# EVALUATION OF THE DENTINAL TUBULE PENETRATION OF AN ENDODONTIC BIOCERAMIC SEALER AFTER THREE FINAL IRRIGATION PROTOCOLS.

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## ABSTRACT

Introduction: This study aimed to evaluate the dentinal tubule penetration of an endodontic bioceramic sealer, Sealer Plus BC, after three final irrigation protocols. Methods: Thirty distobuccal roots of maxillary molars were selected. Root canal preparation was performed up to an #40.06 instrument (X1 Blue) under 2.5% sodium hypochlorite irrigation. Specimens were randomly divided into three groups (n=10), according to the final irrigation protocol: G-NaOCl (2.5% sodium hypochlorite + PUI), G-SS (0.9% saline solution + PUI) and G-H20 (Deionized water + PUI). After final irrigation protocols, all specimens were irrigated with phosphate buffer solution. Root canal obturation was performed using the single cone technique and Sealer Plus BC, stained with a specific fluorophore. Specimens were transversely sectioned and each root third was evaluated in a confocal scanning laser microscopy. Images obtained were analyzed for sealer penetration in the dentinal tubules.

Results: Dentinal tubule penetration of Sealer Plus BC was not observed in any root third, regardless of the final irrigation protocol investigated.

Conclusions: Sealer Plus BC dentinal tubule penetration was not observed after none of the protocols tested. Dentinal tubule penetrability of Sealer Plus BC may be related to other factors rather than the final irrigation protocol.

*KEYWORDS:* Bioceramic. Calcium silicate-based sealer. Dentinal tubule penetration. Endodontics.

## INTRODUCTION

Root canal treatment aims to eliminate microorganisms and to prevent recontamination. This is usually achieved by a combination of chemical-mechanical preparation of the root canal system, followed by three-dimensional filling.[1] The presence of infection in anatomical complexities can be the cause of treatment failure, thus, the penetration of endodontic sealers in these complexities can improve disinfection.[2,3] In addition, during obturation, it is important to use non-toxic materials that allows tissue repair.[4]

Bioceramic sealers, containing calcium silicate and/or calcium phosphate, have recently being studied due to their chemical, physical and biological properties,[4,5] and the capacity to produce hydroxyapatite, providing a link between the root dentin and the filling material.[6] Bioceramic sealers presents favorable properties such as radiopacity, flow, high calcium ion release, alkaline pH, low cytotoxicity and genotoxicity, and adequate antibacterial efficacy.[2]

An important property that a sealer must present is the ability to penetrate into the dentinal tubules, enabling the formation of a physical barrier, improving root filling retention, and burying residual bacteria. This property is related to some physical and chemical properties of the sealers, such as granulometry, solubility, viscosity and surface tension.[7] In addition, the sealer's penetration into the dentinal tubules depends on an effective irrigation of the root canal system, by improving the removal of debris and smear layer,[8,9] and on the type of irrigant used.[10,11]

To evaluate the dentinal tubule penetration of endodontic sealers, confocal laser scanning microscopy has been used. This technique associates endodontic sealers with fluorophores presenting specific wavelengths and binding mechanisms, allowing the visualization and evaluation of the sealer.[12] In relation to the fluorophores, it is recommended for hydrophobic sealers to be used in association with hydrophilic fluorophores (rhodamine B), and for hydrophilic sealers to be used in association with hydrophobic fluorophores.[13]

Mainly due to the importance of the endodontic sealer to penetrate into the dentinal tubule, and the relation of this capacity to the irrigants and irrigation protocols used during treatment, this study aimed to evaluate the dentinal tubule penetration of a bioceramic sealer after using three different substances (sodium hypochlorite, saline solution and deionized water) as irrigants during final irrigation protocols. The null hypothesis of the study was that there would be no differences in the dentinal tubule penetration capacity of the bioceramic sealer regardless of the irrigant tested.

## MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Federal University of Rio Grande do Sul (CAEE nº 23173219.0.0000.5347).

### *Teeth selection*

Thirty distobuccal roots of maxillary molars, extracted from patients aged between 45 - 70 years, for reasons not related to the study, with completely formed apexes, and curvatures of less than 5 degrees, were included in this study. The specimens were stored in 0.9% saline solution (Farmax, Divinópolis, Brazil) after extraction and then submerged in 2.5% sodium hypochlorite (Asfer, São Caetano do Sul, Brazil) during 48 hours for disinfection.

Digital periapical radiographs were performed to confirm the presence of only one root canal, absence of internal root resorptions, calcifications and previous endodontic treatment. Then, specimens were stored in saline solution until testing.

### *Root preparation*

All steps were performed by a single operator (AFL), previously calibrated for the experiments performed in this study.

The dental crowns were removed at the cement-enamel junction and roots were standardized to a 10 mm in length, using a diamond disc (FGM, Joinville, Brazil) at low-speed rotation. Apical patency was visually determined by passing a size #10 instrument (C-Pilot, VDW, Munich, Germany) through the apical foramen. Root canal preparation was performed with a #25.06 instrument (X1 Blue, MK Life, Porto Alegre, Brazil) working at the foramen, and with a #40.06 instrument (X1 Blue) working at 1mm from the working length, using an endodontic motor (VWD Silver, VDW, Munich, Germany) set in the WaveOne ALL program, while specimens were irrigated with 2.5% sodium hypochlorite (5 ml) (Asfer, São Paulo, Brazil).

At the end of preparation, canals were irrigated with 3 ml of 17% ethylenediaminetetraacetic acid (EDTA, Asfer, São Caetano do Sul, Brazil), that was ultrasonically activated using the passive ultrasonic irrigation (PUI) protocol. Activation was performed with an E1 ultrasonic insert (Irrisonic, Helse Ultrasonic, Ribeirão Preto, Brazil), coupled to a Satelec piezoelectric device (Acteon, Indaiatuba, Brazil), positioned 2mm shorter from the working length and used in in-and-out movements, during three cycles of 20 seconds each.

### *Experimental groups*

After root canal preparation, the specimens were randomly (www.random.org) divided into three groups, each containing 10 specimens, according to the final irrigation protocol: G-NaOCl = 2.5% sodium hypochlorite group + PUI; G-SS = 0.9% saline solution group + PUI; G-H2O = deionized water group + PUI (control). Each specimen received 5 ml of the solution, according to the group. PUI was performed as previously described. At the end, all groups were irrigated with 5 ml of phosphate buffer solution for 5 minutes.

### *Root canal obturation*

Prior to obturation, canals were only aspirated with suction tips, taking care to not completely dry the root canal, allowing the sealer setting reaction. An analytical balance (Shimadzu, Tokyo, Japan) was used to weigth 1 g of Sealer Plus BC (SPBC, MK Life, Porto Alegre, Brazil).

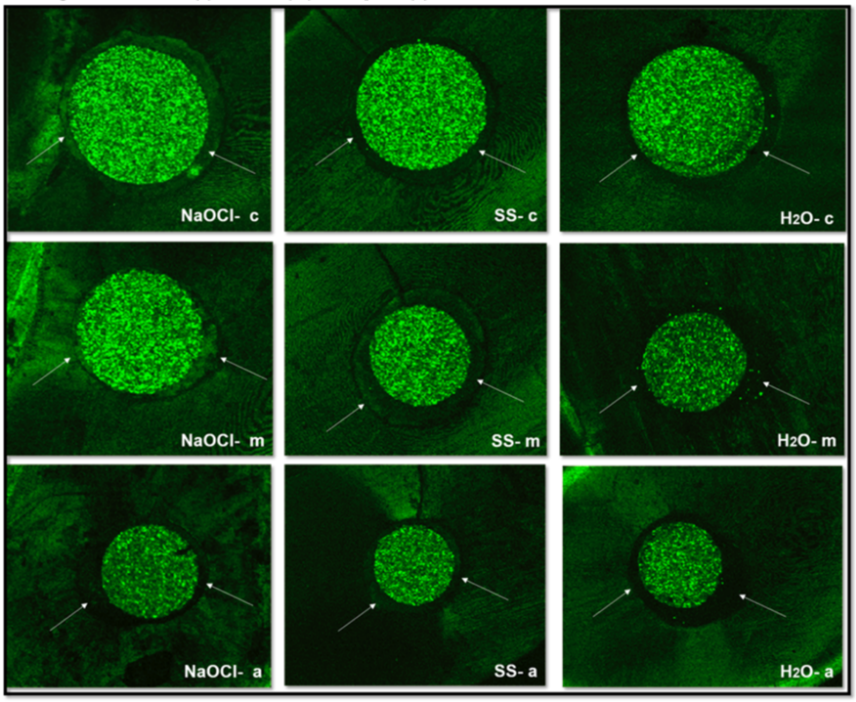
Approximately, 0.1% (weight equivalent of sealer) of the fluorescent dye Fluo-3 AM (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was added to a glass plate, and mixed to the sealer. With the aid of a #25 lentulo spiral (MK Life, Porto Alegre, Brazil), the sealer mixed to the fluorophore was placed inside the root canal. Then, a single #40.06 gutta-percha cone (MK Life, Porto Alegre, Brazil) was placed 1 mm short from the apical foramen. Excess of gutta-percha was removed using a heated plugger, and the remaining material was vertically compacted. Each specimen was sealed with Coltosol (Coltene, Rio de Janeiro, Brazil) and stored wrapped in moistened gauze for 72 h at room temperature for the sealer to set. Digital periapical radiographs were taken to confirm the quality of the obturation.

*Confocal laser microscopy and image analysis*

Forty days after obturation, each specimen was transversely sectioned into 2 mm slices, at 2, 4 and 6mm from the apex, corresponding to the apical, middle and cervical thirds, by using a cut machine (Extec Labcut 1010, Enfield, CT, USA) operating at 280-300 rpm under continuous water refrigeration. All samples were polished with #600-, 1200-, 1500-, 2000- and 2500-grit sandpaper discs (3M, Sumaré, Brazil) with specific paste (Arotec, Cotia, Brazil), and felt discs (FGM, Joinville, Santa Catarina, Brazil).

Samples were evaluated in confocal scanning laser microscopy (CSLM; Olympus Fluoview 100, Olympus Corporation, Tokyo, Japan), with an absorption and emission wavelengths of 494/590 nm, respectively. For correct visualization of all images, the samples were analyzed 10 mm below the surface using a x10 lens. Images for sealer penetration area within the dentinal tubules were generated, recorded and analyzed at x10 magnification using the fluorescent mode to a size of 800 x 800 pixels and a scale set to 70 μm, with the FluoView 10-ASW program (Olympus Corporation, Tokyo, Japan).

**Figure 1:** Representative images of the experimental groups after confocal scanning laser microscopy, referring to the cervical (c), middle (m) and apical (a) thirds.




**RESULTS**

No dentinal tubule penetration of Sealer Plus BC was observed in any group at any root third. For this reason, no statistical analysis was performed.

A different optical density surrounding the sealer was observed in almost all specimens, suggesting the formation of a mineral interface (hydroxyapatite) between the sealer and the root dentin (white arrows - **Figure 1**).

**DISCUSSION**

The penetration of filling materials into the dentinal tubules can prevent the colonization of residual bacteria and the reinfection of the root canal,[14] through a mechanical interlock, that creates a physical barrier that can confine the remaining microorganisms.[15,16] Therefore, it is important to verify the penetration capacity of endodontic sealers within the dentinal tubules.

In this study, Sealer Plus BC, a bioceramic sealer containing calcium trisilicate, calcium disilicate, zirconia oxide, calcium hydroxide and propylene glycol, was used. This sealer has an alkaline pH, a great capacity of calcium ion release, and has adequate flow, setting time and radiopacity.[17]

As for the method of analysis, CSLM is a well-established technique for evaluation of sealers' dentinal tubule penetration.[18-21] It is important to note that the type of fluorophore influences the assessment of penetration into the dentinal tubule when bioceramic sealers sealers are used, but does not have any influence when using epoxy resin-based sealers.[10]

When using Rhodamine B, its hydrophilic nature generates false-positive results when associated with bioceramic sealers.[10] Therefore, this combination must not be performed. In the other hand, Fluo-3 is a hydrophobic dye, that have a high attraction for calcium ions and can be used both in combination with calcium silicate-based sealers and epoxy resin-based sealers.[10] For this reason, Fluo-3 was used in this study, to avoid misinterpretations of our results.

The sealer penetration into the dentinal tubules depend not only on their physicochemical properties, but also in the irrigant used and the effectiveness of the cleaning process of the root canal system. In this study, sodium hypchlorite, saline solution and deionized water were used and ultrasonically activated as final irrigation protocols. Despite the differences on the irrigants used, no sealer penetration was visualized in any groups, regardless of the root third. Therefore, the null hypothesis was accepted.

Several factors can explain these results. An important factor that has to be consider is that only teeth from patients ranging from 45 to 70 years were used in this study. These teeth can present more sclerotic reactional dentin, which can be related to the sealer's penetration difficulty into the dentinal tubules,[22] being one possible explanation for the results presented in this study. Several studies have demonstrated varying degrees of penetrability of the sealers in the root thirds, with the apical root third being the most difficult to be filled.[2,7,15,19,20,22] This varying degree of penetrability can be associated with the density and diameter of the dentinal tubules,[16,22,23] an ineffective supply of the irrigant,[20,22,23] different degrees of dentinal sclerosis,[20,24] and the presence of organic and inorganic content, as well as the anatomical complexity.[2] In addition, the presence of cementum tissue in the apical region of the inner wall of the canal is capable of reducing the sealer's penetration, as the dentinal tubules tend to be obliterated.[21,23,25]

Another possible explanation for these findings can be related to the particle's sizes of the tested sealer,[24,26,27] to its flowability and to its hygroscopic expansion during setting.[7,15,26] In general, dentinal tubule diameter varies from 2 to 3.2 micrometers, thus, to achieve dentinal tubule penetration, the sealer particle size must be smaller than the tubule diameter.[7] According to the manufacturer's information, SPBC has nanometric particles and 0.2% hygroscopic expansion. However, so far, there are no reports on Sealer Plus BC particle size, unabling further discussion.

In addition, it has been reported that precipitates are formed along the cement-dentin and sealer-dentin interfaces, which suggests that they contribute to reduce the sealer's flow not only by filling gaps along these interfaces, but also via interactions within the dentinal structure, such as the deposition of intrafibrillar apatite.[16,28] A previous study observed that Bio Root exhibited a mineral zone devoid of larger particles, but with smaller particles interspersed with the interface, reducing the sealer's penetrability within the dentinal tubules.[29]

A complementary result of the present study was the presence of a fluorescent band in the dentin near to the bioceramic sealer, suggesting the formation of a hydroxyapatite barrier, already observed in previous studies, resulting from the hydration reaction of the sealer.[30-32] After final irrigation protocols, an irrigation with 5 ml of 0.01 M phosphate buffer solution in all groups. A recent systematic review demonstrated that the contact of bioceramic sealers with phosphate buffered solution is capable of increasing the precipitation of calcium hydroxyapatite, confirming its bioactivity and increasing this mineralization effect.[33] It is important to emphasize that the sealer penetrability should had occur before the formation of this mineral interface. Therefore, this cannot be considered a factor that explains the observed results of this study.

This study was limited to evaluate the influence of three different final irrigation protocols, based on the use of different substances. The association of these final irrigation protocols and the activation of the endodontic sealer was not evaluated. Also, this study did not evaluate the same parameters in teeth from younger patients, that could present dentinal tubules of greater diameters. Therefore, further studies are necessary to complement the findings of this study.

**CONCLUSION**

Regardless of the final irrigation protocol, dentinal tubule penetration of Sealer Plus BC was not observed in any root third. This may be related to multiple factors that may had interfered in this outcome.

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The authors deny any potential conflict of interests.

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