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Effect of immersion in distilled water or phosphate-buffered saline on the solubility, volumetric change and presence of voids within new calcium silicate-based root canal sealers

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Abstract

Torres FFE, Zordan-Bronzel CL, Guerreiro-Tanomaru JM, Chávez-Andrade GM, Pinto JC, Tanomaru-Filho M. Effect of immersion in distilled water or phosphate-buffered saline on the solubility, volumetric change and presence of voids within new calcium silicate-based root canal sealers. *International Endodontic Journal*.

Aim To assess the effect of immersion in distilled water or phosphate-buffered saline (PBS) on the solubility, volumetric change and presence of voids of calcium silicate-based root canal sealers (TotalFill BC, Sealer Plus BC and Bio-C), in comparison with the gold standard epoxy resin-based sealer (AH Plus).

Methodology All properties were evaluated after immersion in distilled water or PBS. Solubility was determined by the percentage of mass loss, whereas volumetric change and presence of voids were evaluated by micro-computed tomography, after 7 days of immersion. The volumetric change and percentage of voids between the baseline (after setting) and the experimental period were calculated. Statistical analysis was performed using one-way ANOVA and Tukey's or Student's *t*-tests ($\alpha = 0.05$).

Results The calcium silicate-based sealers had significantly greater solubility and volumetric loss than AH Plus, after immersion in distilled water or PBS ($P < 0.05$). Bio-C had the greatest solubility ($P < 0.05$), followed by TotalFill BC and Sealer Plus BC, which were similar ($P > 0.05$). Regarding the volumetric change, AH Plus had a volume increase, with similar values in distilled water and PBS ($P > 0.05$). TotalFill BC, Sealer Plus BC and Bio-C had a similar volumetric change ($P > 0.05$). The calcium silicate-based materials had the greatest solubility and volume loss after immersion in distilled water ($P < 0.05$). There was no difference in the percentage of voids amongst the sealers, before and after immersion in distilled water or PBS ($P > 0.05$).

Conclusions TotalFill BC, Sealer Plus BC and Bio-C had significantly greater solubility and volumetric loss than AH Plus. Although storage in PBS significantly reduced the solubility and volumetric change of calcium silicate-based sealers, their solubility remained above that recommend by ISO 6876. All the sealers evaluated had low and similar voids, even after immersion in distilled water or PBS.

Keywords: calcium silicate, endodontics, micro-CT, physicochemical properties, root canal filling material.

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Introduction

The development of biocompatible materials able to induce mineralized tissue deposition has been one of the main objectives of Endodontics (Almeida *et al.* 2018). Root canal sealers based on calcium silicates were developed as materials that offer biocompatibility, bioactivity and nongenotoxicity (Donnermeyer *et al.* 2018). Premixed, ready-to-use calcium silicate-based endodontic sealers, such as EndoSequence (Brasseler, Savannah, GA, USA) and TotalFill BC Sealer (FKG Dentaire, La Chaux-de-Fonds, Switzerland), have been studied (Zamparini *et al.* 2019). TotalFill BC has adequate physicochemical properties, but appears to be soluble (Poggio *et al.* 2017, Tanomaru-Filho *et al.* 2017, Colombo *et al.* 2018). Recently, the new calcium silicate-based Sealer Plus BC (MK Life, Porto Alegre, RS, Brazil) and Bio-C Sealer (Angelus, Londrina, PR, Brazil) were developed and introduced. To date, only one study has been conducted evaluating the physicochemical properties of Sealer Plus BC (Mendes *et al.* 2018). The authors observed adequate physicochemical properties, but greater solubility than that recommended by ISO 6876:2012. Regarding Bio-C, there are no studies on its properties in the literature.

Root canal filling materials should have low solubility, in order to prevent dissolution by body fluids (Urban *et al.* 2018). Moreover, the existence of voids inside the sealers can affect the filling quality (Huang *et al.* 2017). Although calcium silicate-based sealers have suitable physical properties, they have been reported to have high solubility when immersed in distilled water (Jafari & Jafari 2017, Urban *et al.* 2018). However, the use of simulated body fluids for immersion provides greater similarity to clinical application (Grech *et al.* 2013). Immersion of calcium silicate-based sealers in phosphate-buffered saline (PBS) led to surface precipitation of calcium hydroxyapatite *in vitro*, evidencing the bioactivity of these sealers (Donnermeyer *et al.* 2018). Nevertheless, information is still sparse, and further investigations are needed to evaluate the clinical relevance of bioactivity and solubility properties (Donnermeyer *et al.* 2018). In addition, the presence of voids in these newly developed bioactive sealers has not yet been fully explored (Huang *et al.* 2017).

It is also important to consider that calcium silicate-based materials can have both solubility and fluid absorption at the same time (Viapiana *et al.* 2014). When solubility is evaluated volumetrically, based on digital image analysis by micro-computed tomography (micro-CT), solubility and dimensional changes can be evaluated by the same test (Silva *et al.* 2017). Although TotalFill BC had high solubility when evaluated according to conventional tests, this material had volumetric stability in the micro-CT assessment (Tanomaru-Filho *et al.* 2017).

Based on this relevant information regarding calcium silicate-based sealers, and considering that the three-dimensional distribution of voids is important for canal filling quality, the aim of this study was to assess the effect of immersion in distilled water or PBS on the solubility, volumetric change and presence of voids of calcium silicate-based root canal sealers (TotalFill BC, Sealer Plus BC and Bio-C), in comparison with the gold standard epoxy resin-based sealer (AH Plus, DeTrey Dentsply, Konstanz, Germany). The null hypotheses were that there is no difference amongst the different sealers placed in the same immersion solution and no difference for the same sealer placed in different immersion solutions.

Materials and Methods

The endodontic sealers and their respective manufacturers, compositions and proportions are described in Table 1.

Solubility evaluation

Based on a previous study (Carvalho-Junior *et al.* 2007), circular plastic moulds 1.5 mm thick and 7.75 mm in diameter ($n = 6$) were filled with the sealers. An

Table 1 Sealers, manufacturers, compositions and proportions used

Sealer	Manufacturer	Composition	Proportion
AH Plus	Dentsply DeTrey GmbH, Konstanz, Germany	Bisphenol A/F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments dibenzyldiamine, aminoadamantane, silicone oil.	1 g : 1 g (Paste/Paste)
TotalFill BC	FKG Dentaire SA, La Chaux-de-Fonds, Switzerland	Zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler and thickening agents.	Ready to use
Sealer Plus BC	MK Life, Porto Alegre, RS, Brazil	Zirconium oxide, tricalcium silicate, dicalcium silicate, calcium hydroxide and propylene glycol.	Ready to use
Bio-C Sealer	Angelus, Londrina, PR, Brazil	Calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide and dispersing agent.	Ready to use

impermeable nylon thread was placed inside the fresh material. The moulds were placed on a glass plate covered with cellophane film and filled; then, another glass plate also covered with cellophane film was placed on the moulds, after which the assembly was placed in an incubator at 37 °C and relative humidity of 95%, for a period corresponding to three times that of their setting time. Since calcium silicate-based sealers require moisture for setting, they were evaluated by placing two pieces of wet cloth between the mould and the glass plates, as proposed by Tanomaru-Filho *et al.* (2017). The specimens were then removed from their moulds and weighed on an analytical balance (Ohaus Adventurer, Model AR2140, São Bernardo do Campo, SP, Brazil). The samples were placed inside plastic flasks containing 7.5 mL of distilled water and kept in an oven at 37 °C for 7 days. The nylon thread kept the specimens suspended and immersed in distilled water during the trial period, without allowing the samples to come into contact with the walls of the containers. After this period, the specimens were placed in the desiccator until they attained final mass stability. The solubility (mass loss) was expressed as a percentage of the original mass. This methodology was repeated for immersion of the samples in PBS.

Volumetric change and presence of voids: micro-CT evaluation

For the purpose of standardization, the samples for the volumetric change and void assessments were made in the same size as those for the solubility test. Circular plastic moulds 1.5 mm thick and 7.75 mm in diameter (n = 6), filled with the sealers, were first placed between two glass plates covered with cellophane film, and then in an incubator at 37 °C and 95% relative humidity, for a period of three times the duration of their setting time. Since the calcium silicate-based sealers require moisture for setting, they were assessed by placing two pieces of wet cloth between the mould and the glass plates, as proposed by Tanomaru-Filho *et al.* (2017). Next, the specimens were removed from their moulds and scanned by micro-CT (SkyScan 1176; Bruker-micro-CT, Kontich, Belgium). After the first scanning, the materials were placed in closed plastic flasks containing 7.5 mL of distilled water and kept in an oven at 37 °C for 7 days. Since the nylon thread for this test was not incorporated into the mass of the material, the position of the sealers was inverted in the plastic flasks after 3.5 days. As a result, the surface of the materials remained in contact with the liquid for the same period of time. After 7 days, the samples were placed in a desiccator under vacuum for 24 h and scanned again. The scanning parameters were as follows: 80 kV voltage, 300 μA current, 18 μm pixel size, copper and aluminium (Cu + Al) filter and 360° rotation. The reconstruction of the images was performed using NRecon software (V1.6.10.4; Bruker-micro-CT). The correction parameters for smoothing, beam hardening and ring artefacts were defined for each sealer. The same parameters were used for the same sealer at the different periods. The reconstructed images were superimposed on the different periods using the Data Viewer software (V1.5.2.4; Bruker-micro-CT). The 3D images were used for quantitative analysis of the samples and allowed the total volume (mm^3) and presence of voids (%) in the materials to be calculated by CTAn software (V1.15.4.0; Bruker-micro-CT). The volumetric change and percentage of voids between the baseline and the experimental period were calculated. This methodology was repeated for immersion of the samples in PBS.

Statistical analysis

All the data were analysed with the GraphPad Prism 7.00 (GraphPad Software, La Jolla, CA, USA) statistical software package. Data were submitted to one-way ANOVA and Tukey's or Student's t-tests (α = 0.05).

Results

Solubility

After immersion in distilled water, all the calcium silicate-based sealers had high solubility values. The

Table 2 Solubility values observed in root canal sealers after storage in distilled water and PBS (mean and standard deviation)

Solubility (% mass loss)	AH Plus	TotalFill BC	Sealer Plus BC	Bio-C Sealer
Distilled water	-0.02 (0.07)^{A,c}	7.82 (0.95)^{A,b}	6.45 (1.36)^{A,b}	20.53 (1.91)^{A,a}
PBS	-0.01 (0.10)^{A,c}	5.24 (2.09)^{B,b}	3.51 (1.12)^{B,b}	17.37 (2.47)^{B,a}

Different upper case letters in the same column represent significant differences for the same sealer after immersion in different solutions ($P < 0.05$). Different lower case letters on the same line represent significant differences between different sealers ($P < 0.05$).

Table 3 Volumetric change values observed in root canal sealers after storage in distilled water and PBS (mean and standard deviation)

Volumetric change (%)	AH Plus	TotalFill BC	Sealer Plus BC	Bio-C Sealer
Distilled water	0.88 (0.02)^{A,b}	−1.71 (0.36)^{A,a}	−1.07 (0.22)^{A,a}	−1.34 (0.29)^{A,a}
PBS	0.77 (0.11)^{A,b}	−0.53 (0.23)^{B,a}	−0.54 (0.27)^{B,a}	−0.58 (0.11)^{B,a}

Different upper case letters in the same column represent significant differences for the same sealer after immersion in different solutions ($P < 0.05$). Different lower case letters on the same line represent significant differences amongst different sealers ($P < 0.05$).

values after PBS immersion were significantly lower than after water immersion ($P < 0.05$). However, both values were higher than the minimum required by ISO 6876. Bio-C had significantly greater solubility ($P < 0.05$), followed by TotalFill BC and Sealer Plus BC, which had similar values ($P > 0.05$). AH Plus had the lowest solubility in both distilled water and PBS ($P < 0.05$), with similar values for both solutions ($P > 0.05$), and complied with the ISO standards. The results are described in Table 2.

Volumetric change

The volumetric changes observed for the root canal sealers are shown in Table 3. Figure 1 shows 3D models that illustrate the different sealers before and after immersion in PBS. AH Plus had the lowest volumetric change, regardless of the immersion liquid ($P < 0.05$). In addition, AH Plus was the only material that had a volume gain, with similar values for distilled water and PBS ($P > 0.05$). TotalFill BC, Sealer Plus BC and Bio-C were similar ($P > 0.05$) and had a significantly greater volume loss when immersed in distilled water ($P < 0.05$).

Presence of voids

The presence of voids observed in the root canal sealers is presented in Table 4 and Fig. 2. AH Plus, TotalFill BC, Sealer Plus BC and Bio-C were similar

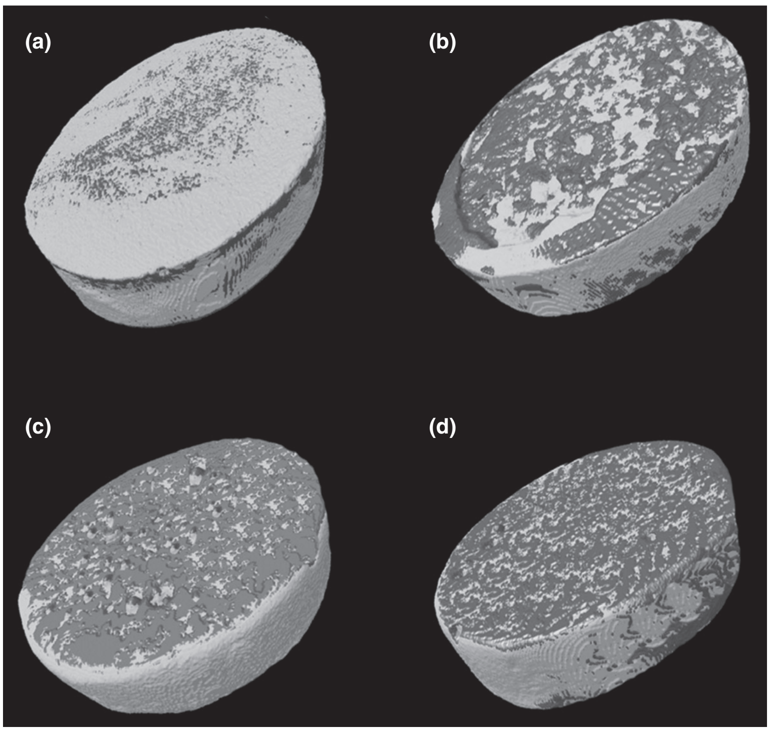

Figure 1 3D models using CTVol software, illustrating sealers (a: AH Plus; b: TotalFill BC; c: Sealer Plus BC; and d: Bio-C Sealer), before (white) and after (grey) immersion in PBS during volumetric change evaluation.

Table 4 Void values observed in root canal sealers after setting (initial) and after storage in distilled water and PBS (mean and standard deviation)

Voids (%)	AH Plus	TotalFill BC	Sealer Plus BC	Bio-C Sealer
Initial	0.79 (0.34)	0.93 (0.13)	0.75 (0.24)	0.81 (0.13)
Distilled water	0.91 (0.13)	1.01 (0.12)	0.68 (0.19)	1.01 (0.28)
PBS	0.67 (0.22)	0.89 (0.15)	0.68 (0.16)	1.01 (0.25)

There was no significant difference for the same sealer after immersion in different solutions ($P > 0.05$). There was no significant difference amongst different sealers ($P > 0.05$).

($P > 0.05$). There was no difference in the percentage of voids for all the materials after immersion in distilled water and in PBS ($P > 0.05$).

Discussion

This study assessed the solubility, volumetric change and presence of voids of new calcium silicate-based sealers, in comparison with AH Plus, after immersion in distilled water or PBS. The null hypotheses were rejected, since several results observed between the sealers and the immersion solution were different and affected the properties of the calcium silicate-based materials.

Root canal filling materials should have low solubility, in order to prevent microbial and fluid leakage (Zarra *et al.* 2018). AH Plus was less soluble than the calcium silicate-based sealers. Moreover, Bio-C had higher values than TotalFill BC and Sealer Plus BC. AH Plus is an epoxy resin-based sealer commonly used as a comparative material, because of its adequate properties (Silva *et al.* 2017). These properties include low solubility, since epoxy resin has strong cross-links (Viapiana *et al.* 2014). On the other hand, calcium silicate-based materials are more hydrophilic, and, therefore, more soluble (Siboni *et al.* 2017).

The current study evaluated the solubility of sealers based on a previous study that proposed smaller sample dimensions without affecting the accuracy of the test (Carvalho-Junior *et al.* 2007). Although the present study evaluated different type of sealers, this methodology has been used to assess endodontic materials with various compositions, including calcium silicate-based materials (Viapiana *et al.* 2014, Tanomaru-Filho *et al.* 2017, Torres *et al.* 2018). This

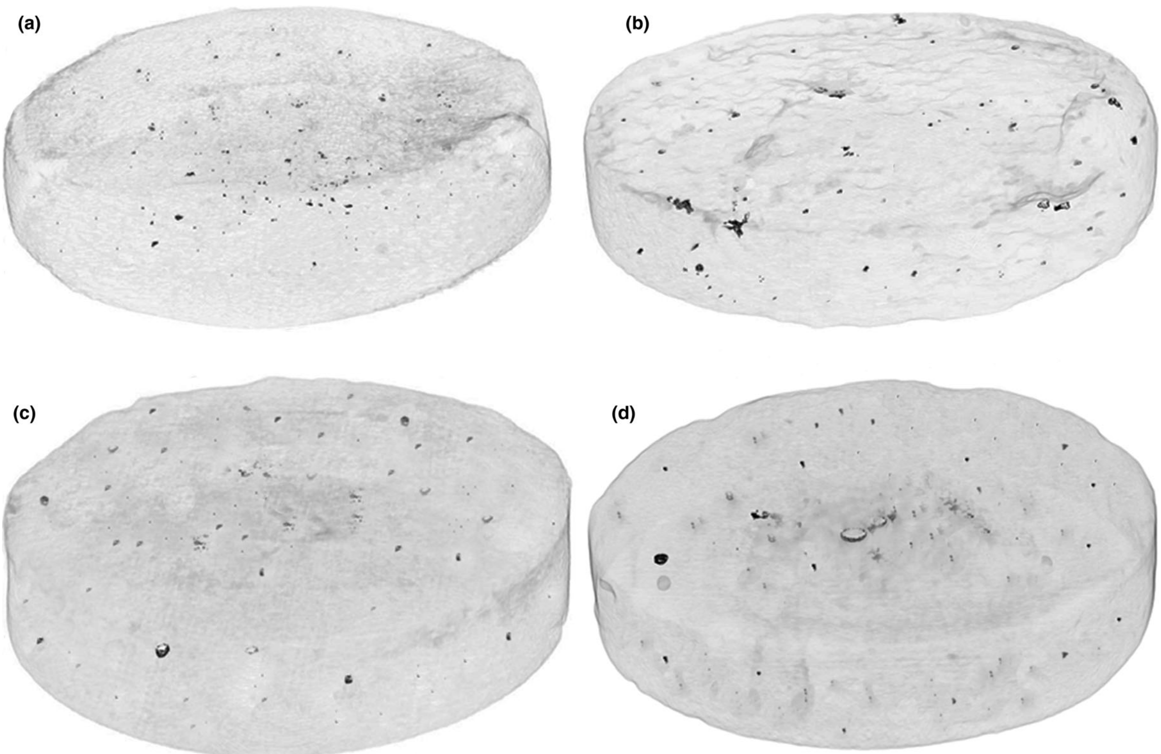

Figure 2 3D models using CTVox software illustrating sealers (a: AH Plus; b: TotalFill BC; c: Sealer Plus BC; and d: Bio-C Sealer) regarding the presence of voids.

conventional test for solubility assessment is based on the measurement of the mass loss of the specimen before and after immersion in distilled water. However, the solubility of calcium silicate materials in distilled water does not express the real condition of the materials *in vivo* (Siboni *et al.* 2017). On the other hand, previous studies reported reduced solubility when calcium silicate-based materials were immersed in a simulated body fluid (Torres *et al.* 2018, Urban *et al.* 2018). Nevertheless, the present findings revealed that even after immersion in PBS, solubility remained above the minimum level recommended by ISO 6876.

Another limitation of conventional solubility tests is that specimens of materials with water uptake capacity may absorb water from the environment (Viapiana *et al.* 2014). Although water sorption is not evaluated using the volumetric change test, the comparison between the initial and final volumes in the digital image analysis allows the evaluation of material properties in a more clinical situation (Silva *et al.* 2017). According to this methodology, solubility and dimensional changes can be evaluated by the same experiment (Silva *et al.* 2017). Moreover, micro-CT allows longitudinal follow-up of the samples (Neves *et al.* 2019). This is the first study to report volumetric change values of endodontic materials after immersion in distilled water or PBS.

The present study showed that calcium silicate-based sealers had greater solubility than AH Plus. Moreover, calcium silicate-based sealers were associated with volume loss whilst AH Plus had volume gain after immersion in distilled water or PBS. For the purpose of standardization of the solubility test, the samples were stored in a desiccator for 24 h after 7 days of immersion, before the final scanning process. This means that the reduction in the original weight or volume obtained by both methods was also caused by evaporation of the free fluids during the final drying of the samples (Gandolfi *et al.* 2015). Moreover, desiccation has a significant effect on sorption, solubility and volumetric expansion of water-based endodontic materials, since the cements not submitted to pre-storage desiccation are subject to positive volumetric expansion after water immersion (Mustafa *et al.* 2018).

In addition to low solubility, endodontic materials should not have a high percentage of voids (Gandolfi *et al.* 2016), since the presence of voids may increase post-treatment apical periodontitis (Başer Can *et al.* 2017). Hence, the current study also investigated the presence of voids using micro-CT. Micro-CT is a highly accurate, nondestructive tool for measuring voids (Huang *et al.* 2018, Kim *et al.* 2018). A low percentage of voids was detected for the materials. The results corroborate previous studies comparing AH Plus and calcium silicate-based sealers (Celikten *et al.* 2016, Wang *et al.* 2018, Kim *et al.* 2019). This finding can be correlated with the small particle size and high viscosity of these sealers (Celikten *et al.* 2016, Huang *et al.* 2017). Nevertheless, a limitation of the present study was the pixel size of 18 μm applied during micro-CT scanning. Although the image resolution allowed voids to be segmented and quantified, smaller pixel sizes improve image quality and detection of voids (Huang *et al.* 2017, 2018, Kim *et al.* 2018, Orhan *et al.* 2018).

The present study revealed that although TotalFill BC, Sealer Plus BC and Bio-C had low voids, their solubility remained above the minimum recommended by ISO 6876, even after immersion in PBS. Therefore, since high solubility can compromise the long-term outcomes of root canal treatment, based on the ISO standard these sealers should not be used clinically. Further studies should be performed to acquire greater knowledge of the performance of calcium silicate-based sealers.

Conclusion

Calcium silicate-based sealers had significantly greater solubility and volumetric loss than AH Plus. All the sealers evaluated had similar low percentages of voids, even after immersion in distilled water or PBS. Although storage in PBS significantly reduced the solubility and volumetric change of TotalFill BC, Sealer Plus BC and Bio-C, their solubility remained above the minimum amount recommend by ISO 6876. Therefore, according to ISO standards, these sealers should not be used clinically.

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Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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