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# Comparative Evaluation of Sealing Ability of Saliva and Blood Contaminated MTA and Biodentine as Root End Filling Materials – An In vitro Study

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#### ABSTRACT

Aim: To compare and evaluate the sealing ability of MTA and Biodentine as root end filling materials contaminated with blood and saliva.

Materials and methodology: 36 single rooted teeth were taken and crowns were resected followed by cleaning and shaping and obturation. Root ends were resected at 3mm level. Samples were divided into two groups one filled with MTA & the other with Biodentine and each group was further subdivided into three groups, i) dry ii) blood and iii) saliva contaminated. Root samples were incubated at 37<sup>o</sup>C, 100 % relative humidity for 24hrs and immersed in 2% methylene blue dye for 48hrs. The depth of dye penetration was evaluated under stereomicroscope. Results were statistically analyzed using one way ANOVA and post-hoc Tukey's test.

Results: Statistically significant difference was observed with mean values of microleakage for Biodentine (0.3211) and MTA (0.8856).

Conclusion: Biodentine has shown the better sealing ability when compared with MTA in dry, blood and saliva contamination.

Keywords: MTA, Biodentine, blood, saliva, sealing ability.

## INTRODUCTION

The goal of endodontic therapy is to achieve hermetic seal between the pulpal and periradicular tissues. A mandatory requirement of root canal therapy is that the obturation and restoration of the tooth must seal the root canals both apically and coronally to prevent leakage and percolation of oral fluids and to prevent recontamination disinfected canals. of The complexity of root canal systems, inadequate instrumentation and presence of physical barriers may result in the failure of non-surgical endodontic therapy in some cases. The objective of endodontic treatment is the elimination of microscopic organisms from the root canal system and the subsequent establishment of an effective barrier to avert further

entry of microorganisms and their by-products to the periapical tissues <sup>[1]</sup>. Conventional endodontic treatment has been indicated to be effective in around 90% of cases. When non-surgical attempts prove unsuccessful or are contraindicated, surgical endodontic therapy is needed to save the tooth. Apicoectomy with retrograde obturation is a widely applied procedure in endodontics, when all efforts for the successful completion of orthograde endodontic therapy have failed <sup>[2]</sup>. Once the root-end preparation has been completed, a suitable root-end filling material is inserted.

According to Gartner and Dorn <sup>[3]</sup> an ideal material to seal the root-end cavities should prevent leakage of -

microorganisms and their by-products into the periradicular tissues. It should also be impervious to moisture, antibacterial, nontoxic, nonresorbable, easy to manipulate, radiopaque, easily adaptable, provide good seal, and promote regeneration of the non-carcinogenic, periodontal apparatus, and biocompatible with the tissue fluids and dimensionally stable. Unlike orthograde root canal filling materials, root-end filling materials are placed in direct contact with vital periapical tissues. The tissue response to these materials, therefore, becomes important and may influence the outcome of surgical endodontic treatment. The presence of moisture should not affect its sealing ability.

A wide variety of materials has been used for root end filling materials like amalgam, zinc oxide eugenol, polycarboxylate cement, zinc phosphate cement, glass ionomer cement, composite resins, calcium phosphate cement, and most recent materials like mineral trioxide aggregate, Biodentine etc. Amalgam has been used as root end material for many years, but its potential disadvantages like staining of tooth, mercury and tin contamination into periradicular spaces. Because of these disadvantages, zinc oxide eugenol based cements such as IRM and Super EBA have been advocated as root end filling materials. However, zinc oxide eugenol based cements have potential disadvantages irritation of vital tissue, solubility of material. Glass ionomer cement bonds physiochemically to dentine, but its sealing ability adversely affects when the root end cavities were contaminated with moisture at the time of placement of cement<sup>[4]</sup>.

During the past several years, special attention has been paid to Mineral Trioxide Aggregate (MTA) as probable alternative to formocresol. MTA had shown the most promising results because of its biocompatibility, mineralized tissue formation, less apical leakage, better marginal adaptation with an excellent long term prognosis, relative ease at which it can be used and with its numerous exciting clinical applications promises to be one of the most versatile cement of the century in the field of dentistry <sup>[5]</sup>. Hence, it is considered as the gold standard material for comparing other materials. However, it also possesses some disadvantages like difficulty to handle and insert into a retrograde cavity and wash out due to long setting time<sup>[5]</sup>.

In the recent times, a Ca<sub>3</sub>SiO<sub>5</sub> based root end material called Biodentine has been developed as probable alternative to MTA. Biodentine is similar to MTA in composition. The powder component of the material consists of tricalcium silicate, dicalcium silicate, calcium carbonate, oxide filler, iron oxide shade, and zirconium oxide. Tricalcium silicate and dicalcium silicate are indicated as main and second core materials, respectively, whereas zirconium oxide serves as a radio opacifier. The liquid contains calcium chloride as an accelerator and a hydrosoluble polymer that serves as a water reducing agent. Compared to other Ca based cements, this material presents two advantages: i) a faster setting time of about 9-12 minutes and ii) higher mechanical properties. The fast setting time, one unique characteristic of the product, is achieved by increasing particle size, adding calcium chloride to the liquid component, and decreasing the liquid content. It also has the added advantage of better handling and biocompatibility and ability to induce odontoblast differentiation and mineralization<sup>[6]</sup>.

Amongst other ideal properties, unaffected by the presence of moisture is the most important characteristics of root end material. Solubility of material is directly related to sealing ability of the material. It is inevitable that moisture including saliva and blood can contaminate the root end when filling material is placed and may affect its sealing ability <sup>[7]</sup>. Farhad et al. stated that the sealing ability of MTA was not affected by blood contamination <sup>[8]</sup>. However, the sealing properties of Biodentine in saliva and blood contaminated environments have not been evaluated. Hence, the purpose of this study was to evaluate and compare the sealing ability of MTA and Biodentine as root end filling materials in dry, saliva and blood contaminated environments.

## METHODOLOGY

The study was conducted on 36 recently extracted single rooted permanent teeth without any caries which were extracted due to orthodontic or periodontal reasons (Fig.1). Ethical clearance was obtained from the ethical committee board of CKS Theja Institute of Dental Sciences And Research Center, Tirupathi. Selection criteria includes anterior permanent teeth with single root canal (straight canal without calcification) and teeth with closed apex and excludes the teeth having developmental anomalies,

cracks, fractures, root caries, open apex, restorations, internal and external root resorption. All the extracted teeth were stored in formalin till the study was commenced. On the day of study, all the 36 teeth were equally treated by removing surface deposits using ultrasonic scaler and kept in 5.25% NaOCl prior to instrumentation<sup>[9]</sup>. Decoronation of teeth was done at CEJ with a diamond disc with water spray coolant (Fig.2). Pre-operative X - ray was taken to check the root canal anatomy. Access cavity preparation was done using a #2 round bur (NSK, Japan) and working length was determined by passing an ISO 15 K file (MANI Inc., Utsunomiya, Tochigi, Japan) into the root canal and x-ray was taken (Fig.3) and then 1 mm was subtracted from that length<sup>[13]</sup>. Teeth were instrumented to a size 25 hand file (MANI Inc., Utsunomiya, Tochigi, Japan). Following this, Protaper Universal rotary files (Dentsply) were used for instrumentation <sup>[9]</sup>. The preparation was started with SX file and followed by S1, S2, F1, and F2 files using RC Prep. The canals were copiously irrigated with 5.25% NaOCl, and normal saline solution. The canals were dried using absorbent points and obturated. Single cone technique was done with Size 40/0.06.Gutta-percha cones (Dentsply)<sup>[13]</sup> and ZnOE sealer (Fig.4), and access cavities were filled with Type II-GIC (GC Fuji II); and then with light cured composite resin (Ivoclar Vivadent; Batch no-CE 0123). Samples were incubated for 24 hours at 37° C and 100% humidity, to allow materials to set.<sup>[9]</sup> Root end resections were made by removing 3mm from the root-end at a 90 degree angle to the long axis of the root with a diamond disc (D&Z,Darmstadt, Germany) under constant water spray irrigation (Fig.5). A 3mm deep root-end cavity was prepared ultrasonically, powered by a minipiezon with DT-043 ultrasonic retrotip (EMS, Nyon, Switzerland) <sup>[14]</sup>. The samples were randomly divided into 6 groups. (Fig.6)

Group 1 – Root end filled with MTA (Angelus) dry (Batch - #000721)

Group 2 – Root end filled with Biodentine (Septodont) dry (Batch-B20503)

Group 3 – Root end filled with MTA contaminated with blood

Group 4 – Root end filled with MTA contaminated with saliva

Group 5 – Root end filled with Biodentine contaminated with blood

Group 6 - Root end filled with Biodentine contaminated with saliva.

Root end cavity was dried with paper points and filled with root-end filling material. (Fig.7)

Group 1 was filled dry with MTA with powder liquid composition of 3:1. Group 2 filled dry with Biodentine, mixing of Biodentine was done using amalgamator with powder liquid composition of 5:1. Group 3 and Group 5, root ends were contaminated with human blood and then blood was removed by aspirating with syringe to leave a coating on the inner wall of cavity <sup>[10]</sup>, and the filling materials were placed. Group 4 and Group 6 root ends were contaminated with human saliva and dried with cotton pellets <sup>[11]</sup> and the filling materials were placed. The samples were incubated for 24 hours at  $37^{\circ}$  C and 100% humidity <sup>[12]</sup> (Fig.8). Two coats of nail varnish was applied, except at the apical end and then allowed to dry for 1 hour. Then, the samples were immersed in 2% Methylene blue dye for 48 hours <sup>[13]</sup>. The roots were removed and rinsed in running tap water for 10 minutes and later dried. Each sample was longitudinally sectioned in a buccolingual direction using microtome (Fig.9). The dye penetration depth along the cavity wall of each prepared segment was evaluated at 20x magnification with stereomicroscope (ProgRes C5 - OLYMPUS model) to evaluate the roots for extent of dye penetration in millimeter (using ProgRes Capture Pro 2.6 software) (Fig.10) and the results were analysed using SPSS software 22 version using independent sample t-test.

## RESULTS

Microleakage values of MTA and Biodentine in dry, contaminated with saliva and blood are summarized in Table: 1

Table 1

Serial No:	DRY		SALIVA		BLOOD	
	MTA	BIODENTINE	MTA	BIODENTINE	MTA	BIODENTINE
1.	1.2 mm	0.38 mm	1.08 mm	0.2 mm	0.9 mm	0.41 mm
2.	1.1 mm	0.37 mm	0.65 mm	0.8 mm	1 mm	0.40 mm
3.	0.6 mm	0.36 mm	0.73 mm	0.2 mm	0.8 mm	0.42 mm
4.	0.58 mm	0.35 mm	0.8 mm	0.23 mm	0.72 mm	0.30 mm
5.	1.1 mm	0.40 mm	0.9 mm	0.4 mm	1.1 mm	0.30 mm
6.	0.9 mm	0.42 mm	1 mm	0.25 mm	0.78 mm	0.28 mm

Data were analysed with SPSS software of 22 version with independent sample t - test. Mean microleakage values of dry Biodentine were greater when compared to Biodentine contaminated with blood and saliva and mean microleakage values of dry MTA were greater when compared to MTA contaminated with blood and saliva.

The microleakage values of Biodentine are less when compared to MTA in dry ,blood and saliva contaminated conditions which is shown in the form of graphs below.





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### DISCUSSION

The success of a periradicular surgery depends on the achievement of a fluid tight hermetic apical seal with a well-adapted root-end filling material. Apical seal during periradicular surgery may be compromised by contamination of root end cavity. Since achieving a dry apical field is not always attainable, this invitro study design was aimed at evaluating the sealing ability of two root end filling materials in saliva and blood-contaminated environment, which is usually a simulation of the virtual clinical scenario. <sup>[14]</sup> Clinical success, apical seal, handling properties, and biocompatibility help in determining the choice of root-end filling material. <sup>[15]</sup>

In the present study, root-end resections were carried out 3 mm from the apex and perpendicular to the long axis of tooth. Mjör et al. showed that in root-end resection, at least 3 mm of the root-end must be eliminated to reduce 98% of the apical ramifications and 93% of the lateral canals and that perpendicular resection minimizes the number of exposed dentinal tubules. <sup>[16]</sup> Ultrasonic tips were used for the root-end cavity preparation. Ultrasonic root-end preparation technique gives excellent results for periradicular surgery and is simple to use. <sup>[17]</sup> Use of ultrasonic tips in apical surgery improved the result of the treatment. <sup>[18]</sup>

The quality of apical seal obtained by root end filling materials has been assessed by the degree of dye penetration, radioisotope penetration, bacterial penetration, electro chemical means and fluid filtration techniques. <sup>[19, 20]</sup> All of these techniques have been shown to have a variety of shortcomings. Dye penetration method is one of the most commonly used and oldest methods to assess the marginal sealing ability of root end filling materials. This method has an advantage of being easy to perform

and less expensive. In the present study, dye penetration method (methylene blue) has been used. Methylene blue is a commonly used dye and has proved to be an important tracer in several endodontic studies assessing microleakage.<sup>[21]</sup>

The results in this present study showed that two root end filling materials exhibited microleakage, but a significantly less microleakage in case of Biodentine. Various microleakage studies have demonstrated the superiority of MTA over the commonly used root-end filling materials and so MTA is used as the gold standard for comparing the sealing ability of Biodentine. <sup>[22, 23-25]</sup> The sealing ability of Biodentine and MTA was comparable although the values were higher for Biodentine which was in agreement with other studies. <sup>[26]</sup> Sealing ability of Biodentine was not affected by blood and saliva contamination, and the values of microleakage were lower in blood and saliva contaminated samples than the dry field. The reason for this is that MTA hydrates in the presence of solutions containing phosphate ions such as blood and saliva. results in the precipitation of hydroxyapatite crystals and formation of tag like structures at the junction of MTA and dentin interface and expands on exposure to moisture.<sup>[27]</sup> However, the least microleakage was exhibited by Biodentine. This may be attributed to: i) the smaller size of Biodentine particles may aid in enhanced adaptation at the cavity surface and filling interface. ii) The decreased pore volume and porosity of Biodentine as compared to MTA may contribute to better sealing. iii) The faster setting time of Biodentine may prevent prolonged leakage when used reducing the bacterial contamination. This may be of significance when it is used in clinical situations. iv) Tags like structures are formed by the interaction of dentine and Biodentine, and there is presence of mineral infiltration zone which is an interfacial layer. The hydration products of calcium provide a highly silicate cements alkaline environment which causes degradation of collagen present in interfacial dentine.<sup>[28]</sup> Sealing ability of Biodentine may be attributed to the tag formation of Biodentine, as seen in the study conducted by Han and Okiji where tag formation was higher in Biodentine than MTA. Therefore, the sealing ability may be enhanced.<sup>[29]</sup>

Malhotra.S et al. (2015) <sup>[13]</sup> conducted a study to evaluate the marginal seal of ProRoot MTA, MTA Angelus, Biodentine and GIC as root-end filling materials, and concluded that the least microleakage was seen in case of Biodentine, which was statistically significant and is in accordance with the present study in which best sealing ability was seen with Biodentine.

Khandelwal. A et al. (2015) <sup>[30]</sup> conducted an invitro study on sealing ability of MTA and Biodentine as root end filing materials and concluded that Biodentine showed best sealing ability than MTA and therefore Biodentine can be used as a replacement for MTA, which is in accordance with the present study.

Radeva. E et al. (2014) <sup>[31]</sup> carried out a study to compare microleakage of MTA and Biodentine for two different apical cavity preparations and by using 0.2% Rhodamine B dye and concluded that Biodentine can be more effective material compared to MTA, which is in accordance with the present study.

Ravi Chandra. PV et al. (2014) <sup>[32]</sup> conducted an invitro study to evaluate marginal adaptation of three root end filling materials namely GIC, MTA and Biodentine and concluded that Biodentine has better marginal adaptation. The better handling properties of this material combined with superior biological, mechanical and physical properties suggest the superiority of Biodentine over other root end filling materials, which is in accordance with present study.

## CONCLUSION

Within the limits of this study it can be concluded that, the sealing ability of Biodentine was better than MTA in dry, blood and saliva contaminated environments, and hence Biodentine can be used as an alternative to MTA as root-end filling material in blood and saliva contaminated environments.

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