



RESEARCH ARTICLE

Comparative Evaluation of Apical Seal Integrity in Retrograde vs. Orthograde Filling Techniques during Endodontic Microsurgery: An *in vitro* SEM-Based Study

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Abstract

Background: Achieving a reliable apical seal is a critical determinant of success in endodontic microsurgery. Recent advances in bioceramic materials have prompted investigation into their sealing effectiveness when applied via orthograde or retrograde techniques. However, comparative data on their performance remains limited.

Aim: To evaluate and compare the apical seal integrity of orthograde and retrograde filling techniques using bioceramic materials through scanning electron microscopy (SEM).

Materials and Methods: An *in vitro* study was conducted on 52 extracted human maxillary central incisors, randomly assigned to four experimental groups (n = 13 per Group). Group 1 received retrograde filling with bioceramic putty; Group 2 received retrograde filling with bioceramic deep putty packing technique (sealer coat + putty). Groups 3 and 4 received orthograde fillings - Group 3 with a bioceramic putty, and Group 4 with a bioceramic deep putty packing technique (sealer coat + putty). Groups 1 and 2 were incubated after retrograde filling; Groups 3 and 4 were incubated before apical resection. All samples were incubated for 7 days at 37° C, then sectioned, and analyzed under SEM to assess number of internal gaps and ratio of marginal gaps in the sample. Statistical analysis was performed using Kruskal-Wallis tests ($\alpha = 0.05$).

Results: The results revealed no statistically significant differences between the orthograde and retrograde techniques in terms of the number of internal gaps or marginal gap ratio ($p = 0.126$ and $p = 0.228$, respectively), indicating that the orthograde approach achieved similar sealing performance to the retrograde method.

Conclusion: Orthograde application of bioceramic putty demonstrated comparable apical sealing to retrograde techniques, with no statistically significant differences observed between groups. These findings suggest that both orthograde and retrograde approaches using bioceramic materials can provide similar sealing performance in endodontic microsurgery.

Keywords

Apical seal, Bioceramic putty, Endodontic microsurgery, Orthograde filling, Retrograde filling, Scanning electron microscopy

Introduction

Endodontic microsurgery, a surgical procedure involving precise root-end resection and cavity preparation under magnification, is a critical approach for managing persistent periapical disease when conventional endodontic treatments fail. Achieving an effective apical seal remains essential, as inadequate sealing can lead to persistent microleakage, bacterial ingress, and subsequent treatment failure [1,2]. Modern advancements in surgical techniques and materials have significantly improved the predictability and success rates of endodontic microsurgery, with success rates exceeding 90% reported in recent studies [3,4].

Historically, retrograde filling techniques using materials such as amalgam, zinc oxide-eugenol

cement, glass-ionomer cements, and resin-based composites were routinely employed following apical resection. However, these materials presented limitations, including poor sealing ability, inadequate biocompatibility, and challenging handling characteristics [5,6]. The introduction of mineral trioxide aggregate (MTA) significantly enhanced sealing capability, biocompatibility, and moisture resistance, rapidly becoming the gold standard for retrograde fillings [7, 8]. Despite these advantages, MTA is associated with drawbacks such as prolonged setting times and difficult handling, prompting the development of newer bioceramic materials like Biodentine, calcium-enriched mixture (CEM), and premixed bioceramic putties, which exhibit superior handling properties, reduced setting time, and comparable sealing effectiveness [9-11].

Recent evidence has questioned the necessity of retrograde filling in all surgical cases, suggesting that well-executed orthograde obturation alone might achieve a comparable or superior apical seal under certain conditions [12,13]. Orthograde filling, performed by placing sealing materials from the coronal aspect through the canal, has traditionally been viewed as less effective when the apex is surgically resected. Nonetheless, some studies have demonstrated that orthograde obturation using bioceramic materials could maintain an adequate seal even after apical resection, potentially simplifying surgical procedures [5,14].

Comparative analyses between retrograde and orthograde techniques remain inconclusive, primarily due to varying methodologies, materials tested, and experimental conditions. A study evaluating microleakage using dye penetration found that retrograde placements generally provide better marginal adaptation than orthograde techniques [15]. Conversely, other investigations utilizing micro-computed tomography (micro-CT) and bacterial leakage models suggest comparable sealing outcomes between orthograde fillings extended beyond the apex and traditional retrograde fillings [9,16]. Furthermore, the method of application (orthograde vs. retrograde) significantly affects material adaptation, bond strength, and leakage resistance, with orthograde placements demonstrating superior mechanical retention [17].

The sealing efficacy of orthograde obturation is influenced significantly by the properties of the sealer used. Epoxy resin-based sealers, historically preferred due to their adhesive qualities, have recently faced competition from bioceramic sealers due to their bioactive potential, chemical bonding with dentin, and reduced shrinkage [18,19]. While some bacterial leakage studies show superior long-term performance of bioceramic sealers, systematic reviews indicate comparable sealing abilities between bioceramic and resin-based sealers, highlighting the necessity for direct comparative studies under standardized conditions [20,21].

Despite numerous studies, there remains a critical knowledge gap concerning the direct comparison of orthograde and retrograde filling techniques using the latest generation of bioceramic putty and sealers. Particularly, there is insufficient evidence on how orthograde obturation materials withstand the mechanical and sealing stresses of subsequent surgical resection. Previous studies have shown that apical resection might affect the sealing integrity of previously set orthograde plugs, raising concerns about potential microleakage and treatment failures [9,22]. Therefore, understanding the comparative sealing efficacy and the potential impact of resection on bioceramic orthograde fillings remains crucial.

Aim of the Research

The aim of this study was to compare the apical seal integrity of orthograde and retrograde filling techniques in endodontic microsurgery using SEM analysis, and to assess the influence of different material combinations on marginal gap formation demonstrated by two variables (number of marginal gaps, and gaps ratio).

Methods and Materials

Study design and sample selection

This *in vitro* experimental study was designed to compare apical seal integrity between orthograde and retrograde root canal filling techniques using scanning electron microscopy (SEM). Sample size estimation was performed using G*Power 3.1 software. Based on an alpha level of 0.05, power of 0.80, and an effect size derived from comparable SEM-based studies, the minimum required sample size was calculated to be 13 teeth per group to achieve adequate statistical power. To compensate for potential sample loss during instrumentation, obturation, or sectioning, the initial number per group was increased to 15 specimens.

A total of 60 extracted human maxillary central incisors with fully formed apices and single straight canals were obtained from Bforbones International (Ajax, Ontario, Canada), a certified provider of human dental specimens for research and education. All specimens were handled in accordance with legal and ethical standards for human tissue use. Teeth were stored in 0.1% thymol and screened to exclude those with fractures, resorption, calcifications, or previous endodontic treatment. Standardization of specimen morphology was achieved by adjusting all samples to a uniform root length of 18 mm, measured with a digital caliper to eliminate variability as a confounding factor.

Sample collection and allocation

The study initially included 60 extracted human maxillary central incisors. During canal preparation and obturation, 8 samples were excluded due to technical complications (e.g., root fracture, perforation, or obturation failure), leaving 52 teeth for SEM processing. Thus, 52 samples were included in the final analysis.

The 52 teeth were randomly divided into four experimental groups (n = 13 per group), Group 1 used retrograde filling (bioceramic putty only), Group 2 used retrograde filling (deep putty packing technique), Group 3 used orthograde filling (bioceramic putty only), and Group 4 used orthograde filling (deep putty packing technique). Figure 1 shows the sample flow chart and the allocation to different groups.

Sample preparation

All teeth underwent initial radiographic examination to confirm a single canal, complete root formation, and absence of caries, resorption, or previous endodontic treatment. Teeth exhibiting cracks, complex canal anatomy, or developmental anomalies were excluded. Part of the crowns were sectioned using a high-speed diamond disc under constant water cooling in order to achieve root lengths standardization to 18 mm based on the shortest sample, measured using a digital caliper (Mitutoyo, Japan).

Each group of four teeth was embedded into a gypsum-based mold mixed with soft wood shavings (2:1 ratio of gypsum to shavings) to simulate trabecular bone radiographically. The molds were stabilized with a metal nut at the base to allow mounting on a simulated clinical phantom head. The roots were isolated with a thin layer of petroleum jelly to facilitate later removal from the mold.

Access cavities were prepared using Endo Z burs (Mani, Japan) mounted on a high-speed NSK turbine (Japan) (Figure 2). Canal patency was established with

a #10 K-file (Mani, Japan), and working length was confirmed using #15 K-file and periapical radiography. Instrumentation was performed using AF Gold rotary files (Fanta, China) up to size 35/.04, and irrigated between each file with 5.25% sodium hypochlorite (Clorox, Saudi Arabia) using side-vented irrigation needles.

Upon completion of preparation, canals were irrigated with 5 mL of 5.25% NaOCl followed by 5 mL of 17% EDTA (META Biomed, Korea) activated for 3 minutes with an ultrasonic device (Ultra-X, Eighteeth, China), and rinsed with distilled water. Canals were dried using size 35/0.04 sterile paper points.

Specimens were randomly assigned into four experimental groups (N = 13 for group), each prepared for different obturation techniques involving bioceramic putty and sealers. Grouping was coded and samples labelled using color-coded markers.

Following obturation procedures - Groups 3 and 4 were incubated for 7 days at 37°C prior to apical resection, while Groups 1 and 2 were incubated after retrograde filling- (described in subsequent sections), specimens were removed from the gypsum blocks and stored in moist cotton in sealed plastic containers at 37°C in an incubator (Carbolite PIF-400, UK) for seven days to simulate clinical setting conditions and ensure complete setting of the filling materials.

Apical 1.5 mm segments of each root were resected using a diamond disc at a 90° angle to the long axis. The internal root surfaces and interfaces between dentin

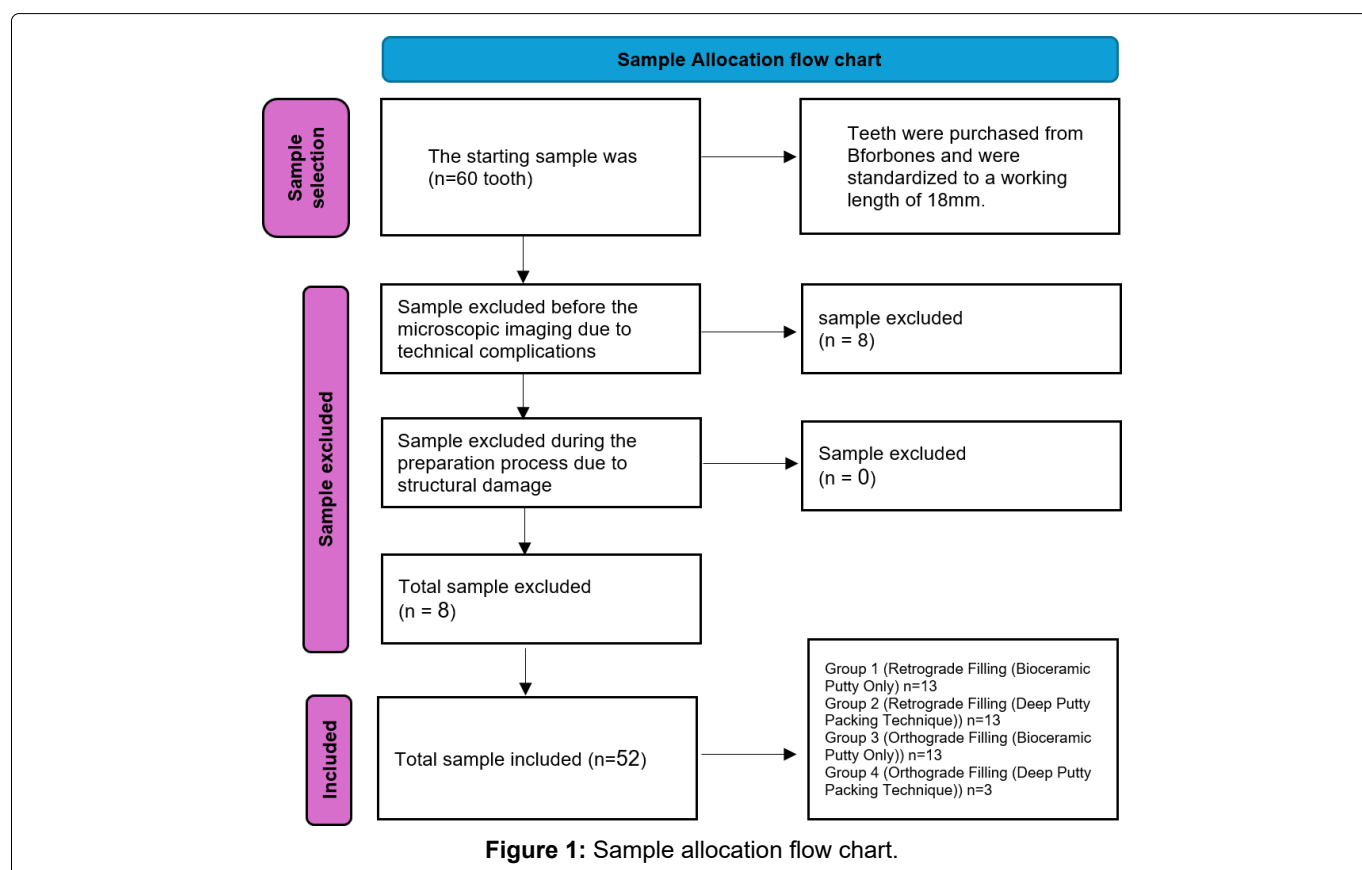




Figure 2: Sample after cavity preparation.



Figure 3: The electronic microscope used in the study.

and filling material were prepared for subsequent analysis under scanning electron microscopy (SEM) as described in the evaluation section (Figure 3).

Root canal instrumentation

Canals were prepared using rotary NiTi files (ProTaper Gold, Dentsply Maillefer, Ballaigues, Switzerland), following the manufacturer's protocol. Apical preparation was standardized at size 0.35 mm at tip. Canals were irrigated with 5.25% sodium hypochlorite (NaOCl) solution during instrumentation, followed by final irrigation with 17% EDTA (Meta Biomed, Korea) for 1 minute, then activation for 3 minutes to remove the smear layer, and finally flushed with distilled water. The canals were dried using sterile paper points.

Figure 4a, Figure 4b, and Figure 4c represent a sample from group 2 after clinical steps which include initial obturation with gutta-percha, sealer, and heat-softened gutta-percha (Figure 4a); preparation of the retrograde cavity and application of a bioceramic sealer layer on the cavity walls (Figure 4b); and the compaction of bioceramic material into the retrograde filling cavity (Figure 4c).



Figure 4a: Sample from group 2 after obturation with gutta-percha, resin sealer, and heat-softened gutta-percha.

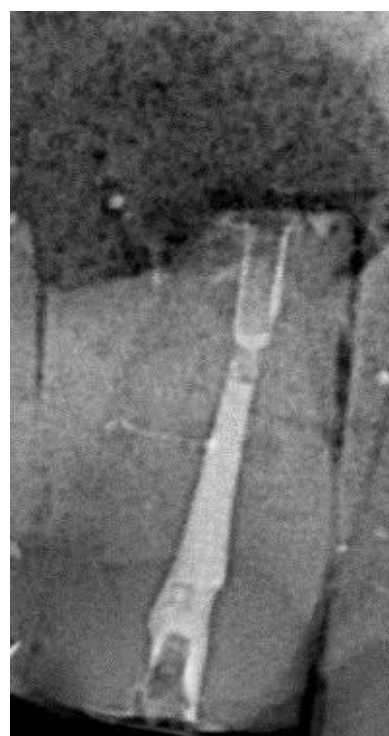


Figure 4b: A sample from group 2 after preparing the retrograde filling cavity and applying a layer of bioceramic sealer on the walls.



Figure 4c: Sample from group 2 after compacting dense bioceramic material into the prepared retrograde filling cavity.

Preparation process per group

Group 1 - retrograde filling (bio ceramic putty only):

In this group, canals were obturated using warm vertical compaction with gutta-percha cones (size 35/0.04) and a resin-based sealer (ADSEAL, META Biomed). After obturation, a 3 mm apical resection was performed at a 90° angle to the long axis, followed by preparation of a retrograde cavity using ultrasonic tips to a depth of 3 mm and a width of 1.5 mm. The cavity was irrigated with 2.5% NaOCl and dried. Dense bio ceramic putty (FKG, Switzerland) was formed in a form of a cone and was inserted into the canal. Radiographs were taken to confirm proper placement. Specimens were incubated for 7 days at 37°C and 100% humidity before analysis.

Group 2 - retrograde filling (deep putty packing technique): Root canals were prepared, resected apically by 3 mm, and retrograde cavities were prepared as in Group 1. A bio ceramic sealer (FKG, Switzerland) was applied to the walls of the retrograde cavity using an ultrasonic tip. The dense bio ceramic putty was then inserted and condensed using a condenser vertically, following the deep putty packing technique. Radiographs were taken to ensure uniform adaptation of the sealer and putty layers. Specimens were incubated similarly to Group 1 before being processed for SEM evaluation.

Group 3 - Orthograde filling (bio ceramic putty only):

A 6 mm orthograde apical plug was placed using dense bio ceramic putty inserted in layers, each condensed with pre-measured pluggers, reaching 1 mm short of the working length. Radiographs were taken after each increment to confirm condensation quality. Once the plug was complete and verified, the remaining canal space was obturated using thermoplasticized gutta-percha.

Specimens were incubated for 7 days at 37°C before the apical resection. Following incubation, apical resection and SEM analysis preparation were performed as described in previous groups.

Group 4 - Orthograde Filling (Deep Putty packing Technique):

This group followed a similar orthograde approach to Group 3 but used the deep putty packing technique. A suitable plugger was selected radiographically to reach 1 mm short of the apex. A bio ceramic sealer was applied with the plugger to cover the walls of the canal cavity, and the sealer layer was unified, then dense bio ceramic putty was incrementally placed and compacted up to 6 mm coronally using alternating pre-selected pluggers. Radiographs confirmed each layer's adaptation. After obturation, the remaining canal space was filled thermoplastically as in Group 3. Specimens were incubated for 7 days at 37°C in a humid environment before being sectioned and examined under SEM.

Scanning electron microscopy (SEM) analysis

SEM imaging (Zeiss EVO MA10, Carl Zeiss, Germany) was conducted at 15 kV, under standardized magnifications ($\times 50$, $\times 500$, $\times 1000$, and $\times 2000$). Marginal adaptation was assessed by calculating the marginal gap ratio (using ImageJ, NIH, USA). Evaluations were performed blindly by two calibrated examiners, and the average of their observations was recorded.

Sections were immersed in 10% nitric acid for 1 minute to remove debris, rinsed in distilled water, and samples were dehydrated using air and ethanol, and mounted on SEM stubs with carbon adhesive. As shown in Figure 5, untreated specimens exhibited surface artifacts and debris that impaired visualization of the filling margins under SEM, and directly Post-treatment imaging (Figure 6) reveals a cleaner dentin-filling interface, allowing more accurate evaluation of marginal adaptation and internal morphology. Samples were examined using a VEGA XMU SEM (TESCAN, Czech Republic) under low vacuum (20 Pa), 7 kV, and 20 μ s scan speed.

Apical Seal assessment

Seal quality was assessed by measuring the number of internal gaps, which is the number of gaps within the filling material (manually counted),

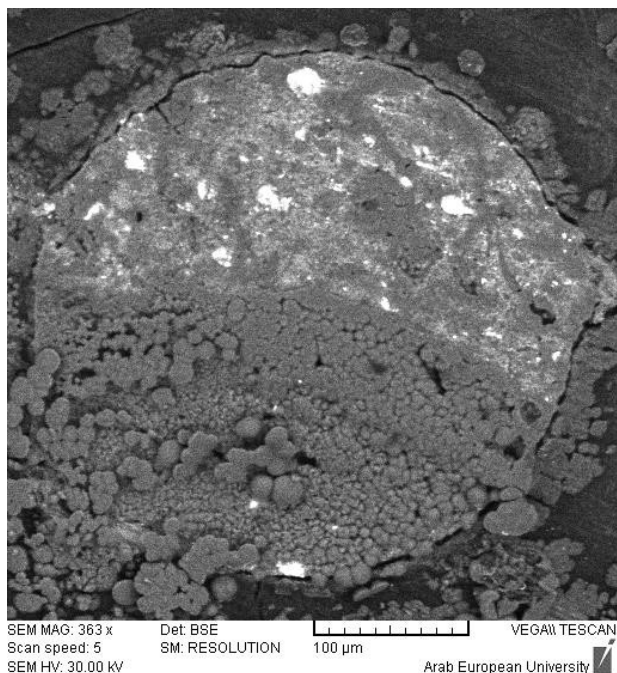


Figure 5: Surface Condition Before Debris Removal (10% Nitric Acid Treatment). SEM image (magnification $\times 363$) of the apical section before cleaning with 10% nitric acid. The surface shows heavy debris accumulation and crystal artifacts obscuring the dentin-filling interface, making marginal gap identification difficult. This emphasizes the necessity of chemical cleaning prior to microscopic analysis. Scale bar: 100 μm .

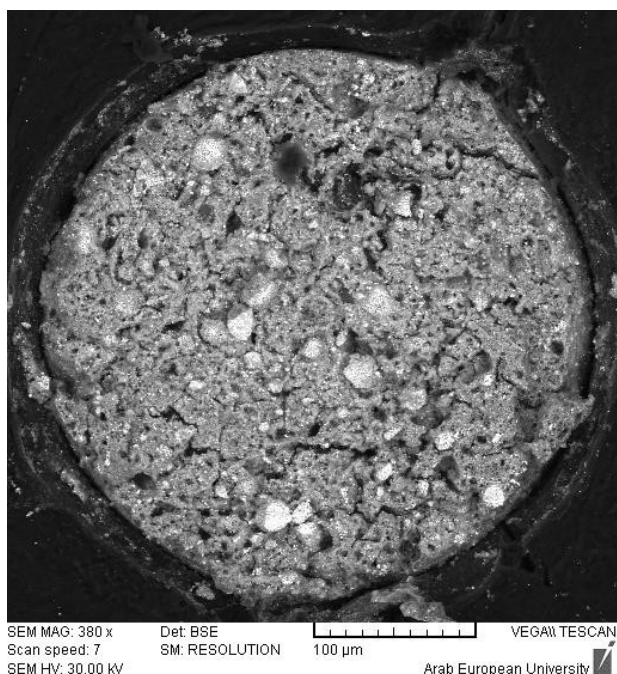


Figure 6: Surface Condition After 10% Nitric Acid Treatment (1 minute). SEM image (magnification $\times 380$) of the same apical section after immersion in 10% nitric acid for 1 minute. The cleaning process effectively removed superficial debris, revealing a clearer interface between dentin and the filling material, and improving contrast for morphological assessment. Scale bar: 100 μm .

and marginal gaps ratio which is the sum peripheral gaps length between dentin and filling material using ImageJ software divided by the total circumferential length of the canal of the sample, therefore the marginal gap ratio was calculated using the formula:

$$\left(\frac{\text{Total length of peripheral gaps}}{\text{Total circumferential length of the canal}} \right) \times 100 \cdot \text{Figure 7}$$

and Figure 8 shows examples of internal marginal gaps.

Statistical analysis

Statistical analyses were performed using SPSS (version 27.0, IBM, Armonk, NY, USA). Normality of data was assessed via the Shapiro-Wilk test. Gap measurements were statistically analyzed using Kruskal-Wallis tests to compare the orthograde and retrograde groups, with significance set at $\alpha = 0.05$.

Results

A total of 52 samples were analyzed for the two variables of this study, 1- number of internal gaps within the filling, and 2- ratio of marginal gaps.

The mean number of internal gaps was highest in Group 1 - Retrograde (Putty Only) ($M = 138.00$, $SD = 57.59$) and lowest in Group 4 - Orthograde (Deep Putty + Sealer) ($M = 96.77$, $SD = 30.90$). The median values followed a similar trend. Descriptive statistics are presented in table 1. Rank-based analysis (Table 1) showed that Group 1 had the highest mean rank (32.35), while Group 4 had the lowest (19.85). The Kruskal-

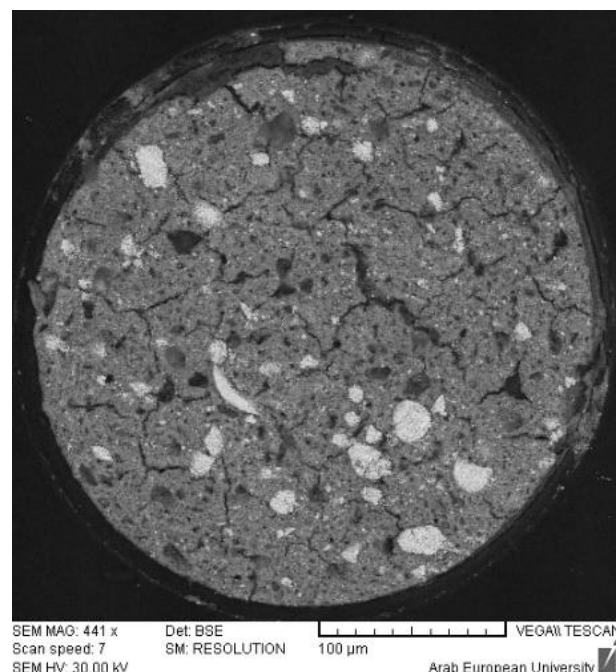


Figure 7: Cross-sectional SEM overview ($\times 441$) of a canal obturated with bioceramic materials. Notably, the bioceramic putty and sealer phases exhibit different granular morphologies. Scale bar: 100 μm .

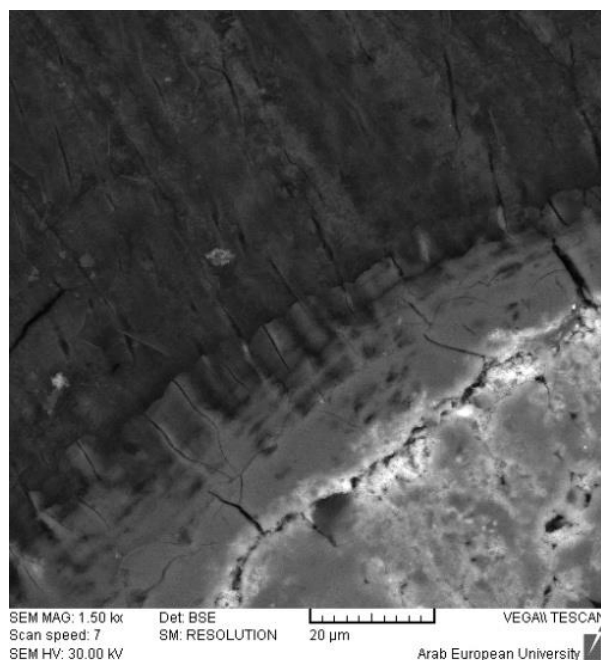


Figure 8: SEM image ($\times 1500$) showing the interface between the sealer and dense bioceramic putty in Group 2. Scale bar: 20 μm .

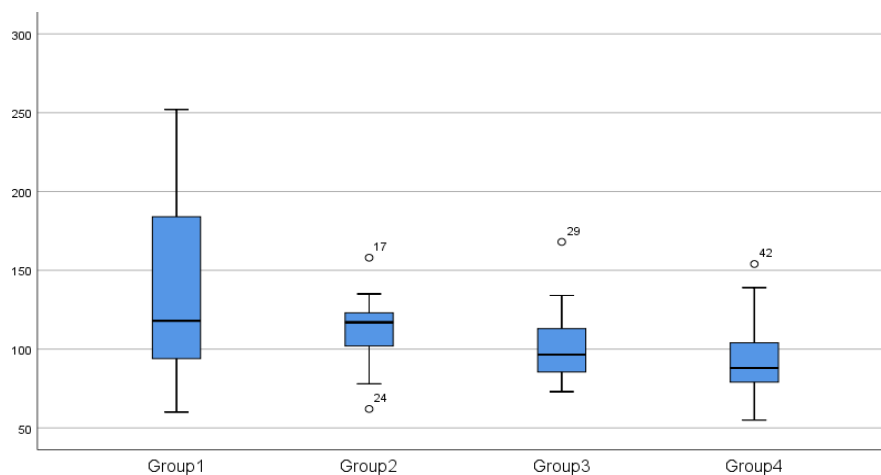


Figure 9: Shows the visualization of the mean with the confidence interval of each of the research groups in terms of the number of internal gaps.

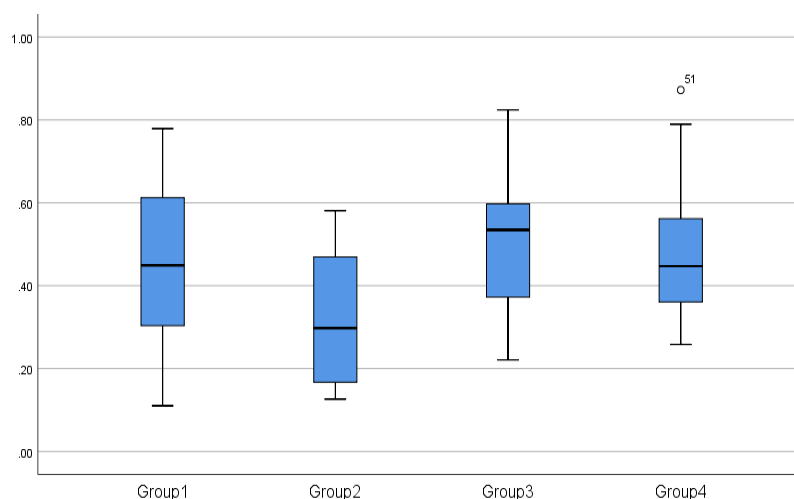


Figure 10: Shows the visualization of the mean with the confidence interval of each of the research groups in terms of the marginal gaps' ratio.

Wallis H test ([Table 1](#)) revealed no significant difference among groups regarding the number of internal gaps ($H = 5.720$, $df = 3$, $p = 0.126$) ([Figure 9](#)).

In terms of marginal gap ratio, the means ranged from 0.33 in Group 2 - Retrograde (Deep Putty + Sealer) to 0.50 in Group 3 - Orthograde (Putty Only). Rank comparison ([Table 2](#)) showed a similar distribution with no statistically significant differences confirmed by the

Kruskal-Wallis test ($H = 4.328$, $p = 0.228$; [Table 1](#)) ([Figure 10](#)).

SEM qualitative findings

The following figures represent an example of how SER imaging was produced and analyzed for both marginal gaps, and for gaps ratio, these visual observations complement the quantitative findings ([Figure 11](#), [Figure 12](#), [Figure 13](#) and [Figure 14](#)).

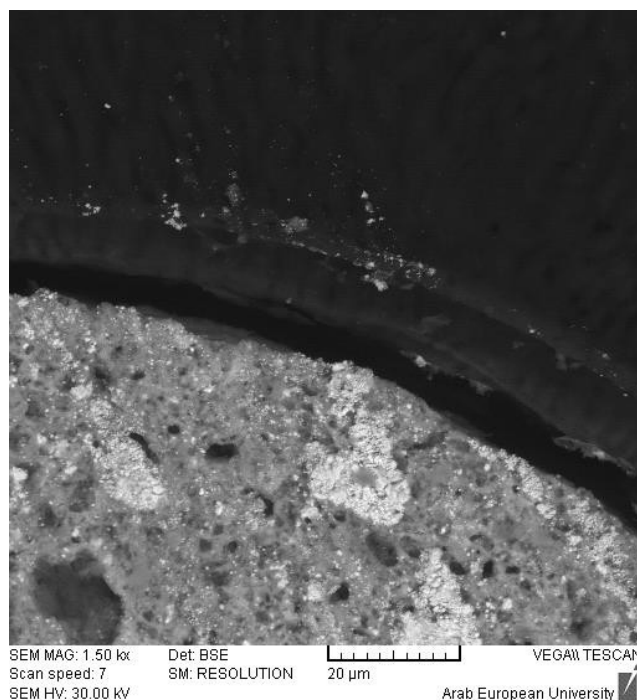


Figure 11: marginal gaps in Group 1. SEM image ($\times 1500$) of a retrograde MTA sample from Group 1. Marginal gaps are clearly visible at the material-dentin interface, indicating incomplete adaptation despite ultrasonic preparation. Scale bar: 20 μm .

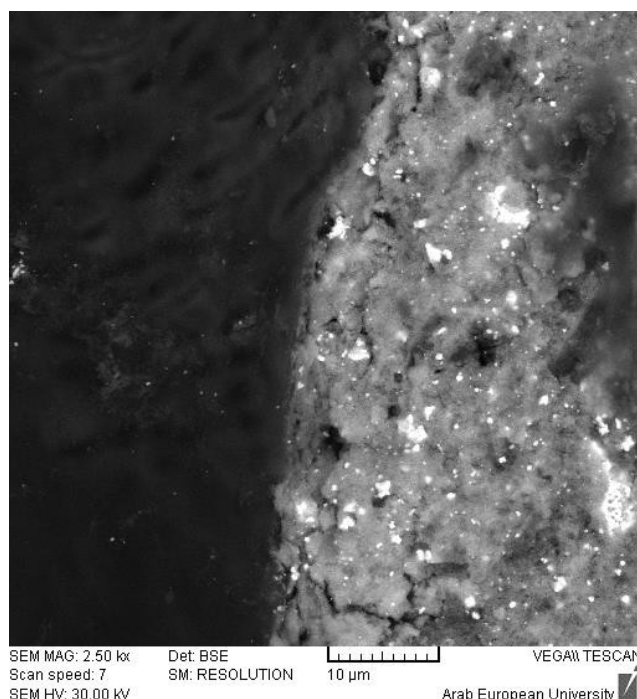


Figure 12: Adaptation in Group 4. High-resolution SEM ($\times 2500$) of a sample from Group 4 (deep orthograde putty packing) showing adaptation along the dentin interface. Scale bar: 10 μm .

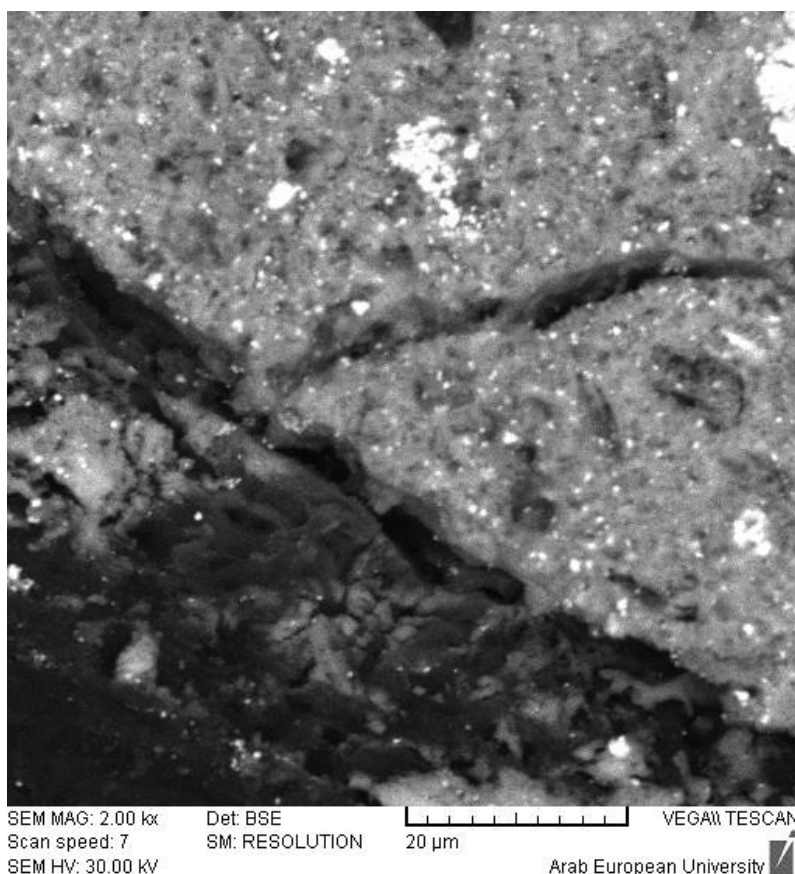


Figure 13: marginal gaps in Group 4. SEM image ($\times 2000$) of another Group 4 sample, revealing peripheral porosity and structural in homogeneity within the apical plug, which may act as potential leakage paths. Scale bar: 20 µm.

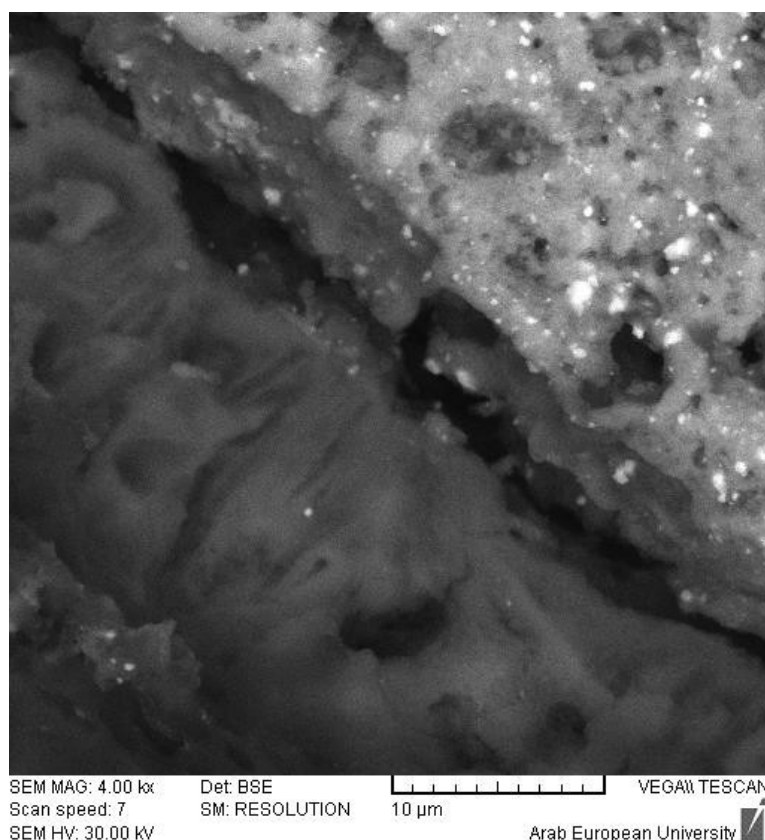


Figure 14: Marginal gaps in Group 3. SEM image ($\times 4000$) from Group 3 (orthograde bioceramic plug) showing minor marginal gaps, yet generally more homogeneous filling compared to Groups 1 and 4. Scale bar: 10 µm.

Table 1: Descriptive and inferential statistics of the number of internal gaps across the research groups.

Group	Descriptivestatistics					Kruskal-WallisHtest		
	N	Mean	SD	Median	95%CI (Lower-Upper)	Mean rank	Statistica value	P-value
Group 1-Retrograde (Putty Only)	13	138.00	57.59	118.00	103.20-172.80	32.35	5.720	0.126
Group 2-Retrograde (Deep Putty + Sealer)	13	112.85	24.90	117.00	97.80-127.89	28.92		
Group 3-Orthograde (Putty Only)	13	103.67	26.39	96.50	86.90-120.44	22.63		
Group 4-Orthograde (Deep Putty + Sealer)	13	96.77	30.90	88.00	78.10-115.44	19.85		

Table 2: Descriptive and inferential statistics of marginal gap ratio across the research groups.

Group	Descriptive statistics					Kruskal-Wallis Htest		
	N	Mean	SD	Median	95%CI(Lower-Upper)	Mean rank	Statistica value	P-value
Group 1-Retrograde (Putty Only)	13	0.45	0.19	0.45	0.33-0.58	24.00	4.328	0.228
Group 2-Retrograde (Deep Putty + Sealer)	13	0.33	0.18	0.30	0.19-0.47	15.67		
Group 3-Orthograde (Putty Only)	13	0.50	0.17	0.53	0.39-0.60	27.67		
Group 4-Orthograde (Deep Putty + Sealer)	13	0.48	0.19	0.45	0.37-0.60	24.62		

Discussion

This study investigated the comparative sealing effectiveness of orthograde vs. retrograde filling techniques using contemporary bioceramic materials. Our findings indicate that orthograde bioceramic materials provide comparable sealing performance to traditional retrograde methods, even after apical resection, as no significant differences were identified between the research groups. This observation aligns with previous studies, suggesting the potential of bioceramic materials to simplify surgical procedures by eliminating the need for additional retrograde filling during surgery [23,24].

We observed similar marginal adaptation and lower microleakage rates for bioceramic materials, consistent with previous literature emphasizing the biocompatibility, moisture resistance, and sealing capabilities of these materials [25-27]. Specifically, Biodentine and MTA demonstrated comparable sealing ability under SEM analysis, affirming their clinical utility [14]. Similar findings were reported by Harinkhere, et al., who demonstrated lower bacterial leakage with MTA and Biodentine compared to traditional materials, reinforcing their effectiveness in maintaining apical integrity [14].

Interestingly, our SEM analyses showed minimal disruption of orthograde material seals following

surgical resection, contrasting some previous studies which suggested resection might compromise orthograde obturation [8,28]. For instance, Moradi, et al. found slightly increased leakage following resection; however, their method differed significantly from ours, notably in sample preparation and evaluation methods, which might explain the variation in findings [8]. This highlights the importance of standardized evaluation methods, like SEM, for accurately assessing sealing integrity post-resection.

The lack of significant difference among the groups may reflect the uniform properties of modern bioceramic materials, which demonstrate favorable adaptation and bioactivity across different application techniques. This outcome challenges earlier assumptions that orthograde fillings are inherently less reliable after apical resection and suggests the technique may remain effective even after surgical intervention [25].

Nonetheless, our study faced several limitations. Primarily, the *in vitro* nature of the research limits direct clinical applicability. Real clinical scenarios involve additional complexities like fluid dynamics, tissue interactions, and patient-specific variables not replicated here.

Additionally, our use of SEM, while highly accurate in visualizing marginal gaps, may be limited by sample preparation artifacts, and SEM-based evaluation does

not directly reflect functional sealing under dynamic clinical conditions. Additional research should integrate methods such as micro-computed tomography (micro-CT), bacterial leakage testing, and longitudinal clinical trials to validate our findings further.

Future studies should also explore the long-term performance of bioceramic materials under clinical conditions, assessing factors such as biodegradability, dimensional stability, and bioactivity effects on surrounding periapical tissues. Such investigations would substantially reinforce our findings, providing comprehensive evidence to support clinical decision-making.

In conclusion, our data suggest that both orthograde and retrograde techniques using bioceramic materials offer comparable apical sealing performance. This may offer clinicians greater flexibility in technique selection without compromising sealing integrity.

Conclusion

This *in vitro* SEM-based study demonstrated no statistically significant difference in apical sealing between orthograde and retrograde techniques using bioceramic materials. The marginal gap percentages and number of internal gaps were comparable across all groups, suggesting that both approaches can provide effective sealing under controlled conditions. These findings indicate that the application method - orthograde vs. retrograde - may not significantly influence the sealing performance of bioceramic putty, allowing clinicians to tailor the approach based on clinical accessibility and preference. Further clinical studies are warranted to confirm these outcomes and explore their long-term implications in endodontic practice.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethical Approval Statement

No ethical approval was required for this study as it is an *in-vitro* study, the teeth included in the study were sourced from an ethically approved source, approval to do this study were obtained from Damascus university higher committee for scientific research.

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Author Contributions

HA: the first and the leading author designed the research, did the experiment, collected the data, and

participated in writing the manuscript. BH: wrote the manuscript, analyzed the data, and finalized the text. HA: supervised the research, helped writing the manuscript. All authors have read and approved the final version of the manuscript.

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