inserted into the canal with an auto-mixture tip. The glass-fiber post was placed in position and the cement was light-cured for 40 s. Samples were stored in distilled water at 37°C for 24 h.

Roots were then transversely sectioned into 1-mm-thick slices (Isomet 1000 South Bay Technology, San Clemente, CA, USA) after removing the first 4 mm of the coronal region. One slice corresponding to the middle third of the root canal *per* tooth was subjected to the push-out evaluation on a testing machine (Emic DL 2000, São José dos Pinhais, PR, Brazil) by applying a

compression load through a cylindrical tip of 0.6 mm diameter at 0.5 mm/min. Data were obtained in MPa and statistically analyzed by one-way ANOVA and Dunnett's T3 test (α =0.05).

The fracture displacement area was analyzed with a photomicroscope (SZX9 Olympus Optical Microscope, Tokyo, Japan) with 16× magnification. Failures were classified as adhesive (cement detachment of the tooth), cohesive (fracture between post and cement), or mixed (adhesive and cohesive failure in the same specimen).

Three specimens of each group were randomly chosen for scanning electron microscopy (SEM) analysis at 500× and 3000× magnification. The qualitative analysis included the observation of the formation of tags into the dentinal tubules.

To compare the antimicrobial effectiveness of the irrigation procedures, eight teeth were contaminated with Enterococcus faecalis (ATCC 19433) for two different periods: 1 h (n=4) or 30 days (n=4). Teeth were decontaminated with 2.5% NaOCI, 2% CHX (Clorhexidina S, FGM, Joinville, SC, Brazil), or sterile distilled water. As control, no decontamination was conducted in one tooth per period. Samples were incubated at 37°C for 1 h or 30 days. Every two days, 0.3 ml was removed from each Eppendorf and replaced with 0.3 ml of sterile BHI broth to maintain viable bacteria.

After contamination, microbial samples were collected from root canals. Three #60 sterile paper points (Dentsply Maillefer, Petrópolis, RJ, Brazil) were used to collect samples from each tooth. Microbial samples were then placed in test tubes containing sterile BHI for the colony forming units (CFU) counting per inoculation on agar plate.

The decontamination was conducted with a sterile 20-ml-syringe and a 17-mm 30-gauge cannula (NaviTip, Ultradent, South Jordan, UT, USA). Each tooth had its root canal irrigated for 1 min with 10 ml of the evaluated solution in movements of insertion and removal to create a flux inside the root canal. The roots were finally irrigated with 10 ml of sterile distilled water.

After decontamination, microbial samples were collected and placed in test tubes containing sterile BHI, agitated in Vortex, and incubated at 35°C for 24 h. CFU/ml were counted through a spread plate technique in which the microbial BHI broth was successively diluted until 10⁻⁷. The count of CFU/ml was conducted after incubation for 48 h at 37°C.

RESULTS

Mean values and standard deviations of shear bond strength are shown in Table 1. There were significant differences between the effect of NaOCI, CHX, and water (p=0.001). The highest bond strength values were observed when CHX was used as the decontamination solution (p<0.05).

For all groups the majority of failures were mixed, occurring between the resin-cement and the dental structure (Table 1). All groups showed formation of resin tags in the dentinal tubules (Figure 1).

The number of CFU/ml increased from 1 h to 30 days of contamination in both control (without decontamination) and sterile distilled water groups. The use of sterile distilled water increased the number of CFU/ml. The decontamination with NaOCI was effective only when the contamination occurred for 1 h, while CHX was effective at both contamination times (Figure 2).

DISCUSSION

The null hypotheses evaluated were rejected, since the irrigation with CHX resulted in higher bond-strength and there were differences between the solutions employed. No statistical difference in the bond-strength between the irrigation with sterile distilled water and the decontamination with NaOCI was observed. It is known that the ionization of NaOCI generates sodium chloride and oxygen, which causes inhibition of interfacial polymerization of dental adhesives4. Moreover, residual reactive free radicals in dentin treated with NaOCI may compete with free radicals generated during the activation of light-cured adhesives, resulting in premature chain termination and incomplete $curing^{6,12,28}$. The idea that NaOCl could reduce the bond-strength of the glass-fiber post to the root canal was not confirmed by this study,

Table 1- Mean values (in MPa), standard deviations and mode of fractures distribution (%) of glass-fiber posts luted to contaminated root canal dentin as a function of the solution employed in the decontamination process

Decontamination solution	Mean±Standard deviation	Adhesive	Cohesive	Mixed
Water	2.70±1.0	10.0%	16.6%	73.3%
NaOCI	2.67±0.7	31.0%	0.0%	68.9%
CHX	3.76±0.6*	13.3%	30.0%	56.7%

^{*} Significant difference (p<0.05)

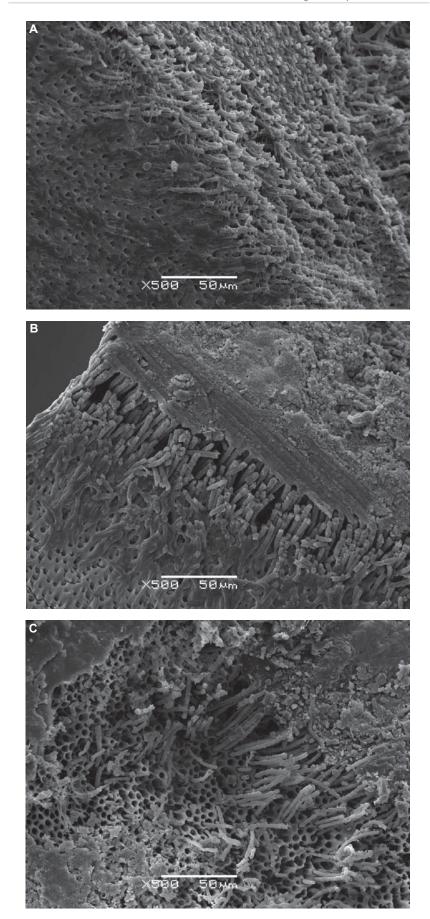


Figure 1- Representative images showing the presence of resin tags in the different groups evaluated: A) Distilled water, B) NaOCI, and C) CHX (500×)

	1 h	30 days
Control	1.06×1010	1.2×1011
(no decontamination)		
Water	1.43×1010	1.8×1011
NaOCI	0	1.74×1010
CHX	0	0

Figure 2- Number of CFU/ml

which is in disagreement with previous studies4,28. The short exposure time to NaOCI plus the action of phosphoric acid could explain these results. While increasing the application time of NaOCI resulted in a progressive decrease in shear-strength12, a 10-minute application results in deproteinization of collagen fibrils and compromises the adhesion process4. Therefore, the irrigation with NaOCl for 1 min would be insufficient to generate significant residues to significantly interfere with the bondstrength. Moreover, it has been suggested that the influence of the irrigation with NaOCI before the cementation of fiber-reinforced posts is adhesivedependent and may not occur with all adhesives8, which may also explain the results of the present study.

In the present study, 1-mm-thick slices obtained from removing the first 4 mm of the coronal region of the roots were used in the push-out evaluation. Although the evaluation of a root canal's coronal, middle, and apical thirds is common in push-out studies, it is also common to observe a regional variation, with the bond-strength decreasing from coronal to the apical region of the root canal^{2,18,29}. For this reason, only a slice corresponding to the middle third was evaluated.

The use of sterile distilled water after E. faecalis exposure, far from decontaminating, increased the number of CFU/ml. This result suggests that an antimicrobial solution should be used when there is contamination during the preparation of root canals to receive posts. It is important however to note that in the teeth used to evaluate the CFU/ml, phosphoric acid was not applied as in the bond-strength evaluation. It should also be pointed out that only eight teeth were used for CFU evaluation. This further evaluation was conducted to ensure that both NaOCI and CHX were effective antibacterial solutions.

The decontamination with CHX was more effective than with NaOCI, when contamination occurred for 30 days, which is in agreement with a previous study¹⁵. NaOCl solutions have the ability to dissolve organic matter as well as antimicrobial action, however, its penetration into the root canal dentin seems to be limited to ≈300 µm^{13,31} while bacteria have been found in deeper layers of ≈500 µm into infected dentin¹. Although the present study was not designed to evaluate the decontamination of deep dentin layers, it can be hypothesized that if bacterial contamination remains in these deep layers, the long-term behavior of the treatment may be jeopardized. Moreover, it has also been shown that at low concentrations, NaOCI may be ineffective against E. faecalis⁷. Thus, the results of the present study could be different if 5% NaOCl was used instead of 2.5% NaOCI. CHX, on the other hand, is an antiseptic of wide antimicrobial spectrum and substantivity. The substantivity is the prolonged association between CHX and the dental surface, with the material remaining active on the tooth surface for prolonged periods²³. This characteristic may add some advantage to CHX when compared to NaOCI solutions.

In the present study, 2% CHX has increased the bond strength. This result is in agreement with another study that evaluated the influence of CHX on the immediate bond strength of fiber-reinforce posts to root canal dentin, and observed a slight improve in bond strength for three of the four posts evaluated14. It should be noted, however, that for coronal dentin, 2% CHX do not affect immediate bond strength values in general, as compared to control groups (without the use of CHX¹⁶). Although CHX was used in the present study due to its potent antimicrobial effect, and only the immediate bond strength was evaluated, the long-term stability of the adhesive bonding to the root canal dentin could also be benefited²⁷. Dentin debonding is currently the main problem when resin-based materials are adhesively bonded to the dental structure. This process of debonding has been shown to be related to the degradation of collagen fibrils due to the activity of enzymes such as matrix metalloproteinases (MMPs) and cysteine cathepsins²⁵. The use of CHX to prevent hydrolysis of the collagen matrix at the hybrid layer is one of the approaches currently employed to improve the durability of dentin bonds^{3,25}.

It should be noted that a steam autoclave was used in the present study. The high temperatures could have altered collagen fibrils, impairing the adhesive process. At the same time, while autoclaving may end MMPs' enzymatic activities in dentin, dentinal gelatinases have detected even after the autoclave treatment of dentin²⁴. Although the irrigation with NaOCI did not influence the push-out bond-strength in the present study, the use of CHX seems to be more appropriate, since it showed a better decontamination property. Further studies with other adhesives and resin cements should be conducted, as the influence of NaOCl and CHX may be different with the use of other resin-based materials, since the use of CHX has also shown controversial results2.

CONCLUSION

The decontamination with NaOCI was effective only when the contamination occurred for 1 h, while CHX was effective at both contamination times. The bond-strength of the glass-fiber posts and resin cement to dentin was not negatively affected by the irrigation with NaOCI.

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