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Sealing ability of mineral trioxide aggregate and Portland cement for furcal perforation repair: a protein leakage study

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Abstract: The purpose of this study was to compare the sealing ability of gray mineral trioxide aggregate (GMTA), white MTA (WMTA), and both white and gray Portland cement as furcation perforation repair materials. A total of 120 human mandibular first molars were used. After root canal obturation and preparation of furcal perforations the specimens were randomly divided into four groups of 25 teeth each. In groups A, B, C, and D furcation perforations were filled with WMTA, GMTA, white Portland cement, and type II Portland cement, respectively. Ten teeth were used as positive controls with no filling materials in the perforations and 10 teeth with complete coverage with two layers of nail varnish were used as negative controls. 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. The controls behaved as expected. Leakage was found in the samples from group A (WMTA), group B (GMTA), and in the two other groups (white and gray Portland cement). There were no statistically significant differences between GMTA and WMTA or white and gray Portland cement, but significant differences were observed between the MTA groups and the Portland cement groups. It was concluded that Portland cements have better sealing ability than MTA, and can be recommended for repair of furcation perforation if the present results are supported by other *in vivo* and *in vitro* studies. (J Oral Sci 51, 601-606, 2009)

Keywords: coronal leakage; bovine serum albumin; mineral trioxide aggregate; perforation; Portland cement.

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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 periradicular tissues. This kind of perforation results in loss of root integrity and further destruction of the adjacent periodontal tissues (1). 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 (2).

In recent decades, a new material known as MTA (mineral trioxide aggregate) has been introduced by Torabinejad, which is capable of creating a thorough seal between root canals and external dental surfaces (3). *In vitro* and *in vivo* studies have shown that MTA has considerable sealing ability and marginal adaptation (4,5). An appropriate tissue reaction to this material has been reported by Shahi et al. (2006) and other authors, although its application has been limited due to its high price (6-8). It has been reported that Portland cement has antimicrobial and physical properties similar to those of MTA (9-11). The main components of MTA are calcium

and phosphate ions. Since these elements are also the main components of dental hard tissues, this may contribute to the biological compatibility of MTA with surrounding tissues (11-13). Sarkar et al. 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 (14). The material comprises calcium oxide in the form of small crystals, and calcium phosphate, which has an amorphous, granular structure (13,14).

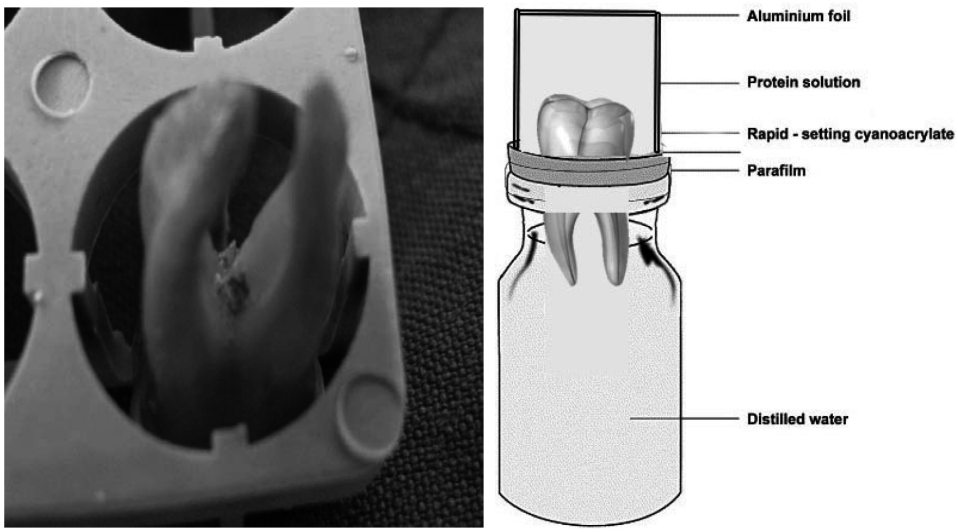
With regard to the physico-chemical properties of MTA, studies addressing the properties of Portland cement have been employed as a reference, since the manufacturer of MTA has confirmed that Portland cement is one of the components. In addition, some studies have demonstrated that both MTA and Portland cement have the same composition and mechanism of action (3,15,16). Portland cement type II is a construction cement with great similarity to MTA, which might offer significant economic advantages if applicable to biological systems (11). Holland et al. (2001) evaluated the reaction of subcutaneous tissue in rats to implantation of dentin tubes filled with mineral trioxide aggregate, Portland cement or calcium hydroxide. The results were similar for all the materials analyzed. Therefore, the authors considered that the mechanisms of action of these materials might be similar (17).

The high biocompatibility of MTA makes it a suitable material for the treatment of root perforations with the goal of regenerating periodontal attachment, and inducing osteogenesis and cementogenesis (3,18,19). In many studies the main challenge of laboratory-based leakage testing models is 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 (20,21). Thus, it is crucial to adopt a standardized, reliable, and reproducible method (22,23). Therefore, the aim of this study was to evaluate protein leakage of white and gray mineral trioxide aggregates in comparison to white and gray Portland cements when used as furcal perforation repair materials.

Materials and Methods

A total of 120 mandibular first molars were selected. The teeth had been extracted for periodontal reasons and had mature roots and crowns. Easy access to the furcation area was a criterion for selection. After root canal obturation and preparation of furcal perforations the specimens were randomly divided into four groups of 25 each; the remaining teeth were randomly assigned to two groups of 10 each as negative and positive controls. Standardized access cavities were prepared. Perforations were made using ISO #0.06 round burs (D & Z, Munich, Germany) (Fig. 1).

In group A, 25 teeth with furcation perforations were filled with WMTA (ProRoot MTA, Dentsply, Tulsa, OK, USA). In group B, 25 teeth with furcation perforations were filled with GMTA (ProRoot MTA, Dentsply, Tulsa, OK, USA). In group C, 25 teeth were filled with white Portland cement (Tehran Cement Company, Tehran, Iran) and in



(a) (b)

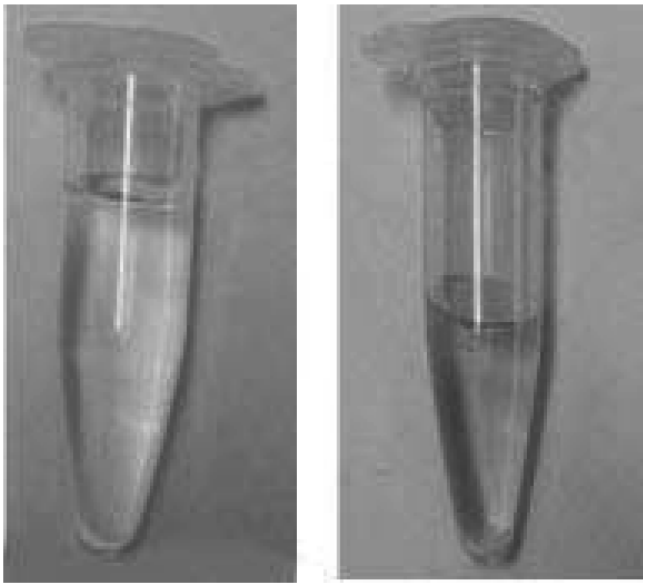
Fig. 1 Perforations prepared using ISO #0.06 round burs (a) and the apparatus used for testing protein leakage (b).

group D, 25 teeth were filled with type II Portland cement (Tehran Cement Company). 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, Detrey, Konstans, Germany). In the positive controls, no filling material was used in the perforations; teeth with complete coverage consisting of two layers of nail varnish were used as negative controls.

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 (Razi Cement Company, Tehran, Iran) to prevent microleakage from the root canals. Before preparing the apparatus for leakage assessment, the temporary filling material was removed from the access cavities of the samples, and the setting of the MTA and Portland cement was checked with an explorer. In order to prepare the leakage assessment apparatus, a hole was created in the rubber stopper of a 10-ml glass vial and the teeth were inserted through it and sealed with cyanoacrylate paste (Razi Cement Company) through the rubber. A plastic cylinder was attached around the crown of the rubber stopper (Fig. 1). The glass vial was filled with 9.5 ml of redistilled water, and the cylinder was filled with 1 ml of 22% bovine serum albumin (BSA) solution (Sigma Chemical Co., St Louis, MO, USA). 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 glass vial was changed and the BSA in the reservoir was replenished daily during the experiment. Presence of protein was detected with a reagent (Coomassive Brilliant Blue) every day for 60 days. Color conversion of the protein reagent was considered to indicate leakage. Protein concentration was quantified with a UV spectrophotometer (GENESYS 10 UV Spectrophotometer, Genesys 10, Madison, USA). The assay is based on observation of maximum absorbance for an acidic solution of Coomassie Brilliant Blue (G-250 Bio-Rad Corporation, Life Science Group, CA, USA) within a range of 465-595 nm when binding to protein occurs. The mass values (ng) of BSA protein that leaked into the space adjacent to the furcal filling material were calculated using absorbance values and a calibration curve coefficient. Data were analyzed by one-way analysis of variance and Tukey test at a 0.05 level of confidence using the SPSS 15 statistical software package.

Results

The specimens in the positive control group showed color conversion of the protein 2 h after the start of the experiment (Fig. 2). In the negative control group, there was no color change throughout the experiment (Fig. 2). Amounts of leakage in the various groups (mean ± standard deviation) ($P < 0.05$) are shown in Table 1.



(a) (b)

Fig. 2 Samples obtained from the specimens after adding the reagent; sample from negative control (a) and sample from positive control (b).

Table 1 Results of protein leakage assessment (mg/ml)

Groups	Number	Mean ± SD (mg/ml)	Minimum (mg/ml)	Maximum (mg/ml)
Control (-)	10	0.0001 ± 0.00032	0.00	0.00
Control (+)	11	0.6327 ± 0.12361	0.49	0.87
White MTA	26	0.3874 ± 0.07918	0.26	0.58
Gray MTA	26	0.3475 ± 0.14005	0.04	0.59
White Portland cement	20	0.1281 ± 0.12463	0.04	0.51
Gray Portland cement	24	0.1209 ± 0.08662	0.04	0.61

In the white MTA group, leakage was observed in all the specimens after 20 days, and the amounts of leakage were similar to those in the gray MTA group. None of the teeth in the negative control group demonstrated leakage during the entire monitoring period. In the white Portland cement and gray Portland cement groups, the degree of leakage was similar. There were no significant differences in the degree of leakage between GMTA and WMTA or between Portland cement type II and white Portland cement ($P > 0.05$) but there were significant differences between the MTA groups and the Portland cement groups ($P < 0.05$). Leakage was significantly less in the Portland cement groups than in the MTA groups. In the Portland cement groups, color change was evident after 31 days (Fig. 3).

Discussion

It has been suggested that the most important factors determining the success of a perforation repair procedure are the location of the perforation, time elapsed between the occurrence of the perforation and repair, the ability of the material to seal the perforation site, and the biocompatibility of the repair material (24-26). Previous investigators have tried to show that MTA is a suitable material for perforation repair because of its high sealing ability compared with other materials (20,27). In those studies, a dye penetration method was used for assessment of microleakage; however, the limitations of traditional linear dye leakage evaluations have been previously well addressed (28). The phenomenon of capillarity is of utmost importance in passive methods used mainly for assessing apical leakage, as the tooth apex is submerged in the dye that penetrates through any space between the canal walls and the filling material (29).

A large number of studies have employed methylene blue as a dye because it is inexpensive, easy to use, has a high degree of staining, and has a molecular weight even lower than that of bacterial toxins (29,30). With regard to dyes, particle size, pH, and chemical reactivity are believed to affect the degree of penetration (31).

Another widely used dye is India ink. This dye has a few disadvantages such as dissolution during the demineralization and clearing process, in addition to the fact that it is difficult to observe its maximum penetration depth in some cases (28,29). Dye leakage studies measure the degree of leakage in one plane, making it impossible to evaluate the total amount of leakage. It is also important to be able to evaluate the laboratory findings in real clinical settings (32). Thus, it is crucial to adopt a standardized, reliable, and reproducible method. One leakage assessment method that is currently used is the albumin protein leakage procedure (23). Protein assay enables the estimation of root-end filling microleakage in all planes. 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 (33). Hamad et al. compared the ability of GMTA and WMTA to seal furcation perforations in vitro, and found no differences between the two formulations. Their results are consistent with the present ones, in that the amount of leakage with GMTA was similar to that with WMTA (34). De-Deus et al. compared the abilities of Portland cement and MTA to prevent coronal leakage through repaired furcal perforations in molar teeth, and found no significant differences between

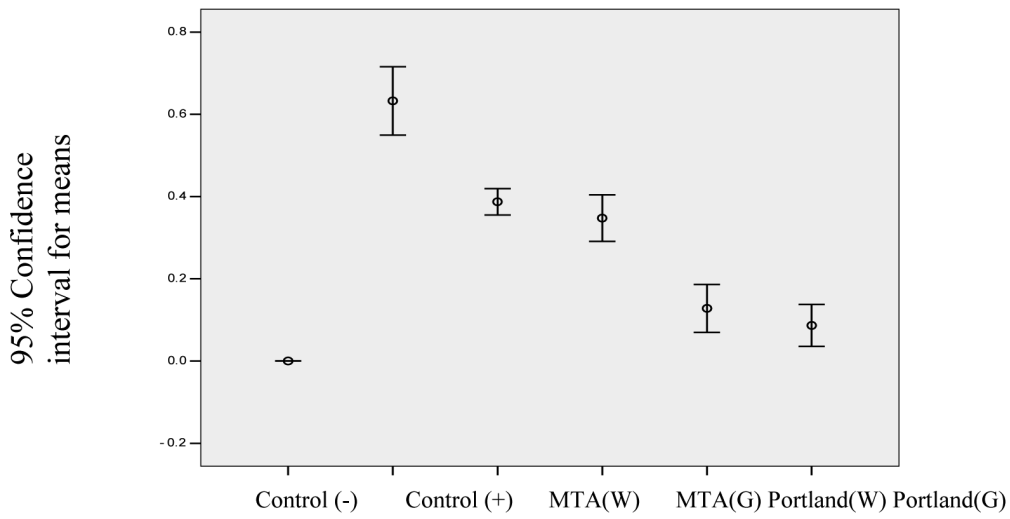


Fig. 3 Comparison of mean values of protein leakage between the experimental and the control groups.

them, although our findings showed that the time needed for leakage to occur in all specimens was significantly longer in the Portland cement samples and the degree of leakage in the Portland cement groups was lower than that in the MTA groups (35). This difference in results may be attributable to differences in the components of the materials. Asgary et al. reported that MTA and PC have the same major components except for bismuth in MTA. They reported that the most significant difference was the presence of higher concentrations of FeO in gray MTA and PC when compared with the white versions. Bismuth oxide particles produce specific phases throughout MTA preparations, resulting in rough amorphous patterns in surface topography near dentinal walls. The fineness of a cement is a major factor influencing its rate of hydration and, consequently, its strength and setting characteristics. These physical properties may account for the superior sealing ability of Portland cement over other perforation filling materials (36,37).

According to Torabinejad et al., the sealing ability of MTA in terms of bacterial leakage depends on its antimicrobial rather than physical properties. However, bacterial leakage studies have employed limited models that did not simulate any of the phenomena occurring in the oral cavity, such as temperature changes, dietary influences, and salivary flow (5,38,39). The protein molecules studied in this experiment are larger than dye molecules and smaller than the majority of bacterial cells. Although our static model enables the measurement of total leakage, it is important to remember that it does not represent the dynamic interaction between the root canal and the periradicular tissue. It is concluded from the present results that Portland cement and MTA may have similar properties in studies that are carried out in vitro, although the methodology we used for leakage assessment differed from that of previous studies. Our results support the idea that Portland cement has the potential to be used in clinical situations similar to those in which MTA is currently being used, although there is a need to add bismuth and remove arsenic from Portland cement for clinical perforation repair. However, further studies involving Portland cement will be necessary to examine its clinical applications.

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