Original Article

Comparative Scanning Electron Microscopic Study of the Marginal Adaptation of Four Root-End Filling Materials in Presence and Absence of Blood

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Abstract

Objectives: The aim of this study was to evaluate marginal adaptation of mineral trioxide aggregate (MTA), calcium enriched mixture (CEM) cement, Biodentine and BioAggregate in presence of normal saline and human blood.

Materials and Methods: In this in-vitro experimental study, 80 extracted single-rooted human teeth were instrumented and filled with gutta-percha. After resecting the root-end, apical cavity preparation was done and the teeth were randomly divided into four groups of 20 (a total of eight subgroups). Root-end filling materials were placed in three-millimeter root-end cavities prepared ultrasonically. Half the specimens in each group were exposed to normal saline and the other half to fresh whole human blood. After four days, epoxy resin replicas of the apical portion of samples were fabricated and scanning electron microscopy analysis was performed to find gaps in the adaptation of the root-end filling materials at their interface with dentin. The Kruskal-Wallis and Mann-Whitney tests were used for statistical analysis of data with $P<0.05$ level of significance.

Results: There were no significant differences in marginal adaptation of the eight tested groups ($P>0.05$).

Conclusion: Based on the results, blood contamination does not affect the marginal adaptation of MTA, CEM cement, Biodentine or BioAggregate.

Keywords: Dental Marginal Adaptation; Mineral Trioxide Aggregate; BioAggregate

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INTRODUCTION

Retrograde root canal filling following apicoectomy is an important procedure, aimed at hermetically sealing the root canal against the leakage of irritants from the canal into the periapical tissues. Insufficient retrograde seal is considered a major cause of treatment failure. Thus, it is important to choose a biocompatible root-end filling material with high sealing ability. Evaluation of the marginal adaptation of filling materials with dentinal walls can provide valuable information about their sealing ability [1]. Several root-end filling materials have been used such as amalgam, gutta-

percha, zinc oxide-eugenol cements, glass ionomer, composite resins, calcium hydroxide cements, and Mineral Trioxide Aggregate (MTA). Mineral trioxide aggregate is a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, tetra calcium aluminoferrite, bismuth oxide, gypsum and some other ingredients [2]. Since its introduction, MTA has shown superior sealing properties and marginal adaptation to other root end filling materials [3]. New root end filling materials are now available in dental market such as calcium enriched mixture (CEM) cement, Biodentine and BioAggregate. The manufacturers claim that these materials have biological properties similar to those of MTA. Calcium enriched mixture cement is composed of calcium hydroxide, calcium oxide, calcium phosphate, calcium sulfate, calcium carbonate and calcium silicate [4]. This material is similar to MTA but has much easier handling [4,5]. BioAggregate is a modified version of MTA. Most of its constituents are similar to those of white MTA; the main difference is that BioAggregate is aluminum-free. It is composed of tricalcium silicate, dicalcium silicate, calcium phosphate monobasic, and amorphous silicone dioxide with the addition of tantalum pentoxide, instead of bismuth oxide in MTA, for radiopacity [6,7].

Leal et al. concluded that BioAggregate and MTA had the same leakage properties [8]. Also, the antibacterial properties and biocompatibility of BioAggregate are the same as those of MTA [9]. It is believed that Biodentine has the same applications as MTA and it may play a role in dentinal repair. The powder includes tricalcium silicate, calcium carbonate and zirconium oxide as radiopaque agent and the liquid consists of water, calcium chloride as an accelerator of setting and water reducing agent, and a modified poly carboxylate as superplasticizer [10,11]. With respect to the available dental literature and lack of information on the marginal adaptation of BioAggregate and Biodentine and the effect of blood contamination on their properties, this study sought to evaluate and compare marginal adaptation of MTA, CEM cement, BioAggregate and Biodentine.

MATERIALS AND METHODS

Sample selection

Eighty single-rooted human teeth with straight roots were selected. Preoperative mesiodistal and buccolingual radiographs of each root were taken to verify that only one root canal was present and that there were no internal or external resorption or calcifications. All the specimens were immersed in 5.25% sodium hypochlorite for 24 hours and then they were stored in saline soaked gauze during the study period. The crowns were cut with a high-speed handpiece (W&H, TREND, Bürmoos, Austria) and a long cylindrical diamond bur (Tizkavan, Tehran, Iran) under water spray to prepare a standardized 15-mm tooth length from the root apex. To ensure that no cracks had been created in the specimens, they were all examined under a microscope (F170, Carl Zeiss AG, Jena, Germany) at ×25 magnification. Six specimens were cracked and replaced.

Root canal treatment

Working length was determined one millimeter short of the length determined by a # 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) with its tip visible at the apical foramen. Canals were shaped using the standard technique with ProTaper rotary files (Dentsply, Maillefer, Ballaigues, Switzerland). The preparation was started with SX file and followed by S1, S2, F1, F2 and F3 files. Between each two files, the canal was rinsed with 2mL of 5.25% sodium hypochlorite and the final rinsing was done with 5mL of normal saline. After drying with paper points (Meta Biomed Co., Ltd., Chungbuk, Korea), the canals were filled with size 30, 0.02 taper gutta-percha (Meta

Biomed Co., Ltd., Chungbuk, Korea) and AH26 sealer (Dentsply Maillefer, Ballaigues, Switzerland) using the conventional lateral compaction technique. Next, the teeth were wrapped in wet gauze and placed in an incubator at 37°C for 24 hours for complete setting of the filling materials.

Root-end preparation

The apical three millimeters of roots were sectioned perpendicular to the long axis of the tooth with a high-speed handpiece and a long cylindrical diamond bur under water spray. Then, a three-millimeter-deep root-end cavity was prepared using a retrotip (E32D, NSK, Tokyo, Japan) attached to an ultrasonic unit (Varios 970, NSK, Tokyo, Japan) under continuous irrigation with saline solution.

Root-end filling

Once the cavities were prepared, the teeth were randomly divided into four groups (n=20). The filling materials, Biodentine (Septodent, Saint Maur des Fossés, France), CEM cement (Bionique Dent, Tehran-Karaj, Iran), ProRoot MTA (Dentsply Maillefer, Tulsa, OK, USA) and Bioaggregate (Innovative BioCeramix, Vancouver, Canada) were mixed according to the manufacturer's instructions. In order to have a homogenous mixture, the mixing procedure was carried out in capsules by means of an amalgamator adjusted at 4500 rpm for 30 seconds [12]. The filling materials were placed into root end cavities by a MTA carrier (Medesy, Maniago, Italy) and condensed with a plugger. Ten specimens in each group were placed in a 1.5 mL volume test tube with a cotton pellet moistened with blood; the remaining 10 specimens were placed in a test tube with a cotton pellet moistened with normal saline. The specimens were in contact with the cotton pellets. All the teeth were evaluated mesiodistally and buccolingually by radiographs. None of the samples had insufficient amount of root end filling materials or voids. Thus, no sample was replaced and the materials in all samples were well condensed. The specimens were stored in an incubator (37°C and 100% humidity) for 96 hours. Next, they were removed from the incubator and their complete setting was ensured using a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland).

Preparation of resin replicas and image analysis:

The impression of the resected root surfaces and cavities was obtained using additional silicone (Panasil®, Kettenbach GmbH & Co. KG., Germany) by means of customized mini-trays made by cutting the tip of test tubes. The technique used for making the impressions was single stage with heavy and extra-light consistency. The replicas were obtained by pouring the mixture of 2/1 w/w epoxy resin (Epoxyran8060, Tehran, Iran) and hardener (Epoxyran1946, Tehran, Iran) into the set impressions. Then, the trays were stored for 24 hours for final setting. Set replicas were evaluated for bubbles and in case of any imperfection, the pouring procedure was repeated. Replicas were mounted on aluminum stubs, sputter-coated with gold and examined and photographed at ×40 to ×2000 magnifications using scanning electron microscopy (SEM) (S4160, Hitachi, Tokyo, Japan). To measure the gap width at the tooth-filling interface, the area of each micrograph was divided into 16 portions; on each portion, the greatest width was determined visually and measured with field emission scanning electron microscope (FESEM) software, and recorded in micrometer in Excel software (version 2010, Microsoft, IL, USA). The mean value of these 16 measurements was calculated and recorded as the maximum mean value of the marginal gap for that specimen. These calculations were repeated for each of the 10 specimens and the mean value of these 10 measurements was reported as the maximum mean value of marginal gap in each subgroup.

Statistical analysis:

The effect of media (normal saline or blood) on the marginal adaptation in each group was analyzed using the Mann-Whitney test. The marginal gap of the four materials in each medium was compared using the Kruskal-Wallis test with $P<0.05$ level of significance. Data were analyzed using SPSS 18 software (Microsoft, IL, USA).

RESULTS

The mean value and standard deviation (SD) of the marginal gap in each group are summarized in Table 1. There was no significant difference in the measured marginal gap between the four filling materials ($P>0.05$). There was no significant difference between the effect of normal saline and blood on the marginal adaptation of the four different filling materials either ($P>0.05$) (Fig. 1). In general, the difference among the measured marginal gap in the eight groups was not significant ($P>0.05$).

DISCUSSION

There are conflicting results reported in the literature about the relationship of marginal adaptation and sealing ability of root end filling materials. Some authors believe that there is no relationship between the marginal adaptation and the sealing properties of root end filling materials [13,14], while some others concluded otherwise [1,15,16]. However, considering the controversial results and limitations, it seems that evaluating marginal adaptation of root end filling materials with root dentin can provide valuable information about their apical sealability [1]. Evidence shows that MTA as a root end filling material has an acceptable marginal adaptation, which is superior to that of other materials like amalgam, IRM®, Super-EBA™ and Vitremer [13,15-21]. Numerous studies have investigated different properties of CEM cement, BioAggregate and Biodentine including their antimicrobial properties, effects on reparative dentin, connective tissue reactions, cytotoxicity, chemotactic effects and bacterial leakage, and the results of these studies have been encouraging [6,8-11,22-44].

Except one [45], there were no other studies on the marginal adaptation of these materials in comparison with one another. In the current study, we used SEM to evaluate marginal adaptation. There are some disadvantages with SEM like its two dimensional nature and the potential separation of the filling material from the root dentin or crack formation in hard tissue when using high vacuum type. In order to overcome the two dimensional limitations, some researchers like Torabinejad et al, [16] and Abdal and Retief [14] used longitudinal sections in addition to cross sections; but conflicting results were obtained using this method. They attributed this to artificial gaps as the result of longitudinal sectioning [14,16,46]. Therefore, longitudinal sections were not made in the current study. Replicas were used in the current study since many reports are available on the successful use of this method. Producing replicas prevents expansion and contraction of tooth structure or root end filling material that occur during sputter coating and alter the results [1,13,15-17,20,47,48]. Torabinejad et al. indicated that replicas like teeth showed artificial gaps at the interface of root end filling material and root dentin as the result of longitudinal sectioning [16].

Table 1. Results of microleakage assessment of the eight experimental groups

Group	Mean (µm)	Variance (µm)
BioD/NS	0.50	0.58
BioD/B	1.58	1.73
CEM/NS	1.13	0.78
CEM/B	1.36	1.17
BioA/NS	0.66	1.11
BioA/B	1.53	0.88
MTA/NS	0.81	1.14
MTA/B	1.36	1.37

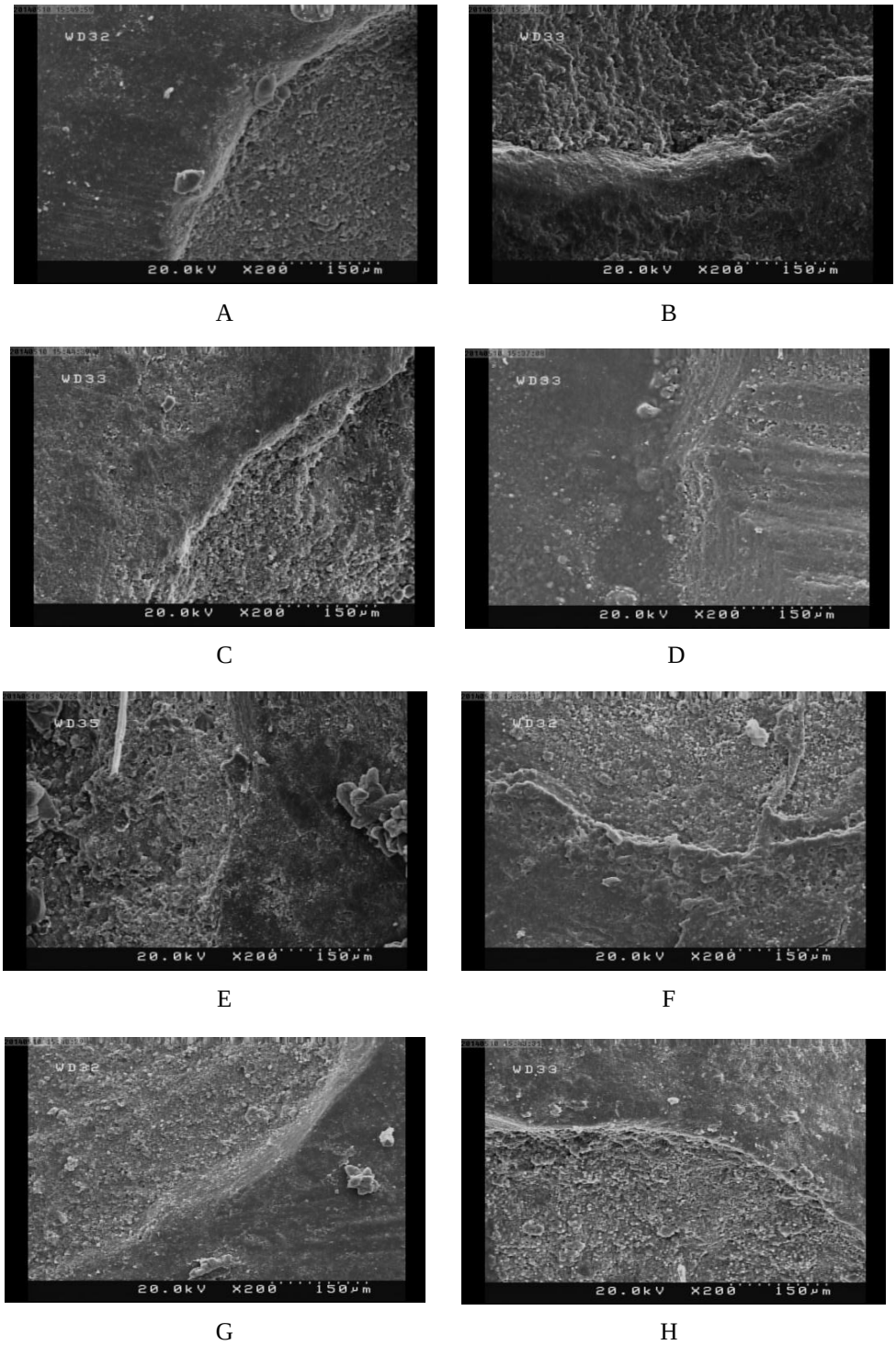

Fig. 1.

A. Micrograph of root dentin-BioAggregate interface exposed to normal saline (×200)
B. Micrograph of root dentin- BioAggregate interface exposed to human blood (×200)
C. Micrograph of root dentin- Biodentine interface exposed to normal saline (×200)
D. Micrograph of root dentin- Biodentine interface exposed to human blood (×200)
E. Micrograph of root dentin- CEM interface exposed to normal saline (×200)
F. Micrograph of root dentine- CEM interface exposed to human blood (×200)
G. Micrograph of root dentine- MTA interface exposed to normal saline (×200)
H. Micrograph of root dentine- MTA interface exposed to human blood (×200)

In a SEM study, Gondim et al. reported that hard tissue details were not lost in replicas when compared to teeth [49].

In order to minimize the risk of crack formation in the specimens, ultrasonic device was used with no hand pressure. Another measure taken to control cracks was preparing the canals up to size F3 file so that after sectioning, the three-millimeter diameter of the root end would fit the retrotip and this would diminish the strokes that could make the root susceptible to cracks. Despite all these measures, the specimens were checked under a dental microscope at ×25 magnification in different steps throughout the study. In a SEM study, Torabinejad et al. measured gap width at the interface of root end filling material and tooth on four points on a micrograph and the mean value of these four measurements was reported in micrometer as the gap width of that specimen. The amount obtained for each specimen was calculated and the mean value was reported as gap width of the study group. The value reported for MTA was 2.68 ± 1.33μm [16].

Badr reported similar results. He studied replicas in both cross sections and longitudinal sections and reported the gap mean value as 1.59±.615 μm and 2.141±0.530 μm, respectively [20]. To be accurate, in the current study, the measurements were made in 16 portions and for each portion, the maximum gap value was reported. Our investigation showed no significant difference among the four root-end filling materials. The explanation may lie in the fact that all of the investigated materials had similar composition with calcium silicate as their main constituent. As in the study conducted by Costa et al, for evaluation of the marginal adaptation of five root-end filling materials, materials containing calcium oxide (MTA and Portland cement) showed similar results [15]. Another explanation for their similar marginal adaptation may be the method of mixing applied in the filling procedure that creates almost the same flow and consistency of filling materials.In the current study, blood did not significantly affect the marginal adaptation of the root end filling materials. According to the dental literature, MTA's physical properties like resistance to displacement and microhardness decrease by blood contamination, which may be due to the effect of blood on the configuration of crystals and prevention of acicular crystal formation. It also has a short-term adverse effect on calcium hydroxide crystals [50-54]. Two in vitro studies investigated the effect of blood on CEM cement and reported its unfavorable effects on physical properties of CEM [50,55].

Torabinejad et al. assessed root-end dye microleakage in dry and blood contaminated environments. They found that the mean dye leakage of MTA contaminated with blood did not change significantly, which was in line with our results [56]. This result regarding the effect of blood cannot be generalized to the in vivo condition; because the duration of exposure of root end filling material to blood cannot be exactly simulated in vitro. By preventing the blood from coagulation in vitro, the clinical situation can be better simulated. But to make it happen, using anticoagulant agents does not seem logical since their effect on root end filling material is not clear. However, some published studies reported unfavorable effects of citrate as an anticoagulant on MTA setting [52,53]. The effect of blood on marginal adaptation needs to be further investigated.

CONCLUSION

This study showed that marginal adaptation of MTA, CEM cement, Biodentine and BioAggregate was not adversely affected by blood contamination.

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