ORIGINAL RESEARCH

An *Ex-vivo* Evaluation of Sealability of Three Bioceramic Physical Variants in Coronal and Apical Thirds of Root Canals

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Abstract

Aim: To assess the coronal and apical microleakage of mineral trioxide aggregate (MTA), Biodentine, and Bioceramic putty when used as coronal and apical seals in endodontically treated teeth.

Materials and methods: The study was conducted on 90 mandibular premolar teeth. The groups were divided into two subgroups. One group assessed the coronal seal ($n = 45$), and the other group assessed the apical seal ($n = 45$). Coronal and apical 3 mm of the root filling was removed and replaced randomly with MTA, Biodentine, and Bioceramic putty in the three experimental subgroups ($n = 15$). All teeth were immersed in methylene blue and after incubation, the degree of dye penetration was assessed under a stereomicroscope of ×10 magnification. Statistical analysis was done using the Kruskal–Wallis test with a p-value set at 0.05.

Results: The mean depth of dye penetration for coronal sealing in the MTA group was 2.91 ± 0.66, in the Biodentine group was 2.12 ± 0.50 and in the Bioceramic putty group was 1.51 ± 0.46. The mean depth of dye penetration for root-end sealing in the MTA group was 0.75 ± 0.55, in the Biodentine group was 1.94 ± 0.88 and in the Bioceramic putty group was 0.29 ± 0.20.

Conclusion: Within the limitations of this study, it can be concluded that Bioceramic putty exhibited better sealing ability in both coronal and apical barriers of root canals.

Clinical Significance: The sealing ability of a material vastly influences the treatment outcome of nonsurgical and surgical root therapy. The study evaluates the coronal and apical seal of three physical variants of Bioceramic material.

Keywords: Bioceramic putty, Biodentine, Microleakage, Mineral trioxide aggregate, Sealing ability.

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Introduction

Periapical pathosis is primarily caused by bacteria and their byproducts from infected dental pulp. Eradication of bacteria and prevention of re-infection are the two main objectives of root canal therapy. According to Ray and Trope, periradicular health depends more on the quality of the coronal restoration seal than the filling of the root canal and apical seal.[1,2] As the primary reason for non-surgical root canal treatment failure most of the time is a poor coronal seal as stated above, an effective technique to minimize the coronal pathway in endodontically treated teeth is the placement of an intra-orifice barrier.[3–12]

Endodontic failure can be caused by a variety of factors, such as inadequate cleaning and shaping that allows microorganisms to persist, under or over obturation, untreated canals, procedural or iatrogenic errors, perforations, ledges, apical transportation, or separated instruments. Additionally, the prognosis of endodontic treatment is linked to the tooth's preoperative condition.[13–17]

One major factor contributing to treatment failure is microleakage. The avoidance of such leakage must be given special consideration during and after root canal therapy by closely monitoring the tooth's sealing. In the future, reducing microleakage might be aided by the application of chemically active, sticky root-canal sealers.

The three-dimensional obturation of root canals to provide a fluid-tight hermetic seal is the final objective for a successful endodontic treatment outcome. "The complete three-dimensional filling of the root canal and all accessory canals should be the goal of root canal procedures," according to Schilder. A well-filled three-dimensional root canal eliminates periapical exudates from percolating and microleaking into the root canal area, inhibits reinfection, creates a favorable biological environment, and promotes complete resolution of periapical pathosis.[9]

Root-end resection is commonly performed after non-surgical methods fail to remove a periapical lesion. An effective apical seal is crucial for this surgical procedure.[1]

Calcium silicate-based bioactive materials have gained considerable importance as root-end-filling materials. A lot of such materials have come into the market on account of their desirable properties and predictable treatment outcomes.[1,12]

The biocompatibility and relatively high material density of MTA have shown better treatment outcomes. However, the

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Flowchart 1: Study design for the methodology followed

90 single rooted mandibular premolars were selected for the study

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Divided into two groups of 45 each (for assessement of coronal and apical leakage)

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Each main group of 45 divided into 3 subgroups of 15 each (MTA, Biodentine and Bioceramic putty)

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The teeth sectioned at a standard measurement of 14 mm coronally from the apex, perpendicular to the long axis of the root

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Cleaning and shaping followed by obturation with gutta-percha and zinc oxide eugenol-based root canal sealer

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MTA, Biodentine and Bioceramic putty placed in coronal and apical parts after removal of 3 mm GP from both the ends in respective groups

↓

Double coating of nail varnish, leaving a 1 mm perimeter around the tooth-restoration site untouched
Specimens were exposed to 2% methylene blue for 48 hours and withdrawn from the dye solution, rinsed with tap water, and longitudinally sectioned

↓

Depth of dye penetration was examined under stereomicroscope and measured in mm using ImageJ software

↓

Statistical analysis performed and results tabulated

time required for conventional MTA to set is four hours, which is considered to be too long, as it might allow the entry of tissue fluid and microorganisms into the root canal space, thus interfering with the prognosis; and the manual manipulation of the material can also lead to differences in treatment outcome.[13]

This study assessed the sealing capability of three bioactive materials when used for root-end filling and coronal seal as well (Flowchart 1).

Materials and Methods

This research was an experimental laboratory study conducted in the year 2024 during the period from October to November.

The sample size for the present study was estimated using G*Power software (latest ver. 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, üsseldorf, Germany).

Ninety extracted single-rooted permanent mandibular premolars with a single root canal were selected for this study.

Two main groups of 45 each were created for coronal seal and apical seal assessment.

Each group was further divided as follows:

- Group A—Mineral Trioxide Aggregate ($n = 15$).
- Group B—Biodentine ($n = 15$).
- Group C—Bioceramic putty ($n = 15$).

The study was conducted after obtaining approval from the Institutional Ethical Committee with IEC Approval Number SRMU/M&HS/SRMDC/2024/S/006.

Inclusion criteria included patent single canal, completely formed root, normal anatomy, absence of caries, and absence of root canal fillings.

Exclusion criteria included a tooth with a fractured root, evidence of craze line, evidence of any resorption, and incomplete apex formation.

The samples were cleaned to remove visible debris, tissue remnants, and calculus. They were then placed in 5.25% sodium hypochlorite for 2 hours and stored in normal saline for further use. The coronal portion above the cementoenamel junction (CEJ) was sectioned with a diamond disk at a standard measurement of 14 mm from the apex, perpendicular to the long axis of the root. The pulp tissue was removed with a barbed broach, and the canals were checked for patency using a #10 K-type file. Cleaning and shaping were performed using the crown-down technique with NeoEndo rotary files up to 3006. A #10 K-type file was used to ensure the patency of the apical foramen. Canals were irrigated with 5.25% sodium hypochlorite and 17% ethylene diamine tetra-acetic acid (EDTA), followed by final irrigation with normal saline. The absorbent paper points were utilized to dry the canals and the obturation was completed with corresponding gutta-percha and zinc oxide eugenol-based root canal sealer.

Ultrasonic tips were used with light pressure to prepare root end cavities of 3 mm depth and 1mm width. The dimensions were checked using a periodontal probe.

Forty-five teeth specimens were allotted in three groups ($n = 15$) at random. Root end preparations were restored with a respective retrograde filling material following the manufacturer's instructions. Except for the resected apical portion, all specimens were painted with two layers of clear nail varnish for sealing all possible portals of communication with the root canals. The groups were divided as follows:

- Group A—Mineral trioxide aggregate (MTA Angelus, Brazil) $n = 15$.
- Group B—Biodentine (Septodont, France) $n = 15$.
- Group C—Bioceramic putty (Endosequence Root Repair Material, Brasseler, USA) $n = 15$.

All samples were placed in vertical positions for Group I, Group II, and Group III, in different containers containing 2% methylene blue dye for 48 hours at 37 °C and 100% humidity. Thereafter, the samples were removed, rinsed for 15 minutes under running tap water, and air-dried. A calibrated stereo microscope was utilized to measure the linear dye penetration to its furthest extent coronally within the root-end cavity.

To assess the coronal seal, the coronal aspect of the gutta-percha was adjusted to terminate 3 mm apical to the level of decoration, as indicated by a periodontal probe. The upper 3 mm of the canal was meticulously debrided of gutta-percha and sealer using an alcohol-moistened pellet. Subsequently, the area was rinsed with sterile saline and dried with an air stream.

Experimental groups were as follows:

- Group I—3 mm barrier of MTA.
- Group II—3 mm of Biodentine.
- Group III—3 mm of Bioceramic putty (BC putty).

Table 1: Comparison of the mean depth of dye penetration for root end sealing b/w three groups using Kruskal–Wallis test

Groups	*N*	*Mean*	*SD*	*Min*	*Max*	*p-value*
MTA	15	0.75	0.55	0.2	2.1	<0.001*
Biodentine	15	1.94	0.88	0.2	3.0	
Bioceramic putty	15	0.29	0.20	0.1	0.8	

*Statistically significant

To establish an effective apical seal, all specimens underwent full immersion in molten sticky wax up to the cementoenamel junction (CEJ). Subsequently, the group samples received a double coating of nail varnish, leaving a 1 mm perimeter around the tooth-restoration site untouched.

Following this, the specimens were exposed to a 2% methylene blue solution under conditions of 100% relative humidity at 37 °C for 48 hours. Post-exposure, the specimens were withdrawn from the dye solution, rinsed with tap water, and longitudinally sectioned in a mesiodistal direction using a low-speed diamond saw after the removal of the wax and nail varnish.

Analysis of Outcome

Dye infiltration was inspected utilizing a stereomicroscope (Zeiss, Munich, Germany) at ×10 magnification, and the extent of dye penetration was assessed by measuring the vertical extent of dye penetration millimeters using ImageJ software. The values were tabulated and subjected to statistical analysis.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses.

Shapiro-Wilk Test, the normality test revealed that the depth of dye penetration for root-end and coronal sealing scores did not follow normal distribution. Hence, the inferential statistics were dealt with using relevant non-parametric tests.

Kruskal–Wallis test followed by Dunn's *post hoc* test was used to compare the mean depth of dye penetration for root-end and coronal sealing between three groups.

The level of significance was set at $p < 0.05$.

Results

The mean depth of dye penetration for root-end sealing within the MTA group was 0.75 ± 0.55, within the Biodentine group, was 1.94 ± 0.88 and within the Bioceramic putty group was 0.29 ± 0.20, as detailed in Table 1 (intragroup comparison).

There was a significant difference in the mean depth of dye penetration between the three groups at $p < 0.001$.

Multiple comparisons of mean differences between groups (Intergroup comparison) revealed that Bioceramic putty showed significantly least depth of dye penetration as compared to MTA and Biodentine and the mean differences were statistically significant at $p = 0.004$ and $p < 0.001$ as depicted in Table 2. This was then followed next by MTA which showed significantly lesser mean depth of dye penetration as compared to Biodentine and the mean difference was statistically significant at $p = 0.001$. This infers that the mean depth of dye penetration for Root-end sealing was significantly least in Bioceramic putty, followed by MTA, and highest in Biodentine.

Table 2: Multiple comparisons of mean diff. in the depth of dye penetration for root end sealing b/w three groups using Dunn's *post hoc* test

(I) Groups	*(J) Groups*	*Mean difference (I–J)*	*95% CI for the difference*		*p-value*
			Lower	*Upper*	
MTA	Biodentine	–1.19	–1.73	–0.65	0.001*
	Bioceramic Putty	0.46	–0.08	1.00	0.004*
Biodentine	Bioceramic Putty	1.65	1.11	2.19	<0.001*

*Statistically significant

Table 3: Comparison of the mean depth of dye penetration for coronal sealing b/w three groups using the Kruskal–Wallis test

Groups	*N*	*Mean*	*SD*	*Min*	*Max*	*p-value*
MTA	15	2.91	0.66	2.0	4.1	<0.001*
Biodentine	15	2.12	0.50	1.6	3.0	
Bioceramic putty	15	1.51	0.46	1.0	2.3	

*Statistically significant

Table 4: Multiple comparisons of mean difference in depth of dye penetration for coronal sealing b/w three groups using Dunn's *post hoc* test

(I) Groups	*(J) Groups*	*Mean difference (I–J)*	*95% CI for the difference*		*p-value*
			Lower	*Upper*	
MTA	Biodentine	0.79	0.31	1.28	0.001*
	Bioceramic putty	1.41	0.92	1.89	<0.001*
Biodentine	Bioceramic putty	0.61	0.13	1.10	0.004*

*Statistically significant

The mean depth of dye penetration for coronal sealing in the MTA group was 2.91 ± 0.66, in the Biodentine group was 2.12 ± 0.50 and in the Bioceramic putty group was 1.51 ± 0.46 as shown in Table 3. There was a significant difference in the mean depth of dye penetration between the three groups at $p < 0.001$.

Inference of the Study

Multiple comparisons of mean differences between groups revealed that Bioceramic putty showed significantly least depth of dye penetration as compared to MTA and Biodentine and the mean differences were statistically significant at $p < 0.001$ and $p = 0.004$ as detailed in Table 4. This was then followed next by Biodentine which showed significantly lesser mean depth of dye penetration as compared to MTA and the mean difference was statistically significant at $p = 0.001$. This infers that the mean depth of dye penetration for Coronal sealing was significantly least in Bioceramic putty, followed by Biodentine, and highest in MTA.

Discussion

Need for the Above Study

The evolution of bioactive, bioceramic materials for endodontics has revolutionized the practice of endodontics and has created a paradigm shift in treatment protocols. However, the continuous improvements in materials and resulting products have encouraged further research to assess the physical and chemical properties along with their biological interactions with the tissues. Hence this study was undertaken to assess a new physical variant of a premixed bioactive bioceramic material with a thicker consistency named as bioceramic putty.

A durable seal is paramount to minimize contamination during and after endodontic therapy. Root-filling materials like gutta-percha and sealer do not offer much resistance to bacterial microleakage. Therefore, it is important to tightly seal the coronal part of the root canal to reduce the failure rate of endodontic treatment.[17–23] The dye penetration test is commonly used to study leakage due to its ease of conduct, low cost, and high staining ability. Low-weight dye molecules can penetrate locations that bacterial cells cannot, making *in vitro* microleakage studies with low-molecular-weight dyes or isotopes more severe than clinically relevant studies. Though the limitation of dye leakage studies is that they only measure leakage in one plane making it hard to assess the total amount of leakage, these studies are still in use on account of their feasibility and have stood the test of time.[4]

Mineral trioxide aggregate consists of 75% Portland cement, 20% bismuth oxide, and 5% gypsum by weight. It was developed at Loma Linda University by MA Torabinejad in 1993 as a root end-filling material, MTA is available as grey and white MTA. It possesses an antimicrobial effect, biocompatibility, superior sealing ability, radiopacity, and tolerance to moisture.[22] The first bioceramic bioactive material to be made available for endodontics was MTA in 1992. Hence on account of its longtime clinical use and predictable treatment outcomes MTA was chosen as one of the materials for this study.

A major disadvantage of MTA is accredited to its long setting time. However recent development of similar improvised materials have overcome this disadvantage.[3,16] Biodentine (Septodont) is one such material and is considered a second-generation bioceramic material. It has properties similar to MTA. Its advantages over MTA are that it sets in a shorter period (approximately 10–12 min) and has a compressive strength similar to dentin.[6]

EndoSequence BC RRM putty was introduced to the market to offer a premixed, ready-to-use option for various endodontic procedures, including root repair, pulp capping, and apexification.[19] The development focused on addressing the need for a material that is not only biocompatible and bioactive but also easy to handle and apply directly from the syringe without the need for mixing.[19] These bioceramic materials have antimicrobial properties and good seal against microleakage which is beneficial when used as intra-orifice barriers.[4,19]

In the present study, MTA, Biodentine, and BC putty were compared in terms of their coronal as well as sealing ability. The results indicated that all materials exhibited some level of leakage. However, BC putty showed the least coronal leakage in both coronal and apical, followed by MTA and Biodentine. This finding suggests that BC putty, with its nanospherical particles (approximately 10^{-3} µm), may offer superior sealing by penetrating dentinal tubules more effectively and forming a micromechanical bond upon setting. This facilitates better adaptation and hydration. The higher surface-to-volume ratio may lead smaller particles to penetrate tubules and hydrate faster than larger particles. This observation is consistent with studies such as those by Ray and Trope[2] who noted that smaller particle sizes in bioceramic materials contribute to better adaptation and reduced leakage.[8]

The improved performance of MTA can be attributed to its exceptional marginal sealing ability, stemming from its hydrophilic properties and the establishment of an interfacial layer between the material and dentin.[8] A recent study by Antunes et al.[11] compared the *in vitro* sealing ability of root-end fillings with MTA and EndoSequence BioCeramic Root repair material-fast set (BC-RRM) putty using bacterial nutrient leakage model which concluded both had similar sealing ability.[21]

Camilleri[6] analysis of composition in MTA and Biodentine found that tricalcium silicate was the main constituent of Biodentine which was denser and showed decreasing porosity over some time. The density and porosity of the sealing materials are crucial elements that determine the extent of leakage and the effectiveness of the treatment. A larger pore diameter leads to greater leakage. Initial porosity is an inherent property of tricalcium silicate-based cement and arises from the gaps between the cement grains that remain before hydration. Upon hydration of the material, the void spaces become saturated with water. Subsequently, as the hydration reaction advances, the resultant products fill these voids, leading to a reduction in porosity. In a study by Soundappan et al.[10] Biodentine which is a fast-setting tricalcium silicate-based material showed inferior marginal adaptation when compared to MTA due to the absence of the possible setting expansion, which is exhibited slightly by MTA. Additionally, Kaur et al.[14] observed that MTA had less microleakage than Biodentine using fluid filtration methods, which supports the finding of superior sealing ability for MTA. This could explain the higher dye penetration in Biodentine and the inferior sealing ability of Biodentine compared to MTA.[18]

Some previous studies comparing the sealing ability, microleakage, and marginal adaptation of Biodentine and MTA have yielded conflicting results. Certain studies have demonstrated that MTA exhibits superior sealing ability and marginal adaptation compared to Biodentine when utilized as a root-end filling material or for furcal perforation repair.[4,15] Conversely, other studies have suggested that the marginal adaptation and sealing ability of Biodentine surpass those of MTA when employed as a root-end filling material.[20] The cause of these conflicts may be due to differences in the methods of investigation.[5]

Long-term clinical studies are needed to assess the material's performance *in vivo* and validate *ex-vivo* findings. Microbial penetration studies can evaluate their ability to prevent bacterial ingress, a critical aspect of root canal treatment success. Evaluating the sealing ability in various root canal morphologies, including complex and curved canals, will ensure broad applicability. Standardizing testing protocols for evaluating sealing ability will ensure consistency and comparability across studies, advancing the understanding and clinical application of bioceramic materials in endodontics, and ultimately improving patient outcomes.

Limitations of the Study

A limitation of this study is that sectioning of the tooth could have led to the development of cracks, and fissures and also caused material drag to appear which may have impacted the resultant marginal adaptation of the materials assessed through stereomicroscope. However, this methodology is commonly employed in many studies as it is reliable, cost-effective, and does not involve elaborate and cumbersome procedures for testing the sealing ability of materials. The outcomes of this *in vitro* study cannot be extrapolated to *in vivo* studies as the conditions are different in clinical scenarios. However, advanced imaging techniques like nano-CT can offer a detailed three-dimensional understanding of their adaptation to root canal walls. Comprehensive biocompatibility and cytotoxicity studies are essential for ensuring patient safety.[20] It can be emphasized that other methods of testing such as bacterial leakage assessment, protein leakage assessment computerized fluid filtration method, and scanning electron microscopy may be used in further studies to validate the findings of this study.

Conclusion

The introduction of bioactive bioceramic materials such as MTA and Biodentine was a breakthrough in dental material science and endodontics. Many modifications to improve formulation and consistency are ongoing to overcome the limitations of these materials. One such improvised material, the pre-mixed bioceramic putty has better-handling properties on account of its consistency with resultant improved qualities over earlier bioactive materials.[20] Because bioceramic putty is new, more studies are needed to gain evidence regarding optimum treatment outcome in the future. Under the limitations of the present study, it can be concluded that bioceramic putty resulted in the best coronal and apical sealing ability when compared to MTA and Biodentine.

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