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ORIGINAL ARTICLE

Dentinal Tubule Penetration and Adaptation of Bio-C Sealer and AH-Plus: A Comparative SEM Evaluation

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ABSTRACT

Objective: The aim of this study was to compare and evaluate the dentinal tubule penetration and adaptation of a premixed bioceramic sealer and an epoxy-resin based sealer in the three radicular thirds.

Methods: 30 wide roots, with single straight canals and totally formed apexes, were endodontically prepared and divided into two groups (n=14) according to the sealer used for root canal filling: AH-Plus (AHP) and Bio-C Sealer (BIOC). Two samples were left as controls. After the canals were filled, the samples were cut and viewed under Scanning Electron Microscopy by taking images to analyse the tubular penetration and adaptation of the sealers. The results were statistically analysed with the Shapiro Wilk, Levene and Mann-Whitney tests ($P<0.05$).

Results: BIOC showed significantly higher penetration in dentinal tubules than AHP in the cervical, middle and apical thirds of the root canal ($P<0.05$) and better adaptation to the dentinal tubule walls.

Conclusion: Under the parameters of this study, BIOC exhibits higher penetration and better adaptation to the dentinal tubules compared to AHP.

Keywords: Bioceramics, dentinal tubules, root canal sealer, scanning electron microscopy

HIGHLIGHTS

- Tubule penetration and adaptation are essential when evaluating the sealing ability of endodontic sealers.
- Comparing new bioceramic sealalers with sealers considered as "Gold Standard" is decisive for evaluating the performance of these materials.
- The results obtained by the SEM images show a better intratubular performance of Bio-C Sealer compared to AH-Plus in the three thirds of the root canal.
- It will be important to have these results complemented by long term studies which will allow the evaluation of the stability of these materials over time.

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INTRODUCTION

Root canal sealers play an important role in the prognosis of endodontic treatment. The ability to seal irregularities and penetrate dentinal tubules are essential when considering a three-dimensional filling of the root canal system (1, 2). This ability is determined by the fluidity of the materials, an essential physicochemical characteristic (3), which allows sealers to reach parts of the root canal not touched by instruments. Accordingly, root canal sealers can penetrate the dentinal tubules, forming a physical barrier between the filling material and the dentine, increasing their retention and isolating possible residual microorganisms in the dentinal tubules, preventing reinfection (3, 4).

AH-Plus (Dentsply DeTrey, Konstanz, Germany) is an epoxy-resin based sealer which is widely used because of its physicochemical characteristics and its ease of handling (5), being used as control of several studies (6, 7). This sealer has minimal polymerization shrinkage and high bond strength to dentine, produced by covalent bonds between the amino group of dentine and the epoxy ring of resin (8), forming a micro-mechanical lock with the root canal (1, 9).

The promising results obtained in recent years with calcium silicate-based sealers have made their use more widespread due to their biocompatibility, small particle size, bactericidal effect

and excellent physicochemical properties (10). Also, these materials have excellent sealing ability, determined by their setting expansion (11) and chemical bond to dentine (12), produced by the formation of hydroxyapatite in the contact zone between the sealer and dentine (13). Although these effects are desirable from an endodontic point of view, this kind of bond and the tubule penetration reached by these materials might interfere in the future restoration of the teeth. Vilas-Boas et al. (14) and Peña Bengoa et al. (15) reported a reduction of the bond strength of fiber posts in teeth filled with bioceramic sealers. They attributed these lower values to the high tubule penetration and the difficulty of removing this type of material from the canal walls.

The aim of this study was to compare and evaluate the dentinal tubule penetration and adaptation of a premixed bioceramic sealer Bio-C Sealer (Angelus, Londrina, Brazil) and an epoxy-resin based sealer AH-Plus (Dentsply) in the three-thirds of the root canal. The null hypothesis was that there is no difference in tubule penetration or adaptation between the sealers.

MATERIALS AND METHODS

This study was subjected to review and analysis by the institutional Scientific and Ethics Committee who gave the authorization number 0112019 for the development of this study.

For the following experimental *in vitro* study, straight and wide roots, extracted for reasons not related to the current study and preserved in 0.1% thymol immediately after extraction were used. Palatal roots of maxillary molars, distal roots of mandibular molars and roots of maxillary incisors were considered. A non-probabilistic sample, based on correlated studies of similar methodology published in the literature (16-18), was used to select 30 roots with single straight canals and a completely formed apex. A Cone-beam tomography (CB gx 500 fed by I-Cat; Gendex, Chicago, IL, USA) was used to verify the anatomy of the canals, discarding samples with more than one canal and medium or severe curvatures according to the Schneider`s classification. Roots with previous endodontic treatment, caries, signs of reabsorption or fracture were discarded.

Preparation of the samples

The samples were cleaned with a P19 periodontal ultrasonic tip (Helse Ultrasonics, Santa Rosa de Viterbo, Brazil) and prophylaxis brushes, to eliminate traces of hard and soft tissue. The samples were cut at 12 mm from the anatomical apex using a 911 HK.104.220 diamond disc (Komet, Lemgo, Germany) in order to standardise samples.

The preparation of the root canals was performed using the Protaper Next system (Dentsply Sirona, Ballaigues, Switzerland) and an X-Smart Plus motor (Dentsply Sirona) using the continuous rotation program for the system. After glide path creation with a K #10 file (Dentsply Sirona), the root canal shaping started with a X1 file (17/0.04), using progressive in and out movements with brushing motion on the withdrawal stroke, up to the working length (WL) was reached (11 mm). The patency was verified with a K #10 file and the canal shaping continued using a X2 (25/0.06), X3 (30/0.07) and a X4 (40/0.06) file. Between each instrument, the canals were irrigated and the foraminal patency was preserved with a K #10 file. During the shaping process, the canals were irrigated with 10 mL of 2.5% NaOCl, using a NaviTip 30-G needle (Ultradent, South Jordan, UT, USA) positioned 2mm short of the WL. Throughout the canal preparation, 10 mL of NaOCl 2.5% were used, followed by 5 ml of EDTA 17% and 5 ml of NaOCl 2.5% for a final rinse. The root canals were dried with #40 paper points.

The samples were randomly divided with an Excel spreadsheet (Microsoft Office 2016, Seattle, WA, USA) into two groups (n=14), leaving two samples as controls to verify the cleanliness and dentinal permeability obtained with the final rinse. The single cone technique was use for root canal filling. For the AHP group the sealer was mixed according to the manufacturer direction and placed into the canal 1 mm short of the WL with a #30 Lentulo spiral (Dentsply Maillefer, Ballaigues, Switzerland) with short in and out movements for 5 seconds. For BIOC group, the canal walls were previously moistened twice with saline carried on a Protaper Next X4 gutta-percha cone (Dentsply Sirona) up to WL. Then, the sealer was placed into the canal 1mm short of the WL with the applicator tips developed for this product until the middle third of the canal was filled. After that, for both groups, a single gutta-percha cone (Protaper Next X4, Dentsply Sirona) was placed in the canal up to WL, cutting the cervical excess with the heated plugger from Calamus Dual (Dentsply Sirona) and compacted vertically with a cold Machtou plugger (Dentsply Maillefer).

The coronal cavity was sealed with Herculite Precis resin composite (Kerr, Texas, USA), after dentine conditioning with 37% Condac 37 orthophosphoric acid (FGM, Santa Catarina, Brazil) and OptiBond Universal adhesive (Kerr). Once the samples were sealed, they were stored in an MRC incubator (Cromtek, Santiago, Chile) for 14 days at 37°C. at 100% humidity to allow the complete setting of the sealers.

The roots were embedded in self-curing transparent acrylic (Marché, Santiago, Chile) and sectioned perpendicularly to the long axis of the root canal at 2, 5 and 8 mm from the anatomical apex, using an IsoMet 5000 metallographic cutter machine (Buehler, Chicago, USA). Thus, samples were obtained of the apical, middle and cervical thirds of the root canal respectively. The samples were submerged in EDTA 17% for two minutes, followed by NaOCl 5.25% for 3 minutes, to eliminate residues produced by the cutting disc.

Scanning electron microscope analysis

The samples obtained were dehydrated using the following regime: 70% alcohol for 12 hours, 80% alcohol for 12 hours, 90% alcohol for 6 hours and 99.7% alcohol for 3 hours. They were mounted on aluminum stubs, and after a gold sputter coating the samples were observed by a scanning electron microscope (SEM) Zeiss EVO 10 (Carl Zeiss Microscopy GmbH, Jena, Germany) using an ultra-high vacuum chamber (UHV) programmed at 15kV. The observations were made at magnifications of 600 x and 8.00 kx. In each of the images obtained, the most representative zone of the sealer-dentine interface was selected. Then, using ImageJ software (National Institute

of Health, Washington DC, USA) the dentine zone was delimited, excluding the canal lumen and any defects in the sample. Three blinded operators, demarcated the dentinal tubules full of sealer in each of the images and by a pixel count, the total percentage of sealer occupation with respect to the dentine area was calculated (Fig. 1). To ensure reproducibility, interobserver agreement were calculated using the Cohen´s kappa statistic. Kappa value was 0.63, demonstrating a good reliability. Finally, a descriptive analysis of the image was made, to evaluate the adaptation of the sealers to the dentinal tubules.

Statistical analysis

The values obtained were tabulated and subjected to statistical interpretation with Stata 11.2 software (StataCorp, College Station, TX, USA) using the Shapiro-Wilk, Levene and Mann-Whitney tests ($P<0.05$).

RESULTS

The images obtained of the control group (n=2) under SEM showed complete smear layer removal produced by the final irrigation. The dentinal walls were smooth with open dentinal tubules (Fig. 2).

The results obtained from the analysis of the SEM images show that Bio-C Sealer presents greater tubule penetration compared with AH-Plus (Fig. 3), with a statistically significant difference in the percentage occupation between both sealers in the three thirds analysed (Table 1). It was seen that the Bio-C Sealer occupied the intratubular space more uniformly than AH-Plus in both the cervical and the middle thirds (Figs. 3b and 3d) while in the apical third, a lower number of tubules and a low intratubular occupation are evident for both sealers (Fig. 3e and 3f).

By qualitatively analysing the adaptation of the sealers in the dentinal tubules, it was observed that Bio-C Sealer occupies the intratubular space in a more uniform and homogeneous way than AH-Plus, which shows fragmented and granular-looking tags. It was observed that the Bio-C Sealer presented longer sealer tags, without interruptions and they are well adapted to the dentinal tubule walls (Fig. 4).

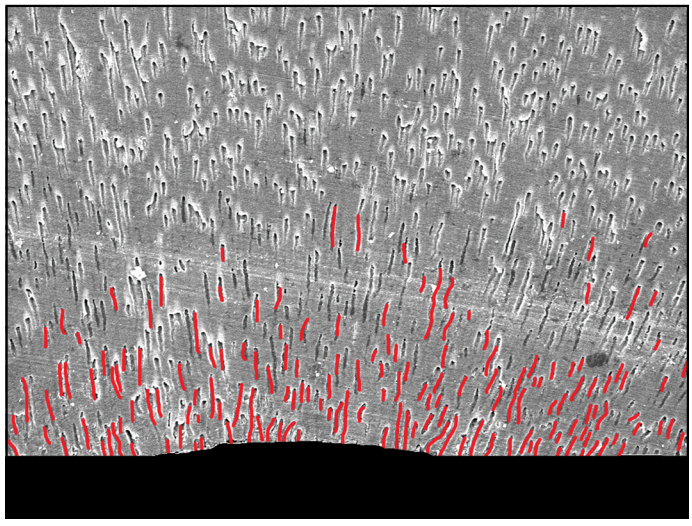

Figure 1. Demarcation of dentinal tubules occupied by the sealers using the ImageJ software. By a pixel count, the occupation percentage of the sealers with respect to the dentin area was calculated

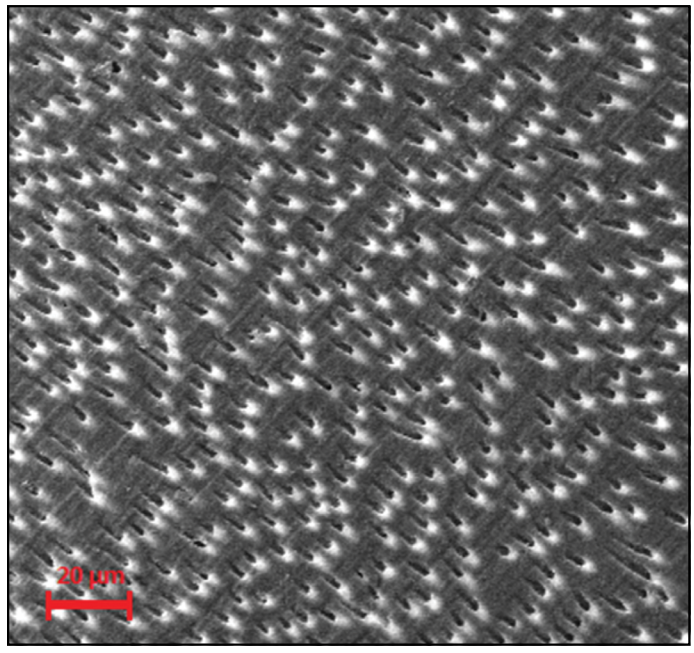

Figure 2. SEM image of control group (600x)
SEM: Scanning electron microscope

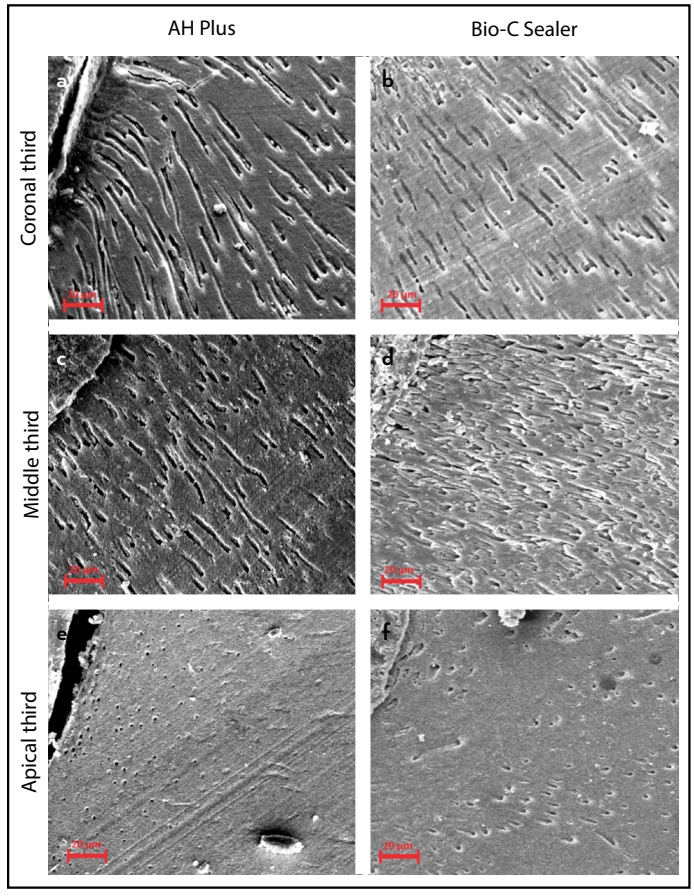

Figure 3. Images obtained under SEM (600x) of the tubular penetration of Bio-C Sealer and AH-Plus in its coronal, middle and apical thirds
SEM: Scanning electron microscope

TABLE 1. Average values of the percentage of tubular occupation between Bio-C Sealer and AH Plus

	Media	SD	Min	Max	P value
Coronal third					
Bio-C Sealer	1.97	1.73	0.71	4.49	P<0.005
AH-Plus	0.81	0.8	0.23	1.45	
Middle third					
Bio-C Sealer	1.87	2.58	0.60	5.16	P<0.001
AH-Plus	0.70	0.56	0.09	1.23	
Apical third					
Bio-C Sealer	0.25	0.11	0.00	1.16	P<0.05
AH-Plus	0.17	0.1	0.00	0.29	

SD: Standard deviation, Min: Minimum, Max: Maximum

DISCUSSION

The tubule penetration of endodontic sealers depends mainly on their physicochemical properties, smear layer removal and dentinal permeability, depending on the anatomical root canal zone (17). Taking these aspects into consideration, this study compared and evaluated the dentinal tubule penetration of Bio-C Sealer and AH-Plus in the three thirds of the root canal, after a final irrigation protocol that included EDTA 17% for smear layer removal.

Maximum tubule penetration was observed for both sealers in the cervical third of the root canal, showing a gradual decrease towards the middle third and an important reduction of these values in the apical third. This is mainly attributed to the histological characteristics of the apical root dentine, described as sclerotic and poorly permeable dentine that has fewer dentinal tubules compared to middle and cervical thirds dentine (17). These results are in agreement with studies by Wang et al. (11), McMichael et al. (19) and Eymirli et al. (20), who reported less sealer tubule penetration in the apical third compared to the middle and cervical root canal thirds.

The results of the present study show that Bio-C Sealer presented greater tubule penetration than AH-Plus in all three thirds analysed. These results agree with those described by El Hachem et al. (5) and Wang et al. (11), who showed that bioceramic sealers have greater tubule penetration than AH-Plus. This could be related to the smaller particle size of the bioceramic sealers, their fluidity and hydrophilicity, which allows them greater penetration and better adaptation to the dentinal tubules (11, 13).

On comparing the tubule adaptation of both sealers, it was seen that Bio-C Sealer tags showed more contact with the tubule walls compared with AH-Plus tags. This might be related to the hydrophilic properties of bioceramic sealers and the chemical bond produced between this type of materials with dentine, as a product of the formation of hydroxyapatite (10, 11, 13). This could favour achieving better adaptation and retention of the sealer, enhancing its sealing ability. In contrast, epoxy-resin based sealers, although having high fluidity and low polymerization shrinkage, are hydrophobic (21, 22), so moisture could negatively affect their ability to penetrate and adapt to dentinal tubule walls.

Analysing the sealer tags inside the dentinal tubules, it could be seen that Bio-C Sealer showed a smooth and consistent tubule penetration with few gaps. This regularity of penetration might be associated, as well as with its hydrophilic nature and premixed presentation, with the nanometric particles present in its composition (11), which would allow a deeper penetration and in a more homogeneous way. In contrast, AH-Plus showed less regularity, with a granular aspect and intratubular gaps, which could be due to its larger particle size, polymerisation shrinkage or problems during the mixing process. The

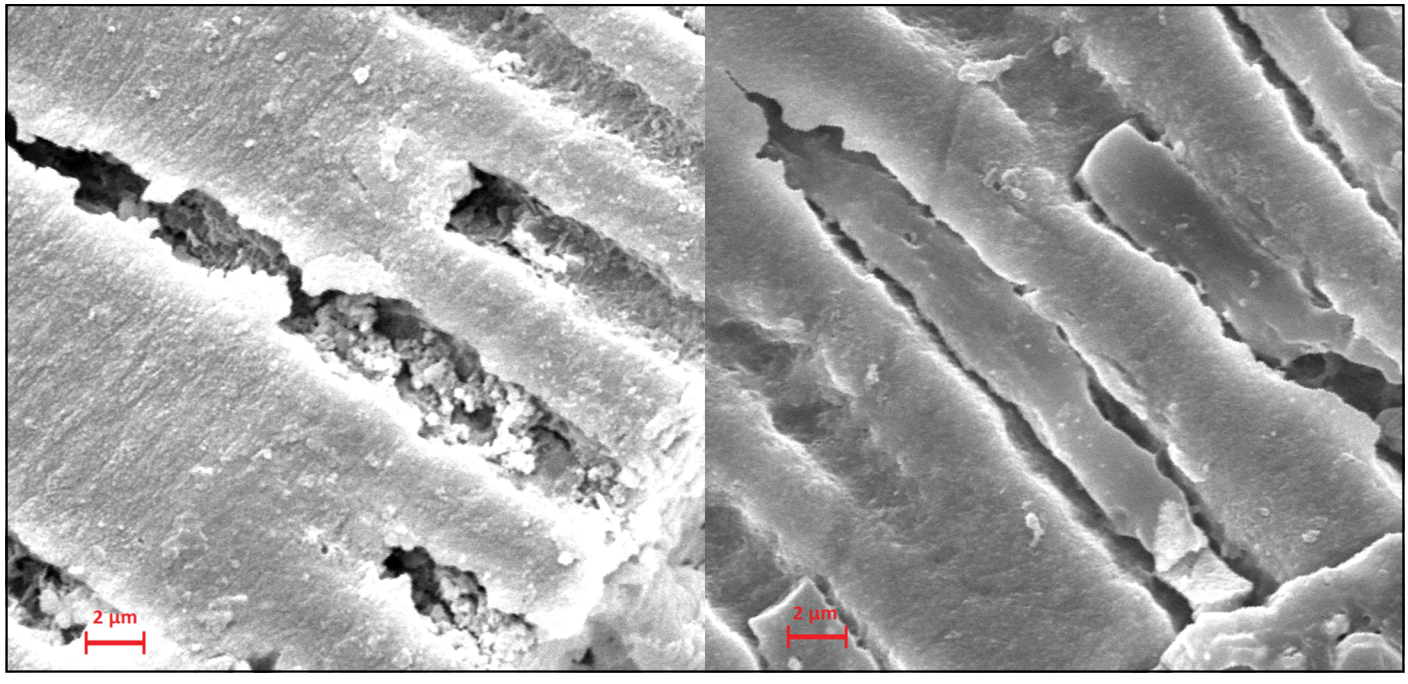

Figure 4. SEM images of tubular adaptation (8.00 kx) of AH-Plus (left side) and Bio-C Sealer (right side)
SEM: Scanning electron microscope

proportions and mixing process of sealers may be a key point in the tubule adaptation of these materials. Arikatla et al. (10) reported that AH-Plus had better tubule adaptation than bioceramic sealers, but the latter were not premixed sealers. Against that, Patri et al. (23) demonstrated a better dentine adaptation of premixed bioceramic sealers than the epoxy-resin based sealers.

The results of this study confirm the good performance of Bio-C Sealer. However, this study only evaluated the dentinal tubule penetration and adaptation of this sealer and it is essential to complement these results with other studies that would allow the evaluation of the all-round performance of this sealer. The literature on bioceramic sealers is still relatively scarce, so more studies are needed to support the clinical relevance of this study, especially the long-term behavior of this type of materials.

CONCLUSION

In conclusion, Bio-C Sealer presents greater penetration and better tubular adaptation than AH-Plus in the three thirds of the root canal.

Disclosures

Conflict of interest: The authors has nothing to disclose.

Ethics Committee Approval: This study was subjected to review and analysis by the Scientific and Ethics Committee of the Faculty of Dentistry of the Andrés Bello University, Viña del Mar, Chile, who gave authorisation for the development of this study. Authorisation number: 011219. Date: December 16, 2019.

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