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Evaluation of intratubular penetration of Bio-C Sealer: a calcium silicate-based sealer

Avaliação da penetração intratubular do Bio-C Sealer: um cimento à base de silicato de cálcio

Evaluación de la penetración intratubular de Bio-C Sealer: un sellador a base de silicato de calcio

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Abstract
This study aimed to evaluate the intratubular penetration of Bio-C Sealer using confocal laser scanning microscopy (CLSM). Sixty canines were divided into four groups (n = 15): Bio-C Sealer, Endo Sequence BC Sealer, Sealer Plus BC, and AH Plus. Three 1-mm thick discs were obtained from each sample and analyzed to determine the area and maximum penetration depth of the sealers. Bio-C Sealer showed maximum penetration in the cervical and middle third, lower than the AH Plus ($p < 0.05$) but similar to the other calcium silicate sealers evaluated ($p > 0.05$). In the apical thirds, there was no difference between the sealers ($p > 0.05$). The area of penetration of Bio-C Sealer was similar to AH Plus and calcium silicate sealers ($p > 0.05$). Hence, it can be concluded that Bio-C Sealer had similar intratubular penetration as the other tested sealers, and it can be used as an endodontic sealer.
Keywords: Calcium silicate; Confocal laser scanning microscopy; Endodontics; Root canal filling materials; Root canal obturation.

Resumo
Este estudo teve como objetivo avaliar a penetração intratubular do Bio-C Sealer utilizando microscopia confocal de varredura a laser (CLSM). Sessenta caninos foram divididos em quatro grupos (n = 15): Bio-C Sealer, Endo Sequence BC Sealer, Sealer Plus BC e AH Plus. Três discos de 1 mm de espessura foram obtidos de cada amostra e analisados para determinar a área e profundidade máxima de penetração dos cimentos. O Bio-C Sealer apresentou penetração máxima no terço cervical e médio, inferior ao AH Plus ($p < 0,05$), mas semelhante aos demais cimentos de silicato de cálcio avaliados ($p > 0,05$). Nos terços apicais não houve diferença entre os cimentos ($p > 0,05$). A área de penetração do Bio-C Sealer foi semelhante ao AH Plus e aos selantes de silicato de cálcio ($p > 0,05$). Assim, pode-se concluir que o Bio-C Sealer teve penetração intratubular semelhante aos demais cimentos testados, podendo ser utilizado como cimento endodôntico.
Palavras-chave: Silicato de cálcio; Microscopia confocal de varredura a laser; Endodontia; Materiais de obturação do canal radicular; Obturação do canal radicular.

Resumen
Este estudio tuvo como objetivo evaluar la penetración intratubular de Bio-C Sealer usando microscopía de escaneo láser confocal (CLSM). Sesenta caninos se dividieron en cuatro grupos (n = 15): Bio-C Sealer, Endo Sequence BC Sealer, Sealer Plus BC y AH Plus. De cada muestra se obtuvieron tres discos de 1 mm de espesor y se analizaron para determinar el área y la profundidad máxima de penetración de los selladores. Bio-C Sealer mostró máxima penetración en el tercio cervical y medio, inferior al AH Plus ($p < 0,05$) pero similar a los demás selladores de silicato de calcio evaluados ($p > 0,05$). En los tercios apicales no hubo diferencia entre los selladores ($p > 0,05$). El área de penetración de Bio-C Sealer fue similar a AH Plus y selladores de silicato de calcio ($p > 0.05$). Por lo tanto, se puede concluir que Bio-C Sealer tuvo una penetración intratubular similar a la de los otros selladores probados, y puede usarse como sellador endodóntico.
Palabras clave: Silicato de calcio; Microscopía de escaneo láser confocal; Endodoncia; Materiales de obturación de conductos radiculares; Obturación del conducto radicular.

1. Introduction

Endodontic sealers are used to fill the space between the dentinal walls and gutta-percha cones to prevent gaps that can enable microbial infiltration (Schilder, 2006). To achieve this, the sealer must have appropriate physicochemical properties, allowing it to penetrate the dentinal tubules through a correct root filling technique (Mamootil & Messer, 2007; Carneiro et al., 2012).

Different root canal sealers have been used for this purpose. Calcium silicate-based sealers show low cytotoxicity and high biocompatibility, along with the potential to promote an osteogenic response (Lee et al., 2019; Alves-Silva et al., 2020). Calcium silicate-based sealers are reported to have a sealing ability comparable to that of resin-based sealers, such as AH Plus (Dentsply Sirona, Ballaigues, Switzerland) (Ersahan & Aydin, 2013). AH Plus is an epoxy resin-based sealer that presents good biological and physical properties and is accepted as the gold standard for evaluating new root canal sealers (De-Deus et al., 2012; Tavares et al., 2013; Hergt et al., 2015; Khalil et al., 2016; Viapiana et al., 2016).

The sealer penetration and the adhesive interface established between the filling material and dentin can be assessed by different methodologies, with confocal laser scanning microscopy (CLSM) being the most accepted (Tedesco et al., 2014; Jardine et al., 2016; Jeong et al., 2017; Piai et al., 2018; El-Hachem et al., 2019; Coronas et al., 2020; De-Bem et al., 2020; Eid et al., 2021; Furtado et al., 2021). Fluo-3 is a fluorescent indicator that traces calcium ions, and it can be used to trace the penetration of calcium silicate-based sealers in the dentinal tubules (Jeong et al., 2017; Coronas et al., 2020; Furtado et al., 2021). When mixed with the sealer, Fluo-3 allows visualization of the material in the dentinal tubules by CLSM images with few artifacts (Jeong et al., 2017; Coronas et al., 2020; Furtado et al., 2021).

To date, no correlation has been found between sealer penetration and sealing ability (De-Deus et al., 2012). However, various studies indicate that the penetration of root canal sealer into the dentinal tubules promotes a physical barrier (White et al., 1984, Kokkas et al., 2004; Kara-Tuncer et al., 2012; Castagna et al., 2013; Jardine et al., 2016), improves retention of the root filling (White et al., 1984), and entomb the residual bacteria (Mamootil & Messer, 2007; Wang et al., 2014; Barbosa et al., 2020).

The physical and chemical properties of the sealer, including particle size, solubility, viscosity, and surface tension, influence the penetration depth (Mamootil & Messer, 2007; Khalil et al., 2016). Several new calcium silicate-based ready-to-use endodontic sealers have been developed. Bio-C Sealer (Angelus, Londrina, Brazil) has been shown to have a short setting time, alkalinization ability, adequate flow, radiopacity (Zordan-Bronzel et al., 2019), biocompatibility, bioactive property (Alves-Silva et al., 2020), and exhibits an antimicrobial potential comparable to that of the other sealers (Barbosa et al., 2020). However, to date, only one study has assessed the intratubular penetration of Bio-C Sealer (Eid et al., 2021), and the purpose of this study was to investigate dentinal tubule penetration of Bio-C Sealer in comparison with AH Plus, Sealer Plus BC (MK Life, Porto Alegre, Brazil) and EndoSequence BC Sealer (Brasseler USA, Savannah, GA), using CLSM, by area (mm^2) and

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maximum penetration depth (µm). The null hypothesis states that there is no difference in the extent of dentinal tubule sealer penetration among the four tested sealers.

2. Methodology

This study was approved by the Institutional Ethics Committee (Protocol 2,823,882). The sample size was estimated using the results of dentinal tubule penetration of different sealers from El-Hachem et al. (2019). An alpha of 0.05, the probability of a type I error, and a power of 0.9, were used.

This lab study of quantitative nature investigated the dentinal tubule penetration of Bio-C Sealer in comparison with AH Plus, Sealer Plus BC, and EndoSequence BC Sealer, using CLSM (Ordinola-Zapata et al., 2009; Jardine et al., 2016; Piai et al., 2018; El-Hachem et al., 2019; De-Bem et al., 2020; Eid et al., 2021).

Sample selection

Sixty extracted human mandibular canines with a single canal were selected for the study. The crowns were removed, and the root length was standardized to 12 mm. Canal patency was checked with a #10 file (Dentsply Sirona, Ballaigues, Switzerland), and the working length (WL) was determined to be 1 mm below the foraminal exit.

Root canal preparation and filling

All treatments were performed by a single operator who was a specialist in endodontics with 20 years of experience. The canals were prepared using WaveOne Gold Large (45.05, Dentsply Sirona, Ballaigues, Switzerland) coupled to an X-Smart Plus endodontic electric motor (Dentsply Sirona, Ballaigues, Switzerland), following the manufacturer's instructions. During preparation, the root canal was irrigated with 2 mL of 2.5% sodium hypochlorite (NaOCl) at each instrument insertion, and the instrument was cleaned with gauze moistened with 2.5% NaOCl after each introduction until the WL was reached. The root canal was filled with 2 mL of 2.5% NaOCl, and the solution was activated with the E1 Irrisonic ultrasonic insert (Helse Ultrasonic, Santa Rosa de Viterbo, Brazil) at power 1 inserted up to 1 mm below the WL. Three cycles of 20 s each was performed. The same protocol was followed for 17% EDTA. The canals were then irrigated with 2 mL of saline and dried with absorbent paper tips.

The teeth were randomly divided into four experimental groups (n = 15) according to the endodontic sealer used: Endo Sequence BC Sealer, Bio-C Sealer, Sealer Plus BC, and AH Plus. Table 1 presents the composition of the endodontic sealer used in this study. AH Plus was handled according to the manufacturer's recommendations. The other sealers did not require handling. Fluo-3 (Sigma-Aldrich, St. Louis, MO, USA) was added to the sealers at a ratio of 1:10,000 (0.0001 g of Fluo-3 dye to 1 g of sealer). Sealers and dyes were weighed using a precision analytical balance (Shimadzu, Tokyo, Japan).

Table 1 - Composition of endodontic sealers used in the present study.

Endodontic Sealer	Composition
AH Plus	Paste A: Bisphenol epoxy resin-A, Bisphenol epoxy resin-F, calcium tungstate, zirconium oxide, silica, iron oxide pigments. Paste B: Dibenzyldiamine, aminodiamantana, tricyclodecane-diamine, calcium tungstate, zirconium oxide, silica, silicone oil.
Sealer Plus BC	Diluting resin, Mineral Trioxide Aggregate, nanoparticulated sílica, pigments, zirconium oxide, tri-calcium silicate, sicalcium silicate, calcium hydroxide, propylene glycol.
Bio-C Sealer	Calcium Silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide and dispersing agent.
Endo Sequence BC Sealer	Zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler and thickening agents.

Source: Authors.

The canals were filled using the single-cone technique using the WaveOne Gold Large cone (Dentsply Sirona, Ballaigues, Switzerland). The excess cone was cut, and vertical condensation was performed. Two millimeters of gutta-percha were removed from the canal entrance and the coronary portion was sealed with temporary filling cement (Cotosol, Coltene, Altstätten, Switzerland). The teeth were then stored for 72 h in an oven set to 37 °C with 100% humidity.

CLSM analysis

Each specimen was sectioned perpendicular to its long axis with a diamond disc in a low-speed cutter (Isomet 1000, Buehler, Illinois, USA) under refrigeration. Three 1 mm thick slices were obtained from each sample at inter-slice distances of 2 mm (apical third), 5 mm (middle third), and 8 mm (cervical third) from the foraminal exit.

The coronal surface of the sections was polished under refrigeration, with sandpaper of decreasing grain to a final grit of 600. For debris removal, the samples were immersed in 17% EDTA for two min and then immersed in 2.5% NaOCl for another two min.

All slices were evaluated using a confocal laser scanning microscope (Olympus Fluoview 1000, Olympus Corporation, Tokyo, Japan), with emission wavelengths of 473/520 nm and ×10 magnification. A set of images ("stacks"), with an interval of 2 µm between them and with a resolution of 512 × 512 pixels, was obtained from each sample.

In each image, the circumference of the root canal wall and the circumference of sealer penetration were measured using Photoshop CC V.19.1.2 software (Adobe Inc., San Jose, California, USA), and the canal and penetration areas were then calculated. The sealer penetration area (μm^2) in the dentinal tubules was obtained by subtracting the value of the root canal area from the penetration area.

The maximum depth of sealer penetration (µm) was measured in each image using ImageJ® V1.46r software (US National Switzerland) and was determined as the longest sealer penetration point between the canal wall and the external root surface.

The Kolmogorov-Smirnov test was used to test the normality distribution of the data. The Friedman test, followed by Dunn's test, was used to compare the root canal thirds in the same group. The Kruskal-Wallis test was used to compare the sealers, followed by Dunn’s test. The level of significance was set at 5%.

3. Results

Considering the maximum linear penetration (µm) of the sealers, the intra-group analysis showed no difference between the thirds in the Bio-C Sealer ($p > 0.05$). Other sealers displayed a greater penetration in the cervical third than in the apical third ($p < 0.05$).

In table 2 presents the median and interquartile distance of maximum penetration (µm) of AH Plus, Endo Sequence BC Sealer, Bio-C Sealer and Sealer Plus BC, in each third. Considering the comparison between the groups, AH Plus showed greater penetration in the cervical third than the other sealers. In the middle third, AH Plus showed significantly better penetration than Sealer Plus BC ($p < 0.05$). In the apical third, there was no significant difference between the groups ($p > 0.05$).

Table 2. Median and interquartile distance of maximum penetration (µm) of AH Plus, Endo Sequence BC Sealer, Bio-C Sealer and Sealer Plus BC, in each third.

	Cervical	Middle	Apical
AH Plus	518.7^{aA}	347.4^{aA}	38.8^{bA}
	(280.6 - 730.9)	(105.4 - 677.8)	(0 - 74.4)
Endo Sequence	121.3^{aB}	127.3^{abAB}	52.9^{bA}
	(81.8 - 185.1)	(61.4 - 307.9)	(7.7 - 107.4)
Bio-C Sealer	128.8^{aB}	113.3^{aAB}	66.6^{aA}
	(97.41 - 321.2)	(37.57-205.8)	(18.6 - 214.7)
Sealer Plus BC	130.3^{aB}	98.0^{aB}	21.7^{bA}
	(95.6 - 194.2)	(55.5 - 120.0)	(4.2 - 48.3)

Different lowercase letters, in each line, indicate significant differences ($p < 0.05$). Different capital letters, in each column, indicate significant differences ($p < 0.05$). Source: Authors.

Considering the sealer penetration area (µm^2), the intra-group analysis showed that the Bio-C Sealer had a greater penetration area in the cervical third compared to the other thirds ($p > 0.05$).

In table 3 presents the median and interquartile distances of the penetration area (µm^2) of AH Plus, Endo Sequence BC Sealer, Bio-C Sealer, and Sealer Plus BC, in each third analysis. The comparison between groups, in each third, indicated that AH Plus and Bio-C Sealer had no difference in the penetration area in the cervical and middle thirds ($p > 0.05$). No differences were observed in the penetration area of the different sealers in the apical third ($p > 0.05$).

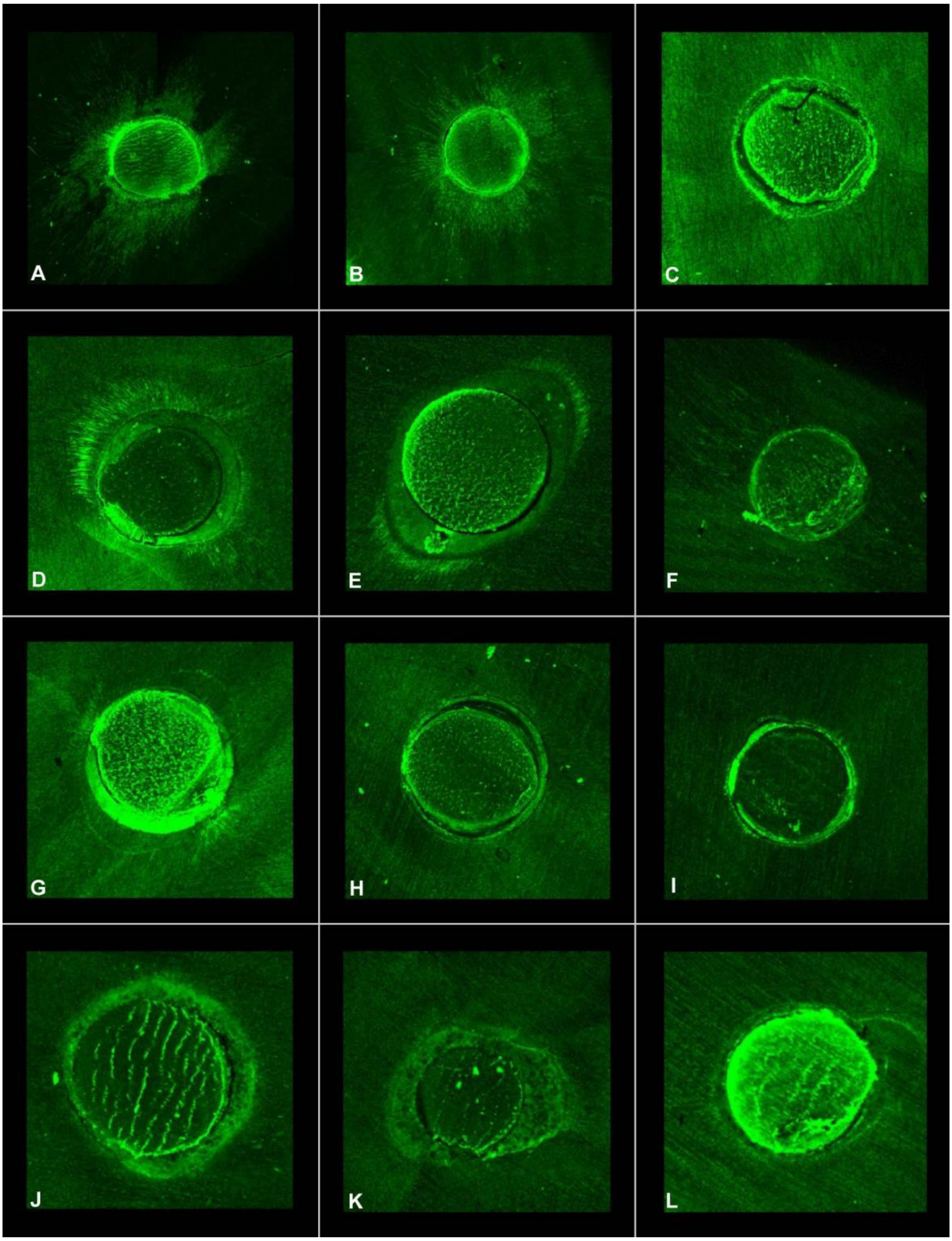
Table 3. Median and interquartile distances of the penetration area (µm^2) of AH Plus, Endo Sequence BC Sealer, Bio-C Sealer and Sealer Plus BC, in each third analyze.

	Cervical	Middle	Apical
AH Plus	466.3^{aA}	187.1^{aA}	6.2^{bA}
	(127.6 - 842.1)	(27.2 - 303.8)	(0 - 11.5)
Endo Sequence	74.3^{aB}	63.6^{aAB}	11.6^{aA}
	(15.9 - 92.6)	(5.2 -124.6)	(0.8 - 22.0)
Bio-C Sealer	60.7^{aAB}	32.7^{bAB}	12.2^{bA}
	(40.1 - 163.7)	(8.3 - 55.5)	(9.0 - 72.4)
Sealer Plus BC	37.0^{aB}	23.0^{aB}	6.3^{bA}
	(11.1 - 70.6)	(9.6 - 36.0)	(1.4 - 9.4)

Different lowercase letters, in each line, indicate significant differences ($p < 0.05$). Different capital letters, in each column, indicate significant differences ($p < 0.05$). Source: Authors.

Figure 1 show representative images from CLSM of the root canal sealers penetrability into dentinal tubule at the cervical, middle, and apical thirds.

Figure 1 – Representative CLSM images showing cervical, middle, and apical canal slices sealed with AH Plus, Endo Sequence BC Sealer, Bio-C Sealer and Sealer Plus BC. AH Plus cervical (A), middle (B), and apical (C); Endo Sequence BC Sealer cervical (D), middle (E) and apical (F); Bio-C Sealer cervical (G), middle (H), and apical (I); and Sealer Plus BC cervical (J), middle (K), and apical (L).



Source: Authors.

4. Discussion

This study investigated the dentinal tubule penetration of Bio-C Sealer in comparison with AH Plus, Sealer Plus BC, and EndoSequence BC Sealer, using CLSM. The null hypothesis of this study was rejected because there was a difference in the intratubular penetrability of endodontic sealers.

To date, only one study has assessed the intratubular penetration of Bio-C Sealer (Eid et al., 2021). Eid et al. (2021) evaluated the tubular penetration of Bio-C Sealer and HiFlow (Brasseler, Savannah, GA, SA) and did not observe differences between the sealers tested.

In this study, Bio-C Sealer was compared with AH Plus, Sealer Plus BC, and EndoSequence BC Sealer, and Bio-C Sealer showed maximum penetration depth in the cervical and middle thirds compared to the apical third, lower than the AH Plus, but similar to the other calcium silicate sealers evaluated. In the apical third, there was no difference in the penetration depth between the sealers. Area of penetration of Bio-C Sealer was similar to AH Plus and other calcium silicate sealers.

The CLSM is an essential technology and a tool to evaluate the penetrability of root canal sealers on dentinal tubule (Ordinola-Zapata et al., 2009; Jardine et al., 2016; Piai et al., 2018). The penetration depth of the sealers in the dentinal tubules can be visualized in horizontal sections, as evidenced by the fluorophore (Rhodamine B or Fluo-3) that was mixed with the endodontic sealer (Ordinola-Zapata et al., 2009).

The most used fluorophore for the assessment of intratubular penetration of sealers is rhodamine B (Ordinola-Zapata et al., 2009; De-Deus et al., 2012; Jardine et al., 2016; Piai et al., 2018; El-Hachem et al., 2019; Eid et al., 2021). However, in contact with calcium-containing sealers, it causes a leaching effect, tracking small degrees of dentin moisture and emitting fluorescence, regardless of the presence of material, which causes a deeper penetration of dye in the dentinal tubules, resulting in inaccurate results (Jeong et al., 2017). Furtado et al. (2021) evaluated whether fluorophores (Rhodamine or Fluo-3) influence the CLSM images of intratubular penetration of the AH Plus, Sealer Plus, Sealer Plus BC, and EndoSequence BC Sealer and concluded that the type of fluorophore influences the tubular penetration of calcium silicate sealers but not of epoxy resin-based sealers when CLSM is used for assessment and that bioceramic sealers should not be used in association with Rhodamine B.

Considering that in the present investigation, all evaluated sealers had calcium in their composition, and hence the Fluo-3 dye was used. Despite being a consolidated marker, previous studies that used Rhodamine B together with materials containing calcium may have overestimated the sealer intratubular penetration (El-Hachem et al., 2019; De-Bem et al., 2020; Eid et al., 2021). This was confirmed by Furtado et al. (2021) and supported by the current study, which showed that the values of sealer penetration were lower than those found in studies that used Rhodamine B (Piai et al., 2018; El-Hachem et al., 2019; Eid et al., 2021).

In this study, in the coronal third, AH Plus had greater intratubular penetration than EndoSequence BC Sealer and Sealer Plus BC. These results differ from those observed by Furtado et al. (2021) who observed similar penetration in the cervical third for the same sealers.

In this study, in the middle third, intratubular penetration of AH Plus was similar to the EndoSequence BC Sealer, which was also observed by De-Bem et al. (2020) and Furtado et al. (2021) and differs from that observed by El-Hachen et al. (2019) showed that AH Plus penetrated less than EndoSequence BC Sealer. In that study, rhodamine B was used as the dye. When AH Plus was compared to Sealer Plus BC, it showed greater intratubular penetration. These results are different from those found in other studies (Coronas et al., 2020, De-Bem et al., 2020; Furtado et al., 2021). In the apical third, there was no difference between the evaluated sealers, which was also similar to the observations in other studies (El-Hachem et al., 2019; Coronas et al., 2020; De-Bem et al., 2020; Furtado et al., 2021).

It is known that the penetration of endodontic sealer into dentinal tubules depends on many factors, including the physical and chemical properties of the sealer, effectiveness of the removal of the smear layer, and anatomy of the root canal system (Kara-Tuncer & Tuncer, 2012).

It has been reported that the flushing effect and hydrodynamic agitation might affect the efficiency of irrigation solutions and smear layer removal. In fact, its adherence forms physical barriers and contamination in the dentinal tubules, blocking the penetration of the sealer (White et al., 1984; Kokkas et al., 2004; Kara-Tuncer & Tuncer, 2012; Castagna et al., 2013; Jardine et al., 2016). Therefore, the irrigation protocol provided in this study was characterized using 2.5% NaOCl during mechanochemical preparation, being agitated with the aid of ultrasound, followed by irrigation with 17% EDTA using the same protocol, which presents good results regarding the removal of the smear layer (Piai et al., 2018; Coronas et al., 2020).

The results presented here agree with the findings in the literature that demonstrated greater sealer penetration in the cervical than in the apical third (Jardine et al., 2016; Piai et al., 2018; El-Hachem et al., 2019; Eid et al., 2021; Furtado et al., 2021). The presence of sclerotic dentin, small number of dentinal tubules, and greater difficulty in smear layer removal in the apical region may explain the difficulty of sealer penetration into the dentin (Ordinola-Zapata et al., 2009).

Another essential characteristic of sealers that contribute to dentinal penetration is the flow rate (Piai et al., 2018). The literature has demonstrated that AH Plus seems to perform its role as a filling sealer quite satisfactorily when considering its physicochemical and biological properties; it is considered the “gold standard” for endodontic sealers (De-Deus et al., 2012; Tavares et al., 2013; Hergt et al., 2015; Khalil et al., 2016; Viapiana et al., 2016). Bio-C Sealer is a new endodontic calcium silicate-based sealer recently introduced into the market. Previous studies have shown that solubility and volumetric variation, setting time, flow rate, adequate radiopacity (Zondan-Bronzel et al., 2019), bioactivity and biocompatibility (Alves-Silva et al., 2020), and capacity to fill the root canals (Tavares et al., 2021; Santos-Júnior et al., 2021) of Bio-C Sealer similar to other bioceramic sealers (Torres et al., 2020). In the present study, dentinal tubule penetration of Bio-C sealer was similar to that of AH Plus and other calcium silicate sealers. Based on these results and previous investigations, the Bio-C sealer is recommended as an effective root-end filling material.

5. Final Considerations

In conclusion, Bio-C Sealer showed maximum penetration in the cervical and middle thirds compared to the apical third, lower penetration than the AH Plus, but similar to the other calcium silicate sealers evaluated. In the apical thirds, no difference in penetration was observed between the sealers. Area of penetration of Bio-C Sealer was similar to AH Plus and other calcium silicate sealers.

More studies are needed to better understand the properties of endodontic sealers and to assist endodontists in choosing the best material during clinical practice.

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