ORIGINAL RESEARCH

Intratubular Sealer Penetration: Scanning Electron Microscopy Associated with Cathodoluminescence Analysis

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Abstract

Aim: The aim of the study was to compare the ability of three endodontic sealers, Endofill (END), AH Plus (AHP), and Sealer Plus BC (SPB), to penetrate dentinal tubules.

Materials and methods: Forty-five human teeth, single-rooted and previously instrumented mandibular premolars, were randomly divided into three experimental groups ($n = 15$): END ($n = 15$), AHP ($n = 15$), and SPB ($n = 15$). After obturation, dental sections were performed horizontally, at 2 and 5 mm from the root apex. The samples were analyzed by scanning electron microscopy associated with cathodoluminescence. Percentage penetration (PP%) and maximum penetration depth (MPD) of the sealers were evaluated by the Kruskal-Wallis and Mann-Whitney tests, for general and paired data, respectively. The Wilcoxon test was applied to analyze the differences between the 5 and 2 mm distances. A 5% significance level was adopted.

Results: As for PP%, AHP and SPB were similar ($p = 0.127$) and presented higher values than END (AHP, $p = 0.024$ and SPB, $p <0.001$); with regard to MPD, AHP and SPB did not differ either ($p = 0.450$), but were higher than END ($p <0.001$); in both analyses, penetration was greater at 5 mm than at 2 mm ($p <0.001$).

Conclusion: SPB showed satisfactory performance in penetrating dentinal tubules, being similar to AHP, and superior to END.

Clinical significance: Greater penetration of sealer into the dentinal tubules may increase the chance of successful endodontic treatment.

Keywords: Endodontics, Root canal filling materials, Scanning electron microscopy.

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Introduction

Endodontic therapy refers to treatment of root canal systems and is influenced by many clinical and biological factors that lead to the success or failure of the treatment performed.[1]

Besides the use of instruments, antibacterial substances such as irrigants and endodontic sealers are essential for a good outcome.[1,2] It is known that mechanical activation of irrigating solutions enhances their action in the dissolution of organic tissues, removal of the smear layer, and combating bacterial infection,[2,3] because the movement of the irrigants against the walls of the root canal favors their penetration into the dentinal ramifications and tubules.[4] Likewise, endodontic sealers, when activated, are capable of more effectively filling flattened canals, isthmus, and dentinal tubules, mainly with the use of ultrasonic devices that promote their agitation and favor their distribution inside the canals, minimizing the formation of gaps that can harbor microorganisms.[5–7] When in direct contact with these microorganisms, in addition to their antibacterial action, sealers increase obturation sealability and quality.[8]

Endofill®—END (Dentsply Sirona, Ballaigues, Switzerland) is a sealer based on zinc oxide and eugenol and is already established in the literature, with radiopacity and impermeability suitable for endodontic needs;[9] likewise, AH Plus®—AHP (Dentsply Sirona, Ballaigues, Switzerland), is a resin sealer, composed of epoxy resin, and is considered as one of the best filling materials, with long-lasting sealing properties, excellent dimensional stability, self-adhesive property, antimicrobial activity, and high radiopacity.[9] Sealer Plus BC®—SPB (MK Life, Porto Alegre, Rio Grande do Sul, Brazil), is a recently developed bioceramic sealer, having characteristics of high flow, radiopacity, antimicrobial activity, biocompatibility and bioactivity, and biomineralization action.[10–13]

Also, it is important to assess the ability of these materials to penetrate the dentinal tubules, once greater penetration may increase the chance of successful endodontic treatment. Several studies were developed with this aim,[14–19] and the cathodoluminescence is a method that may be applied for this purpose.[15] In this technique, electrons impact in a luminescent material results in the emission of photons, that present wavelengths in the visible spectrum.[20]

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The aim of this study was therefore to determine percentage penetration (PP%) and maximum penetration depth (MPD) of three sealers (END, AHP, and SPB) in endodontic therapy. The null hypothesis was that there would be no difference between the sealers regarding the outcomes.

Materials and Methods

Sample Selection

The sample size calculation was based on the method proposed in the article by Oliveira et al.[15] regarding single-rooted teeth, with a 95% confidence interval and 80% power. This generated a final sample of 45 teeth, i.e., 15 per group.

Forty-five extracted human teeth, obtained from the university's tooth bank, mandibular premolars, single-rooted teeth with a single canal, complete apex, and which had not been endodontically treated, were selected for this study. In order to verify the existence of a single canal, the teeth were radiographed in the buccolingual and mesiodistal directions with a digital sensor.

Teeth with multiple canals, incomplete apex, calcifications, or endodontic treatment were excluded. The selected teeth were stored in distilled water, under refrigeration at 9°C, until the beginning of the study.

The study was registered with the institutional Research Ethics Committee (Opinion No. 3.428.728). All teeth had an indication for extraction and the donors signed an informed consent term for donation to the tooth bank.

Root Canal Preparation

All steps of the experiment were performed by just one operator.

The length of the teeth was standardized at 16 mm, while the working length (WL) was set up at 15 mm, by visually checking the passage of a K #10/.02 hand-file (Dentsply Sirona, Ballaigues, Switzerland) through the root apex. The teeth were sectioned with a diamond disk in a micromotor and contra-angle at a speed of 20,000 rpm under irrigation with distilled water. Some teeth needed endodontic access to be complemented with diamond burs 3081 (KG Sorensen, Cotia, São Paulo, Brazil).

Mechanical preparation was performed with the ProTaper Next® system (Dentsply Sirona, Ballaigues, Switzerland), obtaining surgical diameter #30 (X3), following the 25 mm X1, X2, and X3 instruments in the Smart Plus® X endodontic device (Dentsply Sirona, Ballaigues, Switzerland), at a speed of 300 rpm and torque of 2 N cm. Irrigation, using 5 mL syringes (Ultradent, South Jordan, Utah, USA), was performed with 2.5% sodium hypochlorite (NaOCl), 4 mL being used after each file change.

Final Irrigation

In the final irrigation, 3 mL of 17% EDTA and 3 mL of 2.5% NaOCl were used. Each solution was activated with the ultrasonic insert E1-irrisonic® #20 (Helse, Santa Rosa de Viterbo, São Paulo, Brazil) at a length of 14 mm (WL-1 mm), during three cycles of 20 seconds. The canals were dried with suction cannulas (Ultradent, South Jordan, Utah, USA) and #30 absorbent paper points.

Obturation and Storage

Forty-five specimens were divided into three groups (n = 15) according to the sealer: END, AHP, and SPB, manipulates as recommended by their respective manufacturers. At this stage, 0.05 mL of sealer was taken to each root canal using a tuberculin syringe. Each material was inserted and activated for 30 seconds with the E1-irrisonic® device, at 14 mm.

Obturation was performed using the single cone technique, with the same diameter as the preparation (X3). Excess filling material was removed with a heated instrument, followed by gentle vertical condensation with a condenser. The specimens were sealed in the coronal portion with Light-Cured Universal Restorative® Glass Ionomer Cement (GC Corporation, Tokyo, Japan), being stored in an oven at 37°C, with 100% humidity, for 24 hours to allow the sealer to set.

Dental Section

The teeth were sectioned perpendicularly along the long root axis with a diamond disk, using the IsoMet® cutting machine (Buehler, Lake Bluff, IL, USA) at 300 rpm and in a humid environment. The sections were made at 2 and 5 mm from the root apex, obtaining a 3-mm-thick sample. The samples were washed in running water and detergent in order to preserve the surface with minimal scratches for microscopic analysis.[15]

Image Acquisition

Scanning electron microscopy (SEM) was performed with a JSM-6010LA microscope (Jeol, Tokyo, Japan), with the Centaurus detector for cathodoluminescence analysis. Magnification for all samples was 30× and beam acceleration was 20 kV. Evaluation of each section was performed with the ImageJ® software (National Institutes of Health, Bethesda, Maryland, USA), using measurement with a calibrated tool, by only one operator. Initially, the location of the root canal walls was determined by drawing the silhouette of the canal from which the PP% and MPD of the endodontic sealer in the dentinal tubules were identified.

Image Analysis

A continuous line was drawn in order to assess MPD, following the radial penetration of the sealer into the dentin, from the root canal wall to the deepest penetration point. In the PP% analysis, a grid division was applied (horizontal and vertical lines forming squares); the number of squares covering the walls of the main canal corresponded to the total area; then, the number of squares filled with endodontic sealer was verified. Dividing the penetrated area by the total area of the canal-dentin interface allowed the PP% of the endodontic sealer in the dentinal tubules to be determined.

Statistical Analysis

The sample presented non-normal distribution according to the Kolmogorov-Smirnov test (p <0.05), so the statistical analysis was performed using the nonparametric Kruskal-Wallis test for general comparisons and the Mann-Whitney test for paired comparisons. Differences between apical distances of 2 and 5 mm within each group were analyzed using the Wilcoxon test. A 0.05 confidence level was adopted.

Results

PP% and MPD are shown in Table 1 and in Figures 1 and 2.

PP%

The AHP and SPB sealers had a higher PP% than the END sealer (AHP, p = 0.024; SPB, p <0.001); however, no difference was found between AHP and SPB (p = 0.127). At 2 mm, the outcome was significantly smaller than at 5 mm (p <0.001) for the same sealer (Figs 1 and 2).

Table 1: Mean values and standard deviations (SDs) of PP% and MPD of sealers (µm) according to experimental groups ($n = 15$)

Variable	*Distance*	*END*	*AHP*	*SPB*
PP%	2 mm	19.77 ± 12.93^{Aa}	37.11 ± 18.85^{Ab}	27.59 ± 15.02^{Ab}
	5 mm	29.29 ± 21.18^{Ba}	63.64 ± 16.10^{Bb}	57.28 ± 23.01^{Bb}
MPD	2 mm	68.07 ± 39.23^{Ca}	112.67 ± 84.89^{Cb}	315.83 ± 127.18^{Cb}
	5 mm	119.32 ± 73.38^{Da}	116.86 ± 75.26^{Db}	470.94 ± 302.61^{Db}

END, Endofill; AHP, AH Plus; SPB, Sealer Plus BC; SD, standard deviation. Different capital letters indicate significant differences in the column. Lowercase letters indicate significant differences in rows (p <0.05)

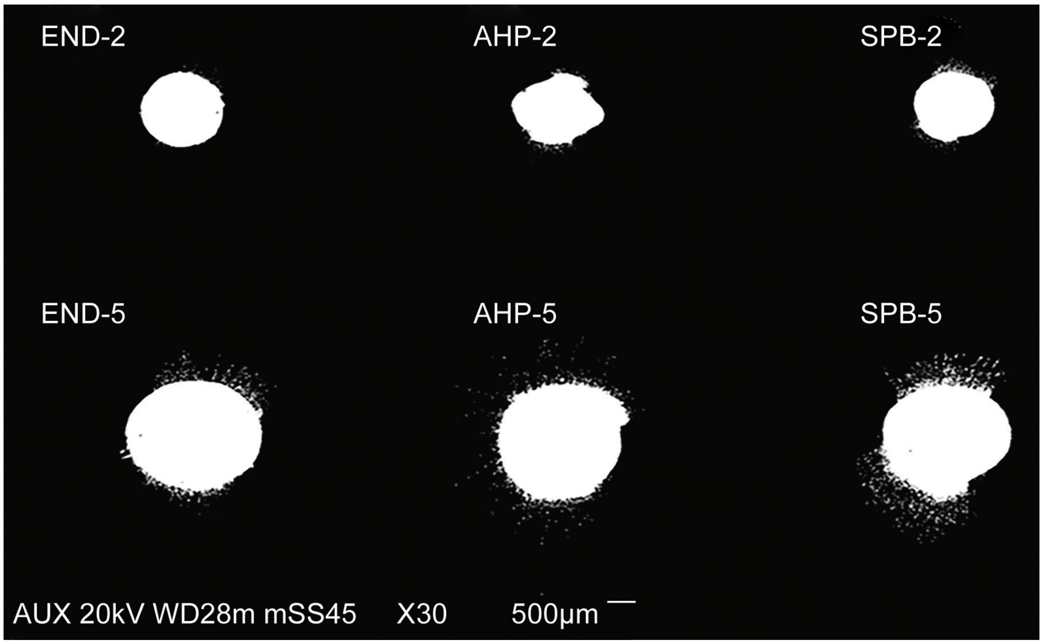

Fig. 1: SEM images associated with cathodoluminescence for the 2 and 5 mm groups. SEM, scanning electron microscopy; EF-2, Endofill 2 mm; EF-5, Endofill 5 mm; AP-2, AH Plus at 2 mm; AP-5, AH Plus at 5 mm; SP-2, Sealer Plus BC 2 mm; SP-5, Sealer Plus BC at 5 mm

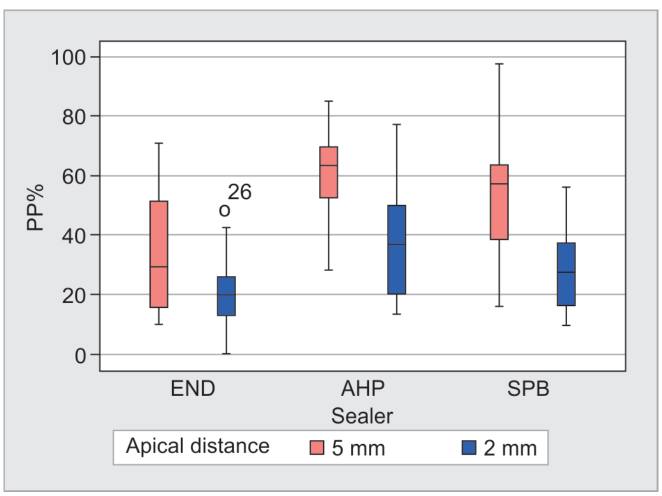

Fig. 2: PP% of the sealers at different distances. Note: PP%, percentage penetration; END, Endofill; AHP, AH Plus; SPB, Sealer Plus BC

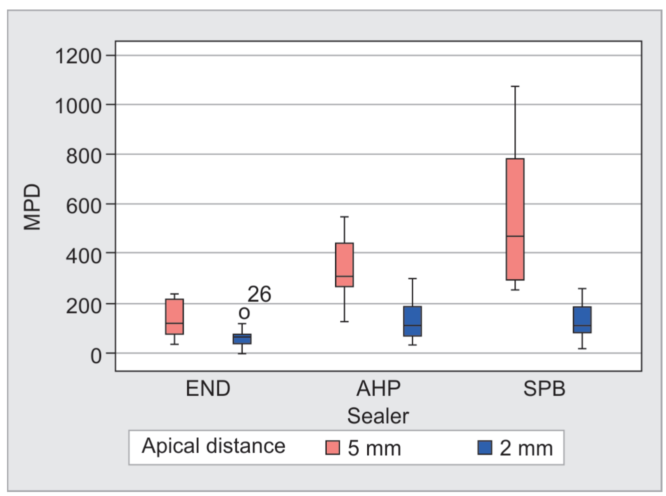

Fig. 3: MPD of the sealers at different distances. Note: MPD, maximum penetration depth; END, Endofill; AHP, AH Plus; SPB, Sealer Plus BC

MPD

AHP and SPB sealers showed greater penetration than END (p <0.001); however, AHP and SPB did not show differences (p = 0.450). Lower penetration was also indicated at 2 mm than at 5 mm (p <0.001) for each sealer (Figs 1 and 2).

Examples of SEM images are shown in Figure 3.

Discussion

The aim of this study was to compare PP% and MPD of END, AHP, and SPB sealers in dentinal tubules, with ultrasonic activation. The null hypothesis was rejected, as the materials showed different performances.

AHP is considered the gold standard among the sealers, while the SPB is commercialized with a proposal to be similar to AHP. Thus, this study aimed to verify if the penetration pattern would be similar between them and, also, in comparison with END, a widely used sealer.

The composition of a sealer affects its physicochemical characteristics, such as consistency, viscosity, and flow capacity, which directly influence tubular penetration.[16] The diversity in terms of results in the present study possibly occurred due to their different compositions: END is based on zinc oxide and eugenol, AHP is composed of epoxy resin, and SPB is a bioceramic material.[9,10–13]

The properties of END and AHP sealers have been investigated in the literature[9,17,18] while SPB has been reported as a biocompatible sealer with low cytotoxicity, the physicochemical properties of which are in accordance with the specifications required by the American Dental Association (ADA).[11–13]

Other factors need to be considered for the sealer to flow into the tubules, such as the inner diameter of the root canal, amount of inserted material, and removal of the smear layer formed after chemical-mechanical preparation.[19–22] For this reason, in this research, the agitation of the irrigating solutions was carried out, as it increases the cleaning capacity.[2–4] Also, ultrasonic activation has begun to be studied in endodontic sealers and has shown an improvement in the distribution of the material along the canal, promoting better sealing, which contributes to quality treatment.[5,6,23]

In the present study, the AHP and SPB sealers presented higher PP% than the END sealer, which is in agreement with De Bem et al.[24] and Piai et al.,[13] with similar penetration values in the apical third. The findings are also in agreement with the study by Tedesco et al.,[25] in which AHP presented better results than END. These results may be explained by the similar physicochemical properties (flowability) of AHP and SPB, resulting in higher dentinal tubule penetration.

De Bem et al.[24] tested AHP ultrasonic activation and passive insertion, and the results obtained showed no significant difference, while Guimarães et al.[5] indicated that ultrasonic activation of this sealer was relevant with effect from 4 mm away from the root apex. However, Khedmat et al.[26] indicated that sealers subjected to different temperatures may change their viscoelastic property, which may result from the ultrasonic activation of these materials. It is postulated that activated endodontic sealers are capable of more effectively filling of anatomical variations that can harbor microorganisms,[5–7] then increasing obturation sealability and quality, improving the antibacterial action.[8]

Regarding sealer MPD in the dentinal tubules, AHP and SPB had similar performance, but were superior in relation to END. The results of Garrido et al.[27] indicated the smallest flow of END in relation to AHP, while Vertuan et al.[12] confirmed that AHP and SPB had similar flows. These results can also be explained by the similarity of the physicochemical properties of these materials.

All the sealers penetrated more at 5 mm than at 2 mm from the root apex, a result expected due to the anatomy of the apical region, which is an area of difficult access, contains fewer tubules and has a smaller diameter, and more sclerotic dentin.[13,19,24,28]

Regarding the methods, SEM has been used to mainly address smear layer removal and endodontic sealer penetration in dental sections.[2,3,22] The sealers, here studied, have a cathodoluminescence property, which makes them contrast when a cathodoluminescence detector is associated with SEM, allowing it to be observed inside the dentinal tubules, in dental slices, which justifies the technique chosen.[15,20]

Finally, from the clinical point of view, sealer PP% seems to be more relevant than sealer MPD.[28]

The obturation of oval canals, here represented by the chosen dental group (mandibular premolars), is a challenge, and the strategies of activating irrigants and sealers must be considered.

The limitations of this study are those related to the *ex vivo* model adopted. The SPB sealer still needs to be studied further, including other physicochemical properties, in addition to clinical studies with longer follow-ups to test its effectiveness.

Conclusion

SPB showed superior performance regarding PP% and MPD in the dentinal tubules in relation to END, but its performance was similar to that of AHP.

Clinical Significance

Greater penetration of sealer into the dentinal tubules may increase the chance of successful endodontic treatment.

Authorship Declaration

All authors have contributed significantly to this manuscript and are in agreement with the submission of it.

References

1. Prada I, Micó-Muñoz P, Giner-Lluesma T, et al. Influence of microbiology on endodontic failure. Literature review. Med Oral Patol Oral Cir Bucal 2019;24(3):e364–e372. DOI: 10.4317/medoral.22907.
2. Orhan EO, Bahadır D, Akgün C, et al. Scanning electron microscopic evaluation of the efficacy of 5% ethylenediaminetetraacetic acid for smear layer removal. Microsc Res Tech 2021;84(2):253–260. DOI: 10.1002/jemt.23582.
3. Akyuz Ekim SN, Erdemir A. Comparison of different irrigation activation techniques on smear layer removal: an in vitro study. Microsc Res Tech 2015;78(3):230–239. DOI: 10.1002/jemt.22466.
4. van der Sluis LW, Gambarini G, Wu MK, et al. The influence of volume, type of irrigant and flushing method on removing artificially placed dentine debris from the apical root canal during passive ultrasonic irrigation. Int Endod J 2006;39(6):472–476. DOI: 10.1111/j.1365-2591.2006.01108.x.
5. Guimarães BM, Amoroso-Silva PA, Alcalde MP, et al. Influence of ultrasonic activation of 4 root canal sealers on the filling quality. J Endod 2014;40(7):964–968. DOI: 10.1016/j.joen.2013.11.016.
6. Wiesse PEB, Silva-Sousa YT, Pereira RD, et al. Effect of ultrasonic and sonic activation of root canal sealers on the push-out bond strength and interfacial adaptation to root canal dentine. Int Endod J 2018;51(1):102–111. DOI: 10.1111/iej.12794.
7. Yamini B, Gali PK, Nagesh B, et al. Effect of indirect ultrasonic activation of modified bioceramic materials on the bond strength and tubular penetration in root canals. Dent Res J (Isfahan) 2021;18:45. PMID: 34429865.
8. Heling I, Chandler NP. The antimicrobial effect within dentinal tubules of four root canal sealers. J Endod 1996;22(5):257–259. DOI: 10.1016/s0099-2399(06)80144-5.
9. Marín-Bauza GA, Silva-Sousa YT, da Cunha SA, et al. Physicochemical properties of endodontic sealers of different bases. J Appl Oral Sci 2012;20(4):455–461. DOI: 10.1590/s1678-77572012000400011.

10. Alves Silva EC, Tanomaru-Filho M, da Silva GF, et al. Biocompatibility and bioactive potential of new calcium silicate-based endodontic sealers: Bio-C sealer and Sealer Plus BC. J Endod 2020;46(1):1470–1477. DOI: 10.1016/j.joen.2020.07.011.
11. Cintra LTA, Benetti F, de Azevedo Queiroz ÍO, et al. Evaluation of the cytotoxicity and biocompatibility of new resin epoxy-based endodontic sealer containing calcium hydroxide. J Endod 2017;43(12):2088–2092. DOI: 10.1016/j.joen.2017.07.016.
12. Vertuan GC, Duarte MAH, Moraes IG, et al. Evaluation of physicochemical properties of a new root canal sealer. J Endod 2018;44(3):501–505. DOI: 10.1016/j.joen.2017.09.017.
13. Piai GG, Duarte MAH, Nascimento ALD, et al. Penetrability of a new endodontic sealer: a confocal laser scanning microscopy evaluation. Microsc Res Tech 2018;81(11):1246–1249. DOI: 10.1002/jemt.23129.
14. De-Deus G, Brandó MC, Souza EM, et al. Epoxy resin-based root canal sealer penetration into dentin tubules does not improve root filling dislodgement resistance. Eur Endod J 2017;2(1):1–5. DOI: 10.5152/eej.2017.16059.
15. Oliveira KV, Silva BMD, Leonardi DP, et al. Effectiveness of different final irrigation techniques and placement of endodontic sealer into dentinal tubules. Braz Oral Res 2017;31:e114. DOI: 10.1590/1807-3107BOR-2017.vol31.0114.
16. Okşan T, Aktener BO, Sen BH, et al. The penetration of root canal sealers into dentinal tubules. A scanning electron microscopic study. Int Endod J 1993;26(5):301–305. DOI: 10.1111/j.1365-2591.1993.tb00575.x.
17. Caceres C, Larrain MR, Monsalve M, et al. Dentinal tubule penetration and adaptation of Bio-C Sealer and AH-Plus: a comparative SEM evaluation. Eur Endod J 2021;6(2):216–220. DOI: 10.14744/eej.2020.96658.
18. Khan MT, Moeen F, Safi SZ, et al. The structural, physical, and in vitro biological performance of freshly mixed and set endodontic sealers. Eur Endod J 2021;6(1):98–109. DOI: 10.14744/eej.2020.36349.
19. Mjör IA, Smith MR, Ferrari M, et al. The structure of dentine in the apical region of human teeth. Int Endod J 2001;34(5):346–353. DOI: 10.1046/j.1365-2591.2001.00393.x.
20. Coenen T, Brenny BJ, Vesseur EJ, et al. Cathodoluminescence microscopy: optical imaging and spectroscopy with deep-subwavelength resolution. Multiscale mechanics of biological, bioinspired, and biomedical materials. MRS Bull 2015;40(4):359–365. DOI: 10.1557/mrs.2015.64.
21. Schilder H. Filling root canals in three dimensions. Dent Clin North Am 1967;723–744. PMID: 5262492.
22. Turkyilmaz A, Erdemir A. Comparison of dentin penetration ability of different root canal sealers used with different obturation methods. Microsc Res Tech 2020;83(12):1544–1551. DOI: 10.1002/jemt.23548.
23. Arslan H, Abbas A, Karatas E. Influence of ultrasonic and sonic activation of epoxy-amine resin-based sealer on penetration of sealer into lateral canals. Clin Oral Investig 2016;20(8):2161–2164. DOI: 10.1007/s00784-016-1737-z.
24. De Bem IA, de Oliveira RA, Weissheimer T, et al. Effect of ultrasonic activation of endodontic sealers on intratubular penetration and bond strength to root dentin. J Endod 2020;46(9):1302–1308. DOI: 10.1016/j.joen.2020.06.014.
25. Tedesco M, Chain MC, Felippe WT, et al. Correlation between bond strength to dentin and sealers penetration by push-out test and CLSM analysis. Braz Dent J 2019;30(6):555–562. DOI: 10.1590/0103-6440201902766.
26. Khedmat S, Momen-Heravi F, Pishvaei M. A comparison of viscoelastic properties of three root canal sealers. J Dent (Tehran) 2013;10(2):147–154. PMID: 23724214.
27. Garrido AD, Lia RC, França SC, et al. Laboratory evaluation of the physicochemical properties of a new root canal sealer based on Copaifera multijuga oil-resin. Int Endod J 2010;43(4):283–291. DOI: 10.1111/j.1365-2591.2009.01678.x.
28. Bolles JA, He J, Svoboda KK, et al. Comparison of Vibringe, EndoActivator, and needle irrigation on sealer penetration in extracted human teeth. J Endod 2013;39(5):708–711. DOI: 10.1016/j.joen.2013.01.006.