Nanoleakage of fiber posts luted with different adhesive strategies and the effect of chlorhexidine on the interface of dentin and self-adhesive cements

The aim of this in vitro study was to evaluate the nanoleakage of fiber posts luted using different adhesive strategies and to investigate the effect of 2% chlorhexidine (CHX) on nanoleakage at the resin-dentin interfaces of self-adhesive cements. The self-adhesive and etch-and-rinse adhesive groups tested demonstrated similar results with regard to nanoleakage. Pretreatment with CHX promoted an adequate seal at the resin-dentin interface for self-adhesive cements.

Fiber posts are widely used to restore endodontically treated teeth. Glass fiber posts are biocompatible, do not corrode, and offer the most favorable optical properties for reproducing the natural aspect of the restored tooth. Resin cements have been widely used for luting fiber posts due to their enhanced mechanical properties. Several factors play a role in the intraradicular bonding of resin-based materials. The peculiar histological characteristics of root dentin, the presence of endodontic smear layers (created either by endodontic instruments or modified by irrigants), and adverse geometric factors—such as the extremely high cavity configuration (C-factor) and the difficult to achieve direct irradiation by light in deep regions of the root canal—are consistent factors that negatively affect the bonding of glass fiber posts to root canal dentin. In addition, the type of bonding system used, the luting cement, and its cure may interfere with hybrid layer formation along the root canal walls, thus affecting post retention. This hybridization is critical in the apical third of the post space due to the difficulty in establishing adhesion in this area.

Traditional resin cements with chemical or dual activation are commonly used to overcome problems in supplying the necessary irradiation of light into the root canal. However, alternative adhesive strategies—such as luting systems with self-etching adhesives—are less complex since these self-etch adhesives are generally applied on dry dentin and do not require the additional step of rinsing with phosphoric acid, thus eliminating the problem of dentin wetness control. Compared to the traditional resin cements, self-etching adhesives require no previous treatment of the dental substrate, since the stages of acid etching and adhesive system application have been eliminated. The bonding mechanism of self-adhesive cements is based on micromechanical retention and chemical adhesion.

When self-adhesive cements are bonded to dentin, there is a rapid change in pH (ranging from 2.0 to 2.4) that causes an early activation of the matrix metalloproteinase (MMP), along with increased collagenolytic activity (approaching maximum levels). During dentin demineralization, latent MMP is denatured as more proteases are exposed. As a result, collagen fibrils that are not completely protected by resin monomers during dentin hybridization become highly susceptible to hydrolytic degradation.

Chlorhexidine (CHX) has been shown to have an inhibitory effect on endogenous collagenolytic activity in dentin. Although CHX diminishes the loss of bond strength over time, not much is known about the influence of a CHX solution—when applied prior to the cementation of indirect restorations—on the integrity of the bonded interface formed by self-adhesive cements to root dentin.

In 2009, Hiraishi et al speculated that the deterioration of the bonding efficacy of commercial self-adhesive luting cements might be related to the presence of moisture contamination on the dentin surface. Hence, it is of interest to examine the influence of different luting systems and the effect of 2% CHX on the nanoleakage at the cement-dentin interface.

**Materials and methods**

Thirty bovine roots were stored for 7 days in a saturated thymol solution at 5°C for disinfection and used within 1 week postextraction. The roots’ inclusion criteria were completely formed apices, without excessive root curvature, and root canals with a diameter smaller than the diameter of a Largo No. 5 bur (DENTSPLY Maillefer), cut to the length of 17 mm. Teeth were divided into 6 experimental groups (n = 3) and restored with different cementation techniques (Table 1).

**Endodontic treatment**

For endodontic treatment, a step-back preparation technique was used with stainless steel K-files and Gates-Glidden burs (No. 3-5) (Miltex, Inc.) at the working length; the roots were irrigated with distilled water after every change of instrument. Roots were dried with paper points and filled with gutta percha cones (DENTSPLY Maillefer) using the lateral condensation technique. The roots were stored in distilled water at 37°C.
Post luting procedures
The next step involved the removal of the gutta percha, leaving at least 5 mm of the endodontic filling at the apex of each canal. The post spaces were prepared to a distance of 10 mm from the cemento-enamel junction, using a No. 4 Largo drill (DENTSPLY Maillefer). The roots were separated randomly into 6 experimental groups (n = 3) according to the luting system used: Group 1, Scotchbond Multi-Purpose Plus (3M ESPE) chemical cure etch-and-rinse adhesive + RelyX ARC dual-cured cement (3M ESPE); Group 2, Clearfil SE Bond (Kuraray America, Inc.) self-etching adhesive + ED Primer (Kuraray America, Inc.) dual cure + Panavia F dual-cured cement (Kuraray America, Inc.); Group 3, Clearfil SE Bond + Panavia F; Group 4, Scotchbond Multi-Purpose Plus chemical cure + RelyX ARC; Group 5, RelyX U100 self-adhesive cement (3M ESPE); Group 6, RelyX Unicem (3M ESPE) self-adhesive cement.

The glass fiber posts (Reforpost, Angelus Industria de Produtos Odontológicos S/A) were cleaned with 70% alcohol for 1 minute, then dried. Afterward, a silane coupling agent (Silano Angelus, Angelus Industria de Produtos Odontológicos S/A) was applied on each post surface for 1 minute; then, the posts in each of the 6 groups were luted following their respective manufacturer’s instructions (Table 2). The materials were manipulated and inserted into the canal with a Centrix syringe with a metallic tip (Centrix, DFL Industria e Comercio). RelyX Unicem was applied with a capsule and elongation tip provided by the manufacturer. For activation purposes, the dual-cure cements and adhesive systems were cured from the top with a halogen curing light unit (Optilux 501, Kerr Corporation) at 600 mW/cm². To simulate clinical conditions, a wax protection barrier was applied to the external surface of the roots to prevent the passage of light.

CHX
To evaluate the effect of CHX in the self-adhesive cements, the prepared root canals were randomly divided into 4 subgroups: Subgroup A, RelyX Unicem; Subgroup B, 2% CHX + RelyX Unicem; Subgroup C, RelyX U100; Subgroup D, 2% CHX + RelyX U100. Prior to cementing the root canals, Subgroups B and D were irrigated with 2% CHX digluconate solution for 1 minute; the excess was dried with absorbent paper points. The luting procedures of the fiberglass posts with self-adhesive cements were performed as described previously.

Nanoleakage test
After cementation procedures were performed, the restored roots were stored in relative humidity for 24 hours at 37°C. Using an Isomet 1000 digital cutting machine (Buehler), the roots were sectioned perpendicular to the long axis.
Table 2. Bonding procedures used in the study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dentin pretreatment</th>
<th>Resin cement application</th>
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<tbody>
<tr>
<td>1 Etch-and-rinse</td>
<td>Apply etch (37% phosphoric acid) for 15 seconds. Rinse with water and dry with paper points. Apply primer. Dry with gentle airflow for evaporation of solvent (5 seconds). Apply adhesive (Scotchbond Multi-Purpose). After each application, remove excess with paper points. Light cure for 10 seconds.</td>
<td>Dispense Rely X Arc cement onto mixing pad and mix for 30 seconds. Apply mixed paste with aid of Centrix syringe and seat post in root canal. Remove excess cement. Light cure for 40 seconds.</td>
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<tr>
<td>2 Self-etching</td>
<td>Clearfil SE Bond: Actively apply primer for 20 seconds. Dry with gentle airflow for evaporation of solvent. Apply adhesive (Clearfil SE Bond). Dry with gentle airflow for 3 seconds. After each application, remove excess with paper points. Light cure for 10 seconds. ED Primer: Mix 1 drop each of Primers ED-A and ED-B. Apply mixture to root canal; leave in place for 60 seconds. Remove excess primer with paper points. Dry with gentle airflow.</td>
<td>Mix Panavia F paste A and paste B for 20 seconds. Apply mixed paste with aid of Centrix syringe and seat post in root canal. Remove excess cement. Light cure for 40 seconds.</td>
</tr>
<tr>
<td>3 Self-etching</td>
<td>Actively apply primer for 20 seconds. Dry with gentle airflow for evaporation of solvent. Apply adhesive (Clearfil SE Bond). Dry with gentle airflow for 3 seconds. After each application, remove excess with paper points. Light cure for 10 seconds.</td>
<td>Mix Panavia F paste A and paste B for 20 seconds. Apply mixed paste with aid of Centrix syringe and seat post in root canal. Remove excess cement. Light cure for 40 seconds.</td>
</tr>
<tr>
<td>4 Etch-and-rinse</td>
<td>Apply etchant (37% phosphoric acid) for 15 seconds. Rinse with water and dry with paper points. Apply Scotchbond Multi-Purpose activator and gently agitate for 5 seconds. Apply catalyst. After each application, remove excess with paper points. Light cure for 10 seconds.</td>
<td>Dispense Rely X ARC cement onto a mixing pad and mix for 30 seconds. Apply mixed paste with aid of Centrix syringe and seat post in root canal. Remove excess cement. Light cure for 40 seconds.</td>
</tr>
<tr>
<td>6 Self-adhesive</td>
<td>No pretreatment.</td>
<td>Rinse with water. Dry by blowing with air syringe and with paper points. Activate capsule and mix Rely X U100 cement in mixer for 15 seconds. Remove excess cement after seating of the restoration. Light cure for 40 seconds.</td>
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</table>

The first slice (1 mm thick) of each root was discarded. The samples were cleaned with 10% liquid phosphoric acid for 10 seconds, washed, and submitted to ultrasound for 10 minutes. Next, each specimen was immersed in a 50% ammoniac silver nitrate solution for 24 hours in dark conditions at 37°C. The specimens were then thoroughly rinsed in distilled water for 2 minutes and immersed in a photodeveloping solution for 8 hours (Kodak Developer D-76, Eastman Kodak Company) under fluorescent light, in order to reduce silver ions to metallic silver grains along the bonded interface, adhesive resin, and cement polymeric structure. Next, the stained specimens were embedded in a polystyrene resin and wet-polished sequentially with aluminum oxide papers (600, 1200, and 2000 grit) and finished with a diamond paste of decreasing grain using a metallographic polisher (PL02, Arotec SA). After each step of the polishing procedure, the specimens were immersed in distilled water and placed in ultrasonic baths (Ultrasone D 1440, Odontobras) for 10 minutes. The specimens were dried with absorbent papers and immersed in a solution of 50% phosphoric acid for 10 seconds, followed by rinsing in distilled water. For deproteinization, a 10% solution of sodium hypochlorite was used for 10 minutes. The specimens were then rinsed, dried at room temperature for 2 hours, and dehydrated with ethanol (at increasing concentrations of 25%, 50%, 75%, 90%, and 100%), for 10 minutes each. The specimens were carbon-coated (SCD-050 Sputter Coater, Leica Microsystems) and analyzed in a scanning electron microscope (SEM) (JEOL Ltd.) at 15 kV. The images of silver-infiltrated specimens were taken in order to calculate the marked area using the computer software Image Tool 3.0 (University of Texas, Health Science Center at San Antonio). The integrity of the interface in each third was then expressed as the percentage of the continuous (gap-free) interface. The percentage of the continuous interface along the entire cement-radicular dentin interface was also calculated.

Statistical analysis
Data were analyzed using ANOVA followed by Tukey’s test at a 5% level of significance (P = 0.05).
Statistical analysis of the data obtained in the nanoleakage test was performed according to a casual split-plot design, in which the factors under study were the cements, the plot, and the root third; Tukey’s test was applied.

Results
Nanoleakage evaluation
ANOVA indicated statistically significant differences between the different luting
systems and between interactions of luting systems and root thirds. The results of Tukey’s test are presented in Table 3.

Group 3 presented greater nanoleakage in the apical third, with statistically significant differences between the middle and cervical thirds (Fig. 1A). A statistically significant difference was also found in the apical thirds of the other groups. The resin tags formed inside the dentinal tubule by the specimens in Group 3 were short and/or not very pronounced (Fig. 1B). A lower mean nanoleakage percentage was achieved by the Group 1 specimens in the apical third, but it did not differ significantly from the other remaining groups (Fig. 2A). It was also possible to observe extensive resin tag formation inside the dentinal tubules in Group 1 specimens (Fig. 2B).

In the specimens of Groups 5 and 6, the SEM images showed no formation of a hybrid layer at the adhesive interface of these cements. This was also true with Subgroups B and D which were pretreated with CHX. All the specimens of self-adhesive cement presented nanoleakage (Fig. 3 and 4).

**Nanoleakage evaluation for CHX**

ANOVA indicated statistically significant differences between the self-adhesive cements not pretreated with CHX and the self-adhesive cements treated with CHX. No difference was observed among the root thirds of the CHX groups. The results of Tukey’s test are presented in Table 4.

**Discussion**

Fiber posts can be cemented using conventional dual-cure resin-based cements in combination with etch-and-rinse or self-etch adhesives, or by using the recently formulated self-adhesive cements that allow simultaneous bonding between the root dentin and the post. Due to the large variety of products and the intrinsic difficulties of bonding within the endodontic space, the use of an adequate luting strategy is particularly important as it directly affects the quality of the tooth-luting interface.\(^{13,20}\)

When the nanoleakage patterns between the root dentin and the luting system were evaluated, a better quality, thicker hybrid layer with long resin tags in the dentinal tubules could be observed for Group 1 at the apical thirds (Fig. 5). With the exception of Group 3, there were no statistically significant differences for the other groups. A 3-step etch-and-rinse adhesive system increases the interfacial adaptation of dual-cure luting cements because it increases

### Table 3. Mean nanoleakage percentage and standard deviation (SD) for the luting systems and the root thirds.

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<tbody>
<tr>
<td>Coronal</td>
<td></td>
<td>17.56 (2.28)(^a)</td>
<td>17.52 (1.12)(^a)</td>
<td>23.64 (2.17)(^a)</td>
<td>21.85 (2.18)(^a)</td>
<td>13.32 (0.71)(^a)</td>
<td>23.26 (2.65)(^a)</td>
</tr>
<tr>
<td>Middle</td>
<td></td>
<td>15.49 (1.99)(^a)</td>
<td>24.09 (4.25)(^a)</td>
<td>23.02 (3.36)(^a)</td>
<td>20.88 (3.86)(^a)</td>
<td>15.79 (4.04)(^a)</td>
<td>21.07 (7.64)(^a)</td>
</tr>
<tr>
<td>Apical</td>
<td></td>
<td>12.23 (3.89)(^a)</td>
<td>22.65 (2.07)(^a)</td>
<td>37.11 (1.13)(^a)</td>
<td>20.42 (7.30)(^a)</td>
<td>16.43 (3.18)(^a)</td>
<td>20.43 (5.30)(^a)</td>
</tr>
</tbody>
</table>

Data with same superscript letters (uppercase for rows, lowercase for columns) are not significantly different (\(P < 0.05\)).
Groups: Group 1, Scotchbond Multi-Purpose Plus chemical cure + RelyX ARC; Group 2, Clearfil SE Bond + ED Prime dual cure + Panavia F; Group 3, Clearfil SE Bond physical cure + Panavia F; Group 4, Scotchbond Multi-Purpose Plus physical cure + RelyX ARC; Group 5, RelyX U100; Group 6, RelyX Unicem.

**Fig. 1.** A. Scanning electron microscope (SEM) image showing a large quantity of nanoleakage in the adhesive interface of a specimen in Group 3. B. SEM image showing that the hybrid layer is extensively infiltrated by silver.

**Fig. 2.** A. SEM image showing low nanoleakage in a specimen from Group 1. B. SEM image at 250X magnification.
adhesive penetration into the dentinal tubules, forming long resin tags in the tubules that are opened by acid etching, thereby improving the pattern of dentin hybridization. However, during the post space preparation, a thick smear layer was created on the root canal walls (mainly in the apical third) which, due to the root’s anatomical configuration, favors the accumulation of debris in the apical region. In this sense, the dentin pretreatment with the use of phosphoric acid in the etch-and-rinse technique may have been the determining factor in the lower nanoleakage percentage at the apical third in Group 1 compared to Group 3 ($P < 0.0001$). It is possible that the self-etch adhesive used in Group 3 (Clearfil SE Bond) was not acidic enough to etch the dentin surface and dissolve the thick root dentin smear layer accumulated in the apical region.

On the other hand, specimens of Group 2 (self-etching adhesive strategy) showed values in the apical third similar to the ones found in Group 1 (etch-and-rinse adhesive strategy). Group 2 incorporated the dual-cured Panavia F cement with the self-etching Clearfil SE Bond and the self-etching and self-curing ED Primer. The use of Clearfil SE Bond without the ED Primer in Group 3 may have contributed to poor infiltration of the resin cement-dentin interface due to possible incompatibility with the dual-cured Panavia F resin cement. After photoactivation of Clearfil SE Bond, a nonpolymerized resin layer (with a pH of 1.35) remained on the top of the polymerized adhesive resin layer due to an oxygen interaction. The acid resin monomers—caused by oxygen inhibition in the nonpolymerized adhesive residual layer—react with the tertiary amine of the resin cement. Moreover, these adhesives promote a permeable hybrid layer, allowing water diffusion from the dentin and forming water droplets along the adhesive resin-cement interface, which may have contributed to the significant interfacial nanoleakage demonstrated in the apical third of the specimens of Group 3. The further application of the ED Primer in Group 2 of this study probably helped eliminate the inherent incompatibility between the self-etch adhesive Clearfil

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### Table 4. Mean nanoleakage (%) and standard deviation (SD) for the self-adhesive cements pretreated with chlorhexidine (CHX).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean % (SD)</th>
<th>Tukey’s</th>
</tr>
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<tbody>
<tr>
<td>RelyX Unicem with CHX</td>
<td>9.17 (2.47)</td>
<td>a</td>
</tr>
<tr>
<td>RelyX U100 with CHX</td>
<td>9.27 (3.16)</td>
<td>a</td>
</tr>
<tr>
<td>RelyX U100 without CHX</td>
<td>15.18 (2.96)</td>
<td>b</td>
</tr>
<tr>
<td>RelyX Unicem without CHX</td>
<td>21.59 (5.00)</td>
<td>c</td>
</tr>
</tbody>
</table>

Data with same lowercase letters are not significantly different ($P < 0.05$).
and the dual-cured Panavia F resin cement, via the \( t \)-isopropylbenzene sulfinate co-initiator that is added to ED Primer liquid B, which reacts with the acidic resin monomers present in the EB Primer liquid A along with the resin cement itself to produce free radicals that can enhance the polymerization reaction.\(^{26,27}\) The importance of ED Primer on the polymerization effectiveness of Panavia F was confirmed by Grande da Cruz et al in 2012.\(^{28}\)

Another aspect that justifies the use of Panavia F with Clearfil SE Bond for fiber post luting is the fact that the 2-step self-etch adhesive Clearfil SE Bond used in this study presents a high amount of viscous hydrophobic monomers on the bonding agent. This increases the viscosity of this adhesive, which can then reduce its diffusion, resulting in lower microretention, decreasing the quality of adhesive interlocking, and eventually compromising the sealing ability of the adhesive. The results in this study showing high values of nanoleakage for this resin luting system in the apical third can be explained by the difficulty in achieving direct irradiation by light in the deep regions of root canals.\(^{24,29}\)

Groups 5 and 6 (self-adhesive cements) were comparable with Groups 1 and 4 (RelyX ARC in combination with etch-and-rinse adhesive groups). Other studies have related satisfactory results using RelyX Unicem cement, which is chemically identical to RelyX U100.\(^{20}\) The favorable adhesion to root canal dentin may have occurred due to the fact that both the RelyX U100 and RelyX Unicem self-adhesive cements are highly compatible with the substrate and can optimize physical interactions, such as micromechanical retention and chemical bonding.\(^{31}\) RelyX Unicem has a chemical interaction with hydroxyapatite; this interaction may be based on the chelation of the calcium ions by acid groups, leading to chemical adhesion to the hydroxyapatite in the tooth. The 10% fluoride content in RelyX Unicem has led to speculation that minor nanoleakage could be related to the water repulsion effect of the fluoride ions, which may help to reduce residual water in the bonding interface and thus improve its resistance to hydrolytic degradation.\(^{32,33}\)

The multifunctional, phosphoric acid-modified, methacrylate monomers of RelyX U100 (pH <2) demineralize root dentin as well as infiltrate the substrate and react with the hydroxyapatite of hard tissues.\(^{34}\) The micromechanical retention associated with the chemical adhesion to hydroxyapatite provides self-adhesiveness to the RelyX U100 cement.\(^{35}\) This chemical interaction produces water, which accelerates neutralization of phosphoric-acid methacrylate, basic fillers, and hydroxyapatite.\(^{36}\) The system likely gained water resistance, and, although water and buffering of the smear layer may have reduced demineralization capacity, the effectiveness of the RelyX U100 cement was not compromised.

The analysis of the results of the effect of 2% CHX pretreatment on the adhesive interface quality of self-adhesive resin cements showed significantly lower nanoleakage on the tooth-luting interfaces that were pretreated with CHX prior to luting with self-adhesive systems. The decreased nanoleakage results of the self-adhesive cements results can be explained by the increase in the dentin surface energy, and the interaction between the dentin surface and the resin cement is strongly dependent upon the equilibrium of high surface energy and high wettability.\(^{36,37}\)

In addition, self-adhesive cements do not require acid etching. Thus, when CHX is applied to smear-covered dentin surfaces, it can partially remove the smear layer and even expose some underlying dentinal tubules. CHX has cationic properties, thereby enabling it to bind to phosphorus- and calcium-containing groups in apatite, and thus producing a strong affinity for tooth surfaces.\(^{19,38}\)

This study assessed the interfacial nanoleakage of fiber posts after 24 hours of water storage. The results showed that the extension of silver nitrate deposition along the bonded interface of fiber posts was significantly influenced by the luting system. However, the study findings do not indicate with certainty that any 1 of the 3 investigated adhesive approaches is better than the others. It is possible that a longer storage time and/or thermal cycling in future studies would give different results. Nevertheless, according to the results observed in this study, the self-etching approach may offer less favorable adhesion to root canal dentin in comparison with etch-and-rinse or self-adhesive approaches.

Based on these findings, clinicians should be aware that, although in the majority of clinical investigations fiber posts are cemented using dual-cured resin cements with etch-and-rinse adhesives, the number of clinical steps in these procedures might favor the occurrence of errors.\(^{1,2,39-41}\) Scientists and manufacturers have been continuously challenged to simplify clinical procedures.\(^{42}\) The self-adhesive cements require no technique-sensitive steps and should therefore be considered as an interesting alternative for luting of intracanal posts because they also present a satisfactory performance, as indicated in the results of the laboratory nanoleakage test in this study. Regarding the effect of dentin pretreatment with CHX, in addition to the inhibitory effects of CHX on dentin proteases, the application of CHX appears to promote an adequate seal at the resin cement-dentin interface.\(^{37,42}\)

**Conclusion**

Within the limitations of this study, it was possible to conclude that Group 3 (Clearfil SE Bond + Panavia F) group showed higher nanoleakage patterns in the apical third compared to the other groups; the specimens of the self-adhesive strategies groups (Groups 5 and 6) demonstrated similar results as compared to the conventional etch-and-rinse adhesive strategies; and in CHX-treated adhesive interfaces of self-adhesive luting cements, reduced uptake of silver particles was observed.

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