



# Calcium silicate-based sealers remnants in isthmuses of mesial roots of mandibular molars: an *in vitro* evaluation

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## ABSTRACT

**Objectives:** Endodontic retreatment aims to address treatment failure through the removal of root canal filling materials. This *in vitro* study evaluated the presence of filling material remnants in the mesial root canals, specifically focusing on the isthmuses, of mandibular molars after retreatment.

**Methods:** One hundred extracted mandibular molar mesial roots with isthmuses were prepared with an R25 file, obturated with one of five calcium silicate-based sealers (BioRoot RCS [Septodont], MTApeX [Ultradent Products Inc.], EndoSequence BC Sealer HiFlow [Brasseler USA], Bio-C Sealer [Angelus]) or an epoxy resin-based sealer (AH Plus Jet [Dentsply Maillefer]), all stained with rhodamine B, and stored at 37°C for 30 days to allow for setting. Retreatment was subsequently performed using R40 and XP-endo Finisher R instruments (FKG Dentaire) with 2.5% sodium hypochlorite irrigation. The presence of remaining filling material was then assessed using confocal microscopy, and setting times were tested per ISO 6876:2012.

**Results:** AH Plus Jet showed the most remnants at 2 mm and the longest retreatment time. Calcium silicate-based sealers exhibited prolonged setting times under dry conditions, with EndoSequence BC Sealer HiFlow showing a particularly extended setting period.

**Conclusions:** Despite retreatment, residues remained in all canals and isthmus regions, particularly Bio-C Sealer and AH Plus Jet in apical areas, emphasizing the difficulty of complete removal and the persistence of filling material.

**Keywords:** Confocal microscopy; Endodontics; Retreatment; Root canal filling materials; Root canal obturation

## INTRODUCTION

Endodontic treatment failure can arise from the per-

sistence of residual bacteria following chemical-mechanical preparation, sustained by fluid percolation from the periapex, leading to intra- and extraradicular

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infections and subsequent periradicular lesions [1]. These microorganisms can resist to the endodontic treatment and remain in regions of anatomical complexities of root canals such as isthmuses, C-shaped canals, lateral canals, ramifications, and dentinal tubules [2,3].

Endodontic sealers, depending on their material characteristic, can influence the patency regaining, tooth retreatability, retreatment time, and the presence of obturation material remnants within the root canals; several previous studies evaluated the effectiveness of removal investigating different types of sealers [4]. Among these endodontic materials, the most evaluated were those based on epoxy resin, calcium silicate-based, and zinc oxide and eugenol-based, but there are some controversial results between these studies. The comparison between the removal of epoxy resin and calcium silicate-based sealers showed similar results between these materials [5–7], while other studies mentioned a greater amount of residue for epoxy resin-based sealers [8,9], whereas there are reports of greater amounts for calcium silicate-based sealers [10]. Besides, there are reports that the calcium silicate-based sealers require a long time for retreatment [8]. There is evidence that the composition of the material is a variable that interferes with root canal retreatment, considering these discrepancies reported.

Complementary endodontic instruments can potentially aid during retreatment in the removal of obturation material. XP-endo Finisher R rotary file (FKG Dentaire, La Chaux-de-Fonds, Switzerland) was designed to be used in regions of anatomical complexities such as root canal isthmuses along with instrumentation during root canal retreatment [11,12]; besides, there are reports of its use in oval root canals [13]. Previous reports also associated the use of the XP-endo Finisher in retreated root canals filled with calcium silicate-based sealers as an improvement for the obturation material removal [5,9]. Therefore, the investigation regarding the use of an additional cleaning instrument is crucial, especially considering anatomical irregularities [4].

The correlation between filling material removal during retreatment and sealer type warrants investigation. Sealers vary in composition, with some requiring mixing to start setting immediately, while “ready-to-use”

sealers rely on assumed moisture contact within root canal dentin to initiate setting and hydration [14].

The hydration process of ‘ready-to-use’ sealers in root canals is still not well elucidated, and whether the total setting reaction takes place, because these materials are moisture-dependent. Thus, it is possible that in areas of anatomical complexities, such as isthmuses, dentin moisture is not sufficient for the complete setting of ‘ready-to-use’ sealers, considering the high volume of material in these regions [15]. A delayed setting reaction in sealers is thought to increase solubility and the risk of failure. However, no studies have addressed the effectiveness of retreatment in root canal isthmus regions focusing on pre-mixed calcium silicate-based materials, particularly in narrow areas like those in maxillary premolars and between the mesiolingual and mesiobuccal canals of mandibular molars [16,17]. In isthmuses, these two forms of calcium silicate-based sealers (powder/liquid and ‘ready-to-use’) behavior regarding their hydration reactions and setting deserve investigation.

Based on the potential influence of local humidity on the setting reaction of calcium silicate-based sealers and their subsequent removal, particularly in areas of anatomical complexity such as isthmuses, we hypothesized that the effectiveness of retreatment would be affected. Therefore, the aim of this study was to evaluate the remaining filling material in canals with isthmuses of mesial roots of mandibular molars using five different sealers. Additionally, to evaluate the time of retreatability and the setting time of the investigated sealers.

## METHODS

### Ethical approval statement

The research project was submitted and approved by the ethics committees of the University of São Paulo and the University of Campinas (CEP 5479358/CEP 5691992).

### Sample size estimation

The sample size for human teeth was determined using G\*Power v3.1 (Heinrich-Heine-Universität Düsseldorf, Germany), based on prior studies [13,15]. For analysis of variance (ANOVA) comparison of four groups, a standard deviation of 0.26, a minimum detectable difference

of 0.15, a test power of 0.80, and an alpha of 0.05 indicated 20 teeth per group. Similarly, the sample size for sealer setting time analysis was calculated using prior studies [18,19] and a pilot study, with an alpha of 0.05, power of 0.80, and an effect size of 2.45, resulting in six samples per group.

### Selection and preparation of teeth

The study included 100 human mandibular molars (first and second) from donors aged 30 to 60 years, stored in 0.9% saline solution. Teeth met the inclusion criteria of no previous endodontic treatment, extensive caries, or incomplete root formation. Isthmuses at 2, 4, and 6 mm from the apex were confirmed, and only Vertucci types II, III, V, VI, and VII canal configurations were selected. Microcomputed tomography (SkyScan 1174; Bruker, Kontich, Belgium) was used to assess canal anatomy and isthmus presence, ensuring consistent mesial canal patterns.

Initially, the coronal access was performed. Afterward, the teeth foramina were sealed with utility wax and inserted into a silicone mold, then filled with colorless acrylic resin up to the enamel-cement junction. After resin hardening, the block with the tooth was removed from the silicone mold. Markings were made on the blocks corresponding to 2, 4, and 6 mm from the apex, and then, cross-sectional cuts were made through these areas using a 0.3-mm-thick diamond disc coupled to an Isomet cutter (Buehler, Lake Bluff, IL, USA), resulting in 2-mm-thick sections. The sections were placed into an ultrasonic bath with distilled water for 7 minutes to remove the debris originating from the cut. The sections were again inserted into the silicone mold and prepared for canal instrumentation up to foraminal patency. The instrumentation protocol started with initial exploration with a #10 C-pilot file (VDW GmbH, Munich, Germany) using exploration movements (introduction of the file with light pressure and 1/4 rotation turn clockwise and counter-clockwise) to check the existence of foraminal patency. In cases where patency was not established after this exploration, reaming movements and light apical-only instrumentation movements were performed.

Then, driven by an electric motor (VDW GmbH), Reciproc Blue R25 system (VDW GmbH) instrumentation was performed with reciprocating motion using

the actual length of the tooth as the working length. For irrigation, each tooth was irrigated with 5-mL sodium hypochlorite 2.5% (Asfer Indústria Química Ltda, São Caetano do Sul, Brazil) using a 30-gauge Navitip needle (Ultradent Products Inc., South Jordan, UT, USA) inserted at 2 mm short of the working length and remaining in the canals for 30 seconds. A final irrigation protocol was performed using 2-mL 17% ethylenediaminetetraacetic acid (EDTA; Maquira, Maringá, Brazil) using a 30-G Navitip needle. Subsequently, solution activation was performed for 20 seconds with an E1-Irrisonic 20.01 ultrasonic insert (Helse Dental Technology, Santa Rosa de Viterbo, Brazil) at 2 mm short of the working length. This procedure was repeated in triplicate, resulting in a total of 6 mL of 17% EDTA and 60 seconds of activation. Finally, root canals were irrigated with 6 mL of saline solution and dried using 35.04 paper points (Dentsply Maillefer, Ballaigues, Switzerland).

To ensure consistent levels of cleanliness among all root canals and achieve sample standardization, a low-vacuum scanning electron microscope (SEM; PSEM eXpress, Aspek Corp., Delmont, PA, USA) was performed on all teeth prior to obturation without any sample preparation. Subsequently, the teeth underwent randomization and were allocated into five groups ( $n = 20$ ) based on the experimental design, according to the materials detailed in Table 1.

The experimental groups underwent the root canal obturation process using the single-cone technique. Initially, the sealers were introduced into the canals using a size 30 Lentulo instrument (Dentsply Maillefer), positioned 2 mm short of the apex. Subsequently, gutta-percha cones 25.08 (VDW GmbH) were inserted 1 mm short of the apex along with the respective endodontic sealer. Periapical radiographs were taken simultaneously to assess the quality of obturation.

According to a previous method [15] for confocal microscopy analysis, the sealers were mixed with rhodamine B 0.1% dye before their insertion into the root canals. After completing the obturation, the teeth were placed in containers with moistened gauze and stored in a humid oven at 37°C for 1 month. In sequence, samples underwent a canal retreatment procedure. For this procedure, all teeth were retreated using R40 (VDW GmbH) and instrumentation supplemented

**Table 1.** Composition and batch number of the root canal sealers used in the analysis

Material	Manufacturer	Batch	Composition
BioRoot RCS	Septodont (Saint-Maur-des-Fossés, France)	Powder B23970 Liquid B23099	Tricalcium silicate, zirconium oxide, and povidone Aqueous solution of calcium chloride and polycarboxylate
MTApex Sealer	Ultradent Products Inc. (South Jordan, UT, USA)	Powder 2019102403 Liquid 2020011401	Tricalcium silicate, tricalcium aluminate, and tantalum oxide Water-based gel
EndoSequence BC Sealer HiFlow	Brasseler USA (Savannah, GA, USA)	2001SPWF	Tricalcium silicate, dicalcium silicate, calcium hydroxide, zirconium oxide, and fillers
Bio-C Sealer	Angelus (Londrina, Brazil)	60406	Calcium silicate, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide and polyethylene glycol
AH Plus Jet	Dentsply Maillefer (Ballaignes, Switzerland)	2204000437	Epoxide paste: bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, aerosol, and pigment Amine paste: 1-adamantane amine N, N'-dibenzyl-5-oxanonandiamine-1, 9 TCD-Diamine, calcium tungstate, zirconium oxide, aerosol, and silicone oil

with XP-endo Finisher R, with 2.5% sodium hypochlorite irrigation using a syringe with a 30-G Navitip needle until reestablishing apical patency.

All chemical-mechanical preparation, obturation, and retreatment procedures were performed by the same previously trained professional (DSBA) with the assistance of an operative microscope.

### Confocal microscope analysis

Images of the 2, 4, and 6 mm of the apex were obtained through confocal microscopy (Confocal Microscope Leica TCS SPE; Leica Microsystems GmbH, Wetzlar, Germany; 50× magnification) with the following parameters: objective lens, N Plan 5.0 × 0.12 DRY; laser, 532 nm (visible range), emission bandwidth, 546 to 740 nm; and image resolution, 512 × 512.

The images were analyzed using the Image Tool 3.0 software (The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA), and the calculation of both the total area of remaining endodontic sealers and the sealer in the isthmuses was performed after retreatment and expressed in percentage according to the following equation :

$$\text{Remnant material } (\%) = \frac{\text{Area of remnant material} \times 100}{\text{Total area of canal or isthmus}}$$

### Retreatment time analysis

The retreatment time during endodontic retreatment procedures was evaluated by recording time (in sec-

onds), with a digital stopwatch. Time measurement started from the moment the instrument encountered the root canal, and the final time for retreatment was recorded when apical patency was reestablished.

### Setting time analysis (moist and dry conditions)

The setting time test was prepared using moist and dry methods, according to the ISO 6876:2012 standards. The moist method used previously manufactured round plaster molds (Durone-IV; Dentsply Maillefer) measuring 10 × 1 mm, which were kept immersed in distilled water for 24 hours at 37°C and filled with endodontic sealers ( $n = 6$ ). For the dry method, the tested endodontic sealers ( $n = 6$ ) were placed inside stainless steel rings (10 × 2 mm), under a glass plate, and stored at 37°C relative humidity.

The setting of the materials was evaluated by placing a 100-G Gilmore needle with a 2-mm tip vertically into the sample surface initially to determine the initial setting time every 30 minutes and at shorter intervals as the setting reaction progressed. The initial setting time was determined from the beginning of material manipulation until the needle indentation was no longer observed on the material's surface.

### Statistical analysis

GraphPad Prism 9 software (GraphPad Software, San Diego, CA, USA) was used for statistical analysis. Normal distributions were assessed using Shapiro-Wilk test.

Mixed ANOVA of the within-and-between effects of the subjects used a *post hoc* analysis with the Tukey test for multiple comparisons. All statistical tests were performed at a significance level of 5% ( $\alpha = 0.05$ ).

## RESULTS

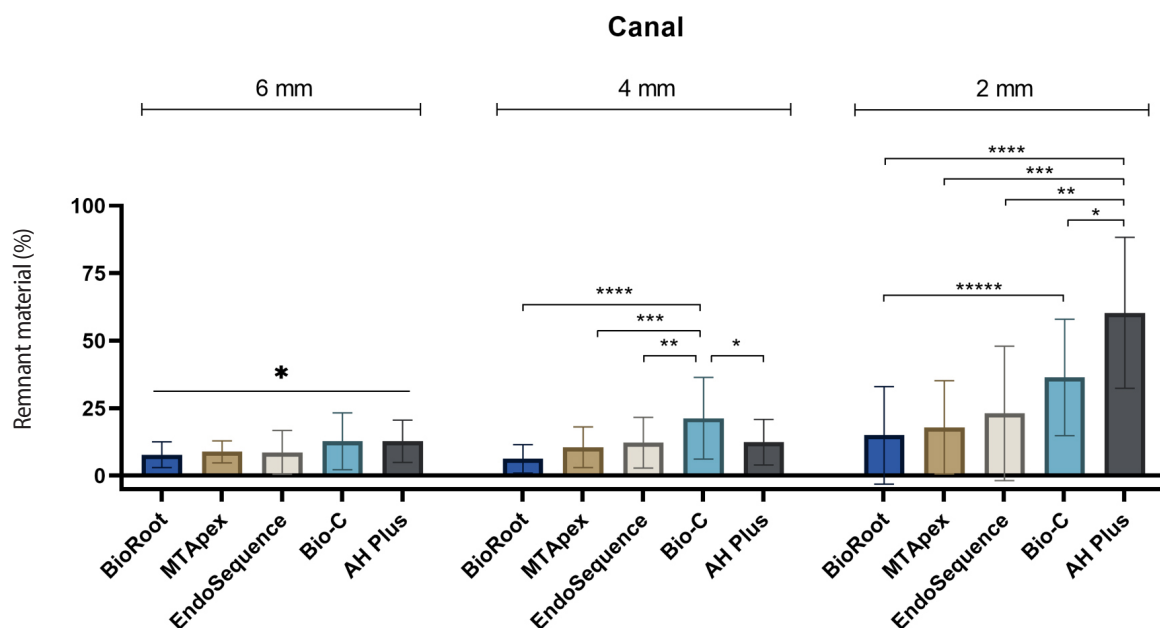
The analysis of the remnant endodontic sealers after the retreatment for both the canals and isthmuses of the mesial roots of mandibular molars, in the regions of 6, 4, and 2 mm, is shown in Figures 1 and 2, respectively.

For the analysis of the remnant filling material inside the canals, in the 6-mm section, there was no statistically significant difference between the remnant filling material in any of the endodontic sealers ( $p > 0.05$ ). In the 4-mm section, the Bio-C Sealer (Angelus, Londrina, Brazil) presented an average of remnant material inside the canals ( $21.3\% \pm 15.1\%$ ) greater than all other sealers tested ( $p < 0.05$ ). At the 2-mm section, AH Plus Jet exhibited the highest mean percentage of remnant in the canals ( $12.4\% \pm 8.4\%$ ) when compared to other materials ( $p < 0.05$ ).

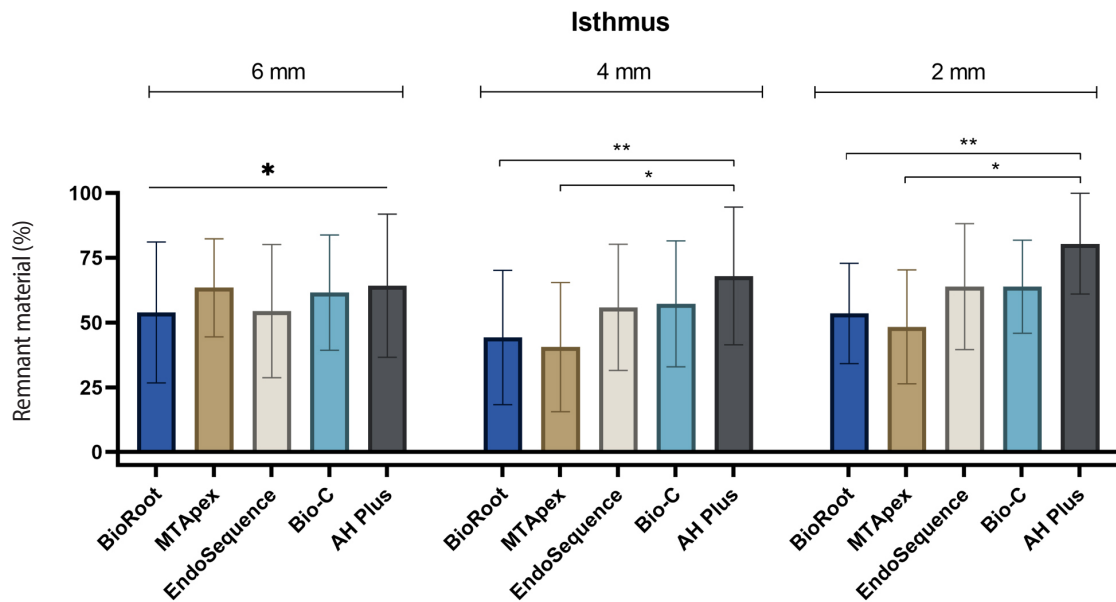
For the remnant filling material inside the isthmuses, in the 6-mm section, there was also no statistically

significant difference between the remaining filling material in the isthmuses between the endodontic sealers evaluated ( $p > 0.05$ ). In the 4-mm section, AH Plus Jet sealer showed a statistically significant difference in relation to the percentage of remnant filling material ( $68.0\% \pm 26.5$ ) when compared to the BioRoot RCS (Septodont, Saint-Maur-des-Fossés, France) and MTApex sealers (Ultradent Products Inc.) ( $p < 0.05$ ). Besides, in the region of 2 mm, AH Plus Jet also presented the highest percentage of remnant filling material ( $80.4\% \pm 19.4$ ) than the BioRoot RCS and MTApex materials ( $p < 0.05$ ). Representative confocal microscopy images of the evaluated samples after the retreatment procedures are shown in Figure 3, indicating a higher degree of remnants within the isthmuses.

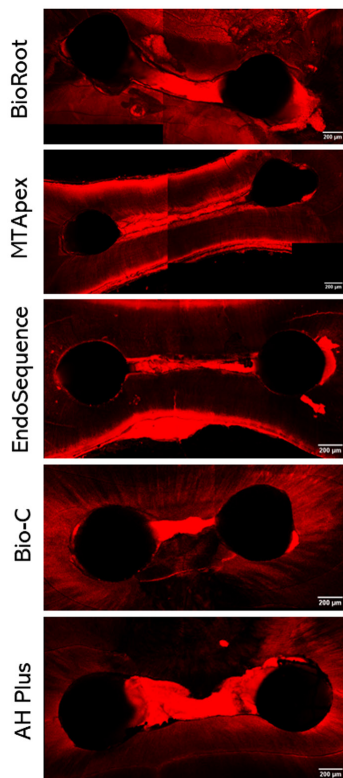
The time elapsed for the endodontic retreatments until the reestablishment of the working length patency was obtained is indicated in Table 2. Patency was reestablished in all specimens. AH Plus Jet demanded the longest mean retreatment time compared to all other materials ( $p < 0.05$ ). There was no statistically significant difference between BioRoot RCS, MTApex, and EndoSequence BC Sealer HiFlow sealers (Brasseler USA, Savannah, GA, USA) ( $p > 0.05$ ), and Bio-C Sealer required the



**Figure 1.** Representation of the mean and standard deviation values in percentage of remnant filling material in canals at the 6-, 4- and 2-mm levels. Bars linking different materials with asterisks indicate statistically significant differences (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). Manufacturer information for each material is provided in Table 1.



**Figure 2.** Representation of the mean and standard deviation values in percentage of remnant filling material in isthmuses at the 6-, 4- and 2-mm levels. Bars linking different materials with asterisks indicate statistically significant differences (\* $p < 0.05$ , \*\* $p < 0.01$ ). Manufacturer information for each material is provided in Table 1.



**Figure 3.** Confocal microscopy representative images of the canal and isthmuses at 4 mm after endodontic retreatment procedures with the tested materials. Manufacturer information for each material is provided in Table 1.

**Table 2.** The retreatment time for all tested materials

Tested material	Retreatment time (sec)
BioRoot RCS	167.3 ± 46.01 <sup>B</sup>
MTApex	161.5 ± 36.46 <sup>BC</sup>
EndoSequence BC Sealer HiFlow	170.2 ± 25.88 <sup>B</sup>
Bio-C Sealer	130.4 ± 11.37 <sup>C</sup>
AH Plus Jet	225.2 ± 33.57 <sup>A</sup>

Values are presented as mean ± standard deviation.

Different uppercase letters indicate statistically significant differences between materials ( $p < 0.05$ ).

Manufacturer information for each material is provided in Table 1.

lowest retreatment time.

Table 3 shows the mean and standard deviation values for the setting times for materials' setting time in moist and dry methods. Regardless of the method of analysis, EndoSequence BC Sealer HiFlow had the highest average setting time among the evaluated calcium silicate-based materials ( $p < 0.05$ ). In the moist method, all calcium silicate-based sealers obtained a lower mean setting time when compared with the dry method ( $p < 0.05$ ). AH Plus Jet was not influenced by the experimental setting time method ( $p > 0.05$ ).

**Table 3.** Representation of the values of setting time of the tested materials

Tested material	Setting time (min) under each condition	
	Moist	Dry
BioRoot RCS	251.8 ± 8.98 <sup>ABCa</sup>	615.8 ± 3.07 <sup>Ab</sup>
MTApex	226.3 ± 40.2 <sup>Ba</sup>	512.9 ± 18.78 <sup>Bb</sup>
EndoSequence BC Sealer HiFlow	351.6 ± 45.19 <sup>Ca</sup>	2751 ± 48.67 <sup>Cb</sup>
Bio-C Sealer	65.77 ± 4.71 <sup>Da</sup>	251.0 ± 52.77 <sup>Db</sup>
AH Plus Jet	615.3 ± 10.29 <sup>Ea</sup>	675.6 ± 138.7 <sup>Aa</sup>

Values are presented as mean ± standard deviation.

Different uppercase letters indicate statistically significant differences between materials ( $p < 0.05$ ). Different lowercase letters indicate statistically significant differences the method for each material ( $p < 0.05$ ).

Manufacturer information for each material is provided in Table 1.

## DISCUSSION

The aim of the study was to evaluate the remaining filling material in canals with isthmuses of mesial roots of mandibular molars. This was achieved by preparing mesial roots of mandibular molars with confirmed isthmuses, obturating them with five different sealers, and performing standardized retreatment procedures. The amount of remaining filling material could be assessed using confocal microscopy, allowing for direct visualization and comparison among the sealers. By focusing specifically on the isthmus areas, the study successfully quantified the remnants left by each material, providing insight into their retreatability and highlighting differences in removal efficiency among the tested sealers.

All endodontic sealers evaluated left remnants of obturation material after the endodontic retreatment procedures. Besides, the percentage of remnant filling material in the root canals directly correlated with the root canal thirds; the closer to the apical region, the greater the amount of remnants. This is the first study analyzing the removal of Bio-C Sealer in a simulated retreatment condition; however, a previous study [20] reported that the use of the XP-endo Finisher R file was more effective in removing Bio-C Sealer cement from oval canals of mandibular premolars when compared to ultrasound-activated irrigation and EndoActivator (Dentsply Maillefer). However, a differentiation between the main root canal and isthmuses was not performed in their work. Studies [21–23] indicate higher solubility for the ready-to-use Bio-C Sealer, theoretically facilitating its removal during endodontic retreatment. Although the setting behavior of calcium silicate-based sealers was

not evaluated during obturation in this study, the expected setting was not observed. This may be related to the influence of isthmuses in mesial roots on local humidity and the possibility of incomplete canal drying before obturation. These factors could have affected the sealer's setting reaction. Further studies are needed to investigate how anatomical variations and different drying protocols impact the clinical setting behavior of calcium silicate-based sealers.

A previous study [24] found a greater amount of remnant for AH Plus in comparison with the EndoSequence BC Sealer HiFlow in the apical 4-mm section; conversely, here, similar results for these materials were observed at this level. At the 2-mm section of the root canal, AH Plus Jet had the highest percentage of remnants when compared to other materials, corroborating with a previous study [9], but diverging from the findings of others [6], since these report no statistically significant difference between the remnants of AH Plus Jet, BioRoot RCS, and EndoSequence BC Sealer HiFlow when removed by different endodontic instruments. This high permanence of AH Plus Jet remnants in root canals and isthmus regions may be associated both with its composition, as it is a resin-based material, and with its low solubility, which is directly correlated with many other physicochemical properties, when compared to other materials, such as BioRoot RCS, Bio-C Sealer, and EndoSequence BC Sealer [23].

The evaluation of the remnant filling material in root canals is a topic previously discussed [4,6,7,11,18]; however, isthmuses were not regarded in previous studies. This anatomical occurrence deserves attention since it is included as a factor for failure in endodontic procedures

due to its insufficient preparation during endodontic instrumentation [19,25]. Here, the isthmus regions of 4- and 2-mm from the apex showed similar results for the remnant filling material. A previous research [26] used microcomputed tomography and passive ultrasonic irrigation with EndoSeal MTA (Maruchi, Wonju, Korea) and AH Plus Jet and showed comparable remnants for both cements, whereas in the present study, which employed XP-endo Finisher R, different results were found, highlighting the influence of instrumentation on results. The XP-endo Finisher R, with its flexibility, tip design, and expansion movement, likely played a key role in improving the removal of filling material, especially in isthmus areas. These mechanical properties are crucial for enhancing cleaning efficiency in complex anatomies, and further development towards this performance to be added to the endodontic armamentarium is highly welcome.

In the present study, ready-to-use materials had similar percentages in relation to the amount of remnant filling material in the isthmus region. No previous works were carried out analyzing canals and isthmuses individually; however, the use of a complementary instrument for cleaning and removing filling material from the root canal system was previously reported [22], mainly focusing on isthmus areas. Individual assessment of root canals and isthmuses is important because these anatomical regions present distinct challenges during retreatment. Evaluating them separately provides a clearer understanding of cleaning effectiveness, highlights persistent difficulties, and helps optimize techniques for more complete filling material removal. The complementation of endodontic instrumentation during retreatment seems to be highly recommended, as reported by previous studies [21,23,24].

The retreatment time analysis showed that canal patency was reestablished in all specimens after endodontic retreatment. Considering the retreatment time, AH Plus Jet had a longer retreatment time when compared to all other evaluated materials, which contrasts with the results of previous studies [8,27]. Although those studies did not relate the retreatment time to the presence or absence of isthmuses, this divergence may lie in their use of single-rooted canals without curvatures.

The association between the composition of end-

odontic sealers and their physicochemical properties is utterly dependent on a predictable setting of the material [24]. The setting time analysis performed here using two methods is justified by the high variances of the setting reaction of ready-to-use sealers, since the humidity of the dentinal tubules is an essential factor for its hydration and the complete setting reaction [28]. Under *in vitro* controlled humidity conditions, it is notable that the ambient humidity condition has significant effects on calcium silicate-based materials. The powder and liquid calcium silicate-based sealers, BioRoot RCS and MTApex, did not show a statistically significant difference between them for the moist method, whereas the opposite occurs in the dry method. Corroborating with the findings for BioRoot RCS [29] sealer, but contrasts with MTApex Sealer [19], probably due to different testing conditions between these studies. For EndoSequence BC Sealer HiFlow and Bio-C Sealer, the results presented here corroborated the results from previous studies [30]. In a clinical setting, this moisture-dependent setting reaction makes it unreliable for use, especially in situations of endodontic procedures where there is no certainty about the remaining moisture in the root canal.

The remnant filling material analysis after endodontic retreatment, here performed, potentially poses a methodological limitation, since a full volume remnant analysis using a three-dimensional method would be applicable [31]. However, to quantify the remnant filling material using confocal microscopy, 2-mm-thick sections were used to overcome the limitation of lacking three-dimensional analysis. Corroborative analysis between confocal microscopy and microcomputed tomography is warranted. Besides, another limitation of this study is the use of rhodamine B as a fluorophore for detecting filling material remnants. Although recent literature highlights that rhodamine B may not be ideal for the tested sealers due to its moisture affinity, potentially leading to overestimated staining, these findings primarily concern moist dentinal tubules. In the present study, the assessment focused on the isthmus, a region intentionally dried with paper cones following each manufacturer's guidelines, including those for ready-to-use sealers. Nonetheless, despite the dry conditions, the possibility of altered staining behavior remains; thus,

this must be taken into consideration while interpreting the results presented here.

## CONCLUSIONS

In conclusion, this *in vitro* evaluation demonstrated that filling material remnants persist in both the canals and isthmus regions of mesial roots of mandibular molars after endodontic retreatment, irrespective of the sealer used. Notably, AH Plus Jet required the longest retreatment time, while Bio-C Sealer exhibited the shortest. Furthermore, the setting time of calcium silicate-based sealers was significantly influenced by humidity conditions, highlighting a potential limitation in clinical applications where moisture control can be challenging. Clinically, this suggests that clinicians should be aware of the potential for residual filling material and the variable retreatability of different sealers when planning endodontic retreatment procedures.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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## AUTHOR CONTRIBUTIONS

Conceptualization, Resources: Marciano MA, Alencar DSB. Data curation, Investigation, Methodology, Validation: all authors. Formal analysis: Alencar DSB, Marciano MA, Pelepenko LE. Funding acquisition, Visualization: Alencar DSB, Marciano MA. Project administration: Marciano MA, Duarte MAH, Alencar DSB, Pelepenko LE. Supervision: Marciano MA. Writing - original draft: Alencar DSB, Marciano MA. Writing - review & editing: Moraes BF, Janini ACP, Pelepenko LE. All authors read and approved the final manuscript.

## DATA SHARING STATEMENT

The datasets are not publicly available but are available from the corresponding author upon reasonable request.

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