Original Article

Could the sealer thickness affect the filling ability of bioceramic endodontic materials? Evaluation in a simulated physiological condition

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Abstract

Context: Bioceramic sealers must provide filling and dimensional stability over time.

Aims: To evaluate by micro-computed tomography (micro-CT), the effect of sealer thickness on the physical properties of Bio-C Sealer (BCS) and BioRoot RCS (BR) after immersion in phosphate-buffered saline (PBS) for 7 and 30 days.

Materials and Methods: Extracted bovine teeth were prepared with ProDesign Logic 40/0.05, and the last 4-mm apical of the roots was sectioned. Then, the specimens were filled using the single-cone technique with BCS or BR (n = 8), providing a smaller sealer thickness (ST) of the apical third and a greater sealer thickness (GT) of the cervical third. The samples were immersed in PBS for 7 and 30 days. After scanning by micro-CT, the volumetric change, material/dentin interface, and filling capacity were assessed.

Statistical Analysis Used: ANOVA/Tukey, paired, and unpaired t-tests were performed (α =0.05).

Results: Sealer thickness did not influence the filling capacity in all conditions tested. After 7 days, BR had a higher volume loss than BCS, with no influence of the sealer thickness. The volumetric loss and percentage of interface gaps were higher for both sealers with GT from 7 to 30 days of immersion. BR with GT showed a higher presence of interface gaps compared to the other groups in all the experimental periods.

Conclusions: GT negatively influences the volumetric change and presence of gaps in the material/dentin interface, especially for BR. Both materials demonstrate adequate filling ability regardless of the sealer thickness used.

Keywords: Biomaterials; endodontics; physical properties; X-ray microtomography

INTRODUCTION

Endodontic sealers must fill the root canal irregularities, ramifications, and apical deltas to provide adequate three-dimensional sealing.[1,2] During the root canal filling, the sealer thickness between the gutta-percha and dentin should be as small as possible since the sealer is susceptible to solubilization and dimensional changes.[3] On the other hand, gutta-percha is a dimensionally stable material, which is considered the strong component of the filling.[4]

Calcium silicate-based materials have been used as endodontic sealers.[5] Previous reports have described excellent results for these materials, such as biocompatibility, bioactive

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potential, and satisfactory physicochemical properties.[6] Furthermore, bioceramic sealers are frequently associated with the single-cone technique, demonstrating promising results.[1,2] Bio-C Sealer (BCS; Angelus, Londrina, PR, Brazil) is a ready-to-use bioceramic sealer showing short-setting time, alkaline pH, radiopacity, and flow according to the ISO 6876:2012.[6,7] Furthermore, excellent bond strength to dentin[8] and filling capacity are described for BCS.[1,2] BioRoot RCS (BR; Septodont, St. Maur-des-Fossés, France) is a calcium silicate-based sealer available in powder/liquid presentation. Proper physicochemical[9] and biological[10] properties have been reported for this material. Furthermore, BR has penetrability capacity in the dentinal tubules,[11] but it is inferior to premixed bioceramic root canal sealers.[12] In addition, BR presented proper filling capacity in the simulated curved canals, regardless of the use of ultrasonic agitation.[13]

Despite the adequate properties of bioceramic sealers that support clinical use,[14] high-solubility values after immersion in distilled water have been reported.[15] High solubility can compromise the quality of endodontic treatment, which may lead to failure.[15] However, previous studies have pointed to a significant decrease in bioceramic sealers' solubility and volumetric change after immersion in alternative physiological fluids containing phosphate.[3,15-17] Therefore, investigations aiming to evaluate the behavior of calcium silicate-based sealers in simulated conditions that can mimic clinical behavior are relevant.[17]

Considering that the sealer thickness after filling should be minimal,[4] this study aimed to evaluate, using micro-computed tomography (micro-CT), the effect of sealer thickness on volumetric change, material/dentin interface, and filling capacity of BCS and BR after immersion for 7 and 30 days in phosphate-buffered saline (PBS). The null hypothesis was that the sealer thickness would not affect the performance of bioceramic sealers regarding the properties evaluated.

SUBJECTS AND METHODS

The materials, composition, manufacturer, proportion, and sealer thickness are shown in Table 1.

Sample size calculation

The G*Power 3.1.7 program for Windows (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) was used for the sample calculation. The ANOVA test was used with an alpha-type error of 0.05 and a beta power of 0.95 for all variables. Previous studies were used to determine the specific effect size for volumetric change, 0.90; filling ability, 0.92;[18] and interface voids, 1.71.[19] A total of seven specimens were indicated as being the ideal size needed. An $n = 8$ was used to compensate for possible losses during methodological implementation.

Root canal preparation

After approval by the ethics committee for the use of animals (Protocol number 05/2021), extracted bovine teeth were used ($n = 16$). The coronal portion was sectioned perpendicularly to the long axis of the tooth, using a carborundum disc (Fabras, São Paulo, SP, Brazil). The digital radiography system Kodak (RVG 6100 Digital Radiography System, Marne-la-Vallée, France) was used to confirm root selection. After immersion in distilled water for 48 h, the roots were irrigated with 2.5% sodium hypochlorite (NaOCl) (Cilco Farma, Serrana, SP, Brazil) and explored manually using #15 and #20 K-files (Dentsply, Maillefer, Ballaigues, Switzerland). The working length (WL) was determined at 1 mm beyond the apical foramen. The roots were molded with condensation silicone (Oranwash, Zhermack SpA, Badia Polesine, Italy) to simulate the periodontal ligament.[1,2] Subsequently, a single previously trained and calibrated operator prepared the root canals using the ProDesign Logic instrument (PDL; Easy Equipamentos Odontologicos, Belo Horizonte, MG, Brazil) operated to VDW. SILVER electric motor (VDW GmbH, Munich, Germany). The file 40/0.05 was used at 950 rpm speed and 4 Ncm torque, in continuous rotation with in-and-out movement up to the WL. During the entire preparation, the root canals were irrigated with 5 mL of 2.5% NaOCl solution. After preparation, the section of the last 4 apical millimeters of the roots was performed with a carborundum disc. The final irrigation was performed with 5 mL of 2.5% NaOCl, 2,5 mL of 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil), which was agitated with a #40 K-file for 3 min, and 5 mL

Table 1: Material, composition, manufacturer, proportion, and sealer thickness used

Material	Composition	Manufacturer	Proportion	Sealer thickness
Bio-C sealer	Calcium silicates, calcium Aluminate, calcium oxide Zirconium oxide, iron oxide, Silicon dioxide, dispersing agent	Angelus, Londrina, PR, Brazil	Ready-to-use	ST: 2 mm apical third GT: 2 mm cervical third
Bioroot RCS	Powder: Tricalcium silicate Zirconium oxide and Povidone Liquid: Aqueous solution of calcium chloride and polycarboxylate	Septodont, Saint Maur-des-Fossés, France	1 scoop powder: 5 drops of liquid	ST: 2 mm apical third GT: 2 mm cervical third

RCS: Root canal sealer, ST: Smaller sealer thickness, GT: Greater sealer thickness

of distilled water. The root canals were aspirated, and only the excess moisture was removed using absorbent paper cones size 40 (Tanari, Manacapuru, Amazonas, Brazil).

Root canal filling

The last 4 mm apical of the root canals was filled using the single-cone technique. For this procedure, principal gutta-percha cones 40/0.04 (Tanari, Manacapuru, Amazonas, Brazil) were used at 1 mm beyond the apical foramen. BCS or BR ($n = 8$) were manipulated following the manufacturer's guidelines [Table 1]. BCS was injected into the root canals using a syringe and plastic needle provided by the manufacturer. For root canals filled with BR, the sealer was inserted into the canal using a Lentulo spiral #40 (Dentsply Maillefer, Ballaigues, Switzerland) and #40 K-file with in-and-out movement up to the WL. After that, the principal cone was inserted into the root canal. The obturation of specimens provided a different sealer thickness: a smaller sealer thickness (ST) in the apical third and a greater sealer thickness (GT) in the cervical third. Then, the excess of gutta-percha was removed and compacted vertically with a heated plugger (Paiva #2; Golgran, São Caetano do Sul, SP, Brazil). Digital radiographs were taken to assess the quality of root canal filling. The samples were placed in an oven at 37°C and 95% humidity for 7 days to ensure the complete setting of the sealers.

Micro-computed tomography scan and immersion of the samples

After completely setting the sealer, all specimens were scanned in micro-CT (SkyScan 1176, Bruker, Kontich, Belgium) using the following parameters: voxel size of 8.74 µm, frame averaging of 4, Cu + Al filter, rotation step 0.5 rotation 180°, 80 kV, and 300 uA. Then, each sample was inserted individually into the Eppendorf tube with 1.5 mL of PBS (Sigma Aldrich, Barueri, SP, Brazil; pH: 7.0) ($n = 8$) and stored in an oven at 37°C for 7 and 30 days. New micro-CT scans were performed after immersion in PBS in different periods using the same parameters described previously.

Micro-computed tomography analysis

The reconstructions of images using individual parameters for each sealer were performed using the NRecon software (v. 1.6.4.7; Bruker micro-CT). Three-dimensional (3D) reconstructed images were registered in different experimental periods using the Data Viewer software through the "3D Registration" tool (v. 1.5.1; Bruker micro-CT), and the quantitative analysis was performed using the CTAn software (v. 1.15.4.0; Bruker micro-CT). Representative images were created using the CTVOx software (v. 3.2; Bruker micro-CT). Each sample was analyzed according to the filling material and sealer thickness: smaller thickness – 2 mm from the apical third and greater thickness– 2 mm from the cervical third.

The volumetric change and material/dentin interface were determined before and after immersion in PBS for 7 and 30 days. The volumetric change was calculated considering the difference in the total volume of the materials (mm^3), before and after immersion in PBS in different periods. The percentage of gaps in the material/interface was assessed based on a previous study.[20] A predefined volume of interest (VOI) was defined in the last 4 mm apical of the root, and the 3D distribution of gaps in the interface with sizes starting at 8.74 µm inside the VOI was detected by a *¨task list¨* with arithmetical and logical operations between the superimposed sections. The filling ability was determined after the setting of the sealers. The percentage of the volume of filling material (sealer and gutta-percha) was quantified in the last 4 mm apical of the root by using adaptive thresholding in the CTAn software. The full study workflow is shown in Figure 1.

Statistical analysis

Data were submitted to the Shapiro–Wilk test and met the normality requirement. Paired *t*-test and repeated-measures ANOVA-tukey were used for comparison between immersion periods. An unpaired *t*-test was used for comparison between the groups. The significance level adopted was 5% for all analyses.

RESULTS

The results are shown in Table 2 and Figures 2 and 3. Materials and sealer thickness did not influence the filling ability ($P > 0.05$). At 7 days, BR had a higher volume loss than BCS ($P < 0.05$) with no influence of the sealer thickness ($P > 0.05$). The volumetric loss and percentage of gaps in the material/dentin interface were higher for both sealers with GT from 7 to 30 days of immersion ($P < 0.05$). BR with GT showed a higher presence of interface gaps compared to the other groups in all experimental periods ($P < 0.05$).

DISCUSSION

The present study assessed the influence of sealer thickness on the physical properties of power/liquid BR or ready-to-use BCS. To the best of our knowledge, the current study is the first to consider the effect of the bioceramic sealer thickness on the outcomes of volumetric change, gaps in the material/dentin interface, and filling ability in a simulated physiological scenario. Considering that the sealer thickness influenced some material properties, our null hypothesis was rejected.

Our results revealed volumetric loss for the evaluated materials regardless of the sealer thickness. These results may be related to the high solubility attributed to bioceramic sealers,[3,15] even after immersion in a simulated

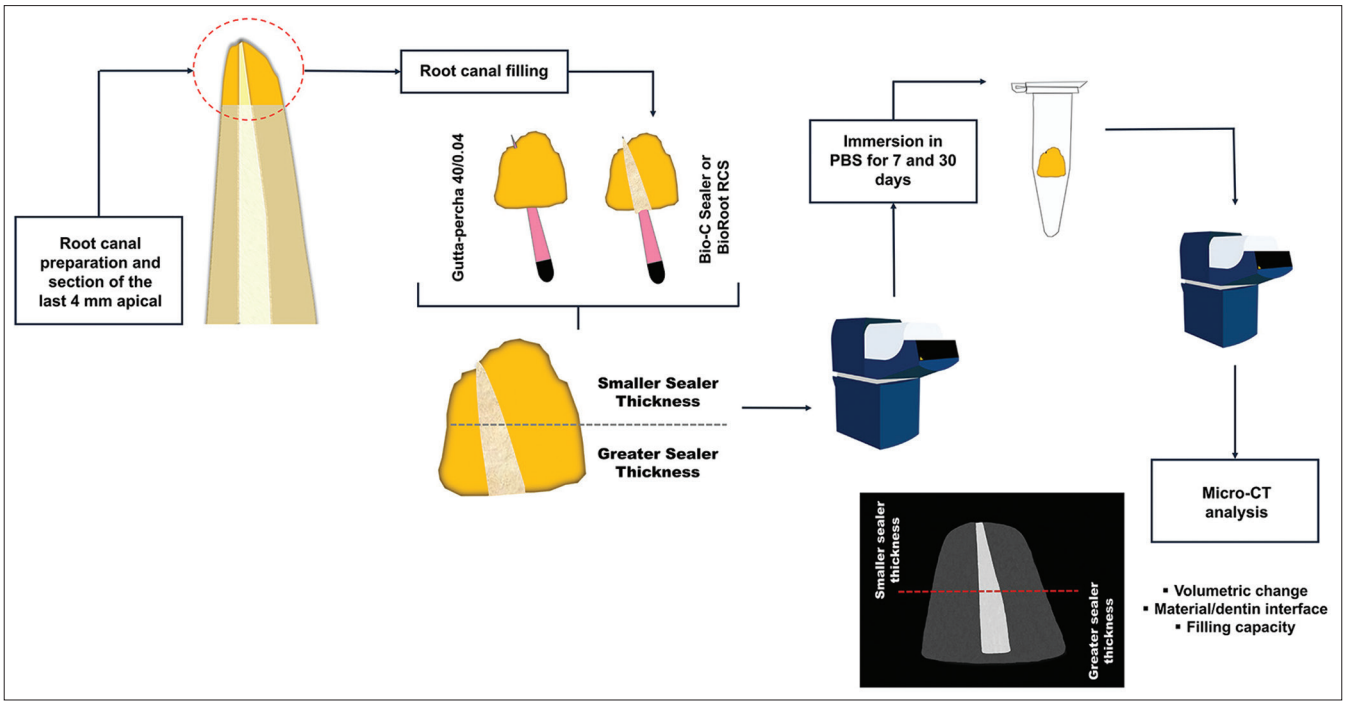

Figure 1: The study´s methodology to assess the variables under investigation (volumetric change, material/dentin interface, and filling capacity) of different calcium silicate-based sealers using micro-computed tomography images

Table 2: Mean and standard deviation of the percentage of volumetric change, gaps in the material/dentin interface, and filling ability of the last 4 mm apical of the roots filling by Bio-C sealer or Bioroot root canal sealer with smaller sealer thickness of the apical third or greater sealer thickness of the cervical third

	BCS/ST	BCS/GT	BR/ST	BR/GT
Volumetric change (days)				
7	$-0.93\pm0.22^{b,B}$	$-1.04\pm0.41^{b,B}$	$-3.17\pm1.21^{a,A}$	$-3.48\pm1.16^{a,B}$
30	$-3.62\pm1.04^{a,b,A}$	$-2.11\pm0.73^{b,A}$	$-3.94\pm1.99^{a,b,A}$	$-5.94\pm1.30^{a,A}$
Material/dentin interface				
Initial	$3.72\pm0.39^{b,B}$	$3.64\pm0.78^{b,C}$	$3.22\pm0.81^{b,C}$	$5.13\pm0.70^{a,C}$
7 days	$4.78\pm1.07^{b,B}$	$5.30\pm0.56^{b,B}$	$5.39\pm1.88^{b,B}$	$10.33\pm1.15^{a,B}$
30 days	$7.96\pm1.73^{b,A}$	$7.98\pm0.89^{b,A}$	$7.46\pm2.98^{b,A}$	$12.89\pm2.17^{a,A}$
Filling ability				
After setting	98.36 ± 0.97^{a}	99.26 ± 0.70^{a}	98.45 ± 1.04^{a}	98.76 ± 0.96^{a}

Volumetric change: Negative values indicate volume loss,The different lowercase letters in the same line indicate statistically significant differences between groups (unpaired *t*-test, $P<0.05$). The different uppercase letters in the same column indicate statistically significant differences between periods of immersion (Paired *t*-test and repeated measures ANOVA-Tukey, $P<0.05$). BCS: Bio-C sealer, BR: Bio-root RCS, ST: Smaller sealer thickness, GT: Greater sealer thickness

body fluid.[3,16] Bioceramic sealers require water to start the hydration process, so the high potential for water absorption can impact the physical properties of these materials.[16,21]

The present study showed that BR with GT exhibited a higher percentage of gaps in the material/dentin interface. This finding reinforces the concept of using a root canal filling predominantly composed of gutta-percha,[4] as it is an inert and dimensionally stable material.[2] Furthermore, this result may be correlated with the different cross-sections of the root canals. As the root canal advances in the cervical direction, the volume increases, and consequently, a greater amount of sealer is required,[1,2] which may result in a greater percentage of gaps in the material/dentin interface. In agreement with our results, a previous study demonstrated lower filling capacity in the cervical third for BR compared to GuttaFlow Bioseal (Coltene Whaledent, Langenau, Germany).[22] Furthermore, BR was associated with a higher percentage of voids when compared to AH Plus.[23] On the other hand, BCS showed a low percentage of voids in oval[5] and flattened root canals,[1,2] showing similarity to AH Plus.

Adequate filling ability (above 98%) was observed for both materials without the influence of sealer thickness. The hydration reaction of bioceramic sealers leads to the formation of hydroxyapatite and consequently aids filling, reducing the incidence of empty spaces inside the filling material.[5] Furthermore, this result may be related to the adequate flow of BCS and BR,[4] in accordance with the ISO standards (≥17 mm).[7] In fact, previous studies

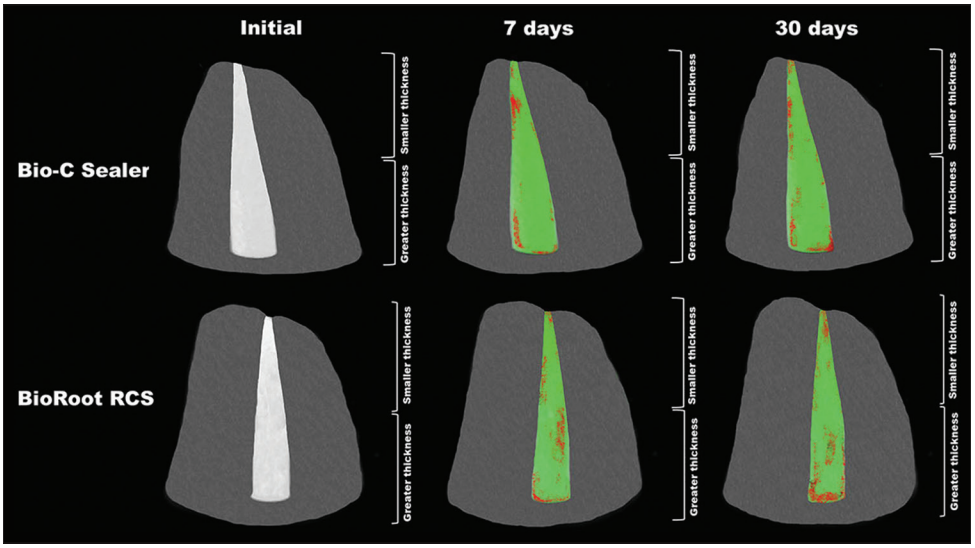

Figure 2: Three-dimensional images showing the volume of Bio-C sealer and BioRoot RCS before (white) and after (green) immersion in phosphate-buffered saline for 7 or 30 days. The volume loss is represented in red

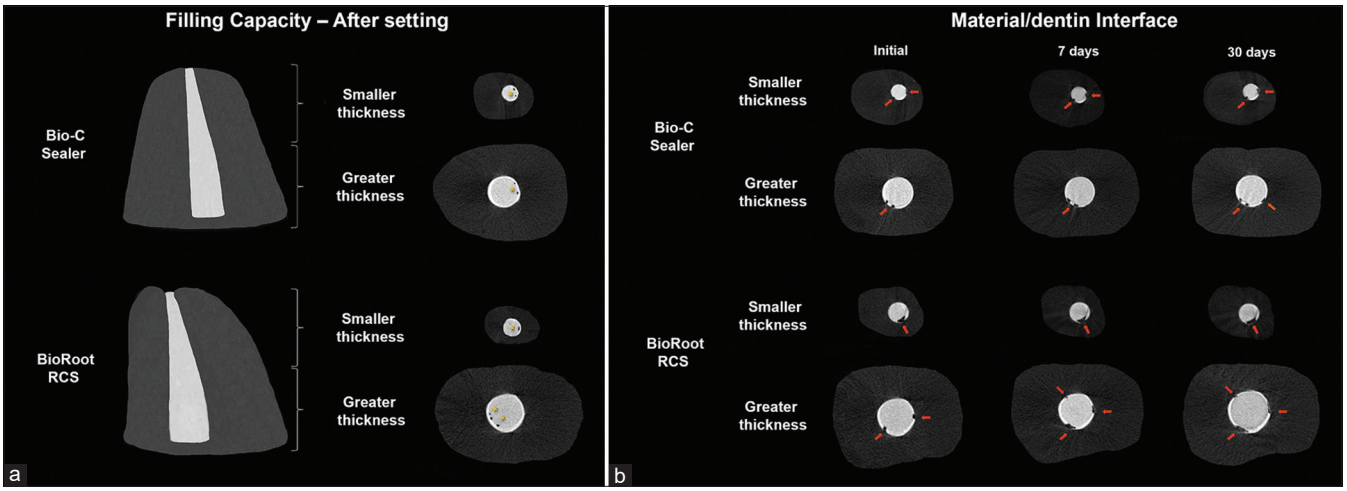

Figure 3: (a) Three-dimensional images and cross-sectional views showing the filling ability of Bio-C sealer and BioRoot RCS after setting. The yellow asterisk indicates the presence of voids for each sealer. (b) Cross-sectional views of the smaller and greater sealer thickness showing the gaps in the material/dentin interface (red arrows) of Bio-C sealer and BioRoot RCS before and after immersion in phosphate-buffered saline for 7 or 30 days

have shown fundamental importance for the flow and other physicochemical properties of bioceramic sealers during root canal filling, especially when the single-cone technique is employed.[1,2,4] Accordingly, previous reports have also shown a low percentage of voids after filling with BCS[1,2,5] and BR.[24]

Assessment of the physical properties of bioceramic sealers using dentin and a simulated body fluid is relevant, as it allows methodological reproduction closer to an *in vivo* condition.[20] Thus, in the present study, bovine teeth and PBS were used to reproduce the clinical scenario at two different moments. In this way, the variables could be quantified in an approximate clinical environment through micro-CT images, which is a highly accurate and gold-standard method for investigating dimensional and morphological changes in endodontic sealers.[25] In addition, future *in vivo* studies should be performed to complement the information obtained in this laboratory study.

The present findings reveal that GT may impair the physical properties of bioceramic sealers. Therefore, especially in the apical critical zone, the establishment of a ST is crucial, as it prevents the solubilization of the sealer and reinfection of the root canal system.

CONCLUSIONS

GT negatively influences volumetric change and material/dentin interface, especially for BR. The ready-to-use

bioceramic BCS had less influence from the sealer thickness when compared to the powder/liquid BR. Both materials demonstrate adequate filling capacity regardless of the sealer thickness used.

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Conflicts of interest

There are no conflicts of interest.

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