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Original Article

Comparison of Marginal Adaptation Between Cold Ceramic Sealer and AH26 Sealer Using Scanning Electron Microscopy: An In Vitro Study

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Abstract
Background: Cold ceramic (CC) is a bioceramic material used for root-end filling. A new bioceramic sealer derived from CC has recently been developed. This in vitro study was conducted to evaluate and compare the marginal adaptation of an experimental bioceramic-based sealer (CC sealer: CCS) with that of an epoxy resin-based sealer (AH26 sealer) using a scanning electron microscope (SEM).
Methods: Twenty extracted human maxillary central incisors were collected, disinfected with 5.25% sodium hypochlorite, and stored in 0.9% saline. The teeth were decoronated at the cementoenamel junction to obtain 13 mm roots. After cleaning and shaping the canals using the crown-down technique, the samples were randomly divided into two groups (n = 10 each). In Group 1, canals were obturated with gutta-percha and AH26 sealer. In Group 2, canals were obturated with gutta-percha and the CCS. After 24 hours of incubation, a 2-mm-thick apical cross-section was prepared from each specimen, and marginal adaptation was assessed using SEM. Statistical analysis was performed using SPSS 20 and the Mann-Whitney test.
Results: The mean marginal gap was 15.6 ± 3.46 µm and 10.47 ± 2.35 µm in the AH26 and CCS groups, respectively. The mean gap of the CCS group was significantly lower in the apical side and coronal side of the specimens compared to the AH26 sealer group ($P < 0.05$).
Conclusion: Within the limitations of this in vitro study, CCS demonstrated significantly better marginal adaptation compared to AH26 under SEM evaluation. It is recommended that further clinical studies validate its performance in clinical settings.
Keywords: Dental marginal adaptation, Endodontics, Root canal obturation

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Background
The three-dimensional obturation of the root canal system is crucial for the success of endodontic treatment. Gutta-percha cones combined with root canal sealers are still the most widely accepted method for canal obturation. Numerous sealers have been developed for use with gutta-percha, differing in composition, sealing ability, biocompatibility, and clinical performance (1).

Among them, bioceramic sealers have gained considerable interest over the past three decades. Krell and Wefel were the first ones to report the use of bioceramic materials as root canal sealers (2); they introduced calcium phosphate cement as an experimental sealer. Since then, advances in calcium silicate-based technology have led to the development of modern sealers, such as iRoot SP in 2007, which marked the beginning of a new generation of endodontic materials labeled as "bioceramic sealers" (3).

Bioceramic sealers possess favorable characteristics, such as small particle size (less than two µm), non-toxicity, high tissue compatibility, hydrophilicity, and low dimensional change upon setting (4). Some of these materials incorporate calcium phosphate, which improves their setting reaction and results in a chemical composition and crystal structure similar to that of dental and bone apatite (5). Their interaction with dentin moisture initiates reactions between calcium silicates and phosphate, forming hydroxyapatite crystals along the mineral infiltration zone and promoting sealing and potential bioactivity (6).

Cold ceramic (CC) is one such bioceramic material initially designed for root-end filling procedures (7-9). This material is known for its biocompatibility, promising sealing performance, alkaline pH, radio-opacity, and moisture-dependent setting properties (7-10).

A novel root canal sealer based on the CC formulation has been introduced recently (11). However, there is currently a lack of data on its physical characteristics, sealing performance, or marginal adaptation when used in root canal obturation.

Among available evaluation tools, scanning electron microscopy (SEM) is highly suitable for analyzing the marginal adaptation of endodontic materials. It provides high-resolution imaging at the submicron level, allowing for the precise measurement of the gap between the sealer and the canal wall, which offers a reliable indicator of sealing ability.

Therefore, this in vitro study aims to compare the marginal adaptation of the CC sealer (CCS) and the AH26 sealer at the dentin–sealer interface using SEM. The null hypothesis is that there is no significant difference in the marginal gap width between the two root canal sealers.

Materials and Methods

This in vitro study included 20 single-canal maxillary centrals extracted due to periodontic and prosthetic reasons in the Shahid Sadoughi dental clinic. The inclusion criteria required fully developed single canal centrals with no calcification, internal or external resorption, root caries, restoration below the cementoenamel junction, and no previous endodontic treatment.

Sample Size

The minimum sample size was calculated using PASS 15 software (NCSS, LLC, Kaysville, Utah, USA), based on the results of a previous study (12). Assuming a 95% confidence interval, 80% statistical power, a standard deviation of 3 µm, and an expected difference of 2 µm between the CC and AH-26 groups in the mean gap at the sealer-dentin interface, the required sample size was determined to be 10 specimens per group.

Specimen Preparation

The collected teeth were cleaned using an ultrasonic scaler and immersed in a 5.25% sodium hypochlorite (NaOCl) solution (Chloran, Iran). The disinfected teeth were then stored in 0.9% sterile saline until further processing.

The crowns were sectioned at the cementoenamel junction using a diamond disc (Tizkavan, Iran) to standardize the root length to 13 mm. The working length was determined by inserting a #10 K-file (Mani, Japan) into the canal until its tip was visible at the apical foramen, followed by a subtraction of 1 mm. Root canal instrumentation was performed using the PROTAPER rotary file system (SX, S1, S2, F1, F2, and F3) with the crown-down technique, driven by the X-Smart motor (Dentsply Maillefer, Ballaigues, Switzerland). After each file, the canals were irrigated with 1 mL of 2.25% NaOCl (Chloran, Iran). Final irrigation involved 17% ethylenediaminetetraacetic acid (Cobalt, Tehran, Iran), normal saline, and 2.5% NaOCl (Chloran, Iran), each applied for 3 minutes to ensure effective removal of the smear layer. The canals were then dried with sterile paper points (Meta Biomed, South Korea) in preparation for obturation.

The prepared roots were randomly divided into two groups (n = 10) using a dice method. Each tooth was assigned a unique numerical code. The operator performing the obturation procedures was aware of the material groups due to the material handling difference, but was not involved in the evaluation phase.

In group 1, canals were obturated with gutta-percha (Meta Biomed, South Korea) and the AH26 sealer, and in group 2, obturation was performed using gutta-percha and the CCS. Both sealers were prepared according to the manufacturer's instructions. For the AH26 sealer, equal amounts of base and catalyst paste were dispensed onto a mixing pad and blended thoroughly for 30 seconds until a homogeneous consistency was achieved. For the CCS, the powder and liquid components were mixed in the recommended ratio (as specified by the manufacturer) on a glass slab using a sterile spatula. The mixture was stirred for approximately 60 seconds until a smooth, uniform paste was obtained. The prepared sealers were immediately used for obturation to ensure optimal properties.

A standardized master gutta-percha cone (size F3, corresponding to the final rotary instrument used) was selected, and its fit was verified within the canal. The master cone was coated with the respective sealer (AH26 or CCS) and gently inserted into the canal up to the working length. The excess sealer was removed from the canal orifice using a sterile paper point.

Subsequently, accessory gutta-percha cones (size #.02 taper) were utilized for lateral condensation. A finger spreader (Dentsply Maillefer) was inserted into the canal alongside the master cone to a depth of 1–2 mm short of the working length. The spreader was then removed, and an accessory cone was inserted into the space it had created. This process was repeated until the spreader could no longer penetrate more than 2–3 mm into the canal. Excess gutta-percha was removed using a heated instrument, and vertical condensation was lightly applied at the canal orifice to ensure a dense fill.

Radiographs were taken to ensure proper obturation. Next, 3 mm of the coronal canal space was filled with temporary material (Coltosol; Ariadent, Tehran, Iran), and all samples were incubated at 37 °C and 100% humidity for 24 hours.

Assessment of Marginal Adaptation

To prepare samples for SEM examination, a two mm-thick transverse specimen was cut 2 mm above the apex of each tooth using a diamond disk perpendicular to the long axis of the roots. The obtained specimens were prepared for analysis using a SEM (TESCAN VEGA3, Czech Republic). The samples were dehydrated using a series of ascending ethanol concentrations (25%, 50%, 75%, and 100%). Finally, the samples were dried and sputter-coated with gold.

Blinding was implemented for the SEM analysis. The evaluator who measured the marginal gaps was blinded to the experimental groups using coded sample labeling. The diameter of the gaps between the obturation materials and canal walls was measured at magnifications of × 500, × 1000, and × 2000. Each sample's surface was divided into four equal parts, and the largest gap between the filling material and the canal wall in each part was measured. The average measurement was recorded as the gap on each side of the sample. This process was performed for both the coronal and apical sides of each sample, and the mean values of both sides were considered the sample gap. Finally, the data were analyzed using SPSS 20 software and the Mann-Whitney test.

Results

The data were not normally distributed based on the Shapiro–Wilk test ($P < 0.05$). Therefore, the non-parametric Mann–Whitney U test was applied for statistical comparisons.

Table 1 presents the mean marginal gaps (μm) in both coronal and apical regions of the CC and AH26 groups. The CC group showed significantly lower marginal gaps than the AH26 group in both the coronal (10.66 ± 2.41 μm vs. 16.50 ± 4.04 μm, $P < 0.001$) and apical (10.27 ± 2.28 μm vs. 14.70 ± 2.87 μm, $P < 0.001$) regions. Similarly, the overall mean gap was significantly lower in the CC group (10.47 ± 2.35 μm) compared to the AH26 group (15.60 ± 3.46 μm), with $P < 0.001$ (Figures 1 and 2).

Table 2 provides the within-group comparison of apical and coronal gaps. No statistically significant differences were found between the apical and coronal sections in either the CC ($P = 0.71$) or AH26 ($P = 0.26$) group.

Table 1. Mean Gap of the Tested Groups

	Mean ± SD (μm)	*P* Value*
Coronal side of CCS	10.66 ± 2.41	0.001
Coronal side of AH26	16.50 ± 4.04	
Apical side of CCS	10.27 ± 2.28	0.001
Apical side of AH26	14.70 ± 2.87	
The overall gap of CCS	10.47 ± 2.35	0.001
The overall gap of AH26	15.6 ± 3.46	

Note. CCS: Cold ceramic sealer; SD: Standard deviation. *Mann-Whitney test.

Table 2. Mean Gap Between the Apical and Coronal Sides of the Tested Groups

	Mean ± SD (μm)	*P* Value*
Coronal side of CCS	10.66 ± 2.41	0.71
Apical side of CCS	10.27 ± 2.28	
Coronal side of AH26	16.50 ± 4.04	0.26
Apical side of AH26	14.70 ± 2.87	

Note. CCS: Cold ceramic sealer; SD: Standard deviation. *Mann-Whitney test.

Discussion

This research evaluated and compared the marginal adaptation of CC and AH26 sealers. Marginal adaptation is considered one of the contributing factors to the sealing ability of endodontic materials, although it does not independently determine the clinical success of root canal fillings. However, this relationship remains influenced by other biological and procedural variables (13).

In this study, an SEM was used to assess marginal adaptation. The advantage of using SEM over various sealing methods is that it allows us to observe defects at the submicron level and evaluate them by preserving microphotographs. Compared to micro-computed

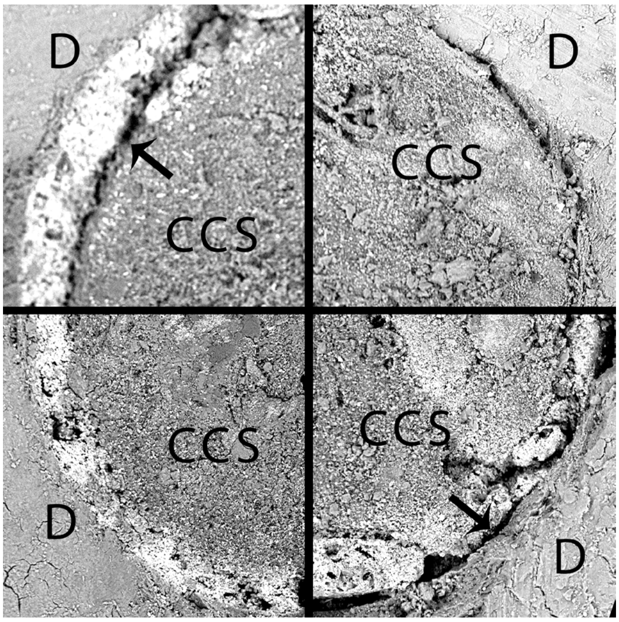

Figure 1. SEM images of CC Sealer group, D: Dentin, CCS: CC Sealer and gutta percha, Black arrows: The gap between dentin and obturation core

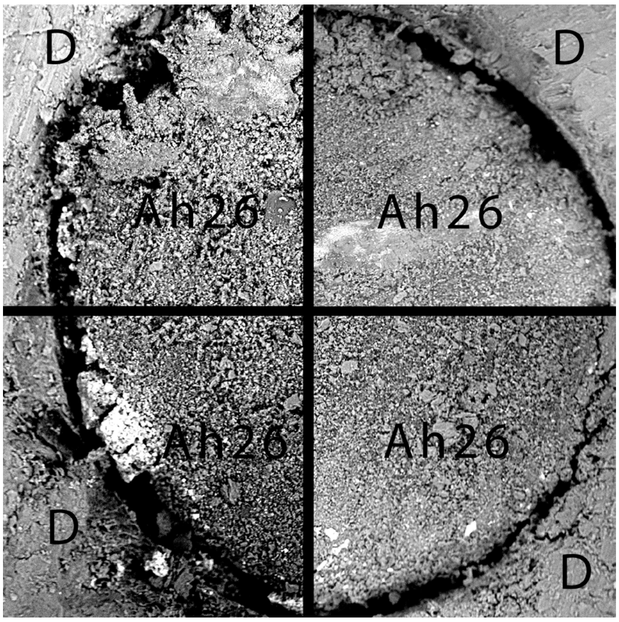

Figure 2. SEM images of AH26 Sealer group, D: Dentin, AH26: AH26 Sealer and gutta percha, Black arrows: The gap between dentin and obturation core

tomography, SEM is a more affordable and attainable method.

The smear layer in root canals, especially in the lower third of the canal, can hinder the effectiveness of sealers. Removing this layer is important for better sealing and bonding to the tooth and reducing the risk of bacterial infection (14,15). In this study, 17% ethylenediaminetetraacetic acid was utilized to remove the smear layer.

Based on our findings, CCS demonstrated significantly narrower marginal gaps compared to AH26 under the specific in vitro conditions evaluated in this study. This difference may be partly attributed to the bioceramic sealer's smaller particle size, hydrophilic nature, and low contact angle, allowing it to easily spread over the dentin walls of the root canal and fill the lateral micro-canals (1). Additionally, according to Polineni et al (16), the bioceramic byproducts are alkaline, causing the collagen fibers of dentin to denature, thereby allowing the sealers to penetrate the dentinal tubules. The reaction among phosphate, calcium silicate hydrogel, and calcium hydroxide creates hydroxyapatite along the mineral infiltration zone due to the reaction between calcium silicates and the moisture present in dentin (5). However, none of these mechanisms has been explicitly investigated in relation to CCS to date. Accordingly, it is recommended that future studies directly evaluate whether these properties also apply to this novel formulation. It is also important to note that marginal adaptation alone does not necessarily equate to long-term sealing ability or clinical success, as multiple biological and procedural factors contribute to endodontic outcomes.

Epoxy resin-based sealers, such as AH26, are known for their acceptable biocompatibility, dimensional stability, and long clinical track record. However, some formulations contain additives, such as silicone oil, which *may lead to polymerization shrinkage or compromised adhesion to dentin*, potentially affecting their sealing ability. *These physicochemical limitations could help explain the relatively larger marginal gaps observed in the AH26 group under the current study's conditions* (17).

A recent study by Mokhtari et al (11) evaluated the antimicrobial activity of the CCS and reported its effectiveness against *Enterococcus faecalis*, comparable to other commonly used sealers, such as AH Plus and Endoseal MTA. The consistency of findings in previous studies suggests that bioceramic sealers, including CCS, may offer advantages in terms of microbial resistance and sealing ability; nonetheless, further clinical and laboratory investigations are needed to confirm these potential benefits in practice.

Despite the laboratory leakage outcomes, many of the physicochemical characteristics of CCS remain to be fully evaluated. Parameters such as setting time, solubility, dimensional stability, flowability, and long-term interaction with dentin have not yet been comprehensively studied in peer-reviewed literature.

Furthermore, additional research is required to evaluate the material's biological responses, including its cytotoxicity, biocompatibility, and potential to induce periapical healing or inflammatory reactions in vivo. Such evaluations are essential for determining its safety profile and clinical reliability. Moreover, clinical trials assessing the material's performance under real-world conditions, along with structured feedback from dental practitioners regarding its handling characteristics and ease of use, will provide critical insights into making more definitive judgments about its suitability in endodontic practice.

Patri et al (18) found that bioceramic sealers are more effective than resin-based sealers in terms of marginal adaptation. Additionally, Padmawar et al (19) compared the marginal adaptation of Endosequence BC RCS (a bioceramic sealer), AH Plus, and EndoRez (a resin-based sealer) to dentin using electron microscopy. Their results showed that the bioceramic sealer had the narrowest gap. These findings are consistent with the results of a previous study conducted by de Miranda Candeiro et al (20), demonstrating that the penetration capacity of the bioceramic sealer in dentin tubules was significantly higher than that of the epoxy resin sealer. Other studies by McMichael et al (21) and Wang et al (22) reported that the use of bioceramic-based sealers resulted in a significantly higher depth of tubular penetration compared to epoxy resin-based sealers across various filling techniques.

It is important to keep in mind that this study had some limitations. For example, when the filled canal was sectioned, there was a high risk that the material could tear or the gutta-percha could smear, concealing the actual gap. Additionally, rough dehydration and drying could cause artifacts, such as volumetric shrinkage, fracturing, or cracking of the samples (23). When cracks form in the dentin and obturated core, they may lead to overestimated gap formation. Furthermore, only a few cross-sections are evaluated, and the gaps are examined in 2 dimensions, while the obturation material should be effective in 3 dimensions. To distinguish genuine gaps between the root filling and dentin from potential artifactual gaps created after vacuum desiccation in conventional SEM, it is necessary to examine fully hydrated specimens using environmental scanning electron microscopy. It is also not clinically established how much the gap difference can affect the clinical prognosis. Therefore, clinical trials and further studies are crucial to confirm the results of the present study.

Conclusion

Within the limitations of this in vitro study, CCS demonstrated significantly narrower marginal gaps at the dentin interface compared to AH26, suggesting improved adaptation under the tested conditions.

These findings suggest that CCS may offer potential advantages as a root canal sealer; however, due to the limited sample size and the absence of clinical parameters, no definitive conclusion can be drawn about its clinical superiority.

Authors' Contribution

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Investigation: Fatemeh Ayatollahi, Erfan Mahmoudian.
Methodology: Fatemeh Ayatollahi, Erfan Mahmoudian.
Project administration: Fatemeh Ayatollahi, Aida Hariri.
Resources: Jalil Modaresi, Erfan Mahmoudian.
Supervision: Jalil Modaresi, Fatemeh Mokhtari.
Validation: Jalil Modaresi.
Visualization: Fatemeh Mokhtari, Aida Hariri.
Writing–original draft: Erfan Mahmoudian, Aida Hariri.
Writing–review & editing: Fatemeh Ayatollahi, Jalil Modaresi, Erfan Mahmoudian, Fatemeh Mokhtari, Aida Hariri.

Competing Interests

The authors declare that there is no conflict of interests.

Ethical Approval

Informed consent was routinely obtained from patients to use their teeth for research purposes. The Ethics Committee of Shahid Sadoughi Dental School approved the study (Ethics approval number: IR.SSU.DENTISTRY.REC.1401.81).

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References

1. Pius A, Mathew J, Theruvil R, George S, Paul M, Baby A, et al. Evaluation and comparison of the marginal adaptation of an epoxy, calcium hydroxide-based, and bioceramic-based root canal sealer to root dentin by SEM analysis: an in vitro study. Conserv Dent Endod J. 2019;4(1):6-13. doi: 10.5005/jp-journals-10048-0046.
2. Krell KF, Wefel JS. A calcium phosphate cement root canal sealer--scanning electron microscopic analysis. J Endod. 1984;10(12):571-6. doi: 10.1016/s0099-2399(84)80103-x.
3. Donnermeyer D, Bürklein S, Dammaschke T, Schäfer E. Endodontic sealers based on calcium silicates: a systematic review. Odontology. 2019;107(4):421-36. doi: 10.1007/s10266-018-0400-3.
4. Camilleri J, Pitt Ford TR. Mineral trioxide aggregate: a review of the constituents and biological properties of the material. Int Endod J. 2006;39(10):747-54. doi: 10.1111/j.1365-2591.2006.01135.x.
5. Al-Haddad A, Che Ab Aziz ZA. Bioceramic-based root canal sealers: a review. Int J Biomater. 2016;2016:9753210. doi: 10.1155/2016/9753210.
6. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against *Enterococcus faecalis*. J Endod. 2009;35(7):1051-5. doi: 10.1016/j.joen.2009.04.022.
7. Hasheminia SM, Loriaei Nejad S, Dianat O, Modaresi J, Mahjour F. Comparing the sealing properties of mineral trioxide aggregate and an experimental ceramic-based root end filling material in different environments. Indian J Dent Res. 2013;24(4):474-7. doi: 10.4103/0970-9290.118399.
8. Modaresi J, Talakoob MH. Comparison of two root-end filling materials. J Isfahan Dent Sch. 2015;11(5):379-86.
9. Akhavan A, Parashos P, Razavi SM, Davoudi A, Shadmehr E. Hard tissue reaction to mineral trioxide aggregate and experimental root-end filling material in guinea pig mandibles. J Dent Sci. 2017;12(2):107-11. doi: 10.1016/j.jds.2016.11.003.
10. Modaresi J, Hemati HR. The cold ceramic material. Dent Res J (Isfahan). 2018;15(2):85-8.
11. Mokhtari F, Modaresi J, Hasanzade Salmasi A, Khamisi N, Zandi H, Lesani K. In vitro comparison of *Enterococcus faecalis* survival in dentinal tubules following root canal therapy with AH plus, endoseal MTA, and cold ceramic sealers. BMC Oral Health. 2024;24(1):1488. doi: 10.1186/s12903-024-05192-8.
12. Mokhtari F, Modaresi J, Javadi G, Davoudi A, Badrian H. Comparing the marginal adaptation of cold ceramic and mineral trioxide aggregate by means of scanning electron microscope: an in vitro study. J Int Oral Health. 2015;7(9):7-10.
13. Xavier CB, Weismann R, de Oliveira MG, Demarco FF, Pozza DH. Root-end filling materials: apical microleakage and marginal adaptation. J Endod. 2005;31(7):539-42. doi: 10.1097/01.don.0000152297.10249.5a.
14. Al Fouzan K, Awadh M, Badwelan M, Gamal A, Geevarghese A, Babhair S, et al. Marginal adaptation of mineral trioxide aggregate (MTA) to root dentin surface with orthograde/retrograde application techniques: a microcomputed tomographic analysis. J Conserv Dent. 2015;18(2):109-13. doi: 10.4103/0972-0707.153069.
15. Tabrizizade M, Asadi Y, Sooratgar A, Moradi S, Sooratgar H, Ayatollahi F. Sealing ability of mineral trioxide aggregate and calcium-enriched mixture cement as apical barriers with different obturation techniques. Iran Endod J. 2014;9(4):261-5.
16. Polineni S, Bolla N, Mandava P, Vemuri S, Mallela M, Gandham VM. Marginal adaptation of newer root canal sealers to dentin: a SEM study. J Conserv Dent. 2016;19(4):360-3. doi: 10.4103/0972-0707.186453.
17. Rouhani A, Ghoddusi J, Naghavi N, Ebadzadeh Z, Akbari M. The sealing ability of resilon and gutta-percha in severely curved root canals: an in vitro study. J Dent (Tehran). 2013;10(2):141-6.
18. Patri G, Agrawal P, Anushree N, Arora S, Kunjappu JJ, Shamsuddin SV. A scanning electron microscope analysis of sealing potential and marginal adaptation of different root canal sealers to dentin: an in vitro study. J Contemp Dent Pract. 2020;21(1):73-7.
19. Padmawar N, Moapagr V, Vadvadgi V, Vishwas J, Joshi S, Padubidri M. Scanning electron microscopic evaluation of marginal adaptation of three endodontic sealers: an ex-vivo study. J Pharm Res Int. 2021;33(28A):49-57. doi: 10.9734/JPRI/2021/v33i28A31510.
20. de Miranda Candeiro GT, Lavor AB, de Freitas Lima IT, de Vasconcelos BC, Gomes NV, Iglecias EF, et al. Penetration of bioceramic and epoxy-resin endodontic cements into lateral canals. Braz Oral Res. 2019;33:e049. doi: 10.1590/1807-3107bor-2019.vol33.0049.
21. McMichael GE, Primus CM, Opperman LA. Dentinal tubule penetration of tricalcium silicate sealers. J Endod. 2016;42(4):632-6. doi: 10.1016/j.joen.2015.12.012.
22. Wang Y, Liu S, Dong Y. In vitro study of dentinal tubule penetration and filling quality of bioceramic sealer. PLoS One. 2018;13(2):e0192248. doi: 10.1371/journal.pone.0192248.
23. Janda R. Preparation of extracted natural human teeth for SEM investigations. Biomaterials. 1995;16(3):209-17. doi: 10.1016/0142-9612(95)92119-q.