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The Effect of *in Vitro* Sample Preparation Methods on Bond Strength of Mineral Trioxide Aggregate to Root Dentin

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ABSTRACT

Introduction: The aim of this study was to compare the effect of sample preparation methods on push-out bond strength of mineral trioxide aggregate (MTA). **Methods and Materials:** Twenty-four extracted human mandibular premolars with single root canal were selected for this *in vitro* study. After instrumentation, irrigation and drying of root canals, they were randomly divided into two experimental groups (n=12). Group 1: After removing the 3 mm of root ends, 2 mm thick sections were established from the remaining roots. MTA was mixed following the manufacturers' recommendations and root sections were filled. Group 2: The whole root canal was used for filling and packing of MTA and like group 1, after removing the 3 mm of root ends, root sections were provided. Push-out bond strength was measured and analyzed by the independent t-test. Level of statistical significance was set at 0.05. **Results:** The highest mean push-out bond strength was in apical section of group 2 (4.86±1.31) and the lowest value was in coronal section of group 2 (3.05±1.15). The results of the independent t-test showed a significant difference between two groups only in the apical section (P=0.02). **Conclusion:** Based on the results of this *in vitro* study, the highest mean push-out bond strength was in apical section of group 2. It seems that using the whole root canal for MTA application is better for MTA *in vitro* analysis of bond strength to root dentin because it is more similar to the clinical conditions.

Keywords: Bond Strength; Endodontics; Mineral Trioxide Aggregate; Root Dentin

Introduction

Mineral trioxide aggregate (MTA) was introduced by Prof. Torabinejad as a biomaterial with favorable properties including the ability to seal the communication path between dental pulp and outer surface of tooth. Other clinical applications of MTA include use in dental perforations and treatment of immature teeth [1-3]. Also, in cases such as apexification [4, 5], internal and external root resorptions [6, 7], re-implanted teeth [8] and the retention of primary teeth [9], MTA can be applied to fill the entire root canal system [10]. One of the critical characteristics of a material used for perforation repair is optimal bonding with dentin and resistance against dislodgement due to occlusal forces and placement of restorative materials [1, 11, 12]. Previous studies have used different methods for assessment of bonding strength to dentin including shear bond strength, tensile bond and push-out bond strength [13, 14]. Push-out test has been appraised as a more reliable and practical approach for the assessment of bond strength [13, 15]. Therefore, different studies have been used for this test for the evaluation of bond strength of MTA to dentin [11, 16]. In common *in vitro* studies, transverse sectioned root segments with different thicknesses (often 2 mm) have been used as a bed for application of MTA. In these studies, after preparation of samples, a mixture of MTA was inserted in the canal cavities of root segments. Although this method has been widely used for the evaluation of push-out bond

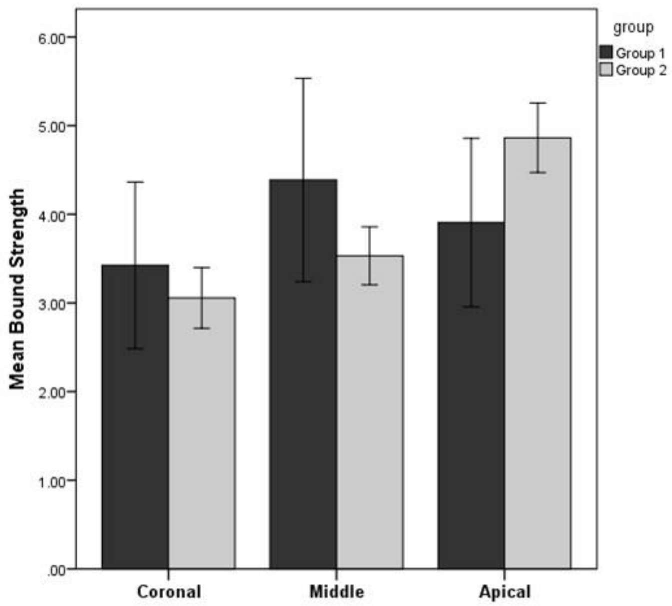

Figure 1. Mean bond strength of study groups (Group 1: using root segments and Group 2: using whole root length for MTA placement) in different root sections has been shown.

strength in several studies [17-20], it seems that the procedure used in this method does not match the clinical conditions and its limitations. For instance, limited access and visibility of the clinician may cause some drawbacks during the application of MTA. Furthermore, the available space in root canal could create restrictions during the packing of MTA [1, 19], which is dissimilar to the *in vitro* experiments. Therefore, the other method used for bond strength evaluation, rather simulating the clinical conditions, is the use of whole root length, which have been prepared before, for the packing of MTA and then providing the root segments for bond strength test [21]. This study was aimed to compare these two methods of sample preparation for the evaluation of MTA push-out bond strength.

Materials and Methods

The study was approved by the Research and Ethics Committee of Tabriz University of Medical Sciences. The sample size of this study was calculated according to Rahoma *et al.* [22] using α=0.05 and study power of 80%. Twenty-four human mandibular premolars with single root canal extracted for periodontal reasons were selected for this *in vitro* study. Subsequent to extraction, the teeth were stored in 3% chloramine-T solution [23]. Teeth were ultrasonically cleaned to remove soft tissues and calculi and stored in normal saline solution until used for this study. Normal saline solution contained 0.1% sodium azide was utilized to inhibit bacterial growth and disinfect the teeth [22]. Teeth were evaluated using mesiodistal and buccolingual periapical radiographs. Those with previous root canal treatment, calcification, developmental abnormalities, immature apices, coronal restorations, deep caries, fractures or cracks, and internal or external resorption were excluded from the study.

Preparation of samples

The tooth crowns were dissected at the level of cemento-enamel junction (CEJ) with a carbide bur in a high-speed handpiece to reach working length of 14 mm in all the samples. The working length was determined with a #15 K-Flexofile (Dentsply Maillefer, Ballaigues, Switzerland), 1 mm away from the apical foramen. Root canals preparation were carried out using #4, #3 and #2 Gates-Glidden drills (Mani, Tochigi, Japan) for coronal enlargement, followed by the use of 40/0.10, 35/0.08 and 30/0.06 RaCe rotary files (FKG Dentaire, La-Chaux-de-Fonds, Switzerland) for final preparation of the root canals. The size of master apical file was #35 file. Canals were irrigated with 1 mL of normal saline after using each file; the irrigation needle was inserted 4-5 mm into the root canal. Subsequently, 1 mL of 5.25% NaOCl (Taj Corp, Tehran, Iran) was used for 3 min, followed by irrigation with 1 mL of 17% ethylenediaminetetraacetic acid (EDTA; Pulpdent Corp, Watertown, MA, USA) for 3 min to remove the smear layer [24]. The final irrigation was carried out with 5 mL of normal saline. The root canals were dried using paper point (Ariadent, Tehran, Iran) size #35. Finally, teeth were randomly divided into two experimental groups (n=12). Group 1: After removing the 3 mm of root ends, 2 mm thick sections were established from the remaining roots using a low-speed water-cooled diamond saw (Isomet; Buehler, Lake Bluff, IL, USA). After that, MTA powder (Angelus, Londrina, Brazil) was mixed with distilled water by the ratio of 1 g powder to 0.33 mL liquid according to the manufacturer's recommendations and placed into the canal space of root sections using MTA carrier, packed with moistened cotton pellets. Group 2: The whole root canal was used. After mixing MTA with distilled water, the cream was packed into the root canal using MTA carrier and packed with endodontic pluggers (sizes 9/11, 10-1/2; Hu-Friedy, Chicago, IL, USA). Also, in this group, after removing the 3 mm of root ends, 2 mm thick sections were established from the remaining roots using a low-speed water-cooled diamond disc. The division of sections into coronal, middle and apical was done according to their locations on the remaining root following the removal of apical 3mm. In both groups, cotton pellets moistened with distilled water and Cavisol (Golchai, Tehran, Iran) were placed over the materials. The samples were incubated at 100% humidity in an incubator at 37°C for 24 h.

Push-out bond strength test

The bond strength of MTA to dentin was assessed by push-out test using a universal testing machine (Hounsfield Testing Equipment, Redhill, UK) based on two techniques. For this purpose, cotton pellets and Cavisol were removed from the root samples and embedded in epoxy resin. The cylindrical plunger of universal testing machine with 1 mm thickness passed through the samples at a speed of 1 mm/min and the least force caused the dislodgment of MTA from the root canal was recorded in Newton(s) (N). The following formula was used to calculate the bond strength in MPa: Bond strength (MPa) = Debonding force (N)/Bonded surface area (mm^2). Bonded surface area was calculated according to the formula $2\pi r\times h$, where π the constant 3.14, r is the root canal radius, and h is the thickness of the root slice in millimeters.

Data analysis

The Kolmogorov-Smirnov test was used to evaluate the normal distribution of data. After calculating the mean ± standard deviation of push-out bond strength, data was analyzed by the independent t-test using SPSS17 (Chicago, IL, USA). Level of statistical significance was set at 0.05.

Results

The results of Kolmogorov-Smirnov test showed the normal distribution of data in the evaluated groups and different sections. Figure 1 presents the mean bond strength of two groups in different sections (coronal, middle and apical) of the root canal. The highest mean push-out bond strength was seen in apical section of group 2 (4.86±1.31) and the lowest value was in coronal section of group 2 (3.05±1.15). According to the results of the independent t-test, although in coronal and middle sections, the bond strength of the first group was higher than the second group and only in the apical section, the amounts of the second group was higher, the difference between two groups was only significant in the apical section (P=0.02). The mean bond strength of group 1 was 0.95 units more than group 2.

Discussion

In the present study, we compared two methods of sample preparation for the evaluation of MTA push-out bond strength. Different techniques can be used to assess the bond strength of a dental material to dentin including tensile, shear and push-out bond strength tests. Push-out bond strength is a valid, reliable and easily interpretable method to determine the adherence of a material to root dentin, stimulating clinical stresses [19, 25-27]. Results of the recent studies that evaluated the limitations of the push-out bond strength test, showed that this test results in parallel fracture of the dentin-cement interface and is an effective test for the evaluation of bond strength [28, 29]. Placing MTA for the whole root length is a more accurate mimic of the clinical situation than using root slices; which is the common method for bond strength assessment of MTA in many studies [2, 17, 19, 30]. When using the whole root length for applying MTA, the clinician may confront difficulties in the apical root segment [13, 31, 32]. The dislodgment resistance and bond strength of MTA with root dentin play an important role in different treatments including root perforations repair, external and internal root resorptions and in cases where MTA is used to fill whole root length [6, 8, 30]. The results of this study showed significant differences between the two methods only in apical sections; moreover, the bond strength of filling the whole root with MTA was greater than in the cross sectional method. Tubular density is different throughout the root thirds and it is the most in the apical thirds [33]. Consequently, the significant difference between two methods of assessing the bond strength of MTA could be attributed to this fact. In contrast, a previous study reported that tubular density is not enough to interfere with adhesion of the root canal sealer [34] and the bond strength of MTA-based sealer was similar among the three root thirds [35].

This study was the first to evaluate the effect of different sample preparation on bond strength of MTA to root dentin and could commence new strategy for evaluation of the bond strength of MTA and other calcium silicate-based cements. One of the limitations of the study was the difficulty in handling MTA; especially when obturating the whole root length, packing of MTA was arduous. Future studies are needed with larger sample size and application of different methods of MTA packing.

Conclusion

Based on the results of this *in vitro* study, the highest mean push-out bond strength was in apical section of group 2 and it seems that, the technique used in this group is more similar to the clinical conditions. Therefore, we recommended using the whole root canal for MTA applying for *in vitro* analysis of the bond strength of MTA to root dentin.

Conflict of Interest: 'None declared'.

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