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An *in vitro* Assessment of the Apical Sealing Ability of MTA Plus and Biodentin

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ABSTRACT

Introduction: The apical vessels may also be severed or damaged enough to interfere with the normal reparative process. Radicular lesions develop when microorganisms of sufficient pathogenicity and number gain access to peri-radicular tissues. Microorganisms may be predominantly resilient to eradication by host defence mechanisms and antimicrobial agents when they are arranging in an extraarticular biofilm.

Objectives: The present study aims to compare the apical sealing ability of two materials Mineral trioxide aggregate (MTA) plus and Biodentin as well as to evaluate bacterial microleakage using a bacterial leakage model for 28 days.

Methods: Sixty single-rooted extracted permanent teeth were selected. All the teeth should have straight pulp canals were included in the study while the tooth with root caries, multiple canals, lateral radicular canals Calcifications, peri-radicular resorptive changes excessive curvatures, developmental defects, root fractures, with internal resorption, previously endodontically treated cracks or root defects were excluded from the study. Samples used in this in-vitro study had been extracted for orthodontic or periodontal reasons.

Results: Biodentine and controls on day 1. There was no leakage observed for MTA plus and Biodentine. Only one sample of positive control leaked. The comparison was done using the Kruskal Wallis test and the p-value was found to be 0.392 which was statistically not significant. Biodentine and control groups on day 5. On day 5, 13.33% of MTA plus group leaked (2 out of 15 samples) against 40% of positive controls leaked (6out of 15 samples).

Conclusion: The resent study concludes that MTA plus and Biodentine have good apical sealing ability against E.faecalis at 28 days. Biodentine was better in performance than MTA plus in terms of apical sealing for accurately measuring the microleakage and quantify it further in-vitro models can be pursued.

Key Words: Apexogenesis, Microorganisms, Periradicular tissues, Pulpal hyperaemia, Pulpotomy

INTRODUCTION

Trauma to a tooth is invariably followed by pulpal hyperaemia, the extent of which cannot be always determined. Congestion and alteration in the blood flow in pulp initiate irreversible degenerative changes, which can result in pulpal necrosis. The apical vessels may also be severed or damaged enough to interfere with the normal reparative process.[1] Radicular lesion develop when microorganisms of sufficient pathogenicity and number gain access to peri-radicular tissues. Once microorganisms are capable to assemble in an extraarticular biofilm, they may be predominantly resistant to removal by host defence mechanisms and antimicrobial agents.[2] Due to the difficulty of the root canal organization and the trouble to clean it using the current techniques and instruments, root canals cannot always be sufficiently treated using a non-surgical orthograde approach.[3] Periradicular surgery, when indicated should be considered an extension of

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nonsurgical treatment as aetiology of the disease process and the objectives of the treatment are the same.

The prime purpose of a root-end filling material is to deliver an apical seal that avoids the movement of bacteria and the dispersion of bacterial contents from the root canal system into the periapical tissues.[4] An ideal root-end filling material should adhere to the preparation walls, which is necessary to create a tight seal in the root canal system.[5] It should be non-absorbable, radiopaque, easy to manipulate, dimensionally stable and can not be exaggerated by the occurrence of moisture.[5]

MTA is ideally suggested as a root-end filling material, but it has been used for pulpotomy, pulp capping procedures, apical barrier formation in teeth with open apexes, repair of root perforations, apexogenesis, and as a root canal filling material. The benefits of Pro root MTA as a root-end filling material, concerning the other stated alternatives, comprise superior sealing ability and improved marginal seal.[6-8] But it has certain clinical disadvantages such as it is tedious to handle and have a long setting time which could be overcome by MTA plus. But the material MTA plus is not much explored.

Biodentine (Septodont USA) was recently introduced to the dental market. This new bioactive cement has dentin - like mechanical properties and can be used as a repair material for root perforations, as a root-end filling material, and resorptions. Biodentine can be indicated in both the crown and root. So the present study aims to compare the apical sealing ability of two materials MTA plus and Biodentin as well as to evaluate bacterial microleakage using a bacterial leakage model for 28 days.

MATERIALS AND METHODS

The present study was conducted in the department of Pedodontics at Vinayaka missions Sankarachariyar Dental College Salem, Tamilnadu. 60 single-rooted extracted permanent teeth were selected. All the teeth should have straight pulp canals were included in the study while the tooth with root caries, multiple canals, lateral radicular canals Calcifications, peri-radicular resorptive changes excessive curvatures, developmental defects, root fractures, with internal resorption, previously endodontically treated cracks or root defects were excluded from the study. Samples used in this in-vitro study had been extracted for orthodontic or periodontal reasons. The study was approved by the institutional research committee and institutional ethical committee. The sample size was determined scientifically. Considering Alpha: 0.05, Power of the study: 0.8 and Effect size: 0.4. Therefore, the estimated sample size for the study was 15 for each group. This was calculated using the software G Power 3.1.

Grouping of samples was done by random allocation of prepared teeth was done.15 samples to each of the experimental group and control group.

Group-1: Mineral trioxide aggregate (MTA) PLUS group (n=15)

MTA plus was manipulated as per the manufacturer's instructions and incrementally placed into the root end cavity and condensed well along the cavity walls and against a flattened file which was placed in the root canal. The initial set was allowed, after 48 hrs the k-file was removed. Each tooth was placed in sterile gauze piece soaked in saline for 48 hrs for initial hard setting.

Group 2: Biodentine group (n=15)

Biodentine was mixed as per the manufacturer's instructions in an encapsulator and incrementally placed into the root end cavity and condensed well along the cavity walls and against a flattened file which was placed in the root canal. The initial set was allowed, after 24 hrs the k-file was removed. Each tooth was placed in sterile gauze piece soaked in saline for 48 hrs for the initial hard setting.

Group 3: Positive control (n=15)

Thermoplasticized gutta-percha (GP) was used without sealer to fill the root end cavity and condensed against the flattened K - file. The file was removed after 48 hrs and placed in moist sterile gauze piece.

Group 4: Negative control (n=15)

Root end preparations were filled with sticky wax and condensed against the k-file. After 48 hrs, the file was removed and placed in moist sterile gauze piece.

Materials

Materials used For control and experimental group were MTA plus (Prevest Denpro Ltd, Jammu, India), Biodentine (Septodont, Saint Maur des Fosses, France), Thermo plasticised Gutta-percha (Bee fill 2 in 1 obturation device, Germany), Sticky wax (Hiflex, UK). Materials used For canal preparation were ultrasonic diamond tips (Kis tips),5.25% sodium hypochlorite (Hyposol, Prevest Denpro),17% ethylenediaminetetraacetic acid (**EDTA**) (Dolo Endogel ™, Prevest Denpro). Materials used For bacterial leakage model were scintillation vials, orthodontic resin, cyanoacrylate, phenol lactose red broth (Sigma Aldrich), E.Faecalis in tryptone soya broth (**TSB**) agar to 1×10^9 CFU/ml. Equipment used in the study were ethylene dioxide sterilization chamber

Statistical analysis was done using Kruskal-Wallis test and post-hoc-tuckey test to compare the intergroup difference and statistical significance was set at the level of P=0.05.

RESULTS

Table 1 indicates the Comparison among groups for apical bacterial leakage. **Table 2** shows the recording of the number of samples leaked in each group and its percentage of failure from day one to 28 days.

Table 1: Comparison among groups for apical bacterial leakage

days	POSITIVE CONTROL							NEGATIVE CONTROL							MTA PLUS							BIODENTINE						
Samples	1	2	5	7	8	14	28	1	2	5	7	8	14	28	1	2	5	7	8	14	28	1	2	5	7	8	14	28
1	×	×	×	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
2	×	×	×	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
3	√	√	√	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
4	×	×	×	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
5	×	×	×	√	√	√	√	×	×	×	×	×	×	×	×	×	√	√	√	√	√	×	×	×	×	×	×	×
6	×	×	×	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
7	×	√	√	√	√	√	√	×	×	×	×	×	×	×	×	×	√	√	√	√	√	×	×	×	×	×	×	×
8	×	×	×	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
9	×	×	√	√	√	√	√	×	×	×	×	×	×	×	×	×	×	√	√	√	√	×	×	×	√	√	√	√
10	×	×	×	√	√	√	√	×	×	×	×	×	×	×	×	×	×	√	√	√	√	×	×	×	√	√	√	√
11	×	×	×	√	√	√	√	×	×	×	×	×	×	×	×	×	×	√	√	√	√	×	×	×	×	×	×	×
12	×	×	√	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	√	√	√	√
13	×	×	√	√	√	√	√	×	×	×	×	×	×	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×
14	×	×	√	√	√	√	√	×	×	×	×	×	×	×	×	×	×	√	√	√	√	×	×	×	×	×	×	×
15	×	×	×	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×

√- samples with bacterial leakage

×-samples without bacterial leakage

Table 2: Distribution of apical bacterial leakage of samples in all the four groups in the percentage

Groups	Positive		Negative		MTA		Biodefinite	
Days	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%
1	1	6.67	0	0	0	0	0	0
2	2	13.33	0	0	0	0	0	0
5	6	40	0	0	2	13.33	0	0
7	15	100	0	0	6	40	3	20
8	15	100	0	0	6	40	3	20
14	15	100	0	0	6	40	3	20
28	15	100	1	6.67	6	40	3	20

Table 3: Comparison of apical bacterial leakage among MTA plus, Biodentine and controls (Day 1)

Group	Apical bacterial leakage	Percentage	Mean Rank	Test Value (Kruskal Wallis Test)	P Value
MTA Plus	0	0	30.00	3.000	0.392
Biodentine	0	0	30.00		
Positive Control	1	6.67	32.00		
Negative Control	0	0	30.00		

Table 3 depicts the comparison of leakage of samples between MTA plus, Biodentine and controls on day 1. There was no leakage observed for MTA plus and Biodentine. Only one sample of positive control leaked. The comparison was done using the Kruskal Wallis test and the p-value was found to be 0.392 which was statistically not significant.

Table 4: Comparison of apical bacterial leakage among MTA plus, Biodentine and controls (Day 5)

Group	Apical bacterial leakage	Percentage	Mean Rank	Test Value (Kruskal Wallis Test)	P Value
MTA Plus	2	13.33	30.50	13.615	0.003*
Biodentine	0	0	26.50		
Positive Control	6	40	38.50		
Negative Control	0	0	26.50		

Table 4 shows the intergroup comparison of leakage of samples between MTA plus, Biodentine and control groups on day 5. On day 5, 13.33% of MTA plus group leaked (2 out of 15 samples) against 40% of positive controls leaked (6out of 15 samples). Statistical analysis was done using Kruskal Wallis test and p-value was found to be 0.003 which was statistically significant.

Table 5: Comparison of apical bacterial leakage among MTA plus, Biodentine and control groups on Days 7, 8 and 14

Group	Apical bacterial leakage	Percentage	Mean Rank	Test Value (Kruskal Wallis Test)	P Value
MTA Plus	6	40	30.50	34.417	0.001*
Biodentine	3	20	24.50		
Positive Control	15	100	48.50		
Negative Control	0	0	18.50		

Table 5 illustrates the comparison of leakage of samples among MTA plus, Biodentine and control groups on day 7, 8 and 14. All the samples showed similar leakage on 7 th, 8th and 14th day. The percentage of samples leaked for, MTA plus was 40% (6 out of 15 samples), whereas 20% of Biodentine showed leakage (3 out of 15 samples). When observed in control groups, 100% of positive controls leaked (15out of 15 samples); and negative control did not leak. Numerically, MTA plus samples leaked more than Biodentine group. Statistical analysis was done using the kruskal Wallis test and the p-value was found to be 0.000 which was statistically significant.

Table 6: Comparison of apical bacterial leakage among MTA plus, Biodentine and control groups (Day 28)

Group	Apical bacterial leakage	Percentage	Mean Rank	Test Value (Kruskal Wallis Test)	P Value
MTA Plus	6	40	30.00	30.950	0.000*
Biodentine	3	20	24.00		
Positive Control	15	100	48.00		
Negative Control	1	6.67	20.00		

Table 6 depicts the comparison of leakage of samples among MTA plus, Biodentine and control groups on day 28. When the percentage of apical leakage was assessed at 28th day MTA plus samples showed 40%, Biodentine showed 20%, positive control showed 100% and negative control showed 6.67% of leakage, in which MTA plus showed more leakage than Biodentine. Statistical analysis showed a significant p-value of 0.000

Table 7: Inter group analysis using post hoc – Tukey test for days 7, 8 and 14

	Comparing Group	Other Group	Mean Difference	Std Error	P Value
Day 7,8,14	MTA Plus	Positive Control	-0.600	0.120	0.000*
		Negative Control	0.400	0.120	0.008*
		Biodentin	0.200	0.120	0.347
	Biodentin	Positive Control	-0.800	0.120	0.000*
		Negative Control	0.200	0.120	0.347
		MTA Plus	-0.200	0.120	0.347
	Positive Control	Negative Control	1.000	0.120	0.000*
		MTA Plus	0.600	0.120	0.000*
		Biodentin	0.800	0.120	0.000*
	Negative Control	Positive Control	-1.000	0.120	0.000*
		MTA Plus	-0.400	0.120	0.008*
		Biodentin	-0.200	0.120	0.347

Table 7 depicts the multiple comparisons among all the four groups. Multiple group comparison was done using post hoc-Tukey test. There was a statistically significant difference between the positive control and the MTA plus group and dentine group and also negative control. MTA plus group showed a significant difference with both the control groups. Biodentine group showed significant difference with positive control. Intergroup comparison between MTA plus and Biodentine group had a mean difference of 0.200 and with a standard error of 0.120, the p-value was 0.347, which was statistically insignificant.

Table 8: Inter group analysis using post hoc – Tukey test on 28th day

	Comparing Group	Other Group	Mean Difference	Std Error	P Value
Day 28	MTA Plus	Positive Control	-0.600	0.128	0.000*
		Negative Control	0.333	0.128	0.057
		Biodentin	0.200	0.128	0.411
	Biodentin	Positive Control	-0.800	0.128	0.000*
		Negative Control	0.133	0.128	0.728
		MTA Plus	-0.200	0.128	0.411
	Positive Control	Negative Control	0.933	0.128	0.000*
		MTA Plus	0.600	0.128	0.000*
		Biodentin	.800	.128	.000*
	Negative Control	Positive Control	-.933	.128	.000*
		MTA Plus	-.333	.128	.057
		Biodentin	-.133	.128	.728

Table 8 depicts the multiple comparisons among all the four groups. Multiple group comparison was done using post hoc-Tukey test. There was a statistically significant difference between the positive control and the MTA plus and the dentine group and also negative control. Intergroup comparison between MTA plus and Biodentine group had a mean difference of 0.200 and with a standard error of 0.128, the p-value was 0.411, which was statistically insignificant.

DISCUSSION

In the present study, two tricalcium silicate types of cement were compared in terms of apical sealing ability. Various approaches have been tried to assess apical microleakage. These include air pressure, neutron activation, radioisotope, electrochemical, fluid filtration, bacteria, and the use of the dyes. Numerous techniques are suggested to measure apical

root canal leakage using transmission electron microscopy, scanning electron microscopy, and electron probe microscope analysis. There is no homogenous leakage test to estimate the sealing capacity of endodontic materials.[9] Bacterial leakage model can be used to study the bacterial penetration across the material. A bacterial leakage model was chosen for the present study because it is most relevant in clinical perspective.[10] Invitro study with bacterial leakage test was conducted for 28 days. The thickness of apical plugin this study is 3mm as supported by Mehmet bani et al.,[11] The amount of apical microleakage was significantly lower for 3 and 4mm apical plugs than 1 and 2mm subgroups of Biodentine and MTA in his study. In the present study, the rood end cavity was prepared with ultrasonic diamond tips. Khandelwal et al.,[12] compared different retro preparations with MTA and Biodentine. Biodentine group prepared using ultrasonics for showed the best sealing than all the other tested groups. Regardless of the preparation techniques employed, Biodentine still presented good sealing than MTA. Preparation of the root end using ultrasonics showed less microleakage than bur prepared teeth for both filling materials. In this study, machine trituration done for biodetine as Gupta PK et al.,[13] reported more microleakage when Biodentine was manually manipulated. The setting time is one of the most clinically relevant factors to be considered. Hence in this study, all the samples were kept in moist gauze piece for a period of 48hrs to allow an initial setting time. Long-setting duration of cement reduces clinical problems since it maintains shape and support stresses during this period.

In the present study, MTA plus showed more apical bacterial leakage than Biodentine. In the negative control group only one sample leaked at the end of the study. The leakage in negative control can be attributed to nail varnish failure. Among the positive control group, there was a 100% apical bacterial leakage indicating the need for an ideal apical sealing material for the retrograde fillings. Under the experimental conditions of this study, dentine showed less leakage than MTA plus which was statistically insignificant. Biodentine and MTA plus showed significant difference than positive control which signifies that both the materials have the good apical sealing ability. The formation of CSH gel also reduces the porosity with time. The crystallization of the dentine continues up to 4 weeks, therefore, improving the strength as well as other mechanical properties (sealing ability). The high mechanical strength of Biodentine may be accredited to the removal of aluminates that reflects weakening and delicateness of the set material as described by the manufacturer. The thickness of the Ca-and Si-rich layers improved over time, and the thickness of the Ca-and Si-rich layer was meaningfully superior in Biodentine equated to MTA after 30 and 90 days, concluding that the dentine element uptake was superior for Biodentine than for MTA.[14]

The sealing capability of a material can be assessed by various criteria such as marginal adaptation, and porosity. When calcium silicate cement are mixed with water; several porosities and micro-channels are created which play a crucial role in the hydration reaction. Kokate and Pawar[15] conducted a study that compared the microleakage of glass ionomer cement, MTA, and Biodentin when used as a retrograde filling material and concluded that Biodentin exhibited the least microleakage when compared to other materials used which supports the current study.[16]

Sulthan[17] carried out a study to evaluate the pH and calcium ion release of MTA and Biodentin when used as root-end fillings. He determined that Biodentine showed alkaline pH and the ability to release calcium ions related to that of MTA. Blood contamination exaggerated the push-out bond strength of MTA Plus nevertheless of the setting time.[18] Formosa et al.,[19] found that the anti-washout gel changed the rheology and properties of the material. In particular, it was noted that while MTA mixed with water had a sandy consistency, MTA mixed with anti-washout gel had a far more vicious and rubbery consistency and are most dough-like. This increased viscosity may explain from a purely physical standpoint, why MTA-AW developed the threshold strength of 3.92 MPa sooner than MTA. The anti washout gel added to MTA did not affect the radiopacity of resultant material the observed an increase in compressive strength of MTA-AW compared to MTA-W.

The present study has to be still explored with detail assessment of the leakage as it has certain experimental limitations invitro. It has certain limitations such as fewer sample size, limitation of in-vitro model, quantifiable evaluation etc. The study can be further directed invitro by extending the longevity of the study and quantifying the microleakage. The above statements, however, should be addressed in future experiments before any conclusive recommendations can be made.

CONCLUSION

The resent study concludes that MTA plus and Biodentine have lesser apical leakage and good apical sealing ability against E.faecalis at 28 days. Biodentine was better in performance than MTA plus in terms of apical sealing for accurately measuring the microleakage and quantify it further *in vitro* models can be pursued.

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Source of funding: Self

Authors contribution

1. Dr. Revathi Bashyam – Data collection
2. Dr. Ramesh Krishnan - Investigation
3. Dr. Kruthika Murali- Investigation

4. Dr. Nandhini.B.Selvarajan- Manuscript preperation
5. Dr. Suresh Kumar Vasaviah- Manuscript writing
6. Dr. Vinola Duraisamy- Editing, Fincial support

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