

Efficacy of Mineral Trioxide Aggregate and Biodentine as Apical Barriers in Immature Permanent Teeth: A Microbiological Study

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ABSTRACT

Aim: To compare the bacterial leakage of mineral trioxide aggregate (MTA) and biodentine when used as an apical plug in immature permanent teeth.

Materials and methods: It was a randomized double-blind *in vitro* study. A total of 60 teeth were divided into 2 groups of MTA and biodentine, which were further divided into 2 subgroups ($n = 30$) each based on the apical plug thickness of 2 and 4 mm. The teeth were cleaned and shaped; root-end resection and canal preparation were done. Mineral trioxide aggregate and biodentine were mixed and filled as apical plugs of 2 or 4 mm thickness. *Enterococcus faecalis* was used to assess the bacterial leakage of the filled samples.

Statistical analysis: The comparison between the two groups was done by Chi-square test for categorical data. All p values <0.05 were considered as statistically significant.

Results: A 4 mm apical plug of biodentine showed the least amount of bacterial leakage followed by 2 mm MTA and 4 mm MTA. A 2 mm apical plug of biodentine showed the maximum bacterial leakage. But this was not statistically significant over a period of 3 months. There was a statistically significant difference (p value = 0.042) among the total number of samples that leaked in the 2 and 4 mm biodentine group.

Conclusion: Mineral trioxide aggregate and biodentine had a similar apical sealing ability. The apical sealing ability of biodentine at 4 mm thickness was greater than 2 mm thickness.

Clinical significance: The apical leakage of the materials used in apexification is one of the main causes of endodontic failures in immature necrotic teeth. Materials like MTA and biodentine have overcome various drawbacks of calcium hydroxide as apexification material. The thickness of the apical plug plays an important role in preventing any microorganism from entering the periapical area, hence maintaining an adequate seal.

Keywords: Apical plugs, Bacterial leakage, Biodentine, *Enterococcus faecalis*, Mineral trioxide aggregate.

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INTRODUCTION

Dental trauma in children and adolescents may cause pulp necrosis in immature teeth.¹ These necrotic immature young permanent teeth have their root development interrupted, leaving unsealed apices and weak dentinal walls that are challenging to deal with conventional endodontic treatment.² Endodontic treatment of immature teeth is a difficult task.³ The inadequate apical stop prevents complete debridement of the canal that limits the procedure of obturation.³ The mainstay of treatment for such immature teeth include apexification procedures.⁴

The most common material used for multiple visit apexification is calcium hydroxide. Although this forms a physiological hard tissue barrier, it also has few shortcomings like increased chances of root fracture, prolonged treatment time, and coronal microleakage.³ These factors have motivated clinicians to look for other alternatives. The use of various materials like dentinal chips, hydroxyapatite crystals, Portland cement, calcium sulfate, mineral trioxide aggregate, and biodentine enables us to overcome the drawbacks of calcium hydroxide.³

Mineral trioxide aggregate (MTA), being an osteoconductive apical plug, has made one visit apexification an increasingly popular procedure.⁵ Properties like a good sealing ability, setting in the presence of blood and biocompatibility makes MTA a right candidate for an apical plug.⁶ Its high success rates reported in the studies have encouraged its use in immature necrotic

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permanent teeth. However, it also has certain handicaps such as extended setting time, poor handling, and inflated cost.⁶ Therefore, surmounting these issues is a new biomaterial named biodentine.

Biodentine has prominent clinical features as improved sealing ability, enhanced compressive strength, reduced porosity, greater density, bioactivity, rapid formation of calcium hydroxide, biomineralization capability, biointeractivity, and color stability compared to mineral trioxide aggregate.^{5,7,8} The endodontic implications of biodentine are similar to MTA. They thus can be used as a root-end filling material.⁵ Nevertheless, there are restricted

studies on its use for apexification and comparison of its sealing ability with MTA.

To achieve a satisfactory outcome after an apexification procedure, the material must have an excellent apical seal. The sealing ability defines the capability of a material to restrict the microleakage throughout its entire thickness. Deficient apical seal leads to microleakage and is one of the major causes of surgical endodontic failure. Various techniques for assessing microleakage include the utilization of dyes, irradiated isotopes, air under pressure, fluid filtration, bacteria, neutron activation analysis, artificial caries, scanning electron microscopy, and other methods.⁸

The molecular size of radioisotope tracers and dye particles being smaller than that of bacteria is a major drawback of the radioisotope and dye penetration technique.⁹ Fluid filtration through a non-destructive method lacks standardization in the materials and methods used. Besides, equipment and devices may not be found everywhere.¹⁰ In the bacterial microleakage technique, bacteria are used as markers. Bacterial culture is placed engaging the coronal part of the tooth, and the apical tip of the sample is in touch with the sterile culture medium, the root canal filling is the only path through which the bacterial culture (upper chamber) can reach the sterile culture medium (lower chamber). This assembly is incubated at 37°C, and the samples are monitored every day to assess turbidity in the lower chamber. Turbidity of the culture medium is a marker of root canal contamination. The bacterial leakage technique is a more clinically relevant approach to assess apical leakage.¹⁰

Enterococcus faecalis (*E. faecalis*) is the organism used to assess bacterial leakage as it is the most common organism to be detected in endodontic failures.⁹ This gram-positive, facultative, anaerobic organism can persist with an inadequate source of nutrition and can invade the dentinal tubules.¹¹ Therefore, the bacterial leakage of the MTA and biodentine apical plugs were carried out using *E. faecalis*. Thus, the main motive of this *in vitro* study is to compare the apical barriers of MTA and biodentine in immature permanent teeth.

MATERIALS AND METHODS

The study protocol was analyzed and accepted by the ethical committee. Teeth were chosen based on the following inclusion criteria: single-rooted mature permanent teeth. Teeth with cracks or fractures, root canal calcification, and external root resorption were excluded.

Sample Size Calculation

A sample size calculator (Sample size Determination in Health Studies, World Health Organization) was used for sample size calculation as follows. Power at 90% and β at 10%, the sample size

proved to be 15 teeth in each group. Therefore, an aggregate of 60 single-rooted teeth was needed for the study.

PROCEDURE

Preparation of Teeth

The chosen samples were immersed in 5.25% sodium hypochlorite (NaOCl) for an hour and then stored in normal saline before the experiment. The apices were cut off by a diamond disk, 2 mm from the apical root end. Root canal lengths were calibrated by manually inserting #15 K-files (Mani, Japan) into the canals. The root canals were enlarged using rotary NiTi files S1, S2, F1, F2, F3 files in compliance with the manufacturer's instructions. The canals were irrigated using 1.0 mL of 3% NaOCl. After mechanical canal preparation, 17% EDTA was used for 30 seconds, followed by a 30 seconds irrigation with 3% NaOCl. Finally, the canal was flushed using 5.0 mL of sterile saline solution.

Experimental Design

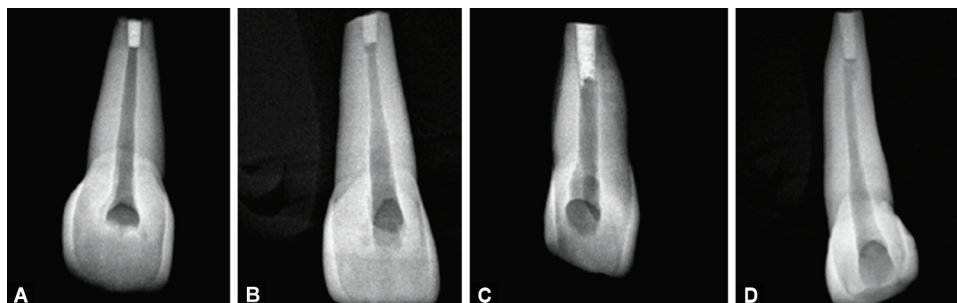
Prepared root canals were allocated to distinct groups by randomization with the help of a computer-generated table of random numbers. All the samples were blinded with the help of coding. Overall, 60 teeth were distributed into 2 groups of (group I) MTA (Angelus, Brazil) and (group II) biodentine (Septodont, India) and 4 subgroups:

- Group IA (MTA 2 mm).
- Group IB (MTA 4 mm).
- Group IIA (biodentine 2 mm).
- Group IIB (biodentine 4 mm).

Retrograde preparation was done using a TF12 bur (Mani, Japan) up to the desired length (2 or 4 mm) needed, based on the group to which the teeth were allocated. Gutta-percha points with 6% taper were inserted into each tooth and were pushed apically till a snug fit was obtained. The excess gutta-percha extruding out from the apical part was cut off with a blade. Based on the 2 or 4 mm group the gutta-percha point was cut off 2 or 4 mm short to act as an individualized plugger. The MTA was carried using the ortho MTA carrier (BioMTA, Korea), whereas biodentine was carried using the plastic carrier and was condensed from the apical end using a graduated hand plugger and placed in 2 or 4 mm thickness as apical barriers. Subsequently, a moistened paper point was positioned into the root canals and, a periapical radiograph was used to confirm the density and thickness of apical barriers formed (Fig. 1).

Storage of Prepared Teeth

All teeth were preserved at 37°C in 100% humidity for 24 hours. Three coats of nail polish were applied to the external root surfaces



Figs 1A to D: Radiographs were taken after placement of biodentine and mineral trioxide aggregate

of the samples, excluding 2.0 mm around the root apex. The prepared teeth were assessed for bacterial leakage after 48 hours.

Bacterial Leakage Technique

First, the bottoms of the Eppendorf test microtubes were cut with the blade. The teeth, along with hot sticky wax on their coronal half of the root surfaces were inserted into the cut microtubes with 2–4 mm of the apical part of root extruding outside the cut portion of the microtube. The intersection between tubes and teeth was sealed internally by sticky wax. The assemblies were sterilized in the ethylene oxide sterilizer for 8 hours. Brain heart infusion broth culture medium was added to the test tube, in a way that the 10–15 mm of the tubes will remain empty for placing the assemblies. Afterward, tubes containing culture mediums are sterilized in an autoclave. The root-microtube assemblies were settled inside test tubes with the root tips in contact with the culture medium. The junction between microtubes and test tubes were sealed using parafilm tape (Fig. 2). Ten microliters of a culture containing 9×10^8 CFU/mL of *E. faecalis* (ATCC 29212) diluted in 1 mL of brain heart infusion broth solution was placed into each tooth (Fig. 3). The bacterial suspension was refreshed every week. The culture medium in the lower assembly was monitored weekly for 3 months for turbidity. To ensure the presence of *E. faecalis* in turbid mediums, samples were picked from the lower assembly using a sterile loop and cultured in blood agar.

Statistical Analysis

The collected data were reviewed by Microsoft Excel and graph pad primer software. The comparison between the two groups was made by Chi-square test for categorical data. All p values < 0.05 were deemed statistically significant.

RESULTS

In this study, 60 single-rooted mature permanent teeth were included. The selected teeth were arbitrarily distributed into two groups: MTA and biodentine. Each group was further split into two subsets of 2 and 4 mm based on the thickness of the apical plug.

Table 1 shows the overall brief details of the study conducted. It depicts the number of samples that showed leakage through a duration of 1 week, 1 month, 2 months, and 3 months in all the four subgroups, i.e., MTA 2 mm, MTA 4 mm, biodentine 2 mm, and biodentine 4 mm. This table differentiates the old samples from

the freshly turbid/new samples that leaked every month. The total number of samples that showed leakage in the MTA 2 and 4 mm apical plug group is 11 and 13, respectively. The total number of samples that showed leakage in the biodentine 2 and 4 mm apical plug group are 15 and 10, respectively.

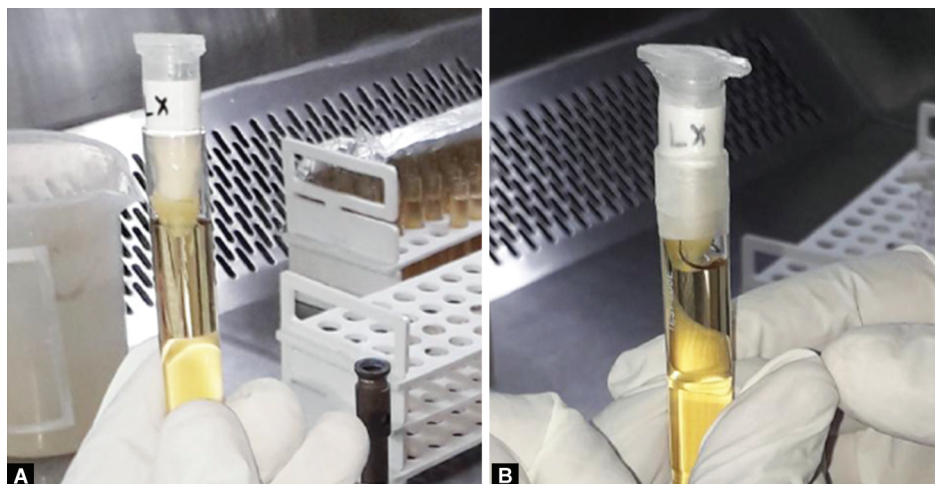
The comparative evaluation of samples that showed leakage in the 2 mm apical plug group of MTA and biodentine over 1 week, 1 month, 2 months, and 3 months disclosed a statistically non-significant p value of 0.486 obtained using the Chi-square test. In contrast, the comparative evaluation of 4 mm apical plugs revealed a p value of 0.231 (Table 2).

The comparative evaluation of samples that showed leakage in the 2 and 4 mm, apical plug group of MTA, over 1 week, 1 month, 2 months, and 3 months disclosed a statistically non-significant p value of 0.851, whereas that of biodentine revealed a p value of 0.149 (Table 3).

All the 15 samples under the 2 mm biodentine apical plug group turned turbid whereas only 11 out of 15 under the 2 mm MTA apical plug group turned turbid over 3 months. No statistically significant difference ($p > 0.05$) was found among 2 mm MTA and biodentine apical plugs. The number of samples that showed leakage over 3 months in the 4 mm MTA and biodentine apical plug group also were statistically insignificant ($p > 0.05$) (Table 4). The comparative evaluation of the total number of samples that



Fig. 3: Inoculation of *E. faecalis* into the root canal



Figs 2A and B: Microtube assembly placed in the widal tube and sealed with parafilm tape

Table 1: The number of samples that showed leakage in relation to the duration of incubation

Group (n = 15)	1 Week	1 Month		2 Months		3 Months		Total
		Old	New	Old	New	Old	New	
MTA 2 mm	3	3	2	5	3	8	3	11
MTA 4 mm	0	0	6	6	6	12	1	13
Biodentine 2 mm	3	3	2	5	4	9	6	15
Biodentine 4 mm	3	3	3	6	3	9	1	10

Table 2: Comparative evaluation of samples that showed leakage in the 2 and 4 mm apical plug groups

Duration	2 mm		4 mm	
	MTA	Biodentine	MTA	Biodentine
1 week	3	3	0	3
1 month	2	2	6	3
2 months	3	4	6	3
3 months	3	6	1	1
p value	0.486	0.231		

Table 3: Comparative evaluation of samples that showed leakage in the MTA and biodentine apical plug groups

Duration	MTA		Biodentine	
	2 mm	4 mm	2 mm	4 mm
1 week	3	0	3	3
1 month	2	6	2	3
2 months	3	6	4	3
3 months	3	1	6	1
p value	0.851	0.149		

Table 4: Comparative evaluation of samples in 2 and 4 mm apical plug groups

Duration	2 mm		4 mm	
	MTA	Biodentine	MTA	Biodentine
Turbid	11	15	13	10
Clear	4	0	2	5
p value	0.100	0.390		

Table 5: Comparative evaluation of samples in the apical plug group of MTA and biodentine

Duration	MTA		Biodentine	
	2 mm	4 mm	2 mm	4 mm
Turbid	11	13	15	10
Clear	4	2	0	5
p value	0.651	0.042		

showed leakage in the 2 and 4 mm, apical plug group of MTA, over 3 months showed a non-significant *p* value of 0.651. However, this was not the case when 2 and 4 mm thickness biodentine apical plugs were evaluated. A statistically significant *p* value of 0.042 was obtained between the 2 and 4 mm thickness apical plugs (Table 5). A 4 mm thickness biodentine apical plug had a better seal than that of 2 mm thickness.

DISCUSSION

The traditionally used material for apexification is calcium hydroxide (CaOH). Over the years, due to its various drawbacks, it has been replaced by materials such as dentinal chips, hydroxyapatite crystals, Portland cement, calcium sulfate, mineral trioxide aggregate, and biodentine.³

The comfort of use and proficiency of MTA plugs over the conventional CaOH has often been endorsed. It was proclaimed that MTA when in direct contact with pulp and periradicular tissues could prevent the microleakage and stimulate the original tissue regeneration. A few surveys showed MTA to have less microleakage than conventional materials.⁴ Mineral trioxide aggregate has exceptional biocompatibility and sealing capability.¹² Moreover, it is less cytotoxic when compared to other materials currently used in pulpal therapy¹² and stimulates cementogenesis;⁵ all these features make it a good option for apical barriers. The reduced technique sensitivity has encouraged clinicians to use MTA in apexification.¹²

Favorable treatment of necrotic teeth with open apices using mineral trioxide aggregate as an apical plug has been recorded by several studies.^{12,13}

Bozeman et al.¹⁴ stated that the compressive strength of white MTA was substantially greater than gray MTA at 24 hours and that gray MTA took a longer time to achieve the final set when compared to white MTA. The greater specific surface area of white MTA suggested by its smaller particle size, triggers a rise in the wetting volume, water-binding capacity, and hydration rate.¹⁵ White MTA will be thicker inspire by the same water-powder ratio. A boost in the cohesion and improved workability is anticipated while using white MTA in comparison with gray MTA.¹⁵ It is proposed by several studies that white MTA is an adequate substitute for gray MTA.¹⁴⁻¹⁶ Studies by Ferris and Baumgartner¹⁷ and Holland et al.¹⁸ have shown them to have similar properties. For the above-stated reasons, white MTA was used for this study.

The current study used MTA-Angelus. It has several advantages, such as good marginal adaptation, good sealing ability in mineralized tissues with complete closure, and has been reported to be inflammation-free in most cases.¹⁹ It has an excellent antibacterial effect and a robust fungicidal effect.²⁰ While both the white and gray MTA-Angelus have arsenic in their structures; the white form has arsenic levels confined to the values stated by the ISO 9917-1 standard.²¹ The composition of MTA-Angelus includes 80% Portland cement and 20% bismuth oxide. It does not contain calcium sulfate, thereby reducing the setting time.²² All these factors promoted its selection for this study.

Though MTA has a satisfactory sealing capability that prevents microbial leakage; however, it has an extended final setting time, potential of dentin discoloration, and trouble in handling³ and compacting it into narrow and curved canals.²³ It has been suggested that its long setting time will result in an increased possibility of bacterial leakage.²⁴ The apical seal obtained could

also be affected by voids that could occur from air bubbles, pores, and capillary channels seen in the set cement.²⁵

Biodentine is an innovative material containing tricalcium silicate that helps in overcoming these drawbacks. An exceptional 3-dimensional (3D) apical obturation combined with the lowest level of microleakage can be obtained when compared with glass ionomers and MTA.²³ Similar to MTA, its bioactivity has been evidenced by the precipitation of hydroxyapatite and the formation of tags that penetrate the dentinal tubules.²⁶ Biodentine is comparable to MTA except for zirconium oxide being added to its powder component. The larger particle size, incorporation of calcium chloride to the liquid constituent, and reducing the overall liquid component contributes to a decreased setting time approximating 12 minutes. Its high compressive strength encourages its use in endodontic treatments. Biodentine has effortless handling properties hence making its placement less time-consuming than MTA.⁵

On the contrary, biodentine has few drawbacks like its higher cost and its availability in the capsular form, which causes increased wastage of material. These factors have led to the use of MTA despite the superior mechanical properties of biodentine. This study aimed at comparing the sealing capability of two calcium silicate containing cement, notably MTA-Angelus and biodentine.

In studies by Ayatollahi et al.²⁷ and Nabavizadeh et al.,²⁸ Peeso Reamer drills were used to simulate open apex conditions. Cechella et al.²⁶ created a standardized open apex using the #6 Gates-Glidden drill. In the present study, root end preparation was done using a bur as in the study by Kim et al.²⁹ to simulate an open apex with thin dentinal walls.

As in the study performed by Moradi et al.¹⁰ high convergence gutta-percha was snugly fit in the coronal part of the apical cavity to provide an intracanal matrix. This supported the condensation of retrograde filling material.

The current study used 2 and 4 mm apical plugs similar to a study by Hachmeister et al.³⁰ In contrast, Hong et al.³¹ used apical plugs measuring 2 mm, and De Leimburg et al.³² used that of 1, 2, and 3 mm in thickness, and found that all had an acceptable seal.

Retrograde placement of MTA and biodentine was done in the study as the leakage of the material does not depend on the delivery technique and instead depends on packing and adaptation of these materials to the dentinal walls.⁴ Radiographs were taken after placing the biomaterials. This ensured the precision of placement and evaluated the thickness of the barrier at the apex in both the groups.¹²

The current study used MTA and biodentine alone in the root canals and the remainder of the canal was left empty as in the study by Nabavizadeh et al.²⁸ Even though this is different from what would have been done conventionally; this design was adopted to evaluate the definite microleakage of obturating materials without interfering with the sealing capacity of gutta-percha and sealer.²⁸

The sealing properties have been tested using various techniques both *in vitro* and *in vivo*. Unfortunately, there is no precise and reproducible leakage test available so far to meet all the requirements.²⁴ Mortensen et al.³³ and Krakow et al.³⁴ stated that the bacterial microleakage technique was more precise in leakage assessment, in comparison to dye penetration or radioisotopes techniques. Torabinejad et al.³⁵ first introduced the method of using bacterial microleakage and named it the Dual Chamber technique. In this technique, the bacterial suspension in the upper chamber is

refreshed every other day. This is important to ensure the vitality and activity of the bacteria. To authenticate the experiment, it is also necessary to determine the bacteria that caused the turbidity in the culture medium (lower chamber).³⁶ A single species was selected to ensure standardization between groups. *Enterococcus faecalis*, frequently seen in secondary infections from endodontic treatment failures, was the apt choice for the study. *Enterococcus faecalis* is a facultative non-motile anaerobic bacteria. It can overpass highly alkaline areas, and glucose deprivation hence can cause persistent infections.¹¹ It can penetrate through the tubules, and it is resistant to irrigation and intracanal medication.³⁶ *Enterococcus faecalis* can form an oral biofilm within the root canal.¹¹ These biofilms are considered a suitable model to evaluate the colonization of bacteria in the root canal.

Apical reactions can occur due to coronal leakage that causes infiltration of microorganisms into the canal. Failure of the endodontic treatment results from the contamination of the pulp chamber, which serves as a reservoir for bacteria.³⁷ As bacterial leakage is more clinically relevant,^{9,28} it is widely used to evaluate the sealing ability of root canal sealers and root canal filling materials.

Sticky wax was used to seal the area between the tooth and the Eppendorf tube as in various bacterial leakage studies.³⁶ To evaluate the efficacy of sticky wax to prevent leakage of the *E. faecalis* inoculum when directly placed into the upper chamber, a similar experimental setup was prepared without placing any teeth. Cut Eppendorf tubes with their open ends sealed with sticky wax were placed into a lower chamber containing nutrient broth. This setup was incubated at 37°C for 3 months. Three out of four samples became turbid, indicating the leakage of the bacteria through sticky wax. The present study ensured that *E. faecalis* was inoculated directly into the pulp chamber and canal space to avoid false-positive results.

Ethylene oxide sterilizer was used to disinfect the experimental setup as in studies by Moradi et al.³⁶ and Rechenberg et al.³⁸ This ensured that there was no melting of sticky wax which might occur with other means of sterilization. Inoculation of *E. faecalis* was done every week; this ensured a continuous supply of microorganisms in contact with the biomaterials.

Bani et al.⁵ compared the microleakage detected from apical plugs of biodentine and MTA. These were placed in an orthograde fashion. The study also assessed the impact of the thickness of these materials on their sealing ability. They evaluated apical plugs of 1, 2, 3, and 4 mm of both these biomaterials and found that a decrease in the apical plug thickness significantly increased the apical microleakage. The study found that 3 and 4 mm apical plugs were superior in seal irrespective of the biomaterials. Results evaluated in the present study have shown no statistically significant variation (p value = 0.651) between the 2 and 4 mm thickness of MTA apical barriers. However, the leakage of 2 and 4 mm thickness biodentine apical plugs was statistically significant with a p value of 0.042. The 4 mm thickness biodentine apical plug had decreased leakage when compared to the 2 mm biodentine apical plug. This might be due to the change in the technique of leakage evaluation used in the study when compared to the fluid filtration technique used by Bani et al.⁵ In the present study, bacterial leakage of the samples was evaluated for 3 months. However, fluid filtration is evaluated for 2-minute intervals over 8 minutes. Hence, this might be one of the significant factors of change in the results when compared to the study by Bani et al.⁵

Shetty et al.⁸ evaluated the sealing ability of MTA-Angelus, MTA Plus, biodentine, and glass ionomer cement at 72 hours, 1 month, and 3 months using fluid filtration technique. This study stated that MTA-Angelus had the least microleakage followed by biodentine and MTA Plus. They found a statistically significant variation was seen among all the materials at different periods. The continuous supply of *E. faecalis* inoculum, which maintains the viability of the organisms, might be another reason for the difference in results of the current study from that executed by Shetty et al.⁸ Inoculation of *E. faecalis* every week for 3 months enhanced the probability of the biomaterials to undergo leakage which was not the case in the study by Shetty et al.⁸ where the samples were tested only thrice, i.e., at 72 hours, 1 month, and 3 months.

Ovsay et al.³⁷ evaluated the microleakage of materials used for repair, namely, MTA, IRM, and biodentine using the bacterial leakage technique. These were applied to perforated furcations in different diameters. The turbidity of the samples was ascertained on the 7th, 15th, 30th, and 45th days. Proroot MTA proved to be the most successful material preventing microleakage as compared to IRM and biodentine. The improved results of Proroot MTA in this study might be since it was mixed in an amalgamator before placement onto the furcal perforations.

Cechella et al.²⁶ evaluated the sealing ability provided by biodentine and MTA apical plugs, with or without phosphate-buffered saline (PBS) intracanal dressing, using a glucose leakage method. They concluded that biodentine had lower sealing ability than MTA. This might have resulted from using PBS that enhanced the seal of MTA. The contact of biodentine with intracanal PBS may have resulted in more excellent water absorption by the cement. This would have caused a change in the powder/liquid ratio, favoring the formation of many pores, hence leading to insufficient sealing capacity.

Yazdizadeh et al.⁴ compared the microleakage of MTA used for apexification in one or two sessions. They inferred that the final obturation should be postponed until the final MTA setting as MTA needs enough time for setting in the presence of the moisture. In the present study, all teeth were preserved at 37°C in 100% humidity for 24 hours. This ensured the complete set of the MTA before bacterial leakage, which could have been the factor that contributed to statistically non-significant results between MTA and biodentine apical plugs.

Naik et al.²⁵ assessed the apical seal acquired with MTA and biodentine after irrigating the root end cavity with MTAD before retrofilling. It concluded that the biodentine had a superior apical seal when compared to MTA. This finding was acquired regardless of the presence of the smear layer. In the current study, the smear layer was formed as a result of retrograde preparation with a bur. This smear layer might have caused increased leakage of MTA 2 mm apical plug to that of biodentine. Another study by Nanjappa et al.¹⁵ evaluating the sealing capability of three root-end filling materials also concluded that biodentine is a better material to prevent apical microleakage in comparison to MTA when used in 2 mm thickness. Another reason for MTA having increased leakage to that of biodentine might be due to the increased air pockets in the set mass which could not be detected in the RVG. This could be avoided if ultrasonic mixing of MTA was done before placement.⁴ Indirect ultrasonic activation is said to improve the adherence of hand-mixed MTA to the dentinal walls.³⁹

Hence, the outcome of the current study might be influenced by the consistency, mixing techniques, and manipulation of materials.

The merits of the study include the use of bacterial leakage, which is more clinically relevant. The study was also done for 3 months, hence mimicking the clinical scenario where follow-up of the patient is done for 3 months. In the clinical scenario, the microorganisms remain viable in the oral environment causing persistent infection, unlike the laboratory conditions where they fade out over time, hence in the current study, the sustainability of the microorganisms was maintained by inoculating the *E. faecalis* strain every week. Another strength of the study is the inoculation of *E. faecalis* into the root canals using micropipettes. This ensures there are no false results due to leakage from the sticky wax.

The small sample size and technique sensitivity of the procedure are a few of the weaknesses in the study. The other drawbacks include it being an *in vitro* study that cannot precisely simulate a clinical scenario and also the use of conventional burs instead of ultrasonic retro tips for retrograde preparation lead to improper smear layer removal, hence, decreased seal of the biomaterials.

Therefore, further clinical studies can be done using larger sample size, using an intracanal medicament, storing the samples in PBS, and using an ultrasonic technique for mixing; as all these additions might have an impact on the seal of these biomaterials.

CONCLUSION

Within the constraints of the study they conclude the following:

- The sealing capability of biodentine was similar to MTA at any apical plug thickness irrespective of the apical plug thickness.
- The sealing capability of the 2 mm thickness MTA apical plug was similar to that 4 mm thickness MTA apical plug.
- Biodentine, when used as an apical plug, has a better seal in 4 mm thickness than the 2 mm thickness apical plug.

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MANUFACTURER NAME

- MTA (ANGELUS, Brazil)
- Biodentine (SEPTODONT, India)
- RVG (CARESTREAM 5100, United States)
- Ortho MTA carrier (BioMTA, Korea)

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