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Original Article

Effect of ultrasonic-assisted iRoot SP single-cone technique on root canal filling efficacy: an in vitro study

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Abstract: Objective: To evaluate and compare the sealing efficacy of three root canal filling techniques: AH plus with warm vertical compaction (WVC), iRoot SP with the single-cone technique, and ultrasonic-assisted iRoot SP single-cone technique. Methods: Ninety premolars extracted for orthodontic purposes at Dalian Stomatological Hospital between March 2022 and March 2024 were collected. After standardized root canal preparation, specimens were randomly assigned to three groups: AH plus group (WVC technique + AH plus), iRoot SP group (single-cone + iRoot SP), and US-iRoot SP group (ultrasonic-assisted single-cone + iRoot SP). Apical microleakage was assessed using dye leakage tests. Sealer penetration at 3 mm, 5 mm, and 8 mm from the apex was evaluated using optical microscopy and confocal laser scanning microscopy (CLSM). Stereomicroscopy and scanning electron microscopy (SEM) were used to analyze sealer distribution within the root canal. Results: The US-iRoot SP group exhibited significantly less apical microleakage than the AH plus and iRoot SP groups ($P<0.05$). CLSM analysis revealed significantly greater average and maximum sealer penetration depths in the US-iRoot SP group at all evaluated levels ($P<0.05$). Stereomicroscopy showed the smallest gap between sealer and gutta-percha in the US-iRoot SP group. SEM confirmed improved adaptation of the sealer to the dentin wall and deeper penetration into dentinal tubules. Conclusion: The ultrasonic-assisted iRoot SP single-cone technique significantly enhances root canal obturation quality compared to both the conventional iRoot SP single-cone technique and the AH plus with WVC technique.

Keywords: Ultrasonic activation, iRoot SP, AH plus, warm vertical compaction, root canal filling, bioceramic sealer

Introduction

Root canal therapy is the primary approach for managing pulp and periapical diseases, with its success reliant on thorough debridement, precise canal shaping, and effective three-dimensional obturation of the root canal system [1]. Root canal filling is a critical step that requires complete sealing of all anatomical structures while preserving canal morphology to ensure intimate adaptation of the filling material to the canal walls [2]. The reported success rate of root canal therapy ranges from 87% to 97%, with microleakage due to inadequate filling being a major cause of failure [3, 4]. Root canal sealers play a key role in obturation by filling the gaps between dentin walls and gutta-percha, blocking residual bacteria and their metabolites, and preventing periapical reinfection [5].

Traditionally, warm vertical compaction (WVC) has been the predominant obturation technique. This method heats gutta-percha to improve its adaptability to the canal walls and is commonly used in combination with AH plus, a resin-based sealer known for its strong sealing ability [6, 7]. However, WVC requires considerable technical expertise. Viapiana et al. reported that improper control of pressure or heating duration during WVC could result in thermal damage to periodontal tissues or structural compromise of the root canal walls, thereby affecting treatment outcomes [8].

With advancements in materials and techniques, the single-cone technique has gained popularity due to its procedural simplicity and the superior characteristics of bioceramic-based sealers [9]. In this approach, the sealer

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acts as the primary obturation material, while gutta-percha serves as a carrier. It involves the insertion of a single gutta-percha cone, substantially reducing treatment time and minimizing risks of thermal injury associated with heated gutta-percha methods [10]. iRoot SP, a novel bioceramic sealer primarily composed of calcium silicate, calcium phosphate, and zirconia, has shown excellent biocompatibility, antibacterial properties, and ease of handling [11]. Studies by Mak and Yan et al. have demonstrated its clinical advantages and increasing adoption in endodontic practice [12, 13].

Ultrasonic-assisted technology has emerged as a valuable adjunct in both irrigation and obturation phases of root canal treatment [14]. Paixão et al. demonstrated that ultrasonic irrigation is effective in cleaning lateral canals and isthmuses, significantly enhancing the removal of debris and bacteria [15]. Furthermore, high-frequency ultrasonic vibrations promote deeper penetration of sealers into complex canal anatomies, improving sealing quality and the long-term success of the obturation. Coşkun et al. reported that ultrasonic activation enhances sealer flow, reduces microleakage, improves bond strength, and enhances overall filling quality [16]. While numerous studies have confirmed the effectiveness of ultrasound in canal preparation and irrigation, its role in obturation, especially in conjunction with bioceramic sealers, remains underexplored.

This study aims to provide both theoretical insight and practical guidance for improving root canal obturation outcomes. This study aimed to evaluate the impact of different root canal obturation techniques, with and without ultrasonic activation, on the quality of root canal sealing. Specifically, the sealing performance of AH Plus combined with WVC, iRoot SP with the single-cone technique, and ultrasonic-assisted iRoot SP single-cone technique was compared. By assessing microleakage and sealer-dentin interface integrity, this research provides both theoretical and practical insight into optimizing root canal obturation protocols.

Materials and methods

Sample selection

This study was approved by the Medical Ethics Committee of Dalian Stomatological Hospital (Approval No. DLKQLL20240205). A total of 90 premolar specimens extracted during orthodontic procedures at Dalian Stomatological Hospital between March 2022 and March 2024 were collected.

Inclusion criteria: (1) Fully developed root apices; (2) Root canal curvature less than 10°; (3) Intact external root structure without signs of resorption, vertical root fracture, or longitudinal cracks; (4) Root length greater than 10 mm; (5) No history of endodontic treatment or pulp therapy; (6) Absence of caries, restorations, or structural defects in both crown and root regions.

Exclusion criteria: (1) Calcified or obstructed canals compromising patency; (2) Presence of previous root canal filling; (3) Carious lesions, resorptive defects, or significant root fractures; (4) C-shaped or other aberrant root canal morphologies.

Methods

Granulation tissue and calculus were removed from the specimen surfaces using a Gracey curette and surgical scalpel. After sterilization, each specimen was assigned a number using a randomization method and immersed in 0.9% saline. Specimens were stored at room temperature in a light-protected environment.

Root canal preparation

Following standard pulpectomy and pulp extirpation, the canal was initially negotiated using #10 and #15 K-files until the apical foramen was reached. The files were then retracted approximately 0.5 mm to establish the working length. Canal preparation was performed using an X-smart motor and ProTaper NiTi rotary instruments. The sequence included initial flaring of the middle and coronal thirds with S1 and SX files, followed by sequential use of S1, S2, F1, F2, and F3 files to the working length (**Figure 1A**). The rotational speed was set at 300 rpm, with torque values as follows: SX and S1 at 3.0 N·cm, S2 at 1.0 N·cm, F1 at 1.6 N·cm, and F2/F3 at 2.0 N·cm. Between each file change, canals were irrigated with 3% sodium hypochlorite (NaClO) using a side-vented needle placed approximately 2 mm short of the apex. Each irrigation cycle lasted 2 minutes with a total volume of 5 mL. Final irrigation

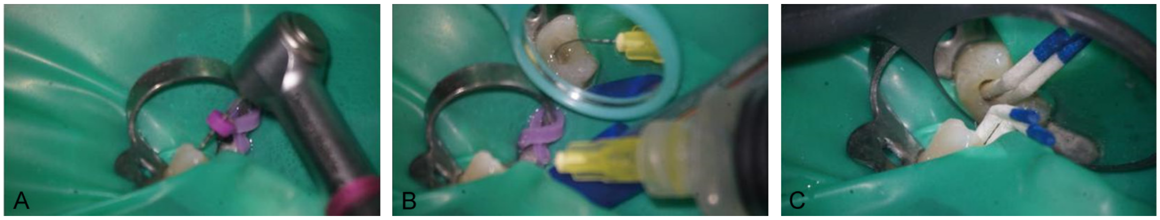


Figure 1. Root canal preparation. A: Establishing root canal patency; B: Root canal irrigation; C: Root canal drying.

involved 30 seconds of ultrasonic activation with 3% NaClO, followed by 30 seconds with EDTA solution, and a final 30-second rinse with sterile distilled water (**Figure 1B**). Before obturation, canals intended for iRoot SP were lightly dried using a single paper point, while other canals were thoroughly dried with multiple paper points (**Figure 1C**).

Experimental groups and root canal filling

Samples were randomly divided into three groups (n=30 each): the AH plus group (WVC technique + AH plus), iRoot SP group (single-cone + iRoot SP), and US-iRoot SP group (ultrasonic-assisted single-cone + iRoot SP).

(1) AH plus group + AH: Root canals were filled using the WVC technique with AH plus sealer (2105000678, Dentsply, USA). The AH plus system consists of a heat carrier (α unit) for cutting gutta-percha and an injection gun (β unit) for backfilling. The master gutta-percha cone was coated with AH plus and inserted into the root canal in 20 randomly selected samples. An additional 10 specimens were filled with AH plus sealer containing rhodamine B (0.1 mg/mL, R6626, Sigma, USA). The cone was gently retracted to ensure uniform sealer coating, then reinserted. The heat carrier was activated to 180°C and used to cut the cone approximately 4 mm from the apex, keeping heating under 10 seconds. Vertical compaction was performed, and backfill was completed with heated gutta-percha injected up to 1 mm from the canal orifice.

(2) iRoot SP group: A 0.04 taper master cone was fitted to 0.5 mm short of the apex, confirmed by resistance. iRoot SP sealer (Innovative Bioceramix, Vancouver, Canada) was injected into 20 randomly selected specimens. The remaining 10 were filled with sealer mixed with Rhodamine B (0.1 mg/mL). The master cone, coated with a small amount of iRoot SP, was inserted into the canal. The heat carrier was used to cut the cone 1 mm below the canal orifice, followed by vertical compaction.

(3) US-iRoot SP group: After selecting the appropriate master cone, iRoot SP sealer was injected into 20 samples, with 10 additional samples receiving sealer mixed with Rhodamine B (0.1 mg/mL). The ultrasonic device (Jet-Sonic Four Plus, Gnatus, Brazil) was set at 5,000 Hz/s and applied using an up-and-down motion for 15 seconds, repeated three times. The master cone was then gently placed to the working length, trimmed 1 mm below the canal orifice using the heat carrier, and compacted vertically to enhance sealer penetration and sealing.

Dye leakage test

The dye leakage test was performed following the protocol described by Antunes et al. [17]. Seven randomly selected specimens from each group, excluding those filled with Rhodamine B-labeled sealers, were used. All external tooth surfaces, except for the apical 3 mm, were coated with nail varnish. The roots were then vertically immersed in 2% methylene blue solution to a depth of 10 mm. After 7 days of dye exposure, the specimens were rinsed under running water to remove residual dye.

Each tooth was sectioned longitudinally along its long axis using a low-speed saw, ensuring the apical foramen was included. Dye penetration was evaluated under a stereomicroscope (SMZ745T, Nikon, Japan). Each sample was measured three times, and the mean value was recorded as the final dye penetration depth.

Scanning electron microscopy (SEM) evaluation

Three specimens from each group, without Rhodamine B labeling, were horizontally sec-

Table 1. Comparison of baseline data ($\bar{x}\pm s$)

	AH plus (n=30)	iRoot SP (n=30)	US-iRoot SP (n=30)	F/χ^2	P
Root canal length (mm)	13.41±0.73	13.26±0.46	13.29±0.55	0.621	0.540
Buccolingual diameter (mm)	7.65±0.41	7.68±0.39	7.61±0.32	0.190	0.827
Root canal curvature	7.57±1.23	7.04±0.97	7.33±1.05	1.783	0.174
Tooth type				0.104	0.950
Maxillary first premolar	21	21	20		
Mandibular first premolar	9	9	10		

tioned 3 mm from the apex using a precision low-speed diamond saw. The specimens were then dehydrated in a vacuum drying oven, mounted on copper stubs, and sputter-coated with a gold-palladium alloy. The interface between the sealer and dentin wall was examined using a scanning electron microscope (Hitachi, Tokyo, Japan).

Cross-sectional observation of samples

Ten randomly selected teeth per group, not labeled with Rhodamine B, were fixed in 10% neutral formalin for 7 days, followed by decalcification in a demineralizing solution for another 7 days. Horizontal sections were made at 3 mm, 5 mm, and 8 mm from the apex to obtain cross-sectional tissue samples. The bonding between the sealer and dentin at the 3 mm level was observed under a stereomicroscope.

After rinsing with tap water for 4 hours, samples underwent gradient dehydration and were cleared in xylene until transparent. They were then embedded and sectioned into 5 μm-thick slices. These sections were mounted on glass slides, covered with resin, and sealed with cover slips. Once the resin cured at room temperature, the samples were examined using an optical microscope (ECLIPSE E100, Nikon, Japan). The average and maximum sealer penetration depths were measured using ImageJ software.

Confocal laser scanning microscopy (CLSM) analysis

Samples filled with Rhodamine B-labeled sealers were stored at 37°C and 100% humidity for 7 days. Thereafter, 1 mm-thick sections were made at 3 mm, 5 mm, and 8 mm from the apex, perpendicular to the tooth's long axis. Sections were rinsed with running water and polished sequentially with 800-, 1000-, and 1200-grit silicon carbide paper, each for 10 seconds.

The polished slices were mounted on slides and examined using CLSM (FV1000, Olympus, Japan). Sealer penetration depth was quantified (average and maximum values) using ImageJ software.

Statistical analysis

All statistical analyses were performed using SPSS version 22.0. The Kolmogorov-Smirnov test was used to assess the normality of continuous data. Variables following normal distribution were expressed as mean ± standard deviation ($\bar{x}$ ± sd). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for multiple group comparisons. Categorical variables, such as tooth type, were expressed as counts and percentages [n (%)], and analyzed using the chi-square (χ^2) test. A *P*-value <0.05 was considered statistically significant. Graphs were generated using GraphPad Prism.

Results

Comparison of baseline data

There were no statistically significant differences among the three groups in terms of root canal length, buccolingual diameter, canal curvature, or tooth type distribution (all P>0.05) (**Table 1**).

Comparison of dye leakage test results

All groups exhibited varying degrees of apical dye leakage. The US-iRoot SP group showed the least leakage, significantly lower than that of the AH Plus and iRoot SP groups (*P*<0.05). No significant difference was observed be-

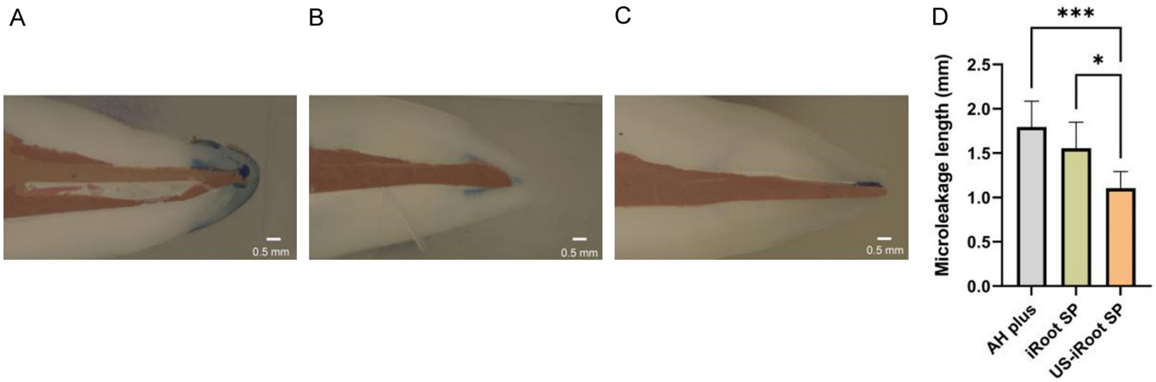


Figure 2. Results of the dye leakage test (20×). A: AH plus group; B: iRoot SP group; C: US-iRoot SP group; D: microleakage length. **P*<0.05; ****P*<0.001.

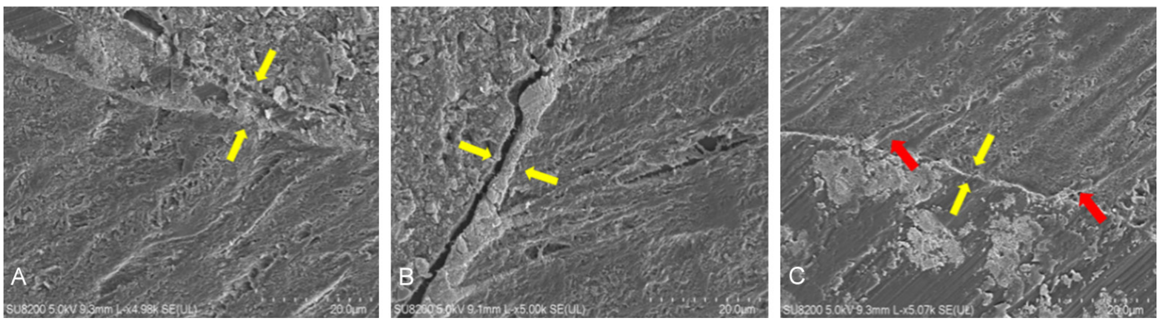


Figure 3. Representative SEM image of the microstructure at 3 mm from the root apex. A: AH plus group; B: iRoot SP group; C: US-iRoot SP group. SEM: Scanning electron microscopy. The yellow arrows denote the interface between the sealer and the dentin wall, whereas the red arrows highlight the penetration of the sealer into the dentinal tubules.

tween the AH plus and iRoot SP groups (*P*>0.05) (**Figure 2**).

SEM imaging and evaluation

SEM analysis at 3 mm from the apex revealed significant differences in sealing effectiveness among the groups. The AH Plus group exhibited poor adaptation, with visible gaps between the sealer and dentin wall. The US-iRoot SP group demonstrated the smallest gaps and the most intimate contact with the dentin, with deep penetration into dentinal tubules. The iRoot SP group showed intermediate sealing quality, between that of the AH Plus and US-iRoot SP groups (**Figure 3**).

Microstructural observation at 3 mm from root apex

At 3 mm from the apex, the US-iRoot SP group exhibited the greatest average and maximum penetration depths, significantly outperforming both the AH plus and iRoot SP groups (both *P*<0.05). No significant difference was found between the AH plus and iRoot SP groups (both *P*>0.05) (**Figure 4**). These findings suggest that ultrasonic activation significantly enhanced sealer penetration at this level.

Microstructural observations at 5 mm from root apex

At 5 mm from the apex, the US-iRoot SP group again demonstrated significantly greater average and maximum penetration depths compared to the other two groups (both P<0.05). No significant differences were found between the AH plus and iRoot SP groups (both *P*>0.05) (**Figure 5**), further supporting the positive effect of ultrasound on sealer penetration.

Microstructural observation at 8 mm from root apex

At 8 mm from the apex, the US-iRoot SP group maintained the highest average and maximum

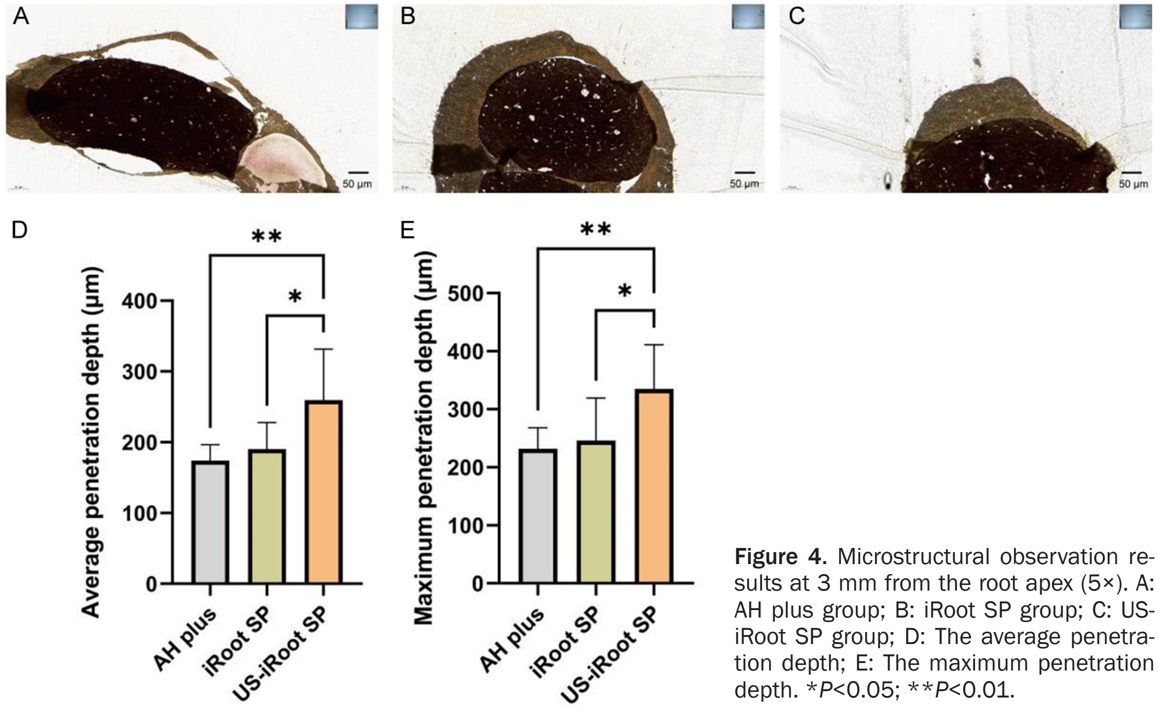


Figure 4. Microstructural observation results at 3 mm from the root apex (5×). A: AH plus group; B: iRoot SP group; C: US-iRoot SP group; D: The average penetration depth; E: The maximum penetration depth. *P<0.05; **P<0.01.

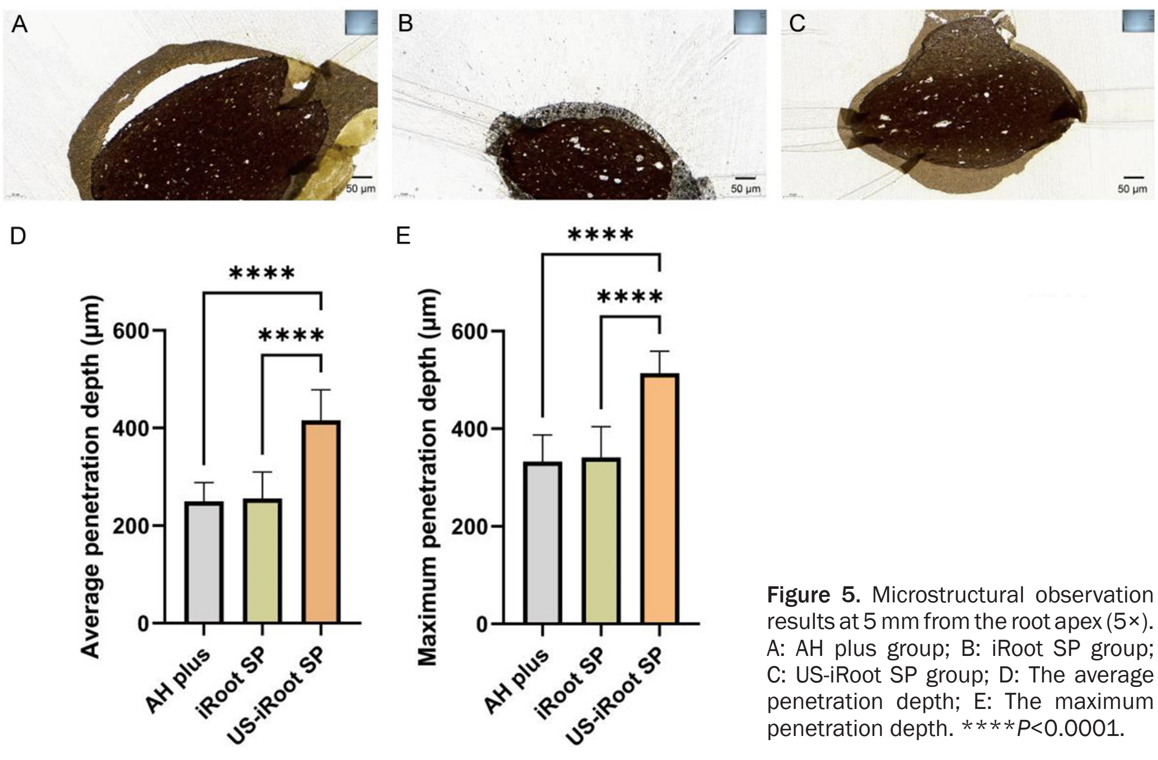


Figure 5. Microstructural observation results at 5 mm from the root apex (5×). A: AH plus group; B: iRoot SP group; C: US-iRoot SP group; D: The average penetration depth; E: The maximum penetration depth. ****P<0.0001.

penetration depths, significantly greater than those in the AH Plus and iRoot SP groups (both P<0.05). No significant differences were observed between the AH plus and iRoot SP groups (both P>0.05) (**Figure 6**). These results indicate that ultrasonic activation enhances sealer penetration even in the coronal third of the root.

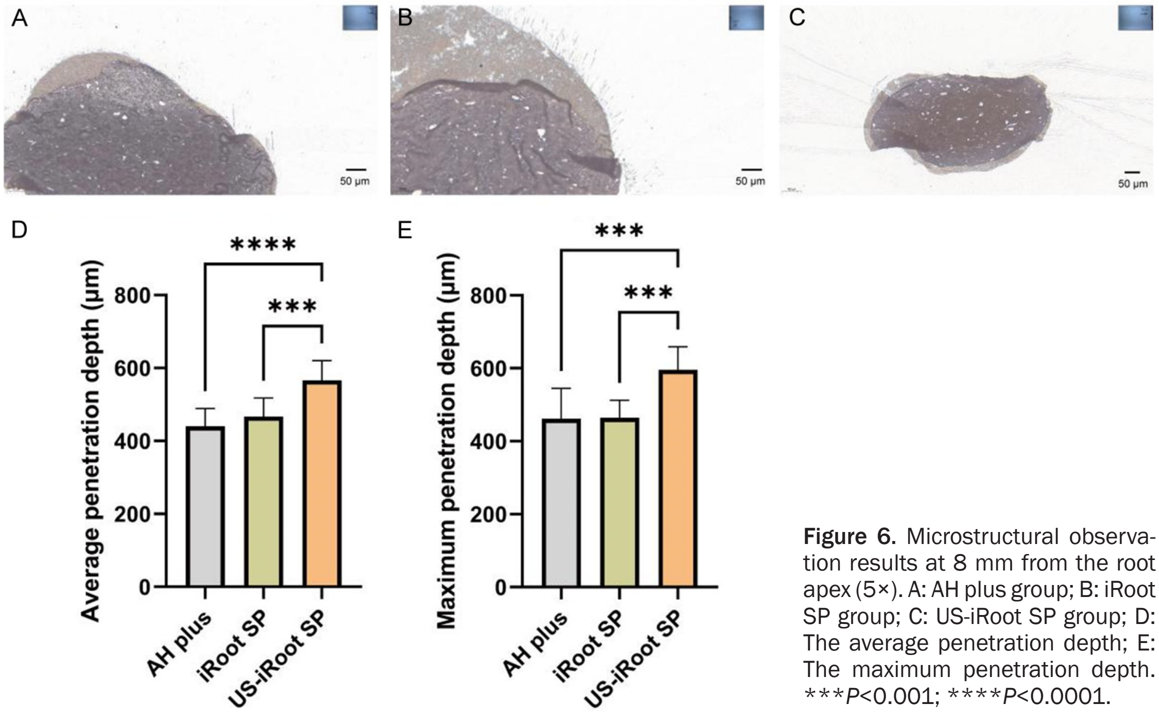


Figure 6. Microstructural observation results at 8 mm from the root apex (5×). A: AH plus group; B: iRoot SP group; C: US-iRoot SP group; D: The average penetration depth; E: The maximum penetration depth. ***P<0.001; ****P<0.0001.

Stereomicroscopic observations

Stereomicroscopic analysis revealed that the gap between gutta-percha and sealer was significantly larger in the AH Plus and iRoot SP groups compared to the US-iRoot SP group, indicating better adaptation in the latter (**Figure 7**).

Results of CLSM analysis

CLSM imaging of Rhodamine B-labeled sealers demonstrated sealer penetration at 3 mm, 5 mm, and 8 mm from the apex in all groups. However, significant intergroup differences were observed. The AH plus group exhibited limited penetration, mainly confined to the central canal region. In contrast, the iRoot SP and US-iRoot SP groups showed wider sealer distribution and greater peripheral diffusion. With increasing depth (from 8 mm to 3 mm), penetration decreased in the AH Plus and iRoot SP groups, while the US-iRoot SP group maintained consistent penetration throughout. These results suggest that ultrasonic activation enhances the sealer's ability to infiltrate complex root canal anatomies. Quantitative analysis of average and maximum penetration depths confirmed the following ranking: US-iRoot SP> iRoot SP>AH plus (**Figure 8**).

Discussion

The success of root canal treatment is closely related to the quality of root canal obturation [18]. Achieving a tight seal and durable bond between the sealer and the inner dentin wall is therefore essential [19]. Clinically, AH plus is widely regarded as the gold standard for evaluating root canal sealers, particularly when used in combination with thermoplastic gutta-percha techniques [7, 20]. With the emergence of bioceramic sealers and the development of adjunctive methods, ultrasonic-assisted techniques have garnered attention for their ability to significantly improve sealer penetration and sealing efficacy [9, 21]. This study compared AH plus and iRoot SP across different filling techniques, incorporating ultrasonic activation to systematically evaluate its impact on root canal filling performance. The findings offer a theoretical basis for improving root canal therapy outcomes and provide new insights for optimizing clinical filling strategies.

The dye leakage test, a widely used method due to its simplicity, cost-effectiveness, and reproducibility, was employed to evaluate apical microleakage [11]. The US-iRoot SP group exhibited the least dye penetration, significantly outperforming both the AH plus and

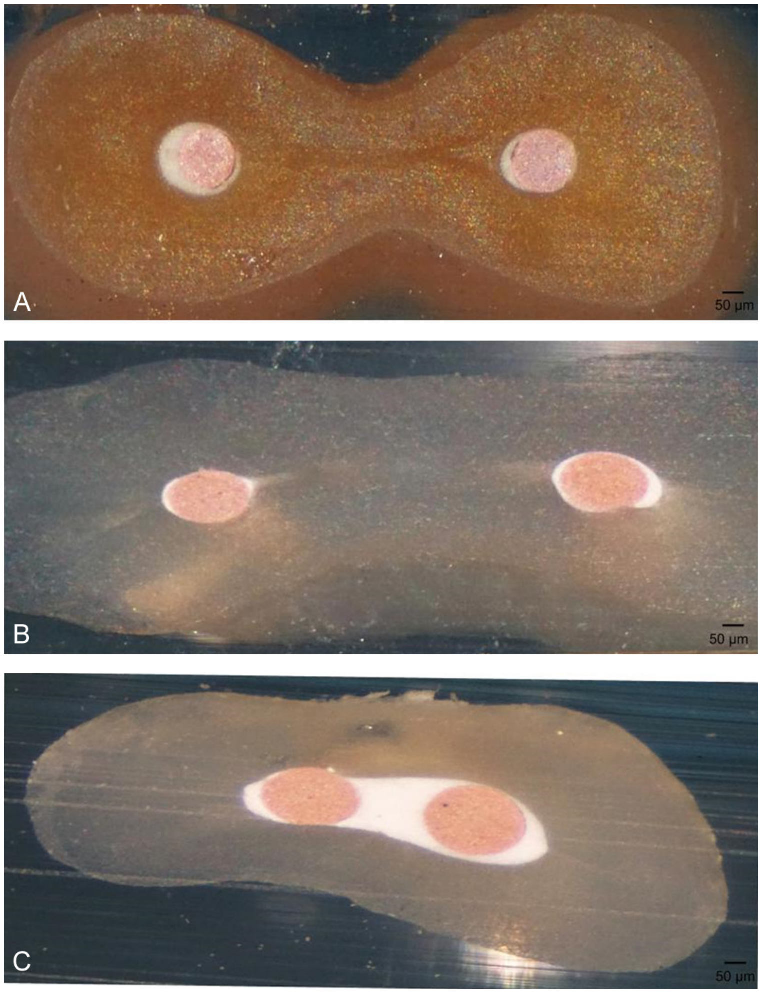


Figure 7. Representative stereomicroscopic images (20×). A: AH plus group; B: iRoot SP group; C: US-iRoot SP group.

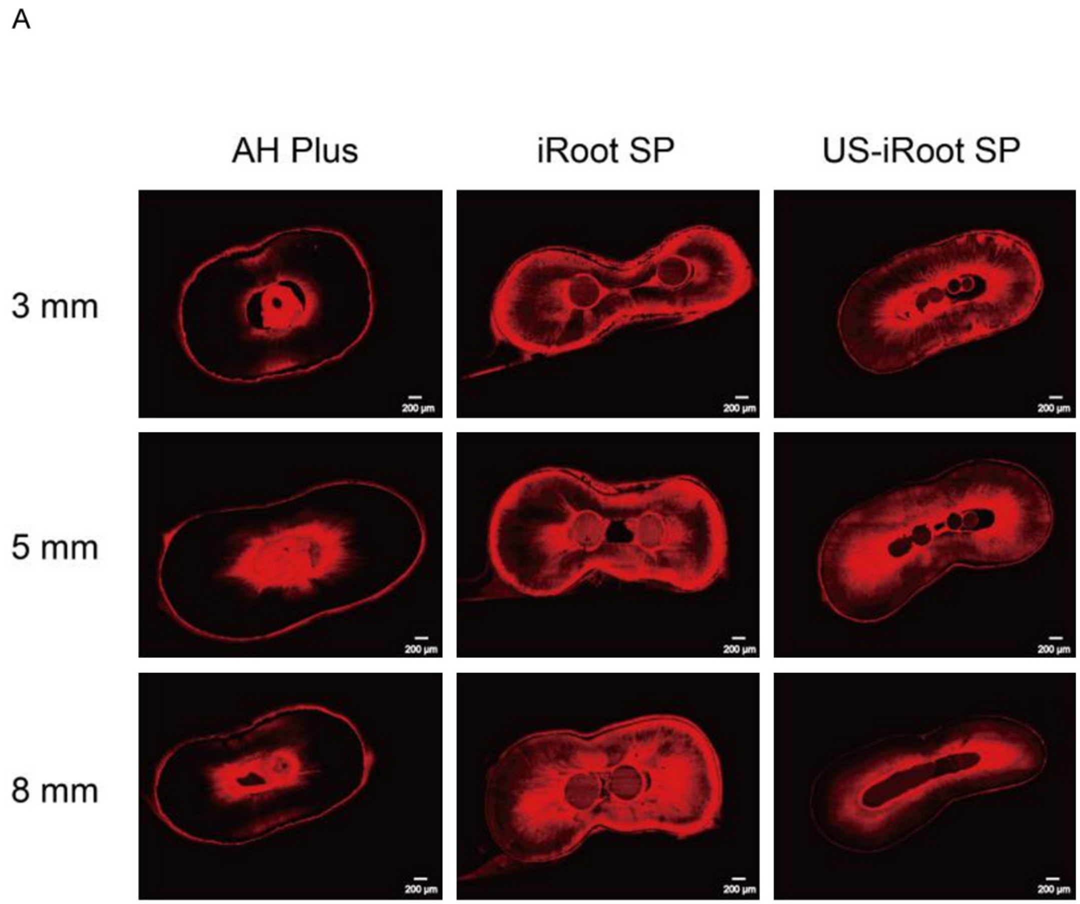
iRoot SP groups. No significant difference was observed between the latter two. These findings suggest that ultrasonic activation effectively reduces apical microleakage and improves the seal integrity. Consistent with these results, microscopic and CLSM analyses confirmed significantly deeper sealer penetration in the US-iRoot SP group, especially at apical sections, indicating enhanced sealing performance with ultrasonic assistance.

This improvement may be attributed to the continuous mechanical energy delivered by ultrasonic vibrations, which facilitates deeper sealer flow into dentinal tubules by overcoming intratubular resistance [22]. Yang et al. demonstrated that the acoustic streaming generated during ultrasonic activation enhances irrigant penetration into complex anatomical regions, improving cleaning efficacy [23]. Similarly, Paixão et al. showed that ultrasonic activation increased the pH and calcium ion release of calcium hydroxide pastes under simulated external resorption conditions, further supporting its role in promoting deeper penetration [15].

Amplitude, a key factor in ultrasonic transmission, is also linked to energy intensity [24]. Although the amplitude may diminish through intermediate materials (e.g., ultrasonic tips, tweezers, gutta-percha), it appears sufficient to displace air bubbles within the sealer without adversely affecting its properties. This could further enhance sealing. Pauletto et al. supported this hypothesis, demonstrating that ultrasonic activation reduces air voids and improves dentinal tubule infiltration, ultimately increasing sealing performance [25].

In this study, no statistically significant differences were observed between the AH plus and iRoot SP groups in terms of dye leakage or sealer penetration at different root levels. This may be attributed to the limited sample size, reducing the power to detect subtle differences. Moreover, potential procedural inconsistencies during in vitro testing may have led to minor gaps between the gutta-percha and iRoot SP sealer, affecting results [26]. Furthermore, the use of thermoplastic gutta-percha in ex vivo conditions may have partially compensated for anatomical irregularities and procedural limitations, thus masking potential differences between these groups [8].

Stereomicroscopic observations offered a macroscopic view of the sealer distribution and its interaction with gutta-percha [27]. Gaps between gutta-percha and sealer were more prominent in the AH plus and iRoot SP groups, while the US-iRoot SP group showed better adaptation. SEM imaging further validated



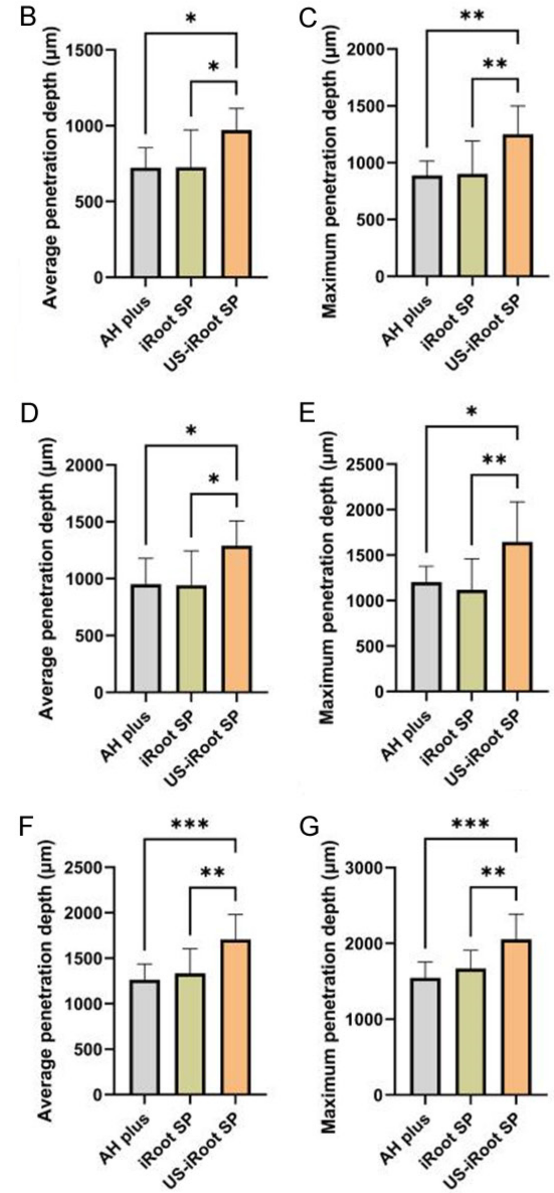


Figure 8. CLSM analysis of sealer penetration depth (5×). (A) Representative CLSM images showing sealer penetration (20×). (B, C) Average (B) and maximum (C) penetration depths at 3 mm from the apex. (D, E) Average (D) and maximum (E) penetration depths at 5 mm from the apex. (F, G) Average (F) and maximum (G) penetration depths at 8 mm from the apex. CLSM: Confocal laser scanning microscopy. *P<0.05, **P<0.01, **P<0.001.

these findings [28]. The iRoot SP group showed better adaptation than the AH Plus group, while the US-iRoot SP group exhibited the strongest bond to dentin and the deepest sealer penetration. These results suggest that iRoot SP offers improved interface compatibility compared to AH plus, and ultrasonic activation further amplifies this effect.

iRoot SP, a calcium silicate-based bioceramic sealer, contains nanoparticles that infiltrate dentinal tubules and form "tag-like" structures, establishing a robust mechanical and chemical bond with the dentin [29]. This enhances the resistance to displacement through micromechanical interlocking [30]. In addition, the residual moisture within dentinal tubules triggers the setting reaction, forming hydroxyapatite and facilitating chemical bonding, which contributes to enhanced structural stability and fracture resistance [31].

Ultrasonic activation further improved the flowability and adaptability of iRoot SP, likely due to cavitation and acoustic streaming effects. These phenomena promote better dispersion of filler particles, improve sealer-dentin interaction, and strengthen interfacial bonding [32]. As a result, the sealer adapted more closely to dentin walls and penetrated more deeply, forming dense resin tags that reinforced micromechanical interlocking, thereby improving both sealing and overall obturation quality [33]. Moreover, ultrasound reduced sealer viscosity and minimized void formation. Celikten et al., using micro-CT, reported significantly fewer voids and better marginal adaptation with the ultrasound-assisted bioceramic single-cone technique [34]. Jordani et al. further emphasized that ultrasonic activation improves sealer distribution in complex root canal anatomies, leading to enhanced sealing [35].

Nevertheless, the risk of overfilling associated with improved sealer flow should not be overlooked, especially in single-cone techniques. Careful placement of gutta-percha cones - slow insertion followed by slight retraction - is essential for ensuring even sealer distribution and avoiding extrusion [36].

In conclusion, ultrasonic activation significantly enhanced root canal filling quality and sealer penetration into dentinal tubules, offering a promising adjunctive method to optimize endodontic treatment. However, this study has several limitations. First, as an in vitro investigation using extracted teeth, it does not fully replicate the complexity of in vivo conditions. Therefore, clinical trials are needed to validate these findings. Second, the relatively small sample size may have limited the statistical power and generalizability of the results. Future studies with larger cohorts and standardized imaging protocols are warranted to provide more robust evidence for clinical practice.

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Disclosure of conflict of interest

None.

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