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Comparative evaluation of ProRoot MTA, MTA Angelus and BioStructure MTA Putty for retrograde filling using dye penetration with stereomicroscopic analysis: An *in vitro* study

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Abstract:
Root-end filling materials must provide an effective apical seal to minimize microleakage and support periapical healing. Therefore, it is of interest to compare the sealing ability of ProRoot MTA, MTA Angelus and Safe Endo Biostructure MTA Putty as retrograde filling materials using dye penetration and stereomicroscopic analysis. Thirty extracted human mandibular premolars were instrumented, obturated, subjected to root-end resection and ultrasonic retrograde cavity preparation and randomly allocated to three groups (n = 10) according to the retrograde filling material used, followed by immersion in 2% methylene blue dye and evaluation of linear dye penetration under a stereomicroscope. Intergroup comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test. ProRoot MTA showed significantly lower dye penetration than MTA Angelus and Safe Endo Biostructure MTA Putty ($p < 0.001$), whereas no statistically significant difference was observed between MTA Angelus and Safe Endo Biostructure MTA Putty ($p = 0.799$). Thus, data shows that ProRoot MTA showed the best apical sealing ability among the materials evaluated and may be considered a more effective retrograde filling material for minimizing microleakage.

Keywords: ProRoot MTA, MTA angelus, bio structure MTA putty, stereomicroscope

Background:
The aim of root canal treatment is to eliminate microorganisms from the root canal system. Achieving complete obliteration of the root canal system and establishing a fluid-tight seal are key aspects of endodontic care **[1]**. The biological aim of root canal treatment is to prevent or cure apical periodontitis by controlled asepsis or disinfection of the root canal system, thereby creating an environment in which periapical repair can occur **[2]**. Despite advancements in endodontic materials, some cases may fail and cannot be managed through non-surgical treatment alone **[3]**. In such cases, surgical procedures including root-end resection, retrograde cavity preparation and root-end filling may become necessary **[4]**. A retrograde filling is intended to establish an apical seal and reduce movement of residual irritants into the periradicular tissues **[5]**. Root-end filling materials must possess suitable biological and physicochemical properties. Biocompatibility is essential to minimize adverse tissue responses **[6]**. Bioactivity is desirable because it may support hard-tissue formation and periapical healing **[7]**. The biological response to mineral trioxide aggregate and related calcium-silicate materials has therefore received considerable attention **[8]**. Their alkaline environment and antimicrobial potential are also relevant to endodontic repair **[9]**. Contemporary calcium-silicate formulations have expanded the range of materials available for root-end procedures **[10]**. Methylene blue remains a commonly used tracer in laboratory microleakage assessment **[11]**. Recent investigations have continued to evaluate the performance of root-end repair materials under different experimental conditions **[12]**. Other studies have examined the adaptation and behavior of calcium-silicate materials at the dentin-material interface **[13]**. Biological compatibility has also been compared among newer endodontic repair cements **[14]**. Laboratory evaluations have further considered their physicochemical and sealing characteristics **[15]**. Material stability and interaction with the surrounding substrate are important determinants of performance **[16]**. The composition and hydration behavior of calcium-silicate cements may influence their clinical handling and final properties **[17]**. Accordingly, newer formulations continue to be investigated as alternatives to established MTA products **[18]**. Comparative studies have assessed root-end filling materials using different leakage models **[19]**. Additional investigations have evaluated the performance of MTA-based materials in endodontic repair **[20]**. The sealing behavior of contemporary materials has been studied under a variety of laboratory conditions **[21]**. Root-end materials have also been compared with respect to adaptation and microleakage **[22]**. Experimental work has examined how material formulation may influence sealing performance **[23]**. Different methods for evaluating leakage have been used to characterize the material-dentin interface **[24]**. Further studies have explored setting behavior and a dimensional characteristic of calcium-silicate cements **[25]**. The effect of material composition on endodontic repair performance has also been investigated **[26]**. Comparisons among commercially available MTA formulations have showed that material properties are not identical **[27]**. This has prompted continued assessment of newer root-end filling products **[28]**. Differences in manufacturing and composition may contribute to variation in material behavior **[29]**. The sealing ability of calcium-silicate materials remains an important focus of experimental endodontics **[30]**. These investigations collectively support continued comparative evaluation of root-end repair materials **[31]**. Therefore, it is of interest to compare the sealing ability of ProRoot MTA, MTA

Angelus and Biostructure MTA Putty as retrograde filling materials using dye penetration and stereomicroscopic analysis.

Materials and Methods:

This is an *in vitro* study carried out in Department of Conservative Dentistry and Endodontics, MES Dental College, Perinthalmanna & National Institute Technology, Calicut. In this study 30 Single rooted mandibular premolars were selected based on the inclusion and exclusion criteria. Teeth were cleaned thoroughly and curetted to remove soft tissue debris. And those teeth were kept in 5 % sodium hypochlorite for 1 hr and stored in normal saline. All the teeth were de-coronated to achieve a standard length of 16mm using diamond bur and water cooling. Apical patency was determined using a #10 K-file (MANI, INC). The file was inserted into the canal so that the tip can be seen slightly through the apical foramen. Working length was determined by subtracting 1 mm from the initial file length. Biomechanical preparation was performed using hand ProTaper instruments (Dentsply) and obturation was performed using F3 gutta-percha points and zinc oxide eugenol sealer. To verify proper obturation, all obturated teeth were radiographed. Samples exhibiting inadequate obturation were re-obturated until satisfactory length, density and adaptation were achieved. The coronal access cavity was filled using GIC (Fuji type IX). This was followed by resection of each sample apically at 90 degrees to the long axis of the root, using a crosscut fissure bur, to remove 3 mm of the apex. A root-end cavity measuring 3 mm in depth and 0.8 mm in diameter was prepared in each specimen using an ultrasonic tip (woodpecker, scaler tip, ED10D, ED11D) powered by an ultrasonic device (UDS-E) at a frequency of 32 KHz. A periodontal probe (Williams's periodontal probe) was used to measure the preparation depth. Samples were randomly allocated into three groups according to the retrograde filling material used.

Groups:

[1] Group 1: Samples filled with ProRoot MTA
[2] Group 2: Samples filled with MTA Angelus
[3] Group 3: Samples filled with Biostructure MTA Putty

Samples were coated with 2 layers of nail varnish to the external surface of each root except for the apical section (2mm) and all the samples were stored in 100% humidity for 24 h. The specimens were suspended in 1% methylene blue for 24 h prior to the analysis. Following these steps, the samples were rinsed under running water for 15 min. longitudinal sections of each tooth was prepared by using a diamond disc (NMD double-sided diamond disc). These specimens were examined using stereomicroscope under 2 x magnifications the NIT, Calicut.

Results:

The collected data were entered into Microsoft Excel and analysed using appropriate statistical software. The normality of dye penetration values in each group was assessed using the Shapiro–Wilk test. Since the data followed a normal distribution, parametric tests were applied **(Table 1)**. Descriptive statistics, including mean, standard deviation, minimum and maximum values, were calculated for each group **(Table 2)**. Intergroup comparison of dye penetration values among ProRoot MTA, MTA Angelus and Biostructure MTA Putty was performed using one-way analysis of variance (ANOVA). When a statistically significant difference was detected, post-hoc pairwise comparisons were carried out using Tukey's Honestly Significant Difference (HSD) test to identify specific group differences. A p-value of less than 0.05 was considered statistically significant. Among the three groups, Group 1 (ProRoot MTA) showed the lowest mean dye penetration (1.273 ± 0.102 mm), while Group 2 (MTA Angelus) exhibited the highest mean value (1.592 ± 0.094 mm). Group 3 (Biostructure MTA Putty) showed a mean dye penetration of 1.558 ± 0.155 mm, which was intermediate between the other two groups. One-way analysis of variance (ANOVA) was performed to compare dye penetration values among the three groups **(Table 3).** The results revealed a statistically significant difference between the groups ($F = 21.204$, $p < 0.001$), indicating that at least one group differed significantly from the others in terms of dye penetration. Post-hoc multiple comparisons using Tukey's HSD test was performed to identify intergroup differences in dye penetration values **(Table 4).** The results showed no statistically significant difference between Biostructure MTA Putty and MTA Angelus ($p = 0.799$). However, ProRoot MTA demonstrated significantly lower dye penetration compared with both Biostructure MTA Putty and MTA Angelus ($p < 0.001$). Additionally, MTA Angelus exhibited significantly higher dye penetration than ProRoot MTA ($p < 0.001$). Post-hoc Tukey analysis revealed that ProRoot MTA had significantly lower dye penetration compared with both MTA Angelus and Biostructure MTA Putty, while no significant difference was observed between MTA Angelus and Biostructure MTA Putty. Effect size analysis demonstrated a large magnitude of difference among the groups, indicating that the type of material had a substantial influence on microleakage. The mean dye penetration values are illustrated in **Figure 1**, while **Figure 2** summarizes the group means ± SD together with the observed minimum–maximum ranges.

Table 1: Normality of dye penetration values

Group	Shapiro–Wilk W	p value	Interpretation
Group 1 — ProRoot MTA	0.949	0.656	Normal
Group 2 — MTA Angelus	0.88	0.13	Normal
Group 3 — Biostructure MTA Putty	0.975	0.934	Normal

Table 2: Dye penetration (mm)

Group	Mean ± SD	Minimum	Maximum
Group 1 — ProRoot MTA	1.273 ± 0.102	1.116	1.411
Group 2 — MTA Angelus	1.592 ± 0.094	1.492	1.733

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Group 3 — Biostructure MTA Putty	1.558 ± 0.155	1.323	1.832

Table 3: ANOVA—Comparison of dye penetration among groups

Source of Variation	SS	df	MS	F value	p value
Between Groups	0.613	2	0.307	21.204	<0.001*
Within Groups	0.391	27	0.014		
Total	1.004	29			

Table 4: Post-hoc Comparison (Tukey HSD)

Comparison	Mean Difference	p value	95% CI (Lower, Upper)	Significance
Biostructure MTA Putty vs MTA Angelus	0.035	0.799	-0.099, 0.168	Not Significant
Biostructure MTA Putty vs ProRoot MTA	-0.285	<0.001*	-0.418, -0.151	Significant
MTA Angelus vs ProRoot MTA	-0.319	<0.001*	-0.453, -0.186	Significant

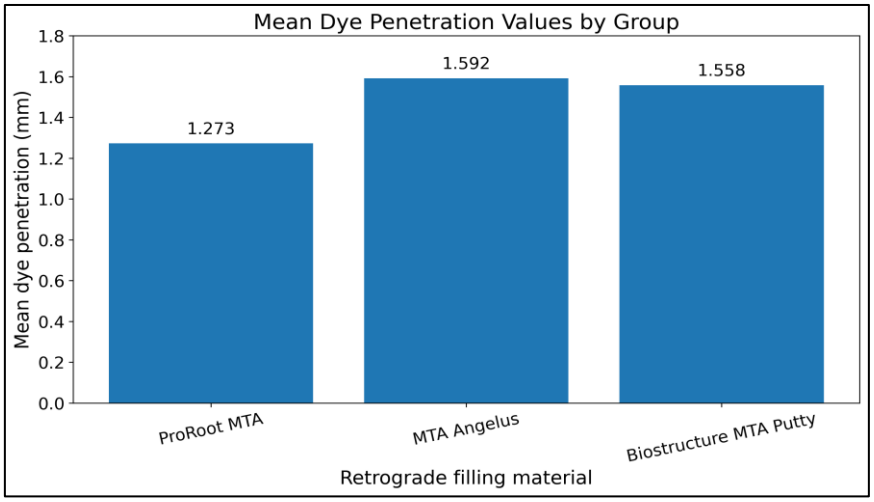

Figure 1: Mean dye penetration values (mm) for ProRoot MTA, MTA Angelus and Biostructure MTA Putty

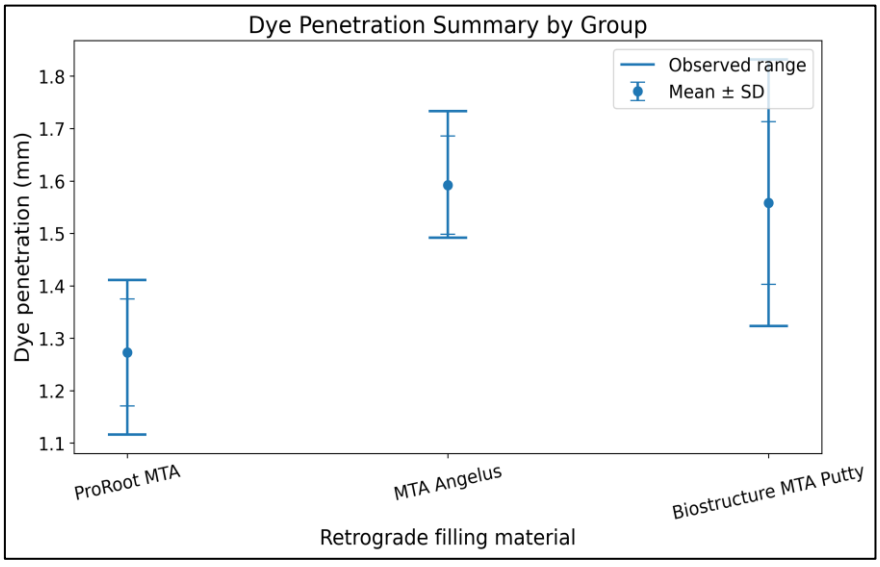

Figure 2: Dye penetration summary showing the group means ± standard deviation and the observed minimum–maximum ranges

Discussion:

MTA contains calcium-silicate phases and has been widely used for vital pulp therapy, apexification, perforation repair and root-end filling. Its biological and sealing properties have been extensively reviewed **[32]**. Clinical and laboratory investigations have also compared MTA with other root-end filling materials **[33]**. Experimental studies have evaluated its marginal adaptation and sealing behavior **[34]**. Other work has examined the physical and biological properties that may contribute to its clinical performance **[35]**. Bacterial leakage models have

provided additional evidence regarding the sealing ability of ProRoot MTA **[36]**. Tissue-response studies have further supported the biological acceptability of MTA-based materials **[37]**. Material characterization research has examined their structural and physicochemical stability **[38]**. Laboratory studies have also investigated leakage and adaptation at the root-end interface **[39]**. The interaction of MTA with surrounding tissues and dentin has been evaluated in experimental settings **[40]**. Clinical comparisons have contributed to the evidence base for calcium-silicate root-end filling materials **[41]**. Earlier endodontic leakage studies used several experimental approaches to compare root-end materials **[42]**. Bacterial penetration models were used to examine the integrity of apical seals **[43]**. Other studies used quantitative methods to compare leakage between endodontic materials **[44]**. Additional laboratory work assessed the sealing behavior of root-end fillings under controlled conditions **[45]**. Foundational studies helped establish the sealing potential of mineral trioxide aggregate **[46]**. Dye penetration methods also contributed to early comparative evaluation of apical microleakage **[47]**. Further investigations compared MTA with established retrograde filling materials **[48]**. Early work on MTA provided evidence regarding its ability to limit leakage at the root end **[49]**. These earlier comparisons provided an important basis for later studies of retrograde filling materials **[50]**. The long-term success of endodontic treatment is largely dependent on the ability of the root-end filling material to establish an effective seal and prevent microleakage. Microleakage allows the passage of microorganisms, fluids and their by-products into the periapical tissues and may compromise treatment outcomes **[51]**. In root-end resection, the resection plane should be as perpendicular as possible to the long axis of the root. Kim and Kratchman emphasized modern microsurgical principles, including appropriate apical resection **[52]**. Related surgical guidance also supports minimizing the bevel angle to preserve root structure and reduce exposed dentinal tubules **[53]**. In the present study, ED10D and ED11D ultrasonic tips were used for root-end cavity preparation. Ultrasonic root-end preparation has been associated with improved access and conservative cavity geometry **[54]**. The present results showed statistically significant differences in dye penetration among the tested materials. ProRoot MTA exhibited the lowest mean dye penetration, indicating superior sealing ability compared with MTA Angelus and Biostructure MTA Putty. This finding is consistent with the favorable sealing characteristics generally described for MTA-based materials **[32]**. The superior performance of ProRoot MTA may be related to its hydrophilicity, setting behavior and close adaptation to dentinal walls **[29]**. Previous bacterial leakage work has likewise supported the ability of ProRoot MTA to provide a stable apical seal **[36]**. In contrast, MTA Angelus showed the highest mean dye penetration in the present experiment, while Biostructure MTA Putty showed an intermediate value. The lack of a significant difference between MTA Angelus and Biostructure MTA Putty indicates broadly comparable sealing performance under the present test conditions. Microleakage was observed in all groups, which is consistent with the limitation that no root-end material can be assumed to create an absolutely hermetic seal **[9]**. Singh *et al.* evaluated the sealing ability of MTA Angelus, MTA Plus and ProRoot MTA using a dye penetration technique and stereomicroscopic assessment. They reported the least microleakage with MTA Angelus, followed by MTA Plus and ProRoot MTA. In contrast, the present study showed significantly lower dye penetration with ProRoot MTA than with MTA Angelus and Biostructure MTA Putty, while no significant difference was observed between MTA Angelus and Biostructure MTA Putty **[55]**. Kumar *et al.* compared the sealing ability of Bio-C Repair, MTA Angelus, GC Fuji VII and TheraCal LC using dye penetration and stereomicroscopic evaluation in furcation repair. MTA Angelus showed an intermediate level of microleakage, while Bio-C Repair showed the lowest microleakage. Although the clinical application and materials differed from the present root-end filling study, these findings further demonstrate that sealing performance varies among calcium silicate-based repair materials **[56]**.

Conclusion:

ProRoot MTA showed the least dye penetration and the best sealing ability among the tested materials. MTA Angelus and Biostructure MTA Putty showed greater and comparable dye penetration. These findings support ProRoot MTA as the most effective retrograde filling material under the present *in vitro* conditions.

Limitations:

The limited evaluation period here as the materials were evaluated after a relatively short experimental period. Long-term changes in sealing ability due to material degradation, dissolution, or interaction with tissue fluids were not assessed. Operator-dependent factors not assessed, though the study was performed under controlled experimental conditions and factors such as operator technique, clinical accessibility and handling characteristics of the materials were not evaluated.

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