Original Article

A Comparative Evaluation of Sealing Ability and Marginal Adaptation of Four Root-End Filling Materials using Stereomicroscope and Scanning Electron Microscope – An *In vitro* Study

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Abstract

Introduction: Root-end filling materials are used to provide an apical seal that prevents the movement of bacteria and bacterial products from the root canal system into the periapical tissues. The present study was conducted to evaluate sealing ability and marginal adaptation of four different root-end filling materials by dye penetration method and scanning electron microscopy (SEM). **Materials and Methods:** Eighty single-rooted human maxillary central incisors were sectioned at cementoenamel junction, prepared, obturated, and resected and 3 mm deep root-end cavity was prepared. Teeth were retrofilled with Biodentine, mineral trioxide aggregate (MTA), MTA Plus, and glass ionomer cement (GIC). Sealing ability was checked by dye penetration method under stereomicroscope. Teeth were split longitudinally and the extent of gap was measured linearly, using SEM to evaluate marginal adaptation. The data were analyzed using SPSS software by Kruskal–Wallis H test and Mann–Whitney U-test. **Results:** Biodentine showed better sealing ability and MTA Plus showed better marginal adaptation compared to all other materials. **Conclusion:** Within the limitations of this study, Biodentine provided better seal and MTA Plus exhibited better marginal adaptation than MTA and GIC when used as retrofilling material.

Keywords: Biodentine, Glass ionomer cement, Mineral trioxide aggregate, Mineral trioxide aggregate plus, Retrofill.

Introduction

The foremost goal of root canal treatment is the elimination and exclusion of all microorganisms and completes obliteration of root canal system to develop a "*fluid tight seal*."[1] Despite the constant evolution of concepts, new endodontic techniques, and the development of more effective materials and instruments, the resolution of periapical pathosis is not achieved in certain cases. In such cases, where conventional endodontic treatment is unsuccessful, surgical endodontic therapy is needed to save the tooth. This procedure includes exposure of the involved apex, resection of apical end of root, preparation of Class I cavity, and insertion of a root-end filling material. The main objective of a root-end filling material is to provide an apical seal that prevents the movement of bacteria and bacterial products from the root canal system into the periapical tissues.[2]

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Many materials have been introduced for this purpose such as amalgam, cavity, super EBA, glass ionomer cement (GIC), composite resins, and mineral trioxide aggregate (MTA). However, no filling material satisfies all the requirements of an ideal material.[3] The rate of the development of new

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retrograde filling materials has been too rapid to allow for clinical evaluation of all materials. Laboratory tests have, therefore, been used to screen new materials for their ability to maintain better seal and marginal adaptation in retrograde cavity filling.

Thus, the aim of this study was to evaluate the sealing ability and marginal adaptation of four different root-end filling materials (GIC, MTA, Biodentine, and MTA Plus) by dye penetration method and scanning electron microscopy (SEM).

Materials and Methods

Eighty single-rooted, human maxillary central incisors with mature apices were selected and decoronated at the cementoenamel junction. The patency of the canal was established by No.10 K-File into root canals until the tip of the apical foramen. Working length was established 1 mm short of the apex. Biomechanical preparation of the root canals was done using step-back technique up to No. 50 K-file (master apical file) with 3% sodium hypochlorite and 17% EDTA as irrigants. Canals were obturated with gutta-percha and zinc oxide eugenol sealer by lateral compaction technique.

The teeth were resected 3 mm from apex 90° to the long axis of the root using a straight fissure bur with copious amount of water. A standardized 3 mm deep and 0.8 mm wide retrograde cavity were prepared using a straight fissure diamond bur (SF 41, Mani, Japan) under dental operating microscope. The cavities were irrigated with saline and dried. The teeth were randomly divided into two Groups A and B of 40 specimens each.

Group A

Samples used for the evaluation of sealing ability by dye penetration method using stereomicroscope.

Group B

Samples used for the evaluation of marginal adaptation by SEM.

Both the groups were divided into four subgroups, restored using GIC, MTA, Biodentine, and MTA Plus, and evaluated for apical leakage and marginal adaptation.

Apical leakage (Group A)

The teeth were coated with three coats of nail varnish except at the apical 1 mm of the resected root and were allowed to dry. These samples were suspended in 1% methylene blue dye for 72 h, rinsed for 15 min under tap water, and were split longitudinally with a diamond disc using water coolant. The depth of dye penetration was examined under stereomicroscope and microleakage was evaluated with the following scoring criteria for depth of dye penetration.[4]

- Score 0: No leakage
- Score 1: Dye penetration involving less than half of the retrograde cavity depth
- Score 2: Dye penetration involving entire depth of the retrograde cavity, but not cavity floor
- Score 3: Dye penetration deeper than retrograde cavity floor.

Marginal adaptation (Group B)

The teeth were split longitudinally with a diamond disc using water coolant. These segments were gold sputtered and analyzed by JEOL JSM T-220 (JEOL, Tokyo, Japan) SEM at ×100–×1000 using the software Image Tool 3.0 and the extent of gap was measured linearly, in micrometers.

The statistical analysis was done using SPSS software by Kruskal–Wallis H test and Mann–Whitney U-test.

Results

Sealing ability

Table 1 shows apical leakage scores in the experimental materials. The Mann–Whitney U-test indicated that there was no statistically significant difference between MTA, MTA Plus, and Biodentine. Whereas, GIC produced significantly inferior seal compared with other materials. The four materials were compared using mean rank which showed that Biodentine has the best sealing ability and GIC has the least sealing ability among four materials tested [Figure 1 and Table 2].

Sealing ability = Biodentine > MTA Plus > MTA > GIC

Marginal adaptation

SEM evaluation showed gap between the dentinal wall and all the materials used in the study. The marginal adaptation

Table 1: Apical leakage scores in the experimental materials (evaluation of sealing ability)

Group	Filling materials	Number of specimens	Apical microleakage scores			
			0	1	2	3
1	GIC	10	0	2	1	7
2	MTA	10	2	4	3	1
3	Biodentine	10	5	4	1	0
4	MTA Plus	10	5	1	3	1

Table 2: Pairwise comparison of apical leakage scores among different filling materials by Mann–Whitney U-test

Filling materials	Median	Sum of ranks	Sum of ranks	Z-value	*P*-value
MTA	1.00	250.50			
MTA Plus	1.00	214.50	94.50	–0.7466	0.4553
MTA	1.00	265.50			
Biodentine	1.00	199.50	79.50	–1.3688	0.1711
MTA	1.00	159.00			
GIC	3.00	306.00	39.00	–3.0486	0.0023*
MTA Plus	1.00	241.50			
Biodentine	1.00	223.50	103.50	–0.3733	0.7089
MTA Plus	1.00	153.00			
GIC	3.00	312.00	33.00	–3.2975	0.0010*
Biodentine	1.00	141.00			
GIC	3.00	324.00	21.00	–3.7952	0.0001*

*$P<0.05$

of different root-end filling materials can be classified in descending numerical order of marginal gap size as follows: GIC (19.752 µm), MTA (16.453 µm), Biodentine (8.734 µm), and MTA Plus (4.898 µm). The study showed statistically significant difference ($P < 0.001$) among all materials tested. MTA Plus showed best marginal adaptation and GIC showed poor marginal adaptation compared to other materials tested [Figure 2 and Table 2].

Marginal adaptation = MTA Plus > Biodentine > MTA > GIC

Discussion

Most endodontic failures occur as a result of a persistent or secondary intraradicular infection. Therefore, a root-end filling material should provide an apical seal to an otherwise unobturated root canal or improve the seal of existing root canal fillings, thereby impeding both the traffic of tissue fluids into the root canal and the egress of microorganisms from the root canal system toward the periradicular tissues.[5]

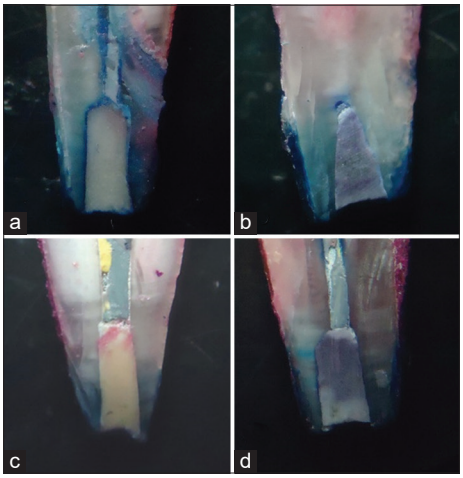

Figure 1: Stereomicroscopic images of longitudinal section of tooth showing the dye penetration along the dentin-restoration interface; (a) GIC, (b) MTA, (c) Biodentine, (d) MTA Plus

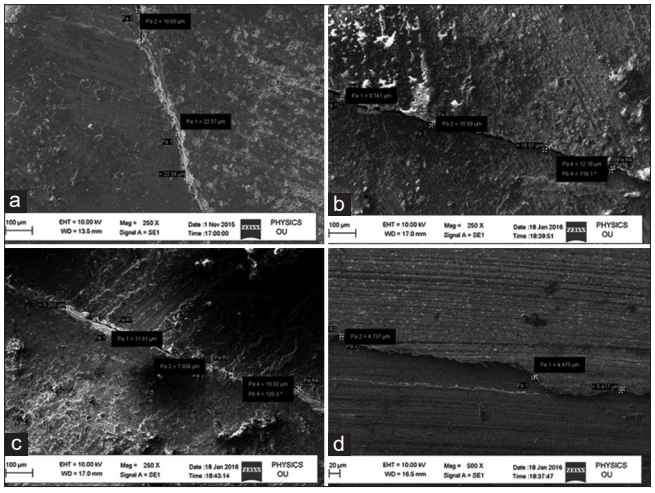

Figure 2: Scanning electron microscope images of longitudinal section of tooth for measurement of gap at the dentin-restoration interface (measured in micrometers); (a) GIC, (b) MTA, (c) Biodentine, (d) MTA Plus

The choice of a retrofilling material could be governed by biocompatibility, apical sealability, handling properties, and long-term clinical success. The clinical significance of microleakage in apical surgery has not been elucidated. However, it seems logical that the lesser leakage would prevent migration of bacteria and toxins into the periradicular tissue.[6] At present, no filling material fulfills all of ideal requirements.

In this study, we chose to compare the commonly used root-end filling materials such as GIC and MTA with recently introduced materials such as Biodentine and MTA Plus.

GIC is a universal material and has been used in root-end fillings as they do not induce tissue inflammatory responses and offer the advantages of biocompatibility, fluoride release, and favorable chemical adhesion to enamel and dentin. However, it is greatly affected by moisture and blood during the initial setting time, resulting in increased solubility and decreased bond strength.[7]

MTA when used as a root-end filling material not only exhibits good sealing ability, excellent long-term prognosis, and good biocompatibility but also favors tissue regeneration[8] and has been considered as a gold standard with which all recently introduced materials are compared. However, the main drawbacks of MTA include difficulty in handling, long setting time, potential discoloration of hard tissues, and high material cost.[9]

New experimental active tricalcium silicate (Ca3SiO5)-based restorative cement is introduced by name of Biodentine™ (Septodont, Saint-Maur-des-Fosses, France). It has certain advantages over MTA like short setting time (12 min), easy manipulation, better compressive strength, and cost-effectiveness and hence can be propagated to be used as an alternative to MTA as a root-end filling material.[10]

Recently, another MTA has been introduced called MTA Plus (Avalon Biomed Inc., Bradenton, FL, USA) which is similar in composition to MTA but is ground finer. It is provided with either a gel or water ampoules for mixing. MTA Plus has been recently introduced so there are not many studies reported on this material.[11]

Root-end resection was done using operating microscope at an angle of 0°–10° which has been proved to be most acceptable by earlier studies. The inclined plane sectioning at 30° or 45° angle could have disadvantages such as open dentinal tubules, errors in post-operative radiographs, more mechanical stresses, more loss of dentin, cementum, and bone that could result in compromised healing.[12] Preferred depth of root resection is 3 mm. Resection at the depth of 3 mm reduces the apical ramifications by 98% and lateral canals by 93%.[12,13] The depth of retrograde cavities in this study was kept to an optimum of 3 mm as more than that does not bestow any greater benefits whereas lesser depth may jeopardize the long-term success

Table 3: Distribution of mean of the gap size of the experimental materials (evaluation of marginal adaptation)

Group	*n*	Mean	Std. deviation	Std. error	Median	Min.	Max	*P*-value
GIC	10	19.752	3.028	0.6772	20.190	12.330	24.540	<0.001
MTA	10	16.453	3.204	0.7165	16.705	10.890	22.240	
Biodentine	10	8.734	2.406	0.5379	8.745	4.370	12.650	
MTA Plus	10	4.898	1.973	0.4412	4.600	1.870	9.280	

of apical seal.[14] This is attributed to the occlusion of apical tubules by retrofilling material.

Dye penetration technique is the most frequently used method to evaluate sealing ability of dental materials and methylene blue dye is widely used for convenience. Its particles are of similar size to that of microorganisms and their metabolites and its small molecular weight provides a high degree of penetrability and sensitivity.[8,15]

In the present study, there is a statistical difference between sealing ability of GIC and other materials tested, where GIC has shown least resistance to microleakage compared to MTA, Biodentine, and MTA Plus. There is no statistical difference between sealing ability of MTA, Biodentine, and MTA Plus, although Biodentine has shown slightly better sealing ability compared to MTA and MTA Plus.

The probable reasons are as follows:

a. When biodentine comes in contact with dentin, it leads to the formation of tag-like structures alongside an interfacial layer called the "mineral infiltration zone," where the alkaline caustic effect of calcium silicate cements hydration products degrades the collagenous component of interfacial dentin[16]
b. The sealing ability of biodentine is most likely through the formation of tags. Han and Okiji showed that calcium and silicon ion uptake into dentin, leading to the formation of tag-like structures in biodentine, were higher than MTA[17]
c. Better seal with biodentine can also be attributed to its modified powder composition, that is, the addition of setting accelerators and softeners, a new pre-dosed capsule formulation for use in a mixing device largely improves the physical properties including sealing ability of the material
d. Biodentine has an advantage of faster setting time (12 min), thereby sealing the interface earlier to avoid further leakage to take place so there is a lower risk of bacterial contamination
e. Porosity and pore volume in set biodentine material are also less than MTA which could be a reason for better sealing ability.[18]

Despite the popularity of dye leakage studies, they have several disadvantages like molecular size of most dye particles is smaller than bacteria, most studies measure penetration in one plane rather than total leakage, they are static and do not reflect the dynamic interaction with periradicular tissues.[19] SEM has also been used to assess the adaptation and the sealing capacity of commonly used root-end filling materials. The inadequacy in the marginal adaptation may influence the sealing ability and the clinical success rate. A few previous studies have evaluated the marginal adaptation of the root-end filling material to the canal wall using resin replicas. Orosco *et al*. stated that for the evaluation of marginal adaptation of the retrofilling material, the samples can be directly viewed under SEM after gold sputtering and there is no need for creation of resin replicas, as direct SEM evaluation of the samples did not result in artificial gap formation.[20]

In the present study, MTA Plus has shown better marginal adaptation followed by Biodentine, MTA, and GIC. A study done by Ravichandra *et al*. to check for marginal adaptation of three root-end filling materials GIC, MTA, and Biodentine concluded that lowest marginal gaps and good marginal adaptation with biodentine followed by MTA and highest marginal gaps with GIC.[21] A SEM study conducted by Martí Bowen *et al*. on sealing ability of Biodentine, MTA, and GIC concluded that biodentine exhibited better marginal adaptation to dentin compared to MTA and GIC[22] which is in agreement with the present study.

In relation to the two types of variables that were analyzed in this study, we can evidence a correlation between apical microleakage rates and gaps at the interface of tooth – root-end filling material. The results were similar to those found by Stabholz *et al*. that also reported a correlation between microleakage and marginal adaptation of the materials.[23] However, these findings are contrary to Abdal and Retief[24] and Yoshimura *et al*.[25]

This study was performed in *in vitro* conditions. Further long-term studies are required to check for sealing ability of Biodentine and MTA Plus before routine clinical usage. Furthermore, further studies need to be done to check for nature and longevity of the bond obtained from dentin-biodentine interface and on the effect of blood contamination on sealing ability of Biodentine and MTA Plus.

Conclusion

The introduction of newer filling materials which are more adaptive, biocompatible, and less technique sensitive has shifted the focus to the use newer materials for the purpose of retrograde filling of the cavity. Within the limitations of this study, Biodentine exhibited better sealing ability and MTA Plus exhibited better marginal adaptation compared to MTA

and GIC. However, results revealed that both sealing ability and marginal adaptation of GIC were inferior compared to all test materials.

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Ethical Approval

Approval from our institution's review board for the use of human tissue used in the study was granted before the study was undertaken.

Disclosure

The authors do not have any financial interest in the companies whose materials are included in this article.

Conflicts of Interest

The authors declare that they have nothing to disclose.

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