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RESEARCH ARTICLE

SEALING ABILITY OF ENDOSEQUENCE ROOT REPAIR MATERIAL, MINERAL TRIOXIDE AGGREGATE AND HIGH STRENGTH GIC AS FURCAL PERFORATION REPAIR MATERIAL: A PROTEIN LEAKAGE STUDY

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ABSTRACT

Aim: The purpose of this study was to compare the sealing ability of Endosequence Root Repair Material (ERRM), Mineral Trioxide Aggregate (MTA) and Ketac Molar easy mix GIC when used as furcal perforation repair materials. **Methodology**: A total of 105 human mandibular molars were used. Root canal treatment was carried out following which standardized furcal perforations were made. The specimen were randomly divided into three groups of 25 teeth each. In groups A, B, and C furcation perforations were filled with ERRM, MTA and GIC respectively. Fifteen teeth were used as the positive controlwith no filling materialin the perforationand fifteen teeth were used as negative control with complete closure of the perforation with two layers of nail varnish. A protein leakage model utilizing 22% bovine serum albumin (BSA) was used for evaluation. Leakage was noted when color conversion of the protein reagent was observed. Leakage was found in all the samples from Group A (ERRM), Group B (MTA), and Group C (GIC). **Result**: There was no statistically significant difference between ERRM and MTA, however, there was a statistically significant difference in GIC as compared to ERRM and MTA. **Conclusion**: Microleakage with ERRM is equivalent to MTA therefore ERRM is a good alternative to MTA for furcal perforation repair.

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INTRODUCTION

Root perforation is a significant complication of endodontic treatment. Such perforation may occur during preparation of access cavities, post space preparation, or as a result of extension of internal resorption into peri-radicular tissues. This kind of perforation results in loss of root integrity and further destruction of the adjacent periodontal tissues (Fuss, 1996). The prognosis of perforation depends on prevention or treatment of bacterial infection at the perforation site. In addition, the use of a non-irritating material that seals the perforation will limit periodontal inflammation (Bryan, 1999) Immediate sealing of the defect allows for the best chance of repair. The material used to seal a perforation should be non-toxic, biocompatible, easy to manipulate, capable of providing an adequate seal, dimensionally stable, radiopaque, and bacteriostatic or bactericidal, moisture insensitive and should be capable of inducing the formation of a calcific barrier (Sluyk, 1998). Different materials have been used to repair these defects, but none fulfill the criteria of an ideal repair material that include sealability, biocompatibility and the ability to induce cementogenesis and osteogenesis. Materials such as light cured glass ionomer, calcium hydroxide, zinc oxide eugenol, super EBA cement, composite resins, amalgam and tricalcium phosphate have been suggested for their ability to repair the defect and allow suitable conditions for the formation of a new periodontal attachment (Lantz, 1970) However, the main disadvantages of these materials include microleakage, varying degrees of toxicity and sensitivity to presence of moisture (Mahmoud Torabinejad, 1999). Endosequence root repair material is composed of calcium silicates, monobasic calcium phosphate, zirconium oxide, tantalum oxide, proprietary fillers, and thickening agents. ERRM as stated by the manufacturers can bond to adjacent dentin, has no shrinkage, biocompatible, hydrophilic, radiopaque, and antibacterial due to a high pH during setting. ERRM is

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available in a premixed form or in a jar as putty or as preloaded syringes in the form of flowable paste that sets within 30 minutes. The major advantage of this material is its improved handling characteristics over traditional MTA and the delivery of a consistent product with each application. However, current research on ERRM is limited and warrants further investigation. Mineral trioxide aggregate (MTA) has been thoroughly investigated in a variety of clinical endodontic applications. MTA has a desirable combination of biocompatibility, hydrophilicity, sealability, strength, and antibacterial action (Walsh, 2014). The clinical applications of MTA include direct pulp capping, apexogenesis, apexification, regenerative endodontic, root perforation repair, and surgical root-end filling (Mahmoud Torabinejad, 1999). Sarkar et al. 2005 reported the propensity of MTA to release Ca and its ability to form hydroxyapatite and concluded that these physicochemical reactions account for its sealing ability, biocompatibility and dentinogenic activity. The clinical success of MTA in these applications is well-studied, but many authors describe the poor handling characteristics of MTA and the resulting technique sensitivity of its application as the major disadvantage of this material (Seung-Jong Lee, 19993).

Ketac Molar (KM) is a posterior glass ionomer with suitable properties for the clinical applications. Ketac Molar Easymix has improved wettability of the powder by the liquid component which is a result of the unique particles that provides highly improved wettability of the powder by the liquid component resulting in for easy and fast mixing. This material meets the operator's strong demand for easy to mix glass ionomer restoratives with high physical– mechanical properties adhesion of Ketac Molar Easymix (KM) to dental tissue relies primarily on a chemical interaction and micromechanical interlocking and the main disadvantage of this material is its low sealing ability and pH[(10)] In many studies the main challenge of laboratory-based leakage testing models are to develop experimental setups that can provide reproducible results and clear-cut conclusions regarding the sealing ability of either the tested materials or techniques. Moreover, it is also important to be able to evaluate laboratory findings in a real clinical setting (Camps, 2003; Matloff, 1982) Thus, it is crucial to adopt a standardized, reliable, and reproducible method. Protein leakage method is more accurate for the estimation of microleakage in all planes, as the molecular size of bovine albumin protein used in protein leakage studies is close to that of bacterial lipopolysaccharide molecules (Bradford, 1976). The aim of this study was therefore to evaluate protein leakage of EndoSequence root repair material, Mineral Trioxide Aggregate and High strength Glass ionomer cement when used as furcal perforation repair materials.

MATERIALS AND METHODS

A total of 105 mandibular first molars were selected for the study. The teeth had been extracted for periodontal reasons and had mature roots and crowns. The teeth were stored at 0.02% thymol solution at 25 ºC until further use. A conventional access cavity was prepared using an Endo access bur (Dentsply, Maillefer, Switzerland). A #10 K file was used to confirm patency and to establish a clinical working length 1mm short of the apical foramen. Root canals were prepared using the Protaper rotary files upto size S1. 2ml of 3% NaOCl was used as an irrigating solution during instrumentation using a 3ml syringe with a 27 gauge needle. A final rinse was done using 17% EDTA.Sterile paper points were used to dry the canals and the canals were obturated with Endoflas and GP points. Standardised Perforations were then made using ISO #0. 09 round burs (BR-48, Mani Inc. Japan)

The Specimens were randomly divided into five groups of 25 as follows

Group 1: n=25, Perforations repaired with ERRM (Brassler, Savannah, USA)

Group 2: n=25, Perforations repaired with MTA (Angelus Repair Endodontic Cement, Brazil)

Group 3: n=25, Perforations repaired withKetac Molar Easymix GIC (3M, ESPE, USA)

Group 4: n=15, Negative Control,Teeth with complete coverage consisting of two layers of nail varnish

Group 5: n=15, Positive Control, No repair filling material was used

The Filling materials were applied in accordance with the manufacturers' instructions, using an MTA carrier (Sybro Endo, Orange, CA, USA), and packed with a cotton pellet. A cotton pellet moistened with sterile distilled water was placed, and the access cavity was filled with IRM (DENTSPLY caulk, DENTSPLY International Inc., US). The teeth were placed on a wet support for 24 h. All surfaces of the teeth except for the furcation areas were coated with two layers of nail varnish and the orifices of all root canals were sealed with cyanoacrylate paste (SUPER GLU, India) to prevent microleakage from the root canals.

Protein Leakage Assessment: In order to prepare the protein leakage assessment apparatus, a hole was created in the rubber stopper and the teeth were inserted through it and sealed with cyanoacrylate paste (Super glue, India) through the rubber. Two test tubes, upper and lower were taken; the upper test tube was attached around the crown of the rubber stopper. The lower test tube was filled with 8 ml of redistilled water and the upper test tube was filled with 1 ml of 22% bovine serum albumin (BSA, Mast diagnostic, India) solution. The apparatus was prepared for all the experimental and control groups, and placed in an incubator at 37°C for 7 days. The water in the lower test tube and the 22% bovine serum albumin (BSA, Mast diagnostic, India) solution in the upper test tube was replenished daily during the experiment.

Quantification Of Protein Leakage: Presence of protein was detected with a reagent (Coomassive Brilliant Blue) every day for 60 days. Blue Color conversion of the protein reagent was considered to indicate leakage. Protein concentration was quantified with a UV spectrophotometer (UV-1800, Shimadzu, Germany). The assay was based on observation of maximum absorbance for an acidic solution of Coomassie Brilliant Blue (Bradford, BioRad, USA) within a range of 465-595 nm when binding to protein occurs. The mass values of BSA protein that leaked into the space adjacent to the furcal filling material were calculated using absorbance values and a calibration curve coefficient.

Statistical Analysis: Data were analyzed by One-Way Analysis of Variance and Posthoc Tukey test at significance level of p< 0.05) level of confidence using SPSS 21 Statistical Software Package.

RESULTS

The amount of microleakage was measured using a Spectrophotometer in all groups for 60 days. GIC showed showed Protein leakage on the 14thday of the experiment (0.015mg/ml), followed by MTA on the 22nd day (0..022 mg/ml)and ERRM on the 23rd day (0.0273 mg/ml). The specimens in the positive control group showed color conversion of the protein 2 h after the start of the experiment and in the negative control group, there was no color change throughout the experiment indicating no Protein leakage. (Table 1). At the end of the 60th day the amount of leakage was 0.5194 mg/ml for GIC; 0.459 mg/ml for MTA ; 0.435 mg/ml for ERRM respectively.

One Way Anova test showed that there was a statistically significant difference between all the groups. (Table 2) However, Post Hoc Tukey Tests showed that there were nostatistically significant differences in the degree of leakage between MTA and ERRM (p = 0.484) but there were significant differences between the MTA group and GIC group (p=0.001) and also between the GIC and MTA group (p = 0.001). (Table 3). Graph 1 shows the comparison of protein leakage observed for 60 days in all the experimental groups while Graph 2, Graph 3, Graph 4 and Graph 5 represents the amount of protein leakage observed at the 14th day, 22nd day,23rd day and the 60th day respectively.

DISCUSSION

In endodontic practice, furcal perforation occurs affecting the prognosis of root canal treatmentand often leads to treatment failure. Hence the repair of furcal perforation is of clinical importance in endodontics. New materials have also been developed to reduce the shortcomings, such as prolonged setting time and difficult manipulation, of MTA. ERRM is a radiopaque hydrophilic material with a particle size of less than 1mm with a unique composition that allows bonding to dentin. ERRM is hydrophilic, aluminum-free, and has a high pH (12.4) (Hirschberg, 2013). Presence of moisture is required for the materials to set and harden. Both Endosequence root repair materials and MTA are hydrophilic and likely to release ionic components that would be more biocompatible. Thus ERRM was used as a first experimental group. Among the various material available Mineral Trioxide Aggregate (MTA) is widely and commonly used for Furcal perforation repair. MTA is considered to be the gold standard for the above mentioned purpose and has received favorable reports in the literature.

In the current study MTA was used as a second experimental group for Furcal repair. GIC has traditionally been used for perforation repair as it bonds chemically to dentine and hence GIC was taken as the third experimental group in the current study. In the present study no statistically significant difference was observed between Mineral Trioxide Aggregate and Endosequence Root Repair Material. However, protein leakage was less in ERRM as compared to MTA and GIC.

EndoSequence Root Repair material showed sealing ability similar to white mineral trioxide in bacterial leakage study; and a better sealing ability than Biodentin and Mineral trioxide aggregate in dye extraction leakage method for furcal perforation repair. It is suggested that because there is a significant difference in the particle size ERRM (. 35µm) and MTA (less than 1µm); ERRM penetrated more into the dentinal tubules and thus bonds better to adjacent dentin (Damas, 2011). However, in a study conducted by Sadullah Kaya et al 2011; MTA showed less leakage than ERRM in root end filling bacterial leakage method. It was put forward that the setting properties of ERRM may be sensitive to the presence or absence of moisture, which could affect the sealing ability and leakage of ERRM. Other possible reasons for less protein leakage in ERRM could be that (i) variations in water-to-powder ratio for MTA could produce a material that can be either too fluid to load in a carrier, or too dry and brittle to place. The ERRM, on the other hand, is available as pre-mixed putty with an ideal consistency for Furcal perforation repair; (ii) MTA is often difficult to condense in the furcal area without void production, whereas the ERRM putty can be easily condensed with root-end pluggers and trimmed paper points to create a dense, void-free filling; (iii)rinsing or burnishing the MTA samples prior to setting would often result in a washout of material from the preparation. The ERRM, however, could be rinsed and burnished with no material loss (Sadullah Kaya, 2011) These differences in handling characteristics were also noticeable during preparation of the samples in this study and could attribute additionally to less leakage observed in ERRM as compared to the MTA.

In this present study, there was a statistically significant difference between MTA and high strength GIC. This is in accordance with other studies in which MTA has shown less microleakage than GIC as Furcal perforation repair material. The reason could be that the MTA is hydrophilic in nature and sets in the presence of moisture (Vajrabhaya et al., 2006) Consequently, when used as a root repair material, presence of moisture on the external surface of the peri-radicular tissue assures proper setting. However GIC is sensitive to moisture contamination for up to 24 hours, (Mount, 1999) during which the cement-forming ions (Ca^{2+}, Al^{3+}) from the glass are transferred to the poly acid, where they are locked up in a resistant gel. Early hydration of GIC leads to absorption of water and subsequent expansion, which causes loss of translucency and erosion. On the other hand, early dehydration of GIC causes loss of water needed for cement formation which lead to fissuring, crazing and cracking of cement surface. Gemalmaz et al. (1998) observed that due to early moisture contamination, the mechanical properties of GICs decreased and their surfaces became more susceptible for leakage (Gemalmaz, 1998) ERRM has similar properties to MTA like hydrophilicity, biocompatibility and good sealing ability. In this study there was statistically significant difference between ERRM and GIC, where in ERRM showed less leakage than GIC. Protein leakage method was used to assess the microleakage which is based on the observation that Coomassie Brilliant Blue G is converted to the blue color when in contact with the protein. The use of protein-dye complex in this experiment provided the advantage of eliminating the problems involved with radioisotope, dye, and bacterial leakage identification method (Bradford, 1976; Lagow, 1994; Malcic, 2006)

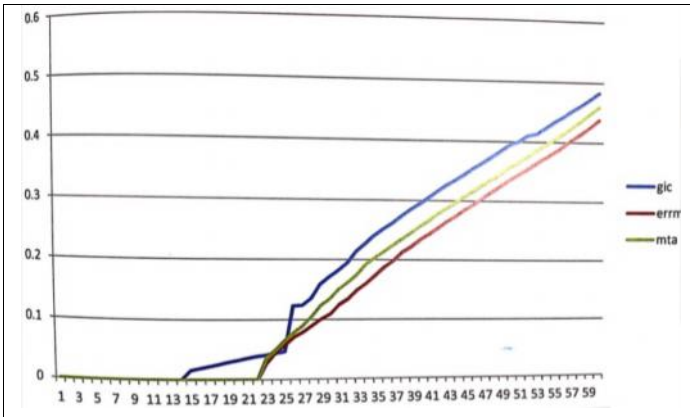
Table 1. Distribution of Mean and Sd for protein microleakage in experimental and control groups

Micro Leakage	N	Mean	Std. Deviation	Std. Error	Minimum (mg/ml)	Maximum (mg/ml)
ERRM (group 1)	25	.1530	.03138	0.00628	0.08	0.43
MTA (group 2)	25	.1661	.02733	0.00547	0.11	0.45
GIC (group 3)	25	.2153	.03820	0.00764	0.15	0.51
NEGATIVE CONTROL (group 4)	15	.0000	0.0000	0.0000	0.00	0.00
POSITIVE CONTROL (group 5)	15	.7545	0.01673	0.00432	0.72	0.78
TOTAL	105	.2350	0.22476	0.02193	0.00	0.78

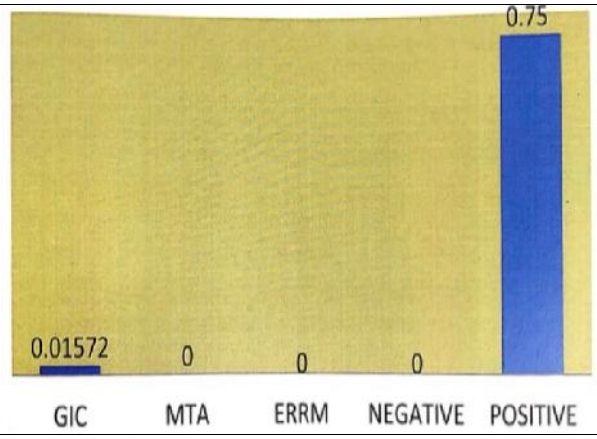
Table 2. One Way Anova Test

MICROLEAKAGE ASSESSMENT	SUM OF SQUARES	Df	MEAN SQUARE	F	*P Value
BETWEEN GROUPS	5.173	4	1.293	1.606E3	0.000
WITHIN GROUPS	0.081	100	0.001		
TOTAL	5.254	104			

MULTIPLE COMPARSION (Microleakage)						
(I)GROUP	(J)GROUP	MEAN DIFFERENCE (I-J)	STD. ERROR	*p VALUE	95% CONFIDENCE INTERVAL LOWER BOUND	95% CONFIDENCE INTERVAL UPPER BOUND
ERRM	MTA	-.01305	.00803	.484	-.0353	.0092
	GIC	-.06226*	.00803	.001	-.0846	-.0400
	NEGATIVE CONTROL	.15304*	.00927	.001	.1273	.1788
	POSITIVE CONTROL	-.60147*	.00927	.001	-.6272	-.5757
MTA	ERRM	.01305	.00803	.484	-.0092	-0353
	GIC	-.04921*	.00803	.001	-.0715	-.0269
	NEGATIVE CONTROL	.16609*	.00927	.001	.1403	.1918
	POSITIVE CONTROL	-.58842*	.00927	.001	-.6142	-.5627
GIC	ERRM	-.06226*	.00803	.001	.0400	.0846
	MTA	.04921	.00803	.001	.0269	.0715
	NEGATIVE CONTROL	.21530*	.00927	.001	.1896	.2410
	POSITIVE CONTROL	-.53921*	.00927	.001	-.5650	-.5135
NEGATIVE CONTROL	ERRM	-.15304	.00927	.001	-.1788	-.1273
	MTA	-.16609*	.00927	.001	-.1918	-.1403
	GIC	-.21530*	.00927	.001	-.2410	-.1896
	POSITIVE CONTROL	-.75451*	.01036	.001	-.7833	-.7257
POSITIVE CONTROL	ERRM	.60147*	.00927	.001	.5757	.6272
	MTA	.58842*	.00927	.001	.5627	.6142
	GIC	.53921*	.00927	.001	.5135	.5650
	NEGATIVE CONTROL	.75451*	.01036	.001	.7257	.7833

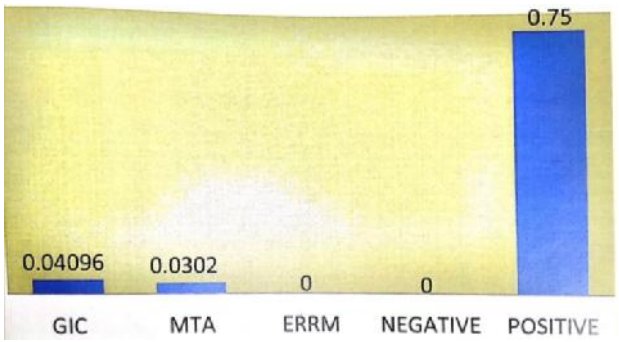


Graph 1. Comparison of protein leakage observed for 60 days in all the experimental groups

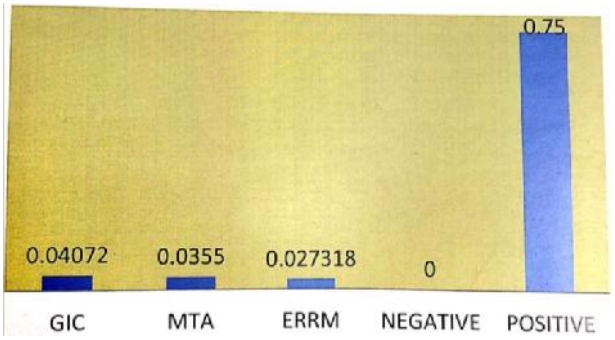


Graph 2. Representation of the amount of protein leakage observed at the 14th day

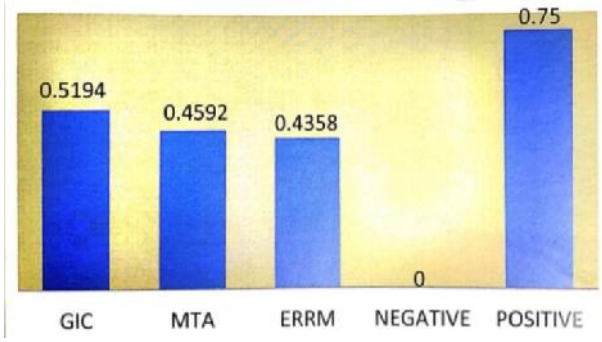
The most widely used methods to test the quality of furcal repair materials are the dye- penetration techniques, which make use of methylene blue and Indian ink dye. However, most dyes have a low molecular weight and can penetrate sites that protein and bacteria cannot. The molecular size of bovine albumin protein used in protein leakage studies is close to that of bacterial lipopolysaccharide molecules. Therefore, it may be advantageous for in vitro studies that simulate clinical situations Most dye leakage studies have measured the degree of leakage in one plane, making it impossible to evaluate the total leakage, (Saidon; Matloff, 1982) whereas protein assay enables the estimation of furcal repair microleakage in all planes. pH and chemical reactivity may also influence the degree of dye penetration. However, the protein-dye complex method had great sensitivity in protein identification and low sensitivity to interference from nonprotein compounds (Kersten, 1989). To overcome the inherent inadequacies of tracer substances and dye, other methods for evaluation of microleakage have been suggested like, saliva or bacterial culture to test the suitability of potential furcal repair materials (Torabinejad, 1995).



Graph 3. Representation of the amount of protein leakage observed at the 22nd day



Graph 4. Representation of the amount of protein leakage observed at the 23rd day



Graph 5. Representation of the amount of protein leakage observed at the 60th day

However, the time needed for the bacteria to grow is within 24 to 48 hours from contamination, which can result in inaccurate data (Fischer, 1998). In addition, normal saliva which usually harbours several different bacterial species may require different conditions of temperature, pH, and oxygen to grow in the laboratory. On the other hand protein Leakage method is rapid (i.e., it takes approximately 2 seconds) and reproducible and the protein-dye complex remains dispersed in the solution for a long period of time (weeks), not requiring critical timing for the assay; therefore, substantial number of samples can be evaluated at the same time (Carpenter, 1972). Ultraviolet-visible spectrophotometry refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means, it uses light in the visible and adjacent (near-UV and near- infrared) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this study, an ultraviolet spectrophotometry was adopted since it is a widely used technique because of its rapid analysis and the cost of the analysis is less expensive (Camps, 2003).

The potential of a material to create a tight seal at the furcal area is highly desirable for the Furcal perforation repair material.

Conclusion

Within the limitations of this study it was concluded that there was no difference in microleakage as measured by Protein leakage method between ERRM and MTA. ERRM can thus be considered as an equivalent and good alternative to MTA for furcal perforation repair.

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