

# An *in vitro* Study to Assess Glucose Microleakage Along Fiber Posts Cemented with 2- and 3-Step Etch-and-Rinse Resin Adhesive Systems

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**Abstract** - The aim of the study is to compare the sealing ability of two adhesive systems. Thirty teeth were endodontically treated and were randomly assigned to 2 groups: **G 1** – 3-step adhesive (Solobond-Plus) and **G 2** – 2-step adhesive (Solobond-M). Posts containing glass fiber were cemented using the adhesive technique recommended by the manufacturer. All samples were mounted on a glucose leakage model. A 10 µL aliquot of solution was drawn from the glass bottle using a micropipette. The samples were then analyzed in a UV-Vis spectrophotometer. The two tested adhesive systems presented a similar capacity to prevent the glucose infiltration.

KEY WORDS: adhesive systems, dentin, dentin sealing, fiber posts, infiltration, sealing ability

## INTRODUCTION

The main role of the posts is to re-establish the biomechanical properties of teeth with the purpose to enable the restoration procedures. Moreover, due to the dehydration of dentin after root canal treatment and the removal of tooth structure, root-filled teeth are widely considered to be more susceptible to fracture than vital teeth<sup>1</sup>. This situation is aggravated when teeth with thin-walled roots are considered<sup>2,3</sup>. As a consequence, in today's dental practice, the employment of posts represents an important point in restorative procedures mainly when involving root filled teeth<sup>4,5</sup>.

Fiber posts were introduced in the 1990s and became popular due to their promising properties<sup>6-8</sup>. To date, fiber posts are considered a viable alternative to cast or prefabricated metal posts in the prosthetic rehabilitation of root-filled teeth with extensive coronal destruction. The quality of the seal provided by a cemented post depends on the cement and dentin adhesive used. A cemented post should be able to provide a seal comparable to the original root canal filling; nevertheless, little information is available on the seal provided by the various luting cements and adhesive systems<sup>9,10,11</sup>.

Currently, fiber-reinforced epoxy resin-based posts are employed with methacrylated-based dentin adhesives and resin cements<sup>12</sup>. The purpose of a post-and-core system is to make a unit from several different materials: tooth structure, post material, core build-up material, and luting cement<sup>13</sup>. This system must be a sealed unit and should

represent a “monoblock” that can resist cyclic chewing forces and prevent leakage of microorganisms and their by-products<sup>14-17</sup>. In that context, the dentin adhesive system has a fundamental role<sup>18</sup>. However, due to the limitation of root dentin bonding, microleakage can occur in the post-dentin interface. While the hermetic seal between several current bonding systems and enamel has been potentially achieved, it is still a great challenge to seal the resin-dentin interface due to the heterogeneous character of dentin structure and surface morphology and/or intrinsic shortcoming of design of modern adhesives<sup>19</sup>.

Several methods have been used to assess the quality of root fillings, root-end fillings, coronal fillings, and cemented posts. However, there is no consensus about the methods used for leakage evaluation<sup>20</sup>. Conventional dye leakage studies provided only limited information and little correlation has been found between the results of apical dye leakage studies and clinical outcome<sup>20-22</sup>. A new and interesting method for evaluating the quality of the fluid-seal was recently developed and labeled as glucose leakage model (GLM)<sup>23</sup>. Since its introduction, the GLM has been used by some studies<sup>23-25</sup> where its advantages were well-outlined. In addition, an established leakage research group has accepted GLM as an evolution of the reliable fluid transport method<sup>24</sup>. The GLM potentially represents an advance in improving the conclusions of *in-vitro* leakage studies.

In recent times, a large number of investigations have been focused on the dentin adhesives properties. Notwithstanding, little information about the influence of the dentin adhesives systems on the sealing ability of cemented fiber posts appears in the literature. Therefore, the present study was designed to evaluate the sealing ability of 2- and 3-step total etching adhesive systems based on the filtration rate of glucose along the cemented fiber posts. The null hypothesis tested was that there is no difference between the sealing ability of 2- and 3-step total etching adhesive systems.

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## METHOD AND MATERIALS

### Specimen selection

This study was revised and approved by the Ethics Committee, Nucleus of Collective Health Studies, Veiga de Almeida University, Rio de Janeiro, Brazil. A sample of 40 well-preserved, extracted human upper canines, with straight roots, complete root formation and no root caries were selected from the tooth bank of Veiga de Almeida University. To improve specimen standardization, selected teeth were  $20 \pm 1$  mm long. All teeth selected for the study presented a single canal. The teeth were cleaned and examined stereoscopically at 10X magnification to verify the absence of cracks. The teeth were disinfected in 0.5% chloramine T, stored in distilled water at 4° C and used within 6 weeks after extraction<sup>26</sup>.

### Endodontic procedures

Five teeth were put aside to serve as the negative control group. Standard access cavities were made in the remaining 35 teeth and the patency of each canal was confirmed by inserting a size 15 file through the apical foramen (AF) before and after completion of the root canal preparation. The working length was determined by subtracting 1 mm from the apical foramen. The coronal and middle third of each canal was prepared using Gates Glidden drills (Dentsply / Maillefer, Ballaigues, Switzerland) sizes 6, 5, 4, 3, and 2 at 1-mm increments creating a 0.02 mm·mm<sup>-1</sup> taper<sup>27</sup>. The apical third was prepared with Flexofiles® (Dentsply, Maillefer) sizes 60, 55, 50, and 45 in a balanced force technique<sup>28</sup>.

The canals were irrigated between each file with 0.5 mL of freshly prepared 5.25% NaOCl (VETEC Química fina LTDA, Rio de Janeiro, RJ, Brazil) using a disposable syringe and 27-gauge needle. In order to remove the smear layer, 3 mL of 17% EDTA (pH 7.7, Herpo Ltd., Petrópolis, RJ, Brazil) was used for 3 minutes as a final rinse. The canals were dried with paper points (Dentsply-Maillefer). Five prepared teeth served as positive control group. The remaining 30 prepared teeth were filled by the same operator using the single cone technique and Grossman sealer (Endofill, Herpo Ltd., Petropolis, RJ, Brazil). A size #40 file was used to pick up a measured amount of sealer (20 µL) which was determined by the employment of fixed-volume semi-automatic micropipette. The sealer was placed into the canal while rotating the tooth counterclockwise. A size 55 0.02-taper gutta-percha cone (Diadent, Chongchong Buk Do, Korea) was placed in the canal up to the full working length.

### Post preparation procedures

The filled roots were stored for 24 hours in physiological saline at 37°C. Subsequently, the coronal-middle gutta-percha fillings were removed of all teeth with Gates Glidden drills to a depth of 14 mm. The working length of the drills was established with silicone stoppers. The root canal space was enlarged with a number 3 low-speed, pre-shaping drill provided by the fiber-post manufacturer (FGM Produtos Odontológicos Ltda., Joinville, Santa Catarina, Brazil) leaving a 6-mm long apical seal. The depth of the post space preparation was 14 mm.

### Post cementation procedures

Thirty roots were randomly assigned to one of two experimental groups that differed in the adhesive system employed for post cementation. Both experimental and controls groups were randomly created using a computer algorithm (<http://www.random.org>).

#### Group 1: Three-step etch-and-rinse adhesive (Solobond Plus)

The post space was etched with 35% phosphoric acid gel (VOCO – Cuxhaven, Germany) for 15 seconds, rinsed thoroughly and dried lightly with paper points. The primer-adhesive was applied into the root canal space by using the small plastic microbrush tip (Cavibrush, FGM Produtos Odontológicos Ltda.). The excess was then air-dried, and the pooled primer remaining in the post space was removed by using two paper points (Endopoints Indústria e Comércio LTDA, Paraíba do Sul, Rio de Janeiro, Brazil). Then the adhesive was applied in the same way, light-cured for 40 seconds with the tip of the LED light-curing unit (Radii SDI Limited, Bayswater, Victoria, Australia) with a 1 mm of standardized distance that was determined with an aid of a glass slab. Each post was cleaned with ethanol and air-dried before the application of the silane (Bifix, VOCO, Cuxhaven, Germany).

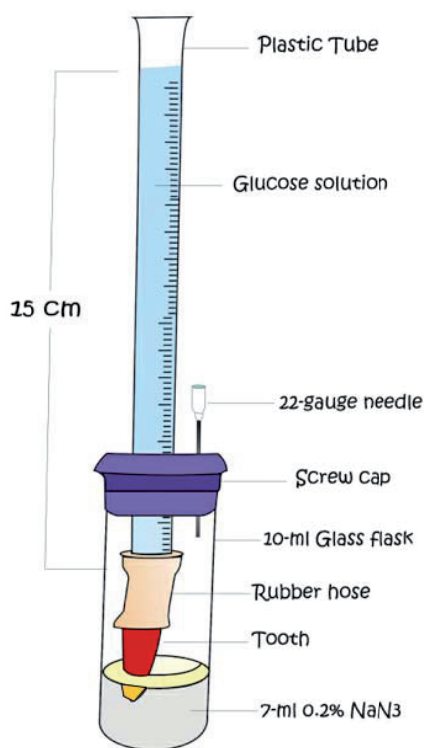
A lentulo bur (Maillefer) was used with a conventional engine of low rotation of 500 rpm to pick up a measured amount of sealer (20 µL) into the post space. White Post number #3 (FGM Produtos Odontológicos Ltda., Joinville, Santa Catarina-Brazil) were cemented with Bifix QM (VOCO – Cuxhaven, Germany) according to the manufacturers' instructions. The dual-cure resin cement was allowed to set by light-curing through the post for 60 seconds with the tip of the LED positioned at the top of the fiber post. The output intensity (1000 mW/cm<sup>2</sup>) was constantly measured during the experiment with the aid of a Demetron light radiometer (DENTSPLY, De Trey, Konstanz).

#### Group 2: Two-step etch-and-rinse adhesive (Solobond M)

Two coats of a Solobond M resin adhesive (VOCO) were applied with a microbrush tip (Cavibrush) to the dentin walls. The pooled primer remaining in the post space was removed by using two paper points (Endopoints). The adhesive light-cure and post cementation process were processed similar to group 1.

### Assembled double-chamber and glucose leakage measuring

The 6-mm gutta-percha filled part of each tooth was removed using an Isomer low-speed saw (Buehler Ltd, Evanston, IL, USA) with copious water coolant, leaving roots 14 mm in length. The objective used just the portion containing the fiber post employed for the leakage evaluation. The specimens of the negative group control were completely coated with nail varnish while in the positive control group, root canal were not filled nor covered. Two coats of nail varnish were applied on the external surface of all experimental specimens, except for the area 3 mm of the apical end.



**Figure 1.** Set-up of the glucose leakage model.

The teeth were placed into a device designed to measure glucose leakage<sup>23,25</sup> (Figure 1). The teeth were individually inserted into a silicone tube ( $\varnothing$  0.5 x 1.5 mm) with the apex (3 mm) protruding through the end. The silicone tube was connected to a plastic tube with a length of at least 15 mm. The assembly was then placed in a sterile 10 mL glass flask with a screw cap. The glass flask contained 3.5 mL of 0.2% NaN<sub>3</sub> so that the root samples were immersed in the solution. NaN<sub>3</sub> was used to inhibit the growth of microorganisms that might influence the readings through the decomposition of glucose<sup>23</sup>. All of the interfaces were sealed with cyanoacrylate adhesive. Four mL of one Mol·L<sup>-1</sup> glucose solution (pH = 7.0 / density =  $1.09 \times 10^3$  g·L<sup>-1</sup> / viscosity =  $1.18 \times 10^3$  Pa·s at 37°C) containing 0.2% NaN<sub>3</sub> was injected into the plastic tube at a level 14 cm higher than the top of the sample, creating a hydrostatic pressure of 1.5 kPa (15 cm H<sub>2</sub>O). The model was transferred to an incubator that provided 100% humidity at 37°C for the duration of the experiment<sup>23</sup>.

A 10  $\mu$ L aliquot of solution was drawn from the glass bottle using a micropipette at 5, 14, 21, 28, 40, and 52 days. After drawing the sample, 10  $\mu$ L of fresh 0.2% NaN<sub>3</sub> was added to the glass bottle reservoir to maintain a constant volume of 7 mL. The sample was then analyzed with a glucose kit (Labtest, Lagoa Santa, SP, Brazil) in a UV-VIS spectrophotometer (Camspec M 330, UK) following a kinetic assay at 505 nm wavelength. The lowest and the highest glucose level for which the glucose assay is believed to be accurate is 0.44 and 26.7 mmol·l<sup>-1</sup>. Therefore, only the values situated between these ranges were considered.

## Data presentation and statistical analysis

Data are presented as mmol·l<sup>-1</sup> of glucose. Data from negative and positive control groups were only used to demonstrate the minimal and the maximal leakage values detected by the experimental model, respectively. The preliminary analysis of the raw pooled data of experimental groups (G1 and G2) shows a non-normal distribution (D'Agostino & Person omnibus normality test). Further, time-dot statistical analysis was performed with nonparametric test methods, using Mann-Whitney test. The concentration of the glucose leakage and each experimental time were used as factors. The alpha-type error set at 0.05. SPSS (11.0 for windows, Statistics 4.0, SPDD, Gorinchem, Netherlands) and Origin 6.0 (Microcal Software, Inc., Northampton, MA, USA) were used as analytical tool.

## RESULTS

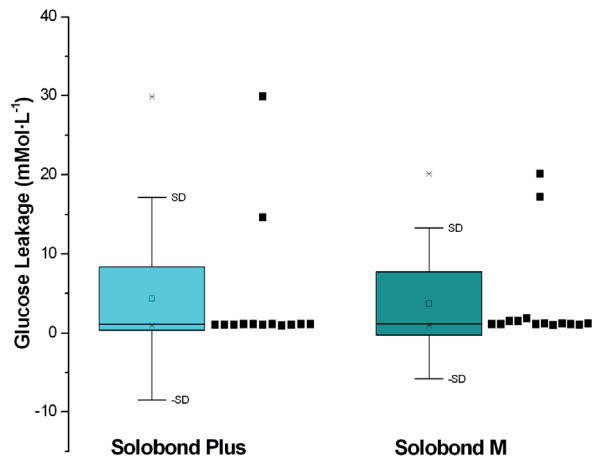
No glucose leakage was recorded for the negative control groups while the positive control group showed substantial glucose leakage from the first day, with the leakage values increasing over time up to 21 days, when all of the samples had maximum leakage concentration (21 mmol·l<sup>-1</sup>). Thus, the results from the control groups confirmed the experimental model.

The box-plot graph in Figure 2 displays the accumulated glucose leakage for each experimental group. The graph in Figure 3 displays glucose leakage over time. Overall, leakage was consistent between the 2 groups for each experimental time. The box plot in Figure 3 illustrates the mean traces, minimal and maximal leakage traces as well as the variability of the cumulative leakage for each experimental group.

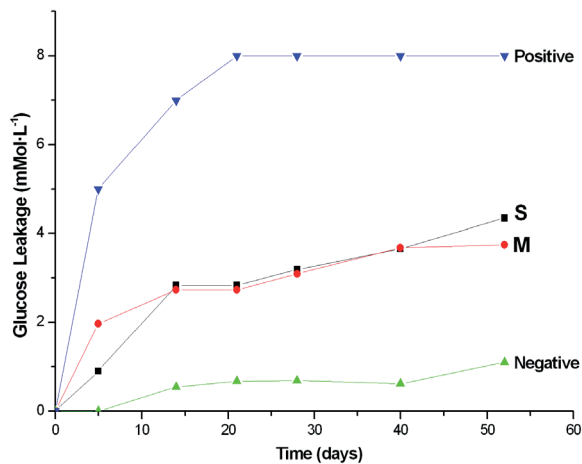
The Mann-Whitney test results show that there were no significant differences among the experimental groups for all experimental times ( $P > 0.05$ ).

## DISCUSSION

Sealing ability has been evaluated by a variety of laboratory-based experimental models including dye leakage, fluid transport, and bacterial penetration methods. The present study used the recent and innovative glucose leakage research set-up, in which a glucose solution was used as tracer. Glucose has some positive points, such as: low molecular weight (180 Da), hydrophilicity, is chemically stable, and also serves as a bacteria nutrient. Xu et al.<sup>23</sup> states that, if glucose could enter the canal from the oral cavity, bacteria that might survive root canal preparation and obturation could multiply and potentially lead to periapical inflammation. Therefore, the use of the GLM was thought to be more clinically relevant than other tests<sup>24</sup>. Moreover, the present model is quantitative, nondestructive, non-labor-intensive, and a time-saving experiment. It is worth mentioning that the coronal low pressure used in the study could help rule out entrapped air or fluid and seemed to be sufficient for a device with high sensitivity<sup>24</sup>. Shemesh et al.<sup>24</sup> suggested that the GLM can be seen as a further development of the fluid transportation concept as a measure of the passage of fluid along root fillings, subjecting them to constant pressure.



**Figure 2.** Time evolution of glucose leakage. Data points are the average from all of the samples.



**Figure 3.** Distribution of the leakage measures in the experimental groups (S- Solobond Plus and M- Solobond M).

In spite of the high sensitivity of the method the present results showed no significant differences on sealability along posts cemented using 2- and 3-step etch-and-rinse adhesive systems. Therefore, the null hypothesis was accepted. Nevertheless, the present study reveals a significant level of glucose leakage in all specimens. Therefore it is correct to affirm that no sample obtained a hermetic closing of the interface dentin-pin. That is in line with the finds of Pashley<sup>27</sup> who stated that most of the dental materials allow microleakage of bacteria and their products. It is worthwhile mentioning that one of the rare studies, Rogic-Barbic et al.<sup>13</sup>, concluded that none of the used protocols were capable to completely prevent the fluid passage. A similar pattern of dentinal microleakage for different adhesive systems was also found by Pradelle-Plasse et al.<sup>29</sup> using the electrolytic method for evaluating the quality of the sealing. On the other hand, Fox & Gutteridge<sup>30</sup> related that roots restored with prefabricated pins and cemented with resinous cement were totally resistant to the Indian ink infiltration. However, this result can be justified for the differences among the methods as well as for the inherent flaws regarding the subjective linear dye leakage model.

In the current stage of the dentin adhesive technology it is clear that the use of an adhesive system is a necessary procedure to minimize microleakage. Mathew et al.<sup>31</sup> have stated that the type of adhesive system represents one of the factors that can influence the sealability. Therefore, the present study proposed to study the influence of the adhesive system in the seal quality is the use of cementation of fiber glass pins. The adhesives tested in the present study belong to two classes of etch-and-rinse adhesives that are available in the market with different operative steps. However, in the present study both systems tested presented a similar capacity to prevent the glucose microleakage, in spite of studies that suggest that the primer combination and adhesive in a same flask for single step application, they may reduce the quality of the hybrid layer of dentin and, as a result, increase the risk of occurrence of larger microleakage<sup>32,33</sup>.

In the only study that uses the fluid transport model, the coronal dentin is not able to block the microleakage<sup>34</sup>. However, Mannocci et al.<sup>35</sup>, using confocal microscopy by laser to observe the Rhodamine-B infiltration, concluded that the use of a traditional 3-step adhesive system provided a better control of the marginal infiltration around pins cemented with self-primers.

The observation of a non-fluid-tight seal specimen in the present study is not a real surprise, because the relative problems to the adhesion in the substratum are a well-documented topic in the literature. Despite the adhesive materials used, their limitations are still considerable being that most of them related to the process of polymerization contraction and humidity control<sup>36</sup>. It should also be emphasized that the root canal possesses an unfavorable geometry cavity for the adhesion process<sup>37</sup>.

Different from the approach of the present study, Pradelle-Plasse et al.<sup>29</sup> stated that long-term sealability is the most important factor to preserve dentin bonding. Both adhesive systems tested have acetone as the solvent in their formulation, which seems to be the most efficient for root dentin treatment. The effectiveness of an acetone solvent in bonding root dentin is related to its evaporation rate and, this is due to the high degree of humidity in the dentin tissue<sup>38</sup>. In fact, this feature provides additional chemical control of the excess of local water, and consequently a better diffusion of the resinous monomers for the collagen fibrils exposed. Even so, the results of the present study stress the need for improvements in the long-term sealing ability inside the root canal space, which would consequently increase the longevity of the current adhesive treatments.

## CONCLUSION

It can be concluded that under the experimental conditions of the present study, the 2- and 3-step etch-and-rinse adhesive systems promoted a similar capacity to prevent the glucose leakage over time.

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