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Effect of Blood and Synthetic Tissue Fluid on Marginal Adaptation and Surface Microstructure of Different Root-end Filling Materials: A Scanning Electron Microscope Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed to evaluate the effect of blood and synthetic tissue fluid on marginal adaptation and surface microstructure of Mineral trioxide aggregate (MTA) and Endosequence root repair material (ERRM Putty).

Materials and Methods: Sixty human single-rooted teeth were endodontically treated, and root-end cavities were prepared to 3 mm depth. The teeth were divided randomly into three groups (n = 20) based on the contamination, and each group was again divided into two subgroups (n=10) based on the root-end filling material used.

Group I (Blood Exposed): The root-end cavities were filled with fresh blood then gently aspirated. The retro cavities were then filled with MTA and ERRM putty and placed in molds containing heparinized blood.

Group II (STF-Exposed): STF was mixed accordingly and the retro cavities were then filled with STF and then gently aspirated. The cavities were now filled with MTA and ERRM putty and placed in molds containing STF.

Group III (Control): The root end cavities were not contaminated with any fluid and were filled with MTA and ERRM putty in this group. All samples were viewed Under SEM at 2000x magnification and statistically analyzed with Tukey post hoc test.

Results: Mean gap width in microns (SEM) was blood with MTA(22.12), followed by blood with ERRM putty (18.89), Control with ERRM putty (14.53), STF with MTA (6.86), Control with MTA (6.85) and STF with ERRM putty (3.78). A significant difference was observed among the three groups (Blood, STF and Control) with gap width in microns (SEM) at 5% level of significance.

Conclusions: The samples contaminated with blood showed more gap width, and ERRM putty exhibited better marginal adaptation.

Keywords: Marginal adaptation; root-end filling; hydroxyapatite; endodontic surgery.

ABBREVIATIONS

ERRM Putty : Endosequence root repair material putty
MTA : Mineral trioxide aggregate
STF : Synthetic tissue fluid
SEM : Scanning electron microscope

1. INTRODUCTION

The main goal of Endodontic therapy is to provide hermetic seal of the endodontium from the periodontium. However, it is necessary to approach retrograde in case of failure due to previous surgery, anatomical problems like non negotiable canals, severe root curvature, iatrogenic errors like ledging of canals, blockage from debris, separated instruments, overfilling of canals, apical canal transportation and horizontal apical root fracture. Other indications for surgery include to establish drainage, for biopsy of a lesion, to repair any iatrogenic, developmental defects, pathologic lesions. "Periradicular surgery includes surgical debridement of pathological periradicular tissue, root-end resection, preparation of a root-end cavity, and placement of a root-end filling to seal the root canal" [1].

"The success of the endodontic surgery depends on the regeneration of a functional periodontal attachment which includes cementum overlying the resected root-end surface, periodontal ligament, and alveolar bone. This regeneration would occur more predictably when the root end cavity is filled with a material that seals the root canal to prevent egress of any remaining bacteria or their by-products and also allows for the formation of a normal periodontium across the exterior surface" [2]. So developed the bioceramic materials like Biodentine, MTA, and Endosequence.

"EndoSequence Root Repair Material (ERRM) (Brasseler, USA) is a new bioceramic material developed recently. It is a premixed product in mouldable putty and preloaded syringeable paste to provide the clinician with a homogeneous and consistent material that sets in the presence of moisture. ERRM proved to be biocompatible, antibacterial, osteogenic, and able to seal root-end cavities" [3]. "However, MTA proved to show the excellent sealing between the dentin and root-end filling material when compared to conventional root-end filling materials" [4]. "During apical surgery, the potential exists for contamination of the root end preparation with blood, saline, and saliva. This contamination may affect the properties of MTA and ERRM putty and, more importantly, maintain an adequate

seal" [5]. So, the present in vitro study aimed to evaluate the effect of blood and synthetic tissue fluid on marginal adaptation and surface microstructure of MTA and ERRM Putty.

2. MATERIALS AND METHODS

In this study, we used sixty freshly extracted human single-rooted teeth. The crowns were resected from the cementoenamel junction (CEJ) using a high-speed diamond bur under water spray by maintaining the root length of 14mm. The canals were cleaned and shaped with step-back technique and obturated with gutta-percha by lateral compaction technique and AH plus sealer. The samples were incubated at 37˚C and 95% relative humidity for 48 hours.

Then, the apical 3-mm of roots were sectioned perpendicular to the long axis of the teeth using a fissured diamond bur. Following root-end cavities were prepared to 3 mm depth using a microsurgical ultrasonic retro tip (Acteon, Satelac) with medium power and water spray and dried with paper points.

The teeth were divided randomly into three groups (n = 20), Group I- Blood Exposed, Group II- Synthetic Tissue Fluid-Exposed, and Group III- Control. Each group was again divided into two subgroups (n=10) based on the root-end filling material used, Subgroup Ia-MTA, Subgroup Ib-ERRM Putty, Subgroup IIa-MTA, Subgroup IIb-ERRM Putty, Subgroup IIIa-MTA, Subgroup IIIb-ERRM Putty.

2.1 Group I-Blood Exposed (n=20)

Collected fresh human blood of 5ml from one of the researchers. Each root-end cavity was filled immediately with 0.1 ml of fresh blood by using the syringe and then gently aspirated with the same (Fig. 1a). All the specimens were filled and aspirated in the same manner. Later the root end cavities of the specimens were filled with the MTA (SUBGROUP Ia) and ERRM putty (SUBGROUP Ib) and placed in a mold containing remaining blood with 50 IU of heparin per one milliliter of blood.

Sub Group Ia (n=10): MTA (MTA Angelus, Brazil) was mixed according to the manufacturer's instructions, incrementally placed into the cavity, and compacted using pluggers. The root end cavities were overfilled slightly, and the excess material was burnished gently. The roots were immediately immersed in molds containing heparinized blood.

Sub Group Ib (n=10): Endosequence (Brasseler, USA) incrementally placed into the cavity and compacted using pluggers. The cavities were overfilled slightly and burnished the excess material. The roots were immediately immersed in molds containing heparinized blood.

2.2 Group II -STF-Exposed (n=20)

STF (synthetic tissue fluid) is a phosphate-containing solution, prepared with 1.7 g of KH_2PO_4, 11.8 g of Na_2HPO_4, 80.0 g of NaCl, and 2.0 g of KCl in 10 L of H_2O [6]. All these ionic compounds were collected in powder form from (Aravind scientific products, Vijayawada) and mixed with 10 L of water [7] and taken only 5ml of prepared solution in the present study. Each root-end cavity was filled with 0.1 ml of STF by using the syringe and then gently aspirated with the same (Fig. 1a). All the specimens were filled and aspirated in the same manner. Later the root end cavities of the specimens were filled with the MTA (SUBGROUP IIa) and ERRM putty (SUBGROUP IIb) and placed in a mold containing remaining STF (Fig. 1b).

Sub Group IIa (n=10): MTA was mixed with distilled water according to the manufacturer's instructions, incrementally placed into the cavity, and compacted using pluggers. The cavities were overfilled slightly, and the excess material was gently burnished, and the samples are placed in a mold containing STF.

Sub Group IIb (n=10): Endosequence incrementally placed into the cavity and compacted using pluggers. The cavities were overfilled slightly, and the excess material was gently burnished, and Samples are placed in a mold containing STF.

2.3 Group III-Control (n=20)

The root-end cavities were not contaminated with any fluid in this group.

Sub Group IIIa (n=10): Root end cavities were prepared, and MTA was placed without blood or synthetic tissue fluid exposure (Fig. 1c).

Sub Group IIIb (n=10): Root end cavities were prepared, and ERRM was placed without blood or synthetic tissue fluid exposure.

All the samples were incubated at 37˚C and 95% relative humidity for 48 hours.

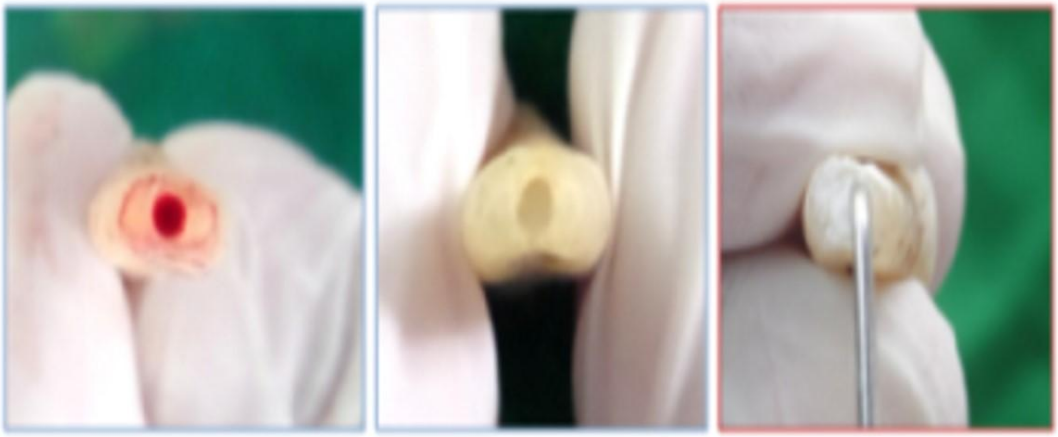

Fig. 1. (Ia) Retrocavity contamination with Blood before filling (Ib) Retrocavity contamination with STF before filling (Ic) Retrocavity filled in the control group

2.4 Scanning Electron Microscopy (SEM) Analysis

The specimens were removed from the molds, rinsed with distilled water, air-dried, and stored at 37^{o}c for 72 hours. The samples were mounted on an aluminum stub, gold-sputtered, and viewed under SEM (Osmania physics lab, Hyderabad). Under SEM, measured the maximum distance between filling and the cavity walls directly at the MTA-dentin interface, Enosequence-dentin interface, and viewed the Surface characteristics of MTA and Endosequence at 2000x magnification.

3. RESULTS

A significant difference in gap width was observed between Blood exposed, STF exposed and Control groups. Blood groups showed more gap width followed by control and STF groups. The maximum gap width among the groups is as follows Ia,Ib,IIIB,IIa,IIIa,IIB in microns (SEM) (Fig. 2). Furthermore, the mean with gap width in microns (SEM) is significantly higher in MTA material in the Blood group than others. However the gap width of MTA in the control group and MTA in STF group is almost same (Table 1).

4. DISCUSSION

“Root-end filling materials are commonly used in surgical endodontics. An ideal material to seal the root end cavity should be dimensionally stable and capable of adhering to the walls of the cavity, and resistant to moisture” [8]. An ideal material should induct or conduct a bone deposition, provide a good seal, capable of set in a wet environment [9].

Bioceramics are biocompatible ceramics introduced to endodontics in the 1990 [10] and are using in endodontics as a root repair material, retro filling material, perforation sealing, endodontic sealers, as these materials have an intrinsic osteoconductive activity that induces regeneration [11].

“Torabinejad developed Mineral trioxide aggregate. It provides an effective seal against the penetration of bacteria and their by-products. So it is recommended as a root-end filling material and as a coronal plug after filling the root canal system” [12]. “An essential feature of MTA as a root-end filling material is cementum-like tissue formation directly on its surface, thus providing a double seal” [13].

“EndoSequence putty Root Repair Material (ERRM) is available as a premixed putty used for perforation repair and in apical surgery like a root-end filling material and pulp capping. For the material to be set, the moisture present in the dentinal tubules is adequate. Properties of Endosequence as stated by the manufacturer are - bond to adjacent dentin, less shrinkage, hydrophilic, highly biocompatible, radiopaque, and antibacterial due to a high pH during setting” [6]. The advantages of this material are improved handling characteristics over MTA.

“The nanosphere particles with a maximum diameter of 1 x 10^{-3}µm in it allow the material to enter dentinal tubules, be moistened by dentin liquid, and create a mechanical bond upon setting” [6]. “ERRM is bioactive due to its ability to form hydroxyapatite or apatite-like layer on its surface when it comes in contact with phosphate-containing fluids” [3].

Table 1. Pair wise comparison of three groups (Blood, STF, and Control) and two materials (MTA and Endosequence) with gap width in microns (SEM) by Tukeys multiple posthoc procedures

Groups with materials	Blood group with MTA	Blood group with Endosequence	STF group with MTA	STFgroup with Endosequence	Control group with MTA	Control group with Endosequence
Mean	22.1160	18.8940	6.8640	3.7830	6.8460	14.5340
SD	3.2141	3.5947	2.0081	0.6589	1.1447	2.5497
Blood group with MTA	-					
Blood group with Endosequence	p=0.0493*	-				
STF group with MTA	p=0.0001*	p=0.0001*	-			
STF group with Endosequence	p=0.0001*	p=0.0001*	p=0.0677	-		
Control group with MTA	p=0.0001*	p=0.0001*	p=0.9999	p=0.0704	-	
Control group with Endosequence	p=0.0001*	p=0.0026*	p=0.0001*	p=0.0001*	p=0.0001*	-

**p<0.05*

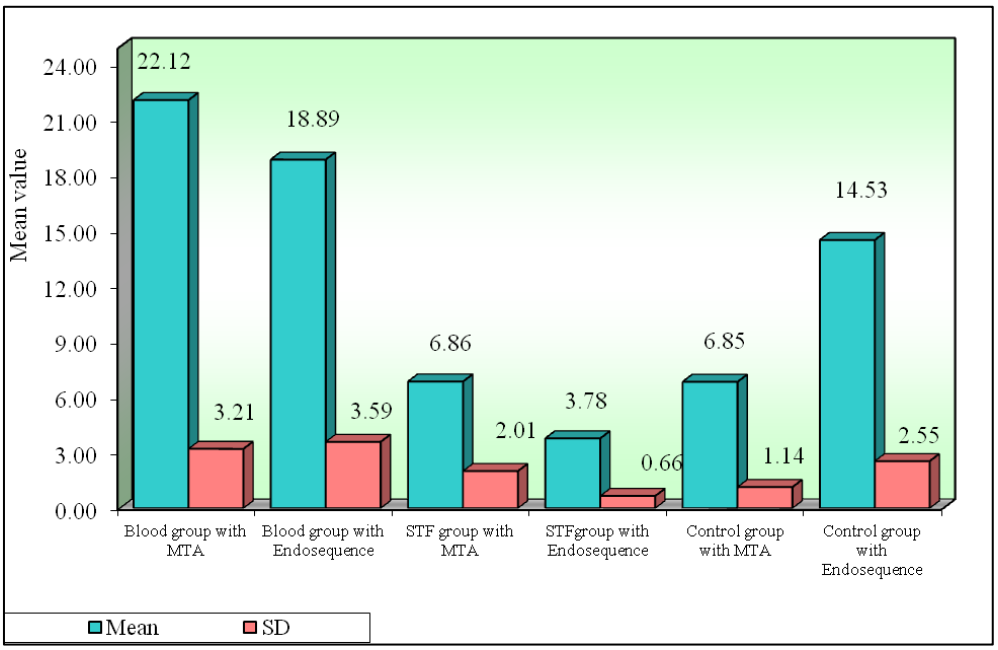

Fig. 2. Comparison of three groups (Blood, STF, Control) and two materials (MTA and Endosequence) with gap width in microns (SEM)

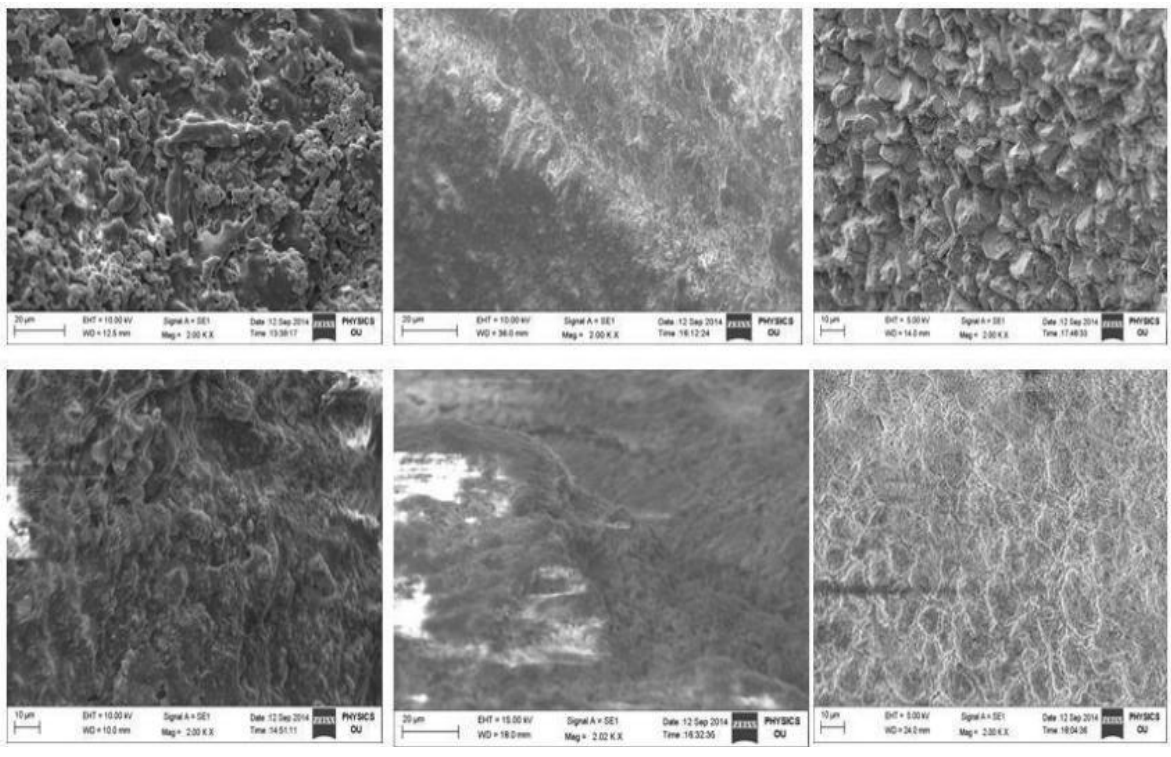

Fig. 3. Surface characteristics of MTA and ERRM putty in three main groups

(3a) MTA In Blood Group (Top right image)
(3b) MTA In STF Group (Top middle image).
(3c) MTA In Control Group (Top left image) (3d) ERRM Putty In Blood Group(Bottom right image).
(3e) ERRM Putty In STF Group (Bottom middle image).
(3f)ERRM Putty in control group (Bottom left image).

"STF (synthetic tissue fluid) is a phosphate-containing solution, does not contain any organic elements. moreover STF has similarities with inorganic components of tissue fluids to some extent and only maintains the mineral contents of tissue fluid. For this study, STF was prepared with: 1.7 g of KH_2PO_4, 11.8 g of Na_2HPO_4, 80.0 g of NaCl, and 2.0 g of KCl in 10 L of H_2O (pH = 7.2)" [7].

4.1 STF Gap width

In the present study, a significant difference was not observed between MTA and Endosequence materials with gap width in microns (SEM) in the STF group (p-value 0.06) at a 5% level of significance. But the gap width in microns (SEM) in the STF group is higher in MTA material than ERRM Putty (Table 1).

"The ability of MTA to precipitate carbonate apatite in contact with STF in the MTA-dentin interfacial area and within dentinal tubules has been proposed as a mechanism of decreasing leakage, which may explain the higher sealing ability of STF-exposed MTA in comparison with saline or distilled water-exposed MTA. In addition, there is evidence of precipitation even within the first hour after immersion in STF. However, complete precipitation over the surface of MTA, MTA-dentin interface has been shown in the long term" [14]. In our study, When compared to MTA, ERRM putty showed less gap when exposed to synthetic tissue fluid, which may be because of precipitation of apatite crystalline structures earlier than MTA.

The present study results are similar to the finding of N. Shokouhinejad, M. H. Nekoofar et al. in which exposure of MTA, ERRM putty, and paste materials to the phosphate-buffered solution (PBS) resulted in no significant difference in the marginal adaptation of these materials. However, ERRM putty and MTA showed superior adaptation compared to ERRM paste in the longitudinal sections under SEM [15].

STF surface microstructure: SEM Photographs of MTA specimens showed crystalline phases, spherical aggregates composed of smaller particles, and a network of acicular crystals. These findings are following the studies conducted by Hashem, Wanees Amin, and Amin Salem Milani [16].EndoSequence Root Repair Material immersed in STF displayed smooth aggregates composed of individual and fused globular particles (Fig. 3e).

4.2 Blood Gap Width

In the present study, gap width in the blood group is significantly higher in MTA material than Endosequence material (Fig. 2). Amin Salem Milani *et al.* [16] studies also showed the negative effect of blood contamination on the marginal adaptation of MTA to dentin than synthetic tissue fluid.

Endosequence showed less gap when exposed to blood than MTA, maybe because finer particle size resulted in better penetration into dentinal tubules and its increased viscosity which makes resistance to dissolution.

Blood surface microstructure: Blood contamination caused fewer hexagonal crystals and a general lack of needle-like crystals. Crystals were more rounded and less angular in MTA-blood samples compared to MTA–STF exposed group (Fig. 3a). The crystals in the Endosequence blood exposed group showed smooth agglomerates (Fig. 3d).

4.3 Control Gap Width

In the present study, gap width in microns (SEM) in the control group is higher in Endosequence material than MTA material (Fig.2). This is due to the better marginal adaptation of MTA to water absorption and expansion of MTA during hydration. The manufacturers of Endosequence material claim that for premixed Endosequence putty, the setting reaction is initiated by moisture. However, in the present study, Endosequence showed more gap in the control group than MTA is because of non-provision of hydration for the complete set of the material by the operator and lack of dentinal fluid in the study specimens.

The results of the present study correlate with the Hirschberg et al. "Study in which comparison of the sealing ability of MTA to the sealing ability of ERRM done using a bacterial leakage model. They concluded that Samples in the ERRM group leaked significantly more than samples in the MTA group" [17]. In contrast to the above result, H. S. Antunes et al. novel bacterial nutrient leakage model showed that MTA and BC-RRM Putty had similar sealing abilities [18].

Control surface microstructure: The surface microstructure of MTA showed the presence of hexagonal crystals and a network of acicular crystals (Fig.3c), and Endosequence showed no agglomerates of crystals in the control group (Fig. 3f).

Clinical studies showed the similar successs rates of MTA and ERRM in modern endodontic surgery [19]. In animal studies ERRM showed histologically better tissue response than MTA on resected root surface [20]. In the present study also ERRM Putty showed better marginal adaptation to root dentin surface.

5. CONCLUSION

In this study, the samples contaminated with synthetic tissue fluid showed less gap width followed by control group and blood group in intergroup comparison. Among the two bioceramic materials the Endosequence putty showed better marginal adaptation to root canal walls when contaminated with blood and also synthetic tissue fluid. ERRM Putty contamination with STF showed good results because of the precipitation of apatite crystals in contrast the ERRM Putty did not show good result in control group, the reason may be the lack of minimum hydration that is necessary for the setting of the material. However, it is worth mentioning that further clinical studies are needed for confirmation of these results.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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