Original Article

DOI: 10.7860/JCDR/2026/80588.23498

Dentistry Section

A Comparative Evaluation of Sealing Efficiency in Two MTA Placement Techniques Using Rhodamine B Dye and Confocal Laser Scanning Microscopy: An In-vitro Study

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ABSTRACT

Introduction: A reliable apical seal is essential for the long-term success of endodontic surgery. Mineral Trioxide Aggregate (MTA) is well known for its excellent sealing properties and is frequently employed as a root-end filling material. The technique of MTA placement-either orthograde through the root canal or retrograde during surgical access-may influence its sealing effectiveness and consequently, the clinical outcome of endodontic procedures. Reported variations in the sealing ability of MTA are primarily attributed to differences in placement techniques and evaluation methodologies. The absence of standardised guidelines continues to limit the establishment of definitive conclusions. Therefore, a direct comparative assessment of orthograde and retrograde MTA placement using Confocal Laser Scanning Microscopy (CLSM) is warranted.

Aim: The present in-vitro study aimed to compare the sealing ability of orthograde and retrograde MTA placement techniques using CLSM.

Materials and Methods: The present in-vitro study was conducted at Vishnu Dental College, Bhimavaram, Andhra Pradesh, India, over a period of four months (January 2025 to April 2025). A total of 24 extracted single-rooted human mandibular teeth with single canals, obtained for periodontal reasons, were randomly allocated into three groups (n=8 per group): Group I (Control): No root-end preparation or filling. Group II (Orthograde): A 6 mm apical MTA plug placed orthogradely using a Disposable Apical Carrier (DAC). Group III (Retrograde): Retrograde root-end preparation followed by MTA filling. All specimens underwent apical resection at 3 mm from the apex. To assess microleakage, samples were immersed in 1% Rhodamine B dye for 24 hours and subsequently examined under CLSM. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp.). Intergroup comparison of apical microleakage (in mm) was conducted using one-way Analysis of Variance (ANOVA). Pairwise comparisons were performed using Tukey's post-hoc test. The level of significance was set at $p<0.001$.

Results: One-way ANOVA revealed statistically significant differences in mean dye penetration among the groups ($p<0.001$), indicating a significant variation in apical microleakage ($F=5.5$, $p<0.001$). Pairwise post-hoc analysis using Tukey's test showed that Group III (Retrograde: 3.2±0.6 mm) exhibited significantly less dye penetration compared to Group I (Control: 9.8±1.6 mm) and Group II (Orthograde: 5.8±1.1 mm) ($p<0.001$). A statistically significant difference was also observed between Group II and Group III ($p<0.001$).

Conclusion: Retrograde placement of MTA demonstrated superior sealing ability compared to the orthograde technique, suggesting that it may be more effective in minimising apical microleakage in endodontic surgery.

Keywords: Apicoectomy, Mineral trioxide aggregate, Root end filling techniques, Sealing ability

INTRODUCTION

The success of endodontic therapy depends on thorough cleaning and shaping of the root canal system and the establishment of a fluid-tight seal [1]. Despite continuous advancements in techniques, materials and instruments, root canal treatment may still fail in certain cases due to persistent periapical pathology. The presence of lateral canals and apical ramifications further complicates the root canal system, making complete debridement difficult. Consequently, conventional root canal therapy is not suitable for all clinical cases [2]. When conventional endodontic treatment fails, surgical endodontic therapy is often required to preserve the tooth [3]. This procedure involves exposure of the root apex, apical resection, root-end cavity preparation and placement of a root-end filling material [4].

Numerous studies have established MTA as one of the most effective retrograde filling materials, demonstrating superior marginal sealing ability compared to alternative materials [3,5-9]. This property is largely attributed to its hydrophilic nature, which enables it to expand during setting in the presence of moisture, thereby filling interfacial gaps between dentin and the filling the precise and enhancing the apical seal [6,10]. As retrograde filling materials come into direct contact with vital periapical tissues, biocompatibility and tissue response play a critical role in surgical success [11].

Retrograde root-end preparation and filling are commonly employed during endodontic surgery due to improved access and enhanced control over material placement at the resected apex [12]. However, in situations where surgical access is limited such as proximity to the maxillary sinus or mental foramen an orthograde approach may be a practical alternative. Orthograde placement of root-end filling material during non-surgical retreatment eliminates the need for surgical root-end preparation and may offer a simpler, less invasive option [13].

Potential approaches include orthograde placement of MTA or the use of bioceramic sealers with a single-cone technique followed by apical resection without additional retrograde filling [12,14]. The technique of material placement may influence the quality of the apical seal. Orthograde MTA plugs placed in a controlled intracanal environment may demonstrate enhanced resistance to leakage after resection, whereas retrograde placement allows direct adaptation to the resected root surface during surgery.

Although MTA is widely used in both techniques, existing literature presents inconsistent findings, largely due to variations in methodology and lack of standardised protocols. To address this limitation, the present study utilised 1% Rhodamine B dye and CLSM to enable high-resolution visualisation and quantitative assessment of microleakage following apicectomy. A Disposable Apical Carrier (DAC) was employed as a novel method to facilitate standardised orthograde MTA placement.

The null hypothesis was that there would be no significant difference in sealing ability between orthograde and retrograde MTA placement techniques, as measured by Rhodamine B dye penetration using CLSM. The alternative hypothesis proposed a significant difference in sealing ability between the two techniques.

MATERIALS AND METHODS

The present in-vitro study was conducted at Vishnu Dental College, Bhimavaram, Andhra Pradesh, India, over a period of four months, from January 2025 to April 2025 (IEC approval no. IECVDC/25/PG01/CE/IVT/56).

Sample size calculation: The sample size was calculated using G*Power software (version 3.1.9.2; Heinrich-Heine-Universität Düsseldorf, Germany). With the level of significance set at 5%, study power at 80% and an expected effect size of 0.765 (derived from a previous study) [12], the required sample size was determined to be eight specimens per group. Accordingly, a total of 24 permanent human mandibular single-rooted teeth extracted for periodontal reasons were collected.

Inclusion and Exclusion criteria: Teeth with single roots and single canals were included. Radiographic evaluation was performed to exclude teeth with multiple canals, anatomical abnormalities, restorations, root caries, previous root canal treatments, cracks, perforations, or canal curvatures exceeding 10° [3,7].

Study Procedure

Sample preparation: The external root surfaces were manually scaled to remove residual calculus. The cleaned teeth were then stored in sealed containers containing normal saline at 37°C until further use [12].

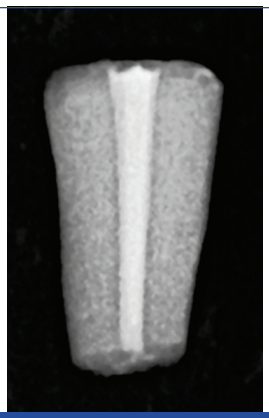
Standardisation and root canal treatment: All procedures were performed by a single operator to ensure consistency and minimise variability among the experimental groups. To standardise root lengths, the teeth were decoronated using a diamond disc to achieve a uniform root length of 12 mm. The Working Length (WL) was established by inserting a size 10 K-file (Mani, Japan) into the canal until it was visible at the apical foramen. The WL was calculated by subtracting 0.5 mm from this measurement. Biomechanical preparation was carried out using Hyflex EDM rotary nickel-titanium instruments (Coltene/Whaledent AG, Altstätten, Switzerland) up to size 40 with a 0.04 taper. During instrumentation, 2.5% Sodium Hypochlorite (NaOCl; 3 mL) was used as the irrigant. The final rinse was activated using ultrasonic files in three cycles of 20 seconds each. Root canals were then dried using International Organization for Standardization (ISO) size 40 paper points [12].

Sample Grouping and Experimental Procedures: The 24 prepared roots were randomly allocated into three experimental groups using random.org software (n=8 per group). Group I (Control Group): A bioceramic sealer (BioStructure RCS, Safe Endo, India) was placed according to the manufacturer's instructions. A gutta-percha cone (size 40, 0.04 taper; ROEKO EDM gutta-percha points, Coltene/Whaledent) was coated with the sealer and inserted into the canal up to the WL. The cone was trimmed at the orifice level using a hot instrument.

Storage: After obturation, the samples were stored in an incubator at 37°C with 100% relative humidity for two months to simulate clinical conditions [12]. This storage protocol is commonly used in endodontic and dental materials research, as it ensures that samples reflect clinically relevant, matured and stable material properties prior to evaluation. It is important for:

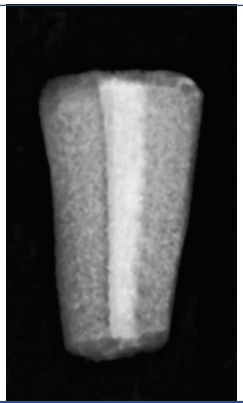
- Simulating the warm and moist oral environment
- Standardising experimental conditions across groups
- Allowing assessment of long-term material stability, including dimensional changes, microleakage and interfacial adaptation

Apicectomy: Following storage, the root tips were horizontally sectioned 3 mm from the anatomical apex using a carbide bur (HM 33IL-316-010, Meisinger, Neuss, Germany) under continuous water irrigation. All apicectomy procedures were performed under 10× magnification using a dental operating microscope (LABOMED Prima DNT, USA) to ensure precision and accuracy [Table/Fig-1] [12].



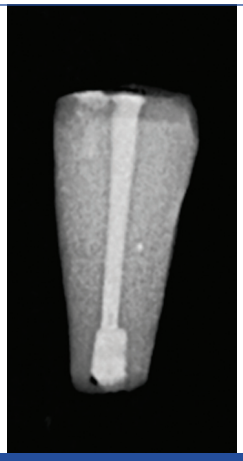
[Table/Fig-1]: Group I: Sample obturated with a single cone technique and without any retrograde cavity preparation and retrograde filling.

Group II (Orthograde MTA Filling): The apical 6 mm of the canal from the WL was filled with MTA cement (ProRoot MTA, Dentsply Maillefer). ProRoot MTA was mixed according to the manufacturer's instructions and delivered to the apical portion of the canal using a custom-made Disposable Apical Carrier (DAC) system (Copyright Registration No. L-123342/2023; Dr. Madhu Varma K). The cement was compacted incrementally using calibrated manual pluggers to ensure a dense and uniform 6 mm apical plug. The remaining canal space was backfilled with thermoplasticised gutta-percha using the Woodpecker Obturation System Fi-G and Fi-P (Woodpecker, China) and further condensed with manual pluggers to ensure complete obturation, as this procedural variation was unavoidable. Storage and apicectomy procedures were identical to those described for Group I [Table/Fig-2].



[Table/Fig-2]: Group II: Sample with orthograde MTA filling and obturated using thermoplasticised gutta-percha.

Group III (Retrograde MTA Filling): Root canal treatment, storage and apicectomy procedures were the same as those described for Group I, with additional steps for retrograde cavity preparation and filling. A 3 mm deep retrograde cavity was prepared using ultrasonic tips (AS3D, Satelec Acteon Group, Mérignac, France) under continuous water spray. The ultrasonic tips were activated with a piezoelectric unit (Newtron P5, Satelec Acteon) at a power setting of 6. The cavities were dried using paper points. ProRoot MTA (Dentsply Maillefer) was mixed according to the manufacturer's instructions and placed incrementally into the retrograde cavities. The material was compacted using manual retrograde condensers to achieve a dense and homogeneous filling [Table/Fig-3].

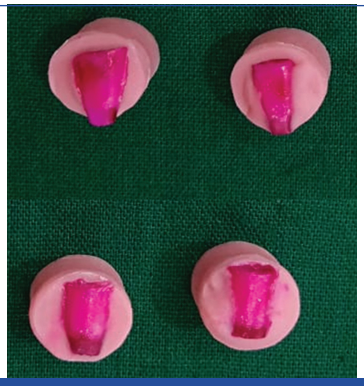


[Table/Fig-3]: Group III: Sample obturated with single cone technique followed by retrograde preparation and retrograde filling material.

Periapical radiographs were obtained for all samples to verify the quality of both retrograde and orthograde fillings. All samples were then stored in an incubator at 37°C with 100% relative humidity for seven days [9].

Dye penetration test: Each sample was coated with nail varnish, leaving the apical 2 mm exposed [Table/Fig-4]. The specimens were immersed in a 1% aqueous solution of Rhodamine B dye for 24 hours to allow dye penetration into any voids or leakage pathways [Table/Fig-5]. After immersion, the samples were thoroughly rinsed with water for 15 minutes to remove excess surface dye [2,3].

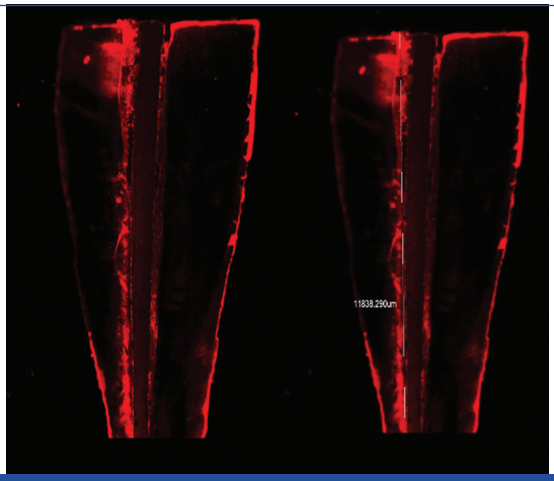
[Table/Fig-4]: Samples coated with nail varnish except 2 mm around root apex.



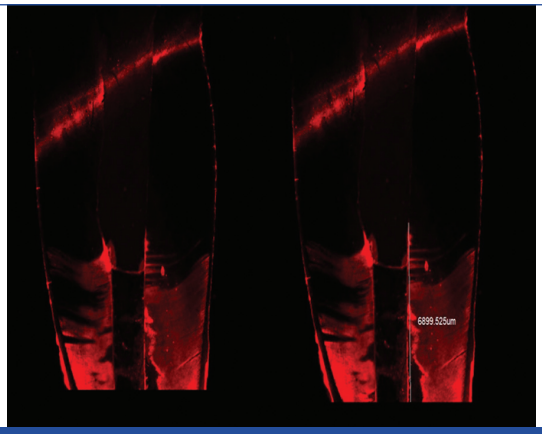
[Table/Fig-5]: Samples after immersion in Rhodamine B dye.

All specimens were buccolingually sectioned using a microtome (Baincut LSS, Chennai) and analysed under a confocal laser scanning microscope (Leica Stellaris Confocal), enabling precise visualisation and quantification of dye penetration within the root canal system [6].

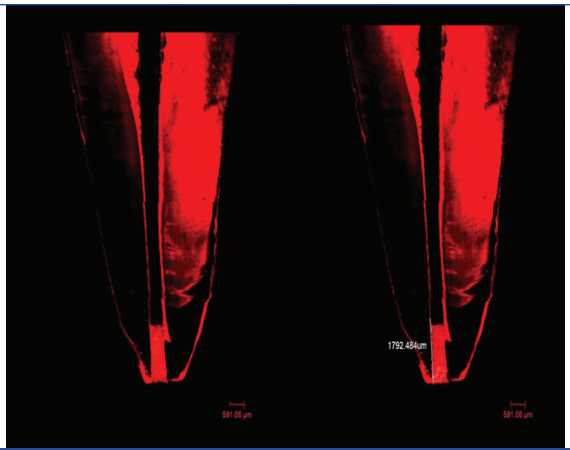
The depth of dye penetration was evaluated using CLSM and microleakage values were initially recorded in micrometres (µm) and subsequently converted to millimetres (mm) using the formula: Micrometres (µm) ÷ 1,000 = Millimetres (mm) [Table/Fig-6-8].



[Table/Fig-6]: Group I: The CLSM image shows a longitudinal section of a tooth root with fluorescent dye. Bright red fluorescence along the canal walls indicates dye penetration, revealing the sealing ability of the filling material. A measurement of 11838.290 µm (11.8 mm) on the right highlights dye infiltration and potential microleakage pathways.



[Table/Fig-7]: Group II: The CLSM image shows a longitudinal section of a tooth root with fluorescent dye. Bright red fluorescence along the canal walls indicates dye penetration, revealing the sealing ability of the filling material. A measurement of 6899.535 µm (6.89mm) on the right highlights dye infiltration and potential microleakage pathways.



[Table/Fig-8]: Group III: The CLSM image shows a longitudinal section of a tooth root with fluorescent dye. Bright red fluorescence along the canal walls indicates dye penetration, revealing the sealing ability of the filling material. A measurement of 1792.484 µm (1.79mm) on the right highlights dye infiltration and potential microleakage pathways.

Quantification was performed by measuring the linear distance from the apical extent to the most coronal extent of dye penetration, represented by a dotted line along the interface between the MTA and canal wall in CLSM images, corresponding to the visualised Rhodamine B infiltration.

Blinding protocol: A single-blinded protocol was implemented to minimise bias. The operator who performed the procedures was not involved in microleakage assessment. Specimens were coded by an independent assistant and the examiner conducting CLSM analysis was blinded to group allocation.

STATISTICAL ANALYSIS

The statistician was also blinded during data analysis. Data were entered and organised using Microsoft Excel (version 2013) and statistical analyses were performed using SPSS software (IBM Corp., version 22.0). Both descriptive and inferential statistics were applied. One-way Analysis of Variance (ANOVA) was used to determine statistically significant differences among the means of the three groups. Post-hoc multiple pairwise comparisons were conducted using Tukey's post-hoc test to identify significant intergroup differences. Statistical significance was set at a 95% confidence level, with a p-value threshold of less than 0.001.

RESULTS

The mean dye penetration values were: Group I: 9.8±1.6 mm, Group II: 5.8±1.1 mm and Group III: 3.2±0.6 mm [Table/Fig-9-11]. Group III exhibited the lowest mean microleakage, followed by Group II, while Group I demonstrated the highest leakage. The overall mean microleakage across all groups was 6.3±3.03 mm. Statistical analysis revealed a significant difference in mean microleakage among the three groups, indicating that the method of MTA placement had a measurable impact on sealing ability. One-way ANOVA confirmed a statistically significant difference (F=5.5, p<0.001). Post-hoc pairwise comparisons using Tukey's test showed statistically significant differences (p<0.001) between: Group I and Group II, Group II and Group III and between Group I and Group III. Group I (control) exhibited significantly higher microleakage compared to both treatment groups, while Group III demonstrated the least leakage.

CLSM images from all groups visually supported these findings [Table/Fig-6-8]. Overall, apical microleakage was minimal in Group III (retrograde), with statistically significant differences compared to Groups I and II, indicating superior sealing ability with retrograde MTA placement [Table/Fig-12].

DISCUSSION

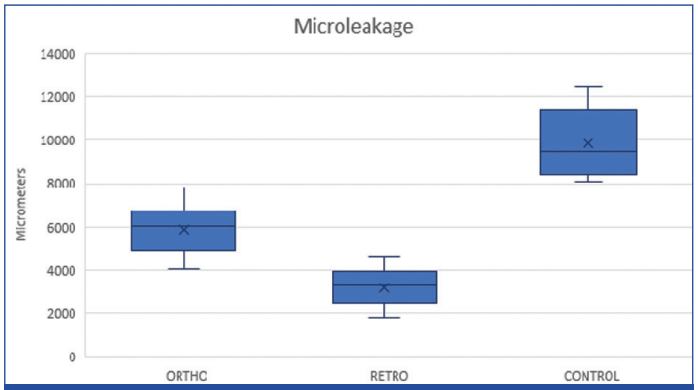
The effectiveness of the apical seal achieved with root-end filling materials has been evaluated using several methods, including dye penetration, radioisotope penetration, bacterial infiltration, electrochemical techniques and fluid filtration. Among these, dye penetration remains the most commonly employed method due to its simplicity, cost-effectiveness and ease of application. Common dyes for this purpose include India ink, basic fuchsin, methylene blue and Rhodamine B [3]. In the present study, 1% Rhodamine B was selected because it is not affected by alkaline materials, penetrates dentin more effectively than methylene blue and exhibits

Intergroup comparsion	Sum of Squares	df	Mean Square	F	p-value
Between Groups	1.78	2	89246376	5.5	<0.001**
Within Groups	3.39	21	1615634		
Total	2.12	23			

[Table/Fig-10]: Intergroup comparison of microleakage (millimetres).
One way ANOVA Test: p-value <0.05 - Significant*; p-value <0.01 - Highly Significant****

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Ortho	Retro	2.69*	6.35	<0.001**	1.09	4.29
	Control	-3.95*	6.36	<0.001**	-5.55	-2.35
Retro	Ortho	-2.69 *	6.36	<0.001**	-4.29	-1.36
	Control	-6.64*	6.36	<0.001**	-8.24	-5.31
Control	Ortho	3.95*	6.35	<0.001**	2.62	5.27
	Retro	6.64*	6.35	<0.001**	5.31	7.96

[Table/Fig-11]: Intergroup pairwise comparison of microleakage (millimetres).
The standard error is the same up to the second decimal place throughout. This has been cross-checked by the author's statistician. Post Hoc LSD Test; p-value <0.05 – Significant; p-value <0.01 – Highly Significant**



[Table/Fig-12]: Graph: Box Plot , X-axis: Represents the three study groups: Ortho, Retro, and Control, Y-axis: Represents the Microleakage values in micrometers.

superior surface activity. Additionally, its fluorescent properties make it particularly suitable for confocal microscopy, allowing easy detection and accurate measurement without additional localisation procedures [3]. This dye is also more sensitive than traditional dyes, enhancing assessment precision [2].

CLSM was utilised to evaluate microleakage due to its advantages over conventional imaging techniques like Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). CLSM eliminates out-of-focus blur and enables three-dimensional visualisation, providing more detailed and accurate information than two-dimensional methods [2,15]. It also produces high-contrast images with reduced artefacts, ensuring more reliable and reproducible results [6]. In the present study, the retrograde group demonstrated the least microleakage compared to the control and orthograde groups, likely due to the superior sealing ability of MTA when used as a retrofilling material. The retrograde technique allows dense compaction of MTA into the root-end cavity, often against a matrix such as gutta-percha under direct visualisation, which facilitates precise adaptation and a more effective seal [13].

Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Ortho	8	5.897	1.18	4.20	4.90	6.89	4.04	7.79
Retro	8	3.207	0.6	3.20	2.44	3.96	1.79	4.64
Control	8	9.848	1.61	5.71	8.49	11.11	8.09	12.49
Total	24	6.317	3.03	6.20	5.03	7.60	1.79	1.24

[Table/Fig-9]: Descriptives of Microleakage (millimeters) across study groups

This enhanced sealing performance is attributed to the formation of hydroxyapatite-like crystals at the interface between MTA and the canal walls, which improve adhesion, reduce dye penetration and minimise microleakage [3,5]. The findings of the present study are consistent with previous research, further supporting the efficacy of MTA in providing a reliable apical seal when used for retrograde fillings [3,5,9].

The DAC is a novel, custom-made and cost-effective system designed for MTA placement. It is typically fabricated chairside using readily available dental materials such as disposable syringes and irrigation needles. The DAC is intended for single use, eliminating the risk of cross-contamination and the need for sterilisation. However, its precision and ease of handling largely depend on the clinician's skill and experience in fabricating and using the device.

In the present study, the orthograde group exhibited greater microleakage than the retrograde group. This may be attributed to the inherent challenges of handling MTA during orthograde placement. The granular texture and loose consistency of MTA make effective placement and compaction difficult, particularly in the apical region of the canal [16,17]. Additionally, its viscosity may hinder adequate condensation, potentially compromising the apical seal. Although MTA possesses excellent sealing properties, its clinical performance is strongly influenced by the delivery technique. Orthograde placement often requires multiple increments and procedural steps, increasing the likelihood of void formation and subsequent microleakage [18]. Furthermore, the orthograde technique is highly technique-sensitive. These factors suggest that the increased microleakage observed is not attributable to the material itself but rather to limitations inherent in the orthograde delivery approach [19].

These findings differ from those reported by Andelin WE et al., [20], who observed no significant difference in leakage between orthograde and retrograde placements. This discrepancy may be due to variations in compaction techniques and difficulties in achieving an effective seal with a 6 mm orthograde MTA plug. This highlights the need for optimisation of orthograde MTA placement methods to improve sealing effectiveness. Al Fouzan K et al., [21] evaluated the marginal adaptation of MTA in both orthograde and retrograde techniques and reported good adaptation in both approaches. Their findings emphasise the importance of proper placement technique to ensure optimal sealing and minimise microleakage.

Adjunctive techniques such as ultrasonic activation, reverse motion of Ni-Ti files and meticulous condensation may enhance material adaptation and reduce microleakage where conventional methods are insufficient [22]. These refinements offer improved control and may significantly enhance sealing in complex anatomical situations. As this was an in-vitro study, it does not fully replicate clinical conditions. In vivo, successful healing typically involves cementum deposition over the resected root surface, which contributes to the re-establishment of periodontal attachment. Cementum formation usually begins at the periphery of the root end and progresses centrally, forming a biological seal, while the root-end filling material provides a physical seal. Together, these create a "double seal" that enhances resistance to reinfection and promotes tissue healing. Long-term clinical studies are necessary to validate these in-vitro findings [2,3,23].

The null hypothesis was rejected, as retrograde MTA placement demonstrated significantly less dye penetration than orthograde placement and the control group ($p<0.001$).

Limitation(s)

The present in-vitro study was limited to single-rooted teeth and a single operator, restricting assessment of complex multi-rooted anatomy and operator-related variability. Microleakage was evaluated after only 24 hours, which may not reflect long-term sealing performance. Future studies should incorporate multi-rooted teeth, multiple operators and extended observation periods to improve clinical relevance.

CONCLUSION(S)

Within the limitations of the present in-vitro study, retrograde placement of MTA demonstrated superior sealing efficacy, suggesting it is a promising approach for optimising outcomes in endodontic procedures involving apical surgery. Future research should focus on refining orthograde placement techniques to enhance their effectiveness in minimising microleakage.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 29, 2025
- Manual Googling: Oct 04, 2025
- iThenticate Software: Oct 06, 2025 (6%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **May 12, 2025**
Date of Peer Review: **Aug 08, 2025**
Date of Acceptance: **Oct 08, 2025**
Date of Publishing: **Jun 01, 2026**