

Influence of the filling technique on the filling quality and intratubular penetration of the sealer in mandibular molars with isthmuses

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Abstract

Objectives: To evaluate the influence of obturation technique, ultrasonic activation, and sealer type on filling quality and intratubular sealer penetration in the mesial roots of mandibular molars.

Materials and Methods: Eighty mandibular molars were scanned using micro-computed tomography and randomly allocated into eight groups according to obturation protocol: single cone, ultrasonic activation with single cone, Tagger hybrid technique, and continuous wave technique, using either epoxy resin-based or bioceramic sealer. After obturation, specimens were rescanned to assess voids and gaps. Laser scanning confocal microscopy was used to evaluate intratubular sealer penetration in root sections. Data were analysed using analysis of variance and Tukey's post hoc test ($\alpha = 0.05$).

Results: Bioceramic sealer associated with ultrasonic activation and Tagger hybrid technique resulted in lower void and gap volumes in the middle third compared with resin-based sealer ($P < 0.05$). Resin-based sealer showed significantly greater intratubular penetration, particularly when combined with ultrasonic activation ($P < 0.05$). Ultrasonic activation significantly enhanced sealer penetration for both materials.

Conclusions: None of the tested techniques completely filled the root canal system and isthmus region. Bioceramic sealer reduced void formation, whereas resin-based sealer promoted greater dentinal penetration.

Clinical Relevance: Ultrasonic activation may improve sealer penetration and distribution within anatomically complex regions, potentially enhancing sealing ability and entombment of residual micro-organisms during endodontic treatment.

INTRODUCTION

Achieving a durable three-dimensional seal is essential to prevent reinfection and promote periapical healing following adequate chemomechanical procedures [1]. However, anatomical complexities, particularly the high prevalence of isthmuses in the mesial roots of mandibular molars, represent a major challenge to complete root canal filling [2]. Isthmuses may present in different configurations along the root thirds, with a reported prevalence ranging from 57% to 83% [3, 4] making these teeth among the most anatomically demanding in endodontic practice. Because gutta-percha does not adhere to dentine and is unable to conform fully to the intricate internal anatomy of the root canal system, the sealer plays a crucial role in filling irregularities and ensuring an effective seal [5, 6].

Various obturation techniques have been proposed to improve sealer distribution within such complex anatomical spaces, yet important limitations remain. Although the single-cone technique is compatible with contemporary shaping systems, it is often associated with thicker sealer layers, limited dentinal tubule penetration, and reduced interfacial strength [7]. To overcome these limitations, ultrasonic activation has been suggested as an adjunct approach, as it may enhance sealer flow through acoustic streaming, reduce void formation, and facilitate penetration into irregular areas such as isthmuses and lateral canals [8, 9].

In addition to the obturation technique, the physicochemical properties of the sealer are critical determinants of obturation quality. Resin-based sealers typically exhibit adequate flow and deeper intratubular penetration, relying primarily on micromechanical interlocking with dentine [5, 6]. In contrast, bioceramic sealers present distinct characteristics, including hydrophilicity and the potential for chemical interaction with dentine, which may improve interfacial adaptation [10]. These material-dependent differences suggest that the performance of obturation techniques, particularly in anatomically complex regions such as isthmuses, may be strongly influenced by the type of sealer used.

Despite increasing interest in optimizing obturation protocols, few studies have simultaneously investigated the combined effects of obturation technique, ultrasonic activation, and sealer type on the filling quality of anatomically complex regions or on intratubular sealer penetration. A clearer understanding of the interaction among these variables is essential to guide clinicians toward more predictable and effective obturation strategies. Therefore, the aim of this study was to evaluate the influence of three obturation techniques (single cone, hybrid Tagger, and continuous wave of condensation) performed with or without ultrasonic activation and combined with either resin-based or bioceramic sealers, on the filling quality of the isthmus region and on intratubular sealer penetration in the mesial roots of mandibular molars. The null hypotheses tested were that: (i) the obturation technique would not influence isthmus filling quality; (ii) ultrasonic activation would not affect isthmus filling quality or intratubular sealer penetration; and (iii) the sealer type would not influence isthmus filling quality or intratubular penetration.

MATERIAL AND METHODS

This study was approved by the Research Committee in Dentistry of the School of Dentistry, Federal University of Rio Grande do Sul (COMPESQ-ODO-UFRGS), as well as by the Institutional Research Ethics Committee (CEP), under protocol no. 39376020.0.0000.5347.

Sample size calculation and selection

The sample size calculation was performed considering a 95% confidence level and 80% statistical power, based on parameters reported in previous studies [11, 12], resulting in a required sample size of 10 per group ($n = 80$).

A total of 80 teeth were scanned using micro-computed tomography (micro-CT) (SkyScan 1275; Bruker-microCT, Kontich, Belgium). Image acquisition was performed at 70 kV and 114 μ A, with a spatial resolution of 14.6 μ m, using a 1.0-mm aluminum filter, 250-ms exposure time, 0.5° rotation step, frame averaging of 5, and 360° rotation. Image reconstruction was performed with NRecon v.1.7.4.6 (Bruker-microCT) applying identical settings for ring artifact correction (value 5), beam hardening correction (40%) and contrast adjustment (0.0035–0.038). Morphological analyses were carried out using CTAAn v.1.23.0.2 (Bruker-microCT) to determine canal volume and surface area.

Root canal procedures

After coronal access, the working length was established by introducing a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal until the tip was visible at the apical foramen; the final working length was set 1 mm short of this measurement. Root canal preparation was performed using the Sequence rotary system: 15/.04, 20/.06, 25/.06, 35/.04, and 40/.04 (MK Life, Porto Alegre, Brazil), operated with a 16:1 reduction electric motor (E-Connect Pro; MK Life) at 300 rpm and 2 Ncm torque. After the use of each instrument, the canals were irrigated with 2 mL of 2.5% sodium hypochlorite (NaOCl), totaling 10 mL per canal.

Following instrumentation, the canals were irrigated with 5 mL of saline solution to neutralize residual NaOCl, followed by 5 mL of 17% EDTA. The chelating solution was ultrasonically activated for 20 seconds using an ultrasonic device (UltraMint; MK Life) in Endo mode at power level 1, with a smooth ultrasonic tip (E1 Irrisonic; Helse, São Paulo, Brazil) measuring 0.2 mm in diameter and .01 taper. Activation was performed with buccolingual movements directed toward the isthmus. Subsequently, the canals were irrigated with 5 mL of 2.5% NaOCl and ultrasonically activated again for 20 seconds following the same protocol. A final rinse was performed with 5 mL of distilled water.

The canals were dried with #40/.04 paper points (MK Life). Master gutta-percha cones #40/.04 (MK Life) were fitted to the working length to confirm adaptation to the apical stop and verified radiographically.

The 80 mandibular molars were randomly allocated into eight experimental groups ($n = 10$), according to the type of sealer, the use or absence of ultrasonic activation, and the obturation technique, as follows: AH Plus with single-cone technique (AHP/SC); AH Plus with ultrasonic activation and single-cone technique (AHP/UASC); AH Plus with hybrid Tagger technique (AHP/THT); AH Plus with continuous wave technique (AHP/CW); Bio-C Sealer with single-cone technique (BC/SC); Bio-C Sealer with ultrasonic activation and single-cone technique (BC/UASC); Bio-C Sealer with hybrid Tagger technique (BC/THT); and Bio-C Sealer with continuous wave technique (BC/CW).

Rhodamine B and Fluo-3 dyes were incorporated into AH Plus and Bio-C Sealer, respectively, to allow visualization under laser scanning confocal microscopy. Both sealers were delivered into the canals using an insulin syringe equipped with capillary-tip needles positioned 2 mm short of the working length.

In the AHP/SC and BC/SC groups, a size 40.04 gutta-percha cone was positioned at the working length and cutted with a heated condenser.

For the AHP/UASC and BC/UASC groups, ultrasonic activation was performed for 20 seconds in each canal, with the insert positioned 2 mm short of the working length and directed toward the isthmus [8]. Next, a size 40.04 gutta-percha cone was positioned at the working length and cutted with a heated condenser

In the AHP/THT and BC/THT groups, after placement a 40.04 gutta-percha cone, a cold lateral compaction was performed using a #25 digital spacer, followed by the insertion of three F accessory gutta-percha cones coated with sealer. Thermoplasticization was then performed using a #60 McSpadden compactor (Dentsply Maillefer) mounted on a contra-angle handpiece (Dabi Atlante; Ribeirão Preto, SP, Brazil) and operated in clockwise rotation toward the apical region. The instrument was activated with short pumping movements of approximately 1 mm amplitude while maintaining contact with the gutta-percha at the canal entrance [7].

In the AHP/CW and BC/CW groups, a 40.04 gutta-percha cone was positioned at the working length and cutted in the apical third using the Fast-pack thermocompactor (MK Life). The middle and cervical thirds were subsequently filled using the Fast-fill thermo-injector (MK Life).

Micro-CT analysis

After completion of the obturation procedures, the roots were stored at 37°C and 100% relative humidity for 7 days to allow complete sealer setting. Subsequently, the specimens were scanned using micro-computed tomography (micro-CT) and reconstructed with the same acquisition parameters described previously. Pre- and post-obturation micro-CT datasets were co-registered using an affine registration algorithm implemented in 3D Slicer software (version 4.3.1; <http://www.slicer.org>).

To assess the filling quality of the root canal system and the isthmus region, the volumes of filling material (mm³) and voids (mm³) were quantified using the 3D Object Counter plugin in ImageJ software [13]. Image binarization was performed to differentiate the filling material (gutta-percha and sealer) from dentine and void spaces within the root filling. The analysis was divided according to root thirds: cervical, middle, and apical. Three-dimensional models were subsequently generated using CTVol software (version 2.2.1; Bruker microCT, Kontich, Belgium).

Laser scanning confocal microscopy analysis

For laser scanning confocal microscopy (LSCM) analysis, the specimens were secured to the metallic platform of a precision cutting machine (LabCut 1010; Extec Corp., Enfield, CT, USA) and transversely sectioned using a diamond disc under continuous water cooling. Three slices were obtained from each root, one from each root third (cervical, middle, and apical), with a thickness of 2.0 ± 0.1 mm. The sections were sequentially polished with silicon carbide sandpapers of increasing grit sizes up to 1200, followed by final polishing with felt discs and a specific abrasive paste (Arotec, Cotia, SP, Brazil). After polishing, the specimens were thoroughly rinsed with distilled water to remove residual debris.

The sections corresponding to the cervical, middle, and apical thirds were examined using a LSCM (FluoView FV1000; Olympus Corporation, Tokyo, Japan) at $\times 4$ magnification. Image acquisition parameters were standardized at a scanning depth of 70 μ m and a resolution of 800×800 pixels. The obtained image stacks were analyzed using image-processing software (Adobe Photoshop; Adobe Systems; San Jose, CA, USA) to quantify intratubular sealer penetration. Initially, the total number of pixels per image was recorded. The root canal lumen was then delineated using the “lasso” tool, and the corresponding pixel count was subtracted from the total to determine the penetrable dentin area. Subsequently, the area infiltrated by the sealer, identified by red fluorescence (Rhodamine B) or green fluorescence (Fluo-3), was selected using the “magic wand” tool. Intratubular sealer penetration was calculated as a percentage, considering the penetrable dentin area as 100%, in accordance with the methodology of previous study [14].

Statistical analysis

The collected data were tabulated in a spreadsheet (Microsoft Excel 2007; Microsoft Corporation, Redmond, WA, USA) and statistically analyzed using BioEstat software (version 5.3; Instituto Mamirauá de Desenvolvimento Sustentável, Belém, Brazil). Normality was assessed using the Shapiro-Wilk test. Because void space data did not follow a normal distribution, comparisons were performed using the Kruskal-Wallis test followed by Dunn's post hoc test. In contrast, intratubular sealer penetration data showed normal distribution and were analyzed using one-way analysis of variance (ANOVA), with Tukey's post hoc test for multiple comparisons. The significance level was set at $\alpha = 0.05$ for all analyses.

RESULTS

Micro-CT analysis

Micro-CT analysis revealed no significant differences in the initial root canal system volumes among the specimens included in the study ($P > 0.05$), confirming adequate randomization prior to the experimental procedures. No significant differences were observed in the overall void volume among the experimental groups, regardless of the obturation technique or the type of endodontic sealer used ($P > 0.05$) (Table 1). Figure 1 presents a three-dimensional reconstruction of a mesial root, highlighting the presence of voids after root canal obturation using each filling technique.

When the root thirds were analyzed separately, no significant differences were detected in the cervical or apical thirds among the groups, irrespective of obturation technique or sealer ($P > 0.05$). In the middle third, however, both BC/UASC and BC/THT groups exhibited significantly lower volumes of voids compared with the other groups ($P < 0.05$). No significant differences were observed among the remaining groups in the middle third ($P > 0.05$).

LSCM analysis

AH Plus sealer showed significantly greater intratubular penetration than the Bio-C Sealer, regardless of the obturation technique employed ($P < 0.05$) (Table 2). Ultrasonic activation of both AH Plus and Bio-C Sealer resulted in significantly greater intratubular penetration compared with the other obturation protocols ($P < 0.05$). No significant differences were observed among the single-cone, hybrid Tagger, and continuous wave techniques when ultrasonic activation was not applied ($P > 0.05$).

When the AH Plus sealer was ultrasonically activated and the canal was obturated using the single-cone technique (AHP/UASC), intratubular penetration was significantly greater than that observed with the other techniques ($P < 0.05$). In the apical third, the highest intratubular penetration values were observed in the AHP/UASC and AHP/THT groups. The single-cone technique without ultrasonic activation resulted in the lowest intratubular penetration values ($P < 0.05$). Figures 2 and 3 present fluorescence indicating the presence of sealer within the root canal lumen, isthmus region, and dentinal tubules.

Table 1
Volume of voids (mm³) after micro-CT analysis.

Root third	AHP/SC	AHP/UASC	AHP/THT	AHP/CW	BC/SC	BC/UASC	BC/THT	BC/CW
Cervical	0.69 (0.36– 1.29) ^A	0.67 (0.31– 1.04) ^A	0.82 (0.41– 1.27) ^A	0.86 (0.55– 1.09) ^A	0.25 (0.13– 0.54) ^A	0.11 (0.08– 0.43) ^A	0.16 (0.08– 0.44) ^A	0.39 (0.23– 1.04) ^A
Middle	0.60 (0.23– 0.77) ^B	0.49 (0.31– 1.22) ^B	0.51 (0.43– 0.80) ^B	0.55 (0.40– 0.83) ^B	0.14 (0.05– 0.33) ^B	0.04 (0.03– 0.13) ^A	0.04 (0.02– 0.12) ^A	0.38 (0.18– 0.57) ^B
Apical	0.09 (0.03– 0.23) ^A	0.07 (0.05– 0.24) ^A	0.05 (0.03– 0.15) ^A	0.16 (0.03– 0.28) ^A	0.07 (0.05– 0.11) ^A	0.01 (0.00– 0.12) ^A	0.06 (0.01– 0.11) ^A	0.03 (0.01– 0.08) ^A
Total	1.55 (0.63– 2.08) ^A	1.35 (0.90– 2.41) ^A	1.49 (0.73– 1.85) ^A	1.55 (1.04– 2.32) ^A	0.64 (0.34– 0.77) ^A	0.19 (0.11– 1.07) ^A	0.40 (0.15– 0.79) ^A	1.26 (0.61– 1.66) ^A

Uppercase letters refer to comparisons between thirds in rows;

Lowercase letters refer to comparisons between thirds in columns.

Table 2
Percentage of intratubular penetration (%) after confocal laser scanning microscopy.

Root third	AHP/SC	AHP/UASC	AHP/THT	AHP/CW	BC/SC	BC/UASC	BC/THT	BC/CW
Cervical	16.23 ± 1.12 ^{Ba}	20.26 ± 6.51 ^{Aa}	12.02 ± 3.67 ^{Ba}	13.98 ± 1.77 ^{Ba}	3.07 ± 3.20 ^{Da}	8.01 ± 2.13 ^{Ca}	4.25 ± 1.49 ^{Da}	1.89 ± 0.57 ^{Da}
Middle	12.79 ± 2.78 ^{Bab}	21.14 ± 7.61 ^{Aa}	14.79 ± 4.70 ^{Ba}	10.80 ± 7.32 ^{Ba}	4.74 ± 4.76 ^{Da}	8.77 ± 2.52 ^{Ca}	3.36 ± 0.89 ^{Da}	1.57 ± 1.02 ^{Da}
Apical	9.40 ± 3.01 ^{Cb}	17.99 ± 4.96 ^{Aa}	16.97 ± 5.49 ^{Aa}	13.93 ± 2.60 ^{Ba}	2.78 ± 3.01 ^{Da}	6.69 ± 4.83 ^{Ca}	3.30 ± 1.70 ^{Da}	2.48 ± 0.93 ^{Da}
Mean	12.81 ± 1.32 ^B	20.14 ± 3.97 ^A	14.59 ± 1.28 ^B	12.77 ± 2.19 ^B	3.54 ± 1.88 ^D	7.83 ± 2.23 ^C	3.64 ± 0.97 ^D	2.05 ± 0.50 ^D

Uppercase letters refer to comparisons between thirds in rows;

Lowercase letters refer to comparisons between thirds in columns.

DISCUSSION

Three-dimensional sealing of the root canal system is essential to prevent reinfection and limit the spread of microorganisms and their byproducts to the periapical tissues [1]. However, residual pathogens are often located in anatomically complex regions such as lateral canals, isthmuses, and dentinal tubules, which are not fully reached by mechanical instrumentation [2, 15]. Consequently, achieving adequate obturation of these areas remains a significant challenge, particularly because voids within the filling mass may favor microbial persistence and the maintenance of apical inflammation [1]. For this reason, the present study focused on mesial roots of mandibular molars with isthmuses connecting the mesiobuccal and mesiolingual canals. Micro-computed tomography (micro-CT) was used to identify this anatomical feature and to standardize the volumetric extent of the canals and isthmuses among specimens. The absence of differences in the initial canal and isthmus volumes confirmed proper sample randomization and strengthened the internal validity of the experimental design.

Overall, no significant differences were observed in the total volume voids among the experimental groups, regardless of obturation technique or sealer type. Although the single-cone technique is conceptually designed to promote intimate adaptation between the master cone and canal walls, previous studies have demonstrated discrepancies between instrument and cone taper, even when produced by the same manufacturer [16]. In addition, the anatomical complexity of the root canal system limits the effectiveness of shaping procedures, resulting in a considerable percentage of untouched canal walls, up to 50% in round canals and up to 80% in oval or flattened canals [15]. As a consequence, the single-cone technique frequently leads to thicker sealer layers [7], which may compromise interfacial strength and increase the risk of void formation compared with thermoplastic obturation techniques [7, 17, 18].

In the present study, the comparable performance of the single-cone technique relative to thermoplastic techniques may be partially explained by the relatively circular cross-sectional anatomy of the mesiobuccal and mesiolingual canals of mandibular molars, which contrasts with the more flattened morphology commonly observed in distal canals, premolars, and canines. This finding reinforces the concept that the suitability of obturation techniques is highly dependent on canal morphology, and that the single-cone technique is less appropriate for oval or flattened canals due to the increased likelihood of thick sealer layers and unfilled spaces.

None of the obturation techniques evaluated resulted in void-free fillings when assessed by micro-CT. However, when a hydraulic sealer was combined with ultrasonic activation or with the Tagger hybrid technique, fewer voids were observed in the middle third. This finding may be related to the favorable flow and adaptation characteristics of bioceramic sealers, which have been shown to exhibit superior adaptation compared with epoxy resin-based sealers [19, 20].

Importantly, the nature of the material occupying the isthmus differed among techniques. While the single-cone and ultrasonically activated single-cone techniques primarily filled the isthmus with sealer, the Tagger hybrid and continuous-wave techniques tended to fill this region predominantly with gutta-percha. Although volumetric analysis did not reveal significant differences in voids, qualitative differences in filling behavior should be considered. In sealer-dominated fillings, the material is in direct contact with dentinal walls,

potentially enhancing adaptation. In contrast, gutta-percha, an inert material lacking adhesion, may be less capable of penetrating and adapting to complex anatomical regions such as isthmuses.

The limited ability of obturation materials to penetrate dentinal tubules was further demonstrated by LSCM. Ultrasonic activation significantly enhanced intratubular sealer penetration regardless of the sealer used. It should be emphasized that confocal microscopy is not suitable for detecting voids or gaps, as gutta-percha does not incorporate fluorescent dyes, making voids indistinguishable from gutta-percha-filled areas [14, 11, 21]. Therefore, LSCM findings in this study were restricted to the evaluation of intratubular penetration.

Passive ultrasonic activation was originally proposed to improve canal cleanliness by inducing acoustic streaming within irrigants [22]. More recently, ultrasonic activation of sealers has been shown to improve marginal adaptation, bond strength, and dentinal tubule penetration [6, 8, 23, 24]. In the present study, ultrasonic activation enhanced intratubular penetration of both epoxy resin-based and hydraulic sealers compared with the single-cone technique alone, corroborating previous reports of limited penetrability of bioceramic sealers under static conditions [25].

Thermoplastic obturation techniques, such as the Tagger hybrid and continuous-wave techniques, aim to reduce sealer thickness and improve material flow by heating gutta-percha. However, consistent with previous studies [26, 27], these techniques did not result in significantly lower void volumes or greater intratubular penetration compared with the single-cone technique. These findings suggest that no obturation technique is capable of completely filling all regions of the root canal system, and that sealer penetration into dentinal tubules may occur independently of the obturation technique itself.

Contrary to previous reports indicating greater sealer penetration in cervical thirds than in apical thirds [28, 29, 30], no significant differences among root thirds were observed in this study. This may be attributed to the ability of the tested techniques to force sealers toward dentinal tubules either through ultrasonic activation or through hydraulic and mechanical compression.

Micro-CT analysis was performed using voxel sizes suitable for detecting voids within the resolution limits established for radiopaque sealers, in accordance with ISO 6876:2012 and previous methodological standards [31, 32]. Alternative methods, such as stereomicroscopic analysis, have been used in previous studies [6], although no technique has yet demonstrated the ability to completely fill the root canal system without defects. Further research is needed to clarify the clinical relevance of different materials and techniques for filling anatomically complex regions such as isthmuses and lateral canals.

Gutta-percha, despite its dimensional stability, does not adhere to dentine and may be displaced under pressure, potentially creating pathways for leakage [33]. In contrast, epoxy resin-based sealers exhibit favorable physicochemical properties, including adequate flow, low solubility, minimal dimensional change, and adhesion to dentine [33, 34]. These characteristics may explain the superior intratubular penetration observed for AH Plus, particularly when combined with ultrasonic activation. The hydraulic sealer evaluated in this study showed comparable void volumes but lower dentinal penetration, which may be related to its higher solubility, wash-out susceptibility, and sensitivity to moisture conditions during setting.

It is well established that current cleaning, disinfection, and obturation protocols are unable to completely disinfect the entire root canal system, allowing bacteria to persist within isthmuses and dentinal tubules [15]. Intratubular sealer penetration plays a critical role in entombing residual microorganisms and limiting their metabolic activity [35, 36, 37]. Epoxy resin-based sealers, such as AH Plus, exhibit antimicrobial activity associated with the release of compounds during polymerization [38], an effect that may be enhanced by ultrasonic activation, particularly against *Enterococcus faecalis* [8].

Finally, although both epoxy resin-based and hydraulic sealers have demonstrated comparable clinical outcomes in terms of treatment success, extrusion rates, and postoperative pain [39], their distinct physicochemical behaviors may influence obturation quality in anatomically complex regions. The present findings suggest that ultrasonic activation, particularly when used with an epoxy resin-based sealer, may improve intratubular penetration and potentially enhance the sealing of challenging anatomical structures.

CONCLUSIONS

Under the conditions of this study, it can be concluded the tested obturation techniques were not capable of completely filling the root canal system and the isthmus region of mesial roots of mandibular molars. No significant differences were observed in obturation quality in terms of voids among the groups. The bioceramic sealer exhibited lower intratubular penetrability than the resin-based sealer. However, ultrasonic activation significantly increased intratubular penetration for both sealers.

Declarations

Conflict of Interest:

The authors declare that they have no conflict of interest.

Author Contribution

L.T.J. wrote the main manuscript text, performed the experimental tests, and conducted the confocal microscopy analysis. W.M.K., G.B.S., and R.A.R. contributed to manuscript writing and performed experimental tests. F.G.B. and E.J.N.L.S. organized the methodology, performed the micro-computed tomography tests, and conducted the software-based data analyses. M.V.R.S. and R.A.R. organized the methodology and supervised the study. All authors reviewed and approved the final manuscript.

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Figures

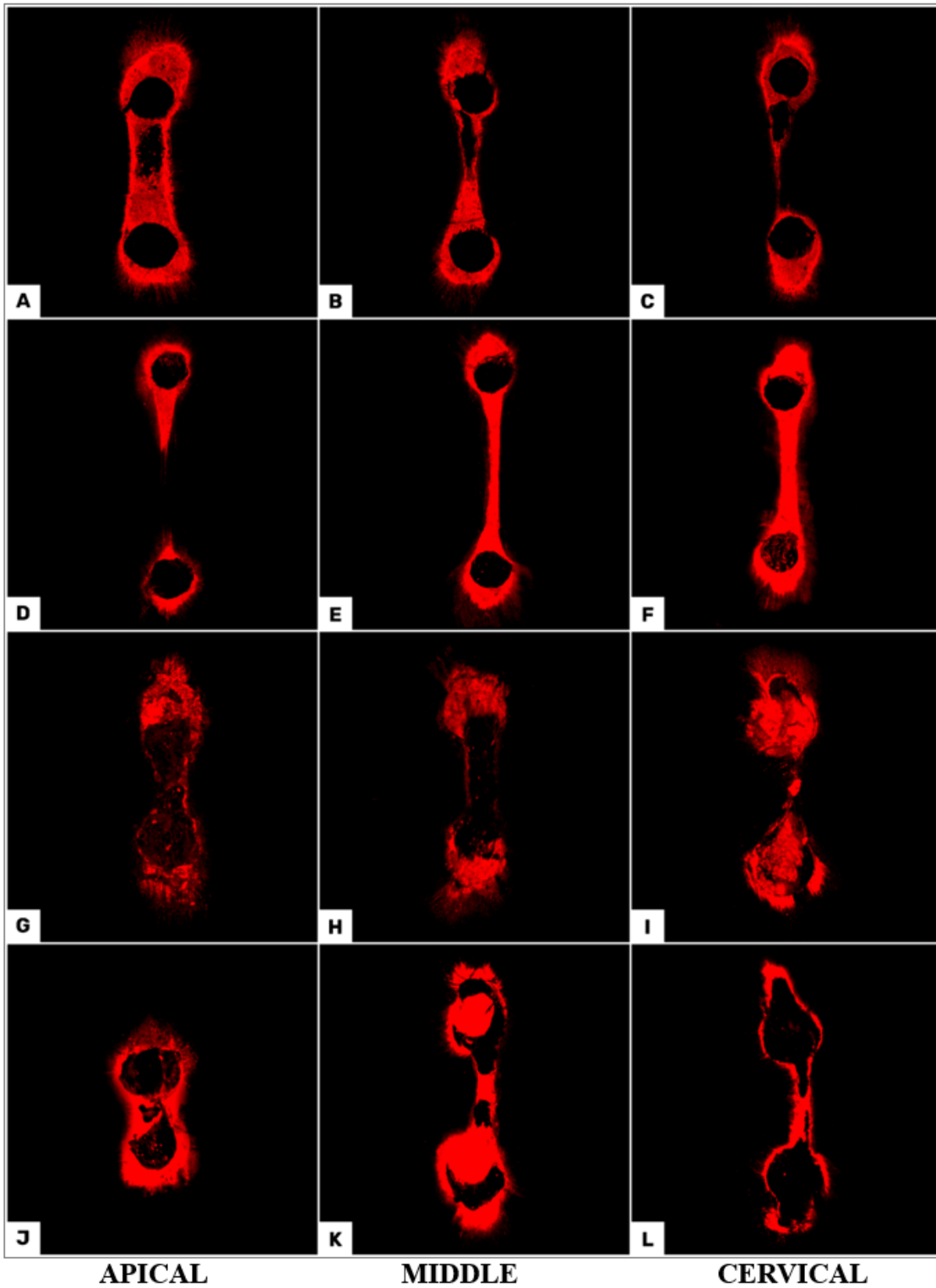


Figure 1

Representative confocal laser scanning microscopy images of root canal fillings performed with AH Plus sealer labeled with Rhodamine B.

(A–C) AHP/SC group; (D–F) AHP/UASC group; (G–I) AHP/THT group; (J–L) AHP/CW group. Red fluorescence indicates the presence of sealer within the root canal, isthmus region, and dentinal tubules.

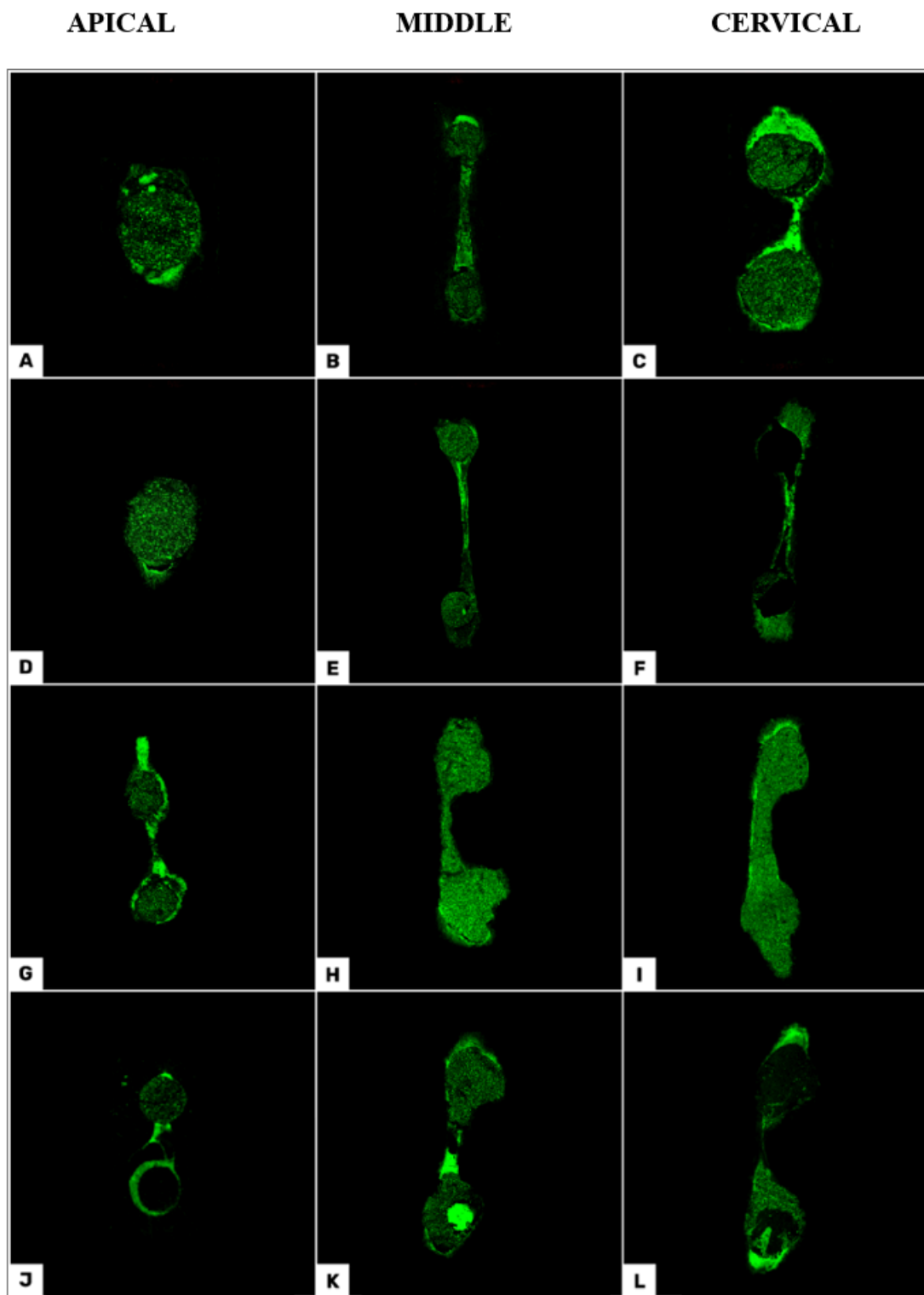


Figure 2

Representative confocal laser scanning microscopy images of root canal fillings performed with Bio-C Sealer labeled with Fluo-3.

(A–C) BC/SC group; (D–F) BC/UASC group; (G–I) BC/THT group; (J–L) BC/CW group. Green fluorescence indicates the distribution of the sealer within the root canal lumen, isthmus region, and dentinal tubules.

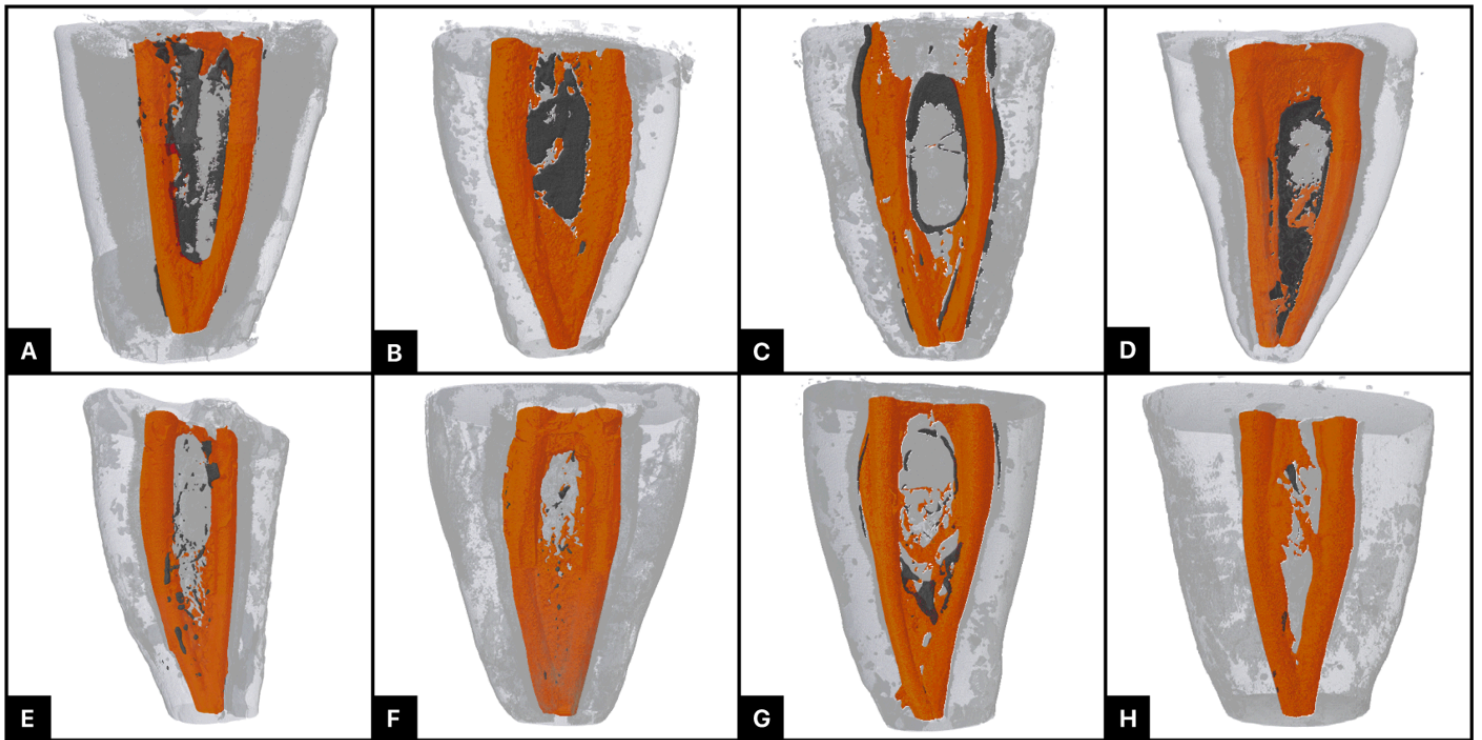


Figure 3

Representative micro-computed tomography (micro-CT) images of the specimens after root canal obturation.

(A) AHP/SC group; (B) AHP/UASC group; (C) AHP/THT group; (D) AHP/CW group; (E) BC/SC group; (F) BC/UASC group; (G) BC/THT group; (H) BC/CW group. The orange areas represent the obturation material (sealer and gutta-percha), whereas the dark gray areas indicate voids within the filling mass.