Effect of acidic environment and intracanal medicament on push-out bond strength of biodentine and mineral trioxide aggregate plus: an in vitro study

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Abstract

Introduction. This in-vitro study aims to evaluate the effect of acidic environment and intracanal medicament on push out bond strength of Biodentine and Mineral Trioxide Aggregate Plus (MTA Plus).

Method. Forty extracted single rooted teeth were sectioned below the cement-enamel junction. The root canals were instrumented using rotary files and then peeso reamer was used to obtain standardized root canal dimension. Specimens were randomly classified into following groups: Group 1: calcium hydroxide in the absence of acidic environment; Group 2: calcium hydroxide in the presence of acidic environment; Group 3: no intracanal medicament in the absence of acidic environment; Group 4: no intracanal medicament in the presence of acidic environment. Specimens were kept for 7 days at room temperature. Thereafter, specimens of each group were transversely sectioned into 1 mm thick slices and divided into 2 sub-groups according to the use of biodentine and MTA Plus. Using Universal Testing Machine, push out bond strength test was carried out and the data were analyzed statistically.

Results. There was no statistically significant difference in the bond strength of biodentine and MTA Plus (P>0.05). For both MTA Plus and biodentine, with or without calcium hydroxide, the push out bond strength was less in acidic environment and this difference was more pronounced without calcium hydroxide. In all the four groups, MTA plus showed comparable bond strength to biodentine.

Conclusion. MTA Plus is a viable option for apexification. The push out bond strength of Biodentine and MTA Plus is impaired by acidic environment. Prior application of calcium hydroxide slightly increased the bond strength, though the difference was statistically insignificant.

Keywords: acidic environment, biodentine, push-out bond strength, intracanal medicament, mineral trioxide aggregate plus

Background and aims

Pulp necrosis in teeth with incomplete root formation can arrest dentin formation as well as hamper the root development [1]. Treatment of such teeth with wide open apices has various complications including endodontic, reparative and restorative problems. The treatment should provide an adequate seal between root canal system and periradicular tissues with the apexification procedure and provide a barrier against which the filling material can be compacted. This has traditionally been carried out using calcium hydroxide (CaOH₂) which also served the purpose of creating bacteria free environment in root canal system.

The combination of apexification and subsequent internal bonding may eliminate treatment problems that arise in case of the pulpless immature tooth [2]. Though calcium hydroxide has been studied widely and has shown success, it has certain disadvantages like prolonged treatment time, need for multiple visits, alteration in hardness of dentin and demineralization of dentin. One alternative to calcium hydroxide apexification is a single step apexification technique using an artificial apical barrier [1]. Its advantages are short treatment time and development of good apical seal. Mineral trioxide aggregate (MTA) has become the material of choice as an artificial apical barrier due to its
biocompatibility, non-mutagenicity, non-neurotoxicity, good sealing properties and induction of hard tissue formation.

Several new calcium silicate-based materials have been developed to overcome the problems like long setting time, difficult handling properties and eliminating the potential of discoloration of MTA, to use as apical barrier materials.

Biodentine (Septodont, Saint-Maur-des-Fosses, France) is a calcium silicate based material. Endodontic uses of Biodentine are similar to MTA and its main advantages are reduced setting time and better mechanical properties than MTA [3].

Mineral Trioxide Aggregate Plus (MTA Plus) (Prevest Denpro Limited, Jammu, India, for Avalon Biomed Inc.) is a cost effective calcium-based silicate cement mainly used for repair of root perforations and root-end filling procedures. Its advantage is the smaller particle size, which is 50% smaller than MTA and <1 μm [3]. MTA Plus had a prolonged capability to release calcium ions and increase the local pH when compared with MTA [4].

In periapical diseases, tissue inflammation and bacterial metabolic acidosis alters the pH of local environment which becomes acidic. This pH ranges from 5 – 6.68 and influences the physical and chemical properties of root filling materials [5-7]. Impeded MTA setting as well as reduced strength and hardness has been reported in an acidic environment [7-9].

Calcium hydroxide is extensively used in endodontics, as an intracanal medicament. It helps in raising the pH in acidic environment created by the presence of butyric acid, perchloric acid in cases of periapical lesions. Because of this property, it is used prior to the single step apexification procedure to reduce the detrimental effects of acidic environment on properties of apical barrier materials.

To date, no research data is available that evaluated and compared the bond strength of the novel cement MTA Plus to dentin after treatment with the intracanal medicament in the presence of acidic environment of periapical lesions. Therefore, this in-vitro study was set to evaluate the effect of acidic environment and intracanal medicament on the push-out bond strength of biodentine and MTA plus.

**Method**

Forty single-rooted sound human permanent teeth with single root canal were collected and stored in 10% formalin until employed in the experiment. The specimens were cleaned off the superficial debris, calculus, tissue tags. The teeth specimens were sanctioned below cemento-enamel junction so that the length of all roots was adjusted to approximately 10 mm (Figure 1a and 1b). Root canals of the specimens were prepared using peeso reamers starting from no. 1 to no. 4 (Figure 2). At every reamer change, root canals were irrigated with 2 ml of 3% sodium hypochlorite (NaOCl) intermittently. After the preparation, root canals were irrigated with 5 ml of 17% liquid ethylene diamine tetra acetic acid (EDTA) for 5 minutes followed by 5 ml of 3% NaOCl for 5 minutes to remove the smear layer. Final irrigation was done with 10 ml of normal saline.
Then root canals of the specimens were dried with absorbent paper points. The specimens were then randomly divided into 4 groups each having a sample size of 10 as follows:

Group 1 (n=10): Teeth specimens with calcium hydroxide in the absence of acidic environment
Group 2 (n=10): Teeth specimens with calcium hydroxide in the presence of acidic environment
Group 3 (n=10): Teeth specimens with no intracanal medicament in the absence of acidic environment
Group 4 (n=10): Teeth specimens with no intracanal medicament in the presence of acidic environment.

Figure 3. Transversely sectioned dentin slices.

For Group 1 and Group 2, calcium hydroxide powder was mixed with distilled water in the ratio 2:1. Specimens of Group 2 and Group 4 were wrapped in gauze pieces soaked in butyric acid to simulate the acidic environment. Acid-soaked pieces of gauze were replaced every day with fresh ones to ensure sufficient acidic environment. Specimens were kept for 7 days at room temperature. After that, Ca(OH)₂ medicament was removed using gentle manual agitation with no. 80 K file & irrigation with 5 ml NaOCl followed by a final flush with 5 ml normal saline.

Each root from each group was transversely sectioned 2 mm away from the anatomic apex into 2 slices of thickness 1 mm each (Figure 3). The slices from each group formed two subgroups for that particular group, based on the filling material used i.e. biodentine and MTA plus. These materials were mixed according to manufacturer’s description and were placed into canal spaces using a plugger on a glass plate. Then all the samples were kept for 48 hours at room temperature and then were subjected to push out bond strength test using Universal Testing Machine (UTM). To allow free motion of the plunger, the samples were placed on a metal slab with a central hole. The jig had a clearance of approximately 0.2 mm from the margin of the dentinal wall to ensure contact only with the material. Maximum load applied to the material at the time of dislodgement was recorded in Newtons. In order to express bond strength in MPa, the recorded value was divided by the adhesion area of root canal filling calculated by the $2\pi rh$ formula; where r is the root canal radius and h is the thickness of the root-dentin slice in millimeters.

**Data analysis**

Data was collected and statistical analysis was done using SPSS version 20.0. Bond strength was expressed in terms of mean and standard deviation for all the four groups. Unpaired ‘t’ test was used to describe the comparison. P-value less than 0.05 was considered as statistically significant.

**Results**

Figure 4 summarizes the comparison of push out bond strength among four groups. MTA plus showed better bond strength in the absence of acidic environment and calcium hydroxide. In the presence of acidic environment and calcium hydroxide, biodentine showed better bond strength than MTA plus. But the results did not reflect any statistical significance (p>0.05). In all the four groups, MTA plus had a comparable bond strength to biodentine (Tables I and II).

Table I and table II describe the push out bond strength of MTA Plus & biodentine respectively, in 4 different groups. When group 1 was compared with group 2 and group 3 was compared with group 4, there was a decrease in bond strength of both the materials.

Also, when the push out bond strength was compared between group 1 and group 2 for both MTA Plus [Group 1 vs Group 2: 78 (32.2) vs 45 (15.9)] and biodentine [Group 1 vs Group 2: 78 (29.0) vs 51 (20.2), the values showed statistical significance (p value <0.05). Thus, for both the filling materials, with or without calcium hydroxide, the push out bond strength was less in acidic environment and this difference was more pronounced without calcium hydroxide.
Table I. Comparison of Push-out bond strength of MTA plus.

| Specimen (Groups)                                          | Mean (SD) MTA Plus | t Value | P Value |
|------------------------------------------------------------|--------------------|---------|---------|
| With calcium hydroxide and without Acidic Environment (group-1) | 78 (32.2)          | 2.905   | 0.012   |
| With Calcium Hydroxide and Acidic Environment (group-2)     | 45 (15.9)          |         |         |
| Without Calcium Hydroxide and without Acidic Environment (group-3) | 75.0 (29.2)        | 2.905   | 0.010   |
| Without Calcium Hydroxide and with Acidic Environment (group-4) | 42.0 (21.0)        |         |         |

Table II. Comparison of Push-out bond strength of Biodentine.

| Specimen (Groups)                                          | Mean (SD) Biodentine | t Value | P Value |
|------------------------------------------------------------|----------------------|---------|---------|
| With calcium hydroxide and without Acidic Environment (group-1) | 78.0 (29.0)          | 2.415   | 0.028   |
| With Calcium Hydroxide and Acidic Environment (group-2)     | 51.0 (20.2)          |         |         |
| Without Calcium Hydroxide and without Acidic Environment (group-3) | 57.0 (22.13)         | 1.55    | 0.137   |
| Without Calcium Hydroxide and with Acidic Environment (group-4) | 42.0 (21.0)          |         |         |

Discussion

An endodontic root filling material should adhere well to root canal dentin to maintain the integrity of the root filling material–dentin interface. It is also significant in resisting the displacement of the filling material during function and operative procedures. There are various tests like tensile, shear, and push-out strength tests used for evaluating the adhesive property of root filling material to dentin. In the present study, push-out test has been used, as it is a reliable, efficient, and practical method [10].

One-mm thin slices of teeth specimens were used in the present study in order to provide uniform placement and to simplify calculations of the bond area. In addition, the specimens were prepared to obtain a standardized root canal diameter of 1.3 mm (corresponding to no. 4 peeso reamer), to simulate wide open apices in clinical condition [11].

In this study, the bond strength of Biodentine and MTA plus were evaluated and compared after exposure to an acidic pH. As shown by previous studies, biodentine has better adhesive property than MTA as it has less porosity, prominent biomineralization ability, less setting time and is a better alternative to MTA due to its less impaired properties when these materials are exposed to acidic environments [1,12,13]. However, results of the present study did not reflect any statistical significance in the bond strength of biodentine and MTA Plus. It signifies the use of MTA Plus as a choice of material for apexification in inflamed environment. Butyric acid, a byproduct of metabolism of anaerobic bacteria, was used in the present study, to simulate the clinical environmental conditions of periradicular infection [7,9]. The results of this study indicated that the presence of acidic environment adversely influenced the bond strength to root
dentin. This is in accordance with the previous studies [14]. In an acidic environment, the development of hydroxyapatite crystals and consequently a hybrid layer at root end filling materials’–dentin interfacial gap, are likely to be disturbed. Also, low pH inhibits the setting reaction, affects adhesion, decreases portlandite formation and increases the solubility and porosity of calcium silicate–based materials [8,15-17].

Placement of root end filling material in an inflamed tissue increases its solubility. To decrease inflammation, placement of some medications, including Ca(OH)₂, is recommended [16]. The result of the present study indicated that prior application of calcium hydroxide increased the bond strength. This can be attributed to the conversion of calcium hydroxide to calcium carbonate at the root end or the reaction of both MTA Plus and Biodentine with residual calcium hydroxide, resulting in improvement of their marginal adaptation [18,19].

Further studies regarding the effect of acidic environment and intracanal medicament on the properties of biodentine and MTA plus are warranted in the near future.

**Conclusion**

Under the conditions of this study, MTA Plus is a viable option for apexification. The bond strength of biodentine and MTA Plus is impaired by acidic environment. Prior application of calcium hydroxide slightly increased the bond strength, though the difference was statistically insignificant.

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