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Fracture resistance of immature teeth filled with mineral trioxide aggregate or calcium-enriched mixture cement: An *ex vivo* study

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ABSTRACT

Background: The ability of mineral trioxide aggregate (MTA) to strengthen the tooth structure has been studied with contradictory results, and there is a lack of data in the case of Calcium-enriched mixture (CEM) cement as a novel endodontic biomaterial. The aim of the present study was to evaluate the reinforcing effect of MTA and CEM cement on simulated immature teeth.
Materials and Methods: This *ex vivo* study was carried out on a total of 46 human maxillary incisors. Access cavities were prepared. Five teeth were randomly selected as negative control. The root length of the remaining teeth was standardized to 9 mm. Rotary files and peeso reamers were used to enlarge the canals. The prepared specimens were randomly assigned into three experimental (n = 12) and a positive control (n = 5) groups. In groups 1 and 2, the canals were filled with MTA or CEM cement, respectively. In group 3, a 5-mm MTA plug was placed, and the remainder of the canal was filled with composite resin. The canals of the positive control were kept unfilled. After 6 months, the teeth were tested for fracture strength in a universal testing machine. The groups were compared using Univariate analysis of variance (ANOVA).
Results: There were significant differences between fracture strength of experimental groups with that of both control groups (P<0.05). However, the differences among the three experimental groups were not statistically significant (P>0.05).
Conclusion: After 6 months, MTA and CEM cement exhibit distinct reinforcing effect on immature teeth.

Key Words: Calcium-enriched mixture cement, fracture resistance, fracture strength, immature teeth, mineral trioxide aggregate

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INTRODUCTION

Endodontic and restorative treatments of necrotic immature permanent teeth are still a challenge in dentistry.[1] Apexification, the traditional endodontic treatment for these teeth, is a long-term procedure. Weak dentinal walls and long duration of the apexification make these teeth more prone to cervical root fracture.[2-4] Recent studies have found that long-term Ca $(OH)_2$ therapy decreases the fracture strength of dentin.[5-8] The most promising alternative to apexification is the use of mineral trioxide aggregate (MTA) as an apical barrier.[9,10] The material has excellent biocompatibility[11,12] and the capacity to induce cementogenesis and osteogenesis.[9,10] Regardless of the excellent biologic properties of MTA, the thin dentinal walls still make these teeth more prone to fracture,[4] and a reinforcing technique in these weak roots is necessary. Several materials including composite resin and different post systems have been used to strengthen these weak teeth.[2,13-16] Based on the results of these studies, it appears that composite resin bonded to the canal walls has great potential to increase fracture resistance.[2,17-19] The

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ability of MTA to strengthen the tooth structure has been studied with controversial results.[8,20-22] White *et al*. showed weakening of dentinal structure in short term and attributed this effect to the structural alteration of proteins caused by the alkalinity of MTA.[8] However, one hypothesis is that with similar elastic modulus to dentin, MTA should theoretically be able to strengthen roots.[23] This strengthening effect has been shown in other studies.[21]

Recently, a new biomaterial, calcium-enriched mixture (CEM) cement has been introduced.[24] This cement consists mainly of CaO, SO_3, P_2O_5, and SiO_2. CEM cement releases calcium hydroxide during and after setting.[24,25] This cement has antibacterial features similar to calcium hydroxide and better than MTA.[26] In comparison with MTA, this novel cement has similar sealing ability and pH, increased flow, but decreased working time and film thickness.[24,27] This cement has also low cytotoxic effect similar to MTA and less than Intermediate restorative material (IRM).[28,29] This cement has excellent biocompatibility and profound capacity to induce hard tissue formation in vital pulp therapies.[30,31] A recent animal study has shown its capacity in regenerating periodontal ligament (PDL) and induction of cementogenesis.[32] The material has also shown favorable results in apexogenesis as well as pulpotomy of permanent teeth, management of furcal perforation, and internal and external root resorption.[33-36] Due to the controversial results about root reinforcement capacity of MTA and lack of data in the case of CEM cement, the present study was conducted to evaluate the strengthening effect of MTA and CEM cement.

METHODS AND MATERIALS

Tooth selection

This *ex vivo* study was carried out on human maxillary incisors without caries or root curvature extracted due to the periodontal reasons. The root surfaces were cleaned, and the teeth were stored in 5.25% sodium hypochlorite (NaOCl) for 10 minutes. Then, they were examined with a ×4 magnifying glass to discard the specimens with any cracks or fractures. Mesiodistal and labiolingual dimensions of the teeth were measured at the CEJ using a Gauge (Buffalo Dental Manufacturing C.D, Syosset, NY, USA). The mean values obtained were 6.7 mm and 6.4 mm for mesiodistal and buccolingual aspects, respectively. The samples presenting a difference of 20% from these values were discarded, leaving a total of 46 teeth available for the study. The teeth were stored in phosphate buffered solution (PBS) until use.

Specimen preparation and open apex simulation

Access cavities were prepared using a water-cooled round bur in a high-speed handpiece (NSK, Japan). Five teeth were randomly selected as negative control, and no further treatment was performed on them. The remaining teeth were subjected to the following procedures: First, the root length was standardized to 9 mm as measured from the facial CEJ to the apex by resecting the root end using a diamond fissure bur mounted on a high-speed handpiece (NSK, Japan). Then, RaCe rotary file (FKG Dentaire, Switzerland) size 40, 0.1 taper and 35, 0.08 taper were used to enlarge the canals to the apex. Two milliliter of normal saline was used to irrigate the canals between two files. To simulate immature teeth, the canals were further enlarged using peeso reamers (No. 1-6) (Mani, Japan) with gentle pressure and under copious water spray until the No. 6 peeso could be passed beyond the apex.[37] The canals were irrigated with 5 ml of normal saline. The specimens were again examined to ensure the absence of cracks. The prepared specimens were randomly assigned into following three experimental (n = 12) and a positive control (n = 5) groups: Group 1 (MTA group): White MTA (WMTA) powder was mixed with distilled water according to the manufacturer's instructions and compacted into the root canal to the facial CEJ using pluggers (Dentsply, USA) [Figure 1]. A glass slab was used to prevent extrusion of the MTA. Group 2 (CEM group): CEM cement (BioniqueDent, Tehran, Iran) was prepared according to the manufacturer's instructions and compacted into the canals as MTA

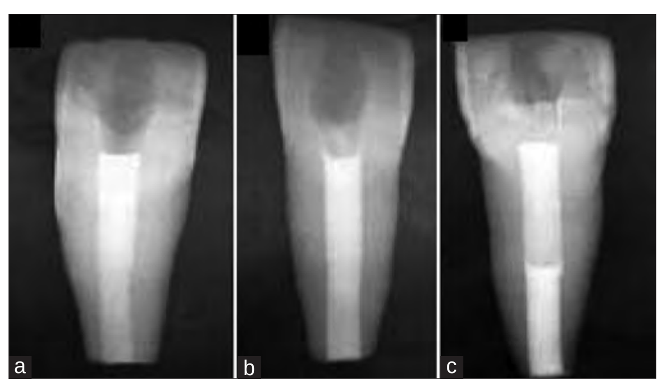

Figure 1: Sample of each group filled with different materials. (a) MTA-filled tooth (Group 1); (b) CEM-filled teeth (Group 2); (c) MTA plug backfilled with composite resin (Group 3)

group. Group 3 (MTA plus composite group): WMTA was mixed and compacted into the canal as MTA group, except that only apical 5 mm was filled. After MTA plug placement, the remainder of the canal was etched using 37% phosphoric acid for 15 seconds, and irrigated with distilled water for 30 seconds. Then, Single Bond was applied to the walls by microbrush and light cured for 20 seconds. Z100 Filtek (3M, ESPE, USA) composite resin was incrementally placed into the canal to the facial CEJ and light cured for 40 seconds. Positive control: The canals of the positive control were kept unfilled. All the teeth were radiographed to verify the homogeneity of the fillings and symmetric dentinal walls [Figure 1]. The teeth were placed in flower arranging sponge moistened by PBS and incubated at 37°C and 100% humidity for 6 months.

Fracture strength test

The teeth were embedded in self-curing acrylic resin to 2 mm apical of facial CEJ in cylinders with 1.5 cm diameter and 2.5 cm height. A surveyor was used to ensure alignment of the tooth long axis with central axis of the resin blocks. To prevent resin penetration into empty canals in positive control group, a plastic bar was placed in the canal prior to embedding. Thereafter, the specimens were mounted in a universal testing machine (Hounsfield testing equipment, UK). A compressive force at a crosshead speed of 5 mm/min was applied at the level of the lingual CEJ using chisel-shaped tip. The force was delivered at 130° to the tooth long axis in a linguolabial direction until fracture. The maximum force leading to fracture was recorded in Newton (N).

Statistical analysis

The mean values of fracture strength was compared using Univariate analysis of variance (ANOVA) followed by Duncan multiple range post hoc test. All analyses were performed using SPSS software. *P* values less than 0.05 were considered as statistically significant.

Table 1: Summary statistics of compressive strength in the groups

Group	N	Mean (N)	Std. Deviation
MTA(1)	12	1963.83	454.25
CEM (2)	12	2199.25	302.76
MTA + Composite(3)	12	2190.27	511.19
Control +	5	1556.60	551.94
Control -	5	1821.40	367.70

RESULTS

Fracture strength values in the groups showed a slight degree of variation [Table 1]. The results of Kolmogorov-Smirnov test confirmed the normality of the data (P>0.05). However, due to discrepancy mentioned above and the low power of this test for small sample size, a log (10) transformation was applied before doing ANOVA. Results of the ANOVA test showed significant differences in the fracture strength among groups ($F_{(4, 40)}$ = 3.104, P=0.026, Partial *Eta* Squared = 0.237 and Observed Power = 0.764). Partial *Eta* Squared as a measure of effect size showed a moderate effect size of difference among groups and the power of test provide a confidence of 76.4% for truly rejecting the null hypothesis of equal means. Results of Duncan multiple range post hoc test showed that there were significant differences among MTA+Composite and CEM groups with both control groups (P<0.01). MTA group also showed significantly higher strength values than positive control (P<0.05). There was no significant difference among three experimental groups [Figure 2].

DISCUSSION

Different materials including composite resins, resin-reinforced glass ionomers, resin-based root canal fillings (Resilon), and different post systems have been used to reinforce the immature permanent teeth with different results.[2,13-16,38] The studies on Resilon failed to show any strengthening effect of this resin-based material.[19,37] On the other hand, numerous studies confirm the reinforcing effect of various bonding techniques.[2,14,18,19,38,39] However, problems in adhesion to dentin and coronal leakage resulting from polymerization shrinkage are some of the drawbacks of these materials.[21,40,41]

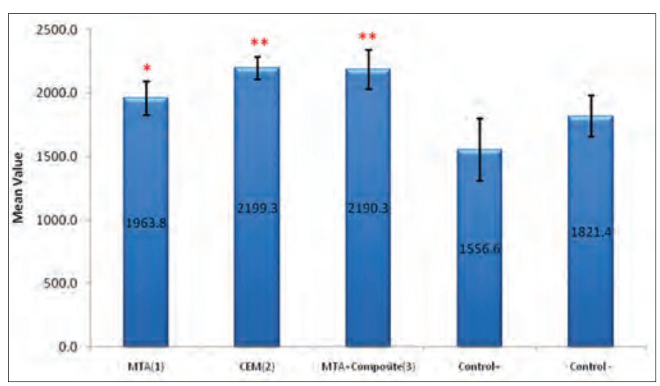

Figure 2: Mean (±SE) values of compressive strength in the groups. **Significantly different from Control+ and Control – (P < 0.01). *Significantly different from Control+ (P < 0.05)

Nowadays, apical MTA plug has become the treatment of choice for immature necrotic teeth.[39] Studies on the effect of MTA on fracture resistance of these teeth have reported contradictory results. Therefore, the present study was designed to evaluate this issue in an *ex vivo* simulated immature tooth model. Cvek[3] classified immature teeth into four groups according to the root maturity. Some studies have used immature tooth model mimicking stage 1 or 2 Cvek classification;[21] however, the roots are very weak at these stages, and simulating these stages *ex vivo* may cause cracks in the dentin which influence the results. In contrast, the nearly mature roots in the final stage may need no reinforcement.[42] Therefore, in the present study, stage 3 was selected for simulation.[14,37] Therefore, the root length was standardized to 9 mm, and the apex was enlarged using peeso reamers (No 1-6). To standardize the specimens, the parameters such as absence of cracks, alignment of the teeth within resin blocks, the gap between top of the resin and the CEJ, dimensions of the teeth before and after preparation were all matched.

Fracture of immature teeth mainly occurs during chewing or biting or as a result of impact trauma.[3] To simulate the force leading to fracture, different amounts of force have been applied to various parts of the teeth in different directions. These studies used forces in labiolingual,[20-22] linguolabial,[2,17,19,37,42] or vertical directions.[38,43,44] In our study, the force was applied at 130° to the tooth long axis in a linguolabial direction, simulating the average angle of contact of maxillary and mandibular incisors in a class I occlusion.[18,37]

The amount of the force also varies from 0.5 to 500 mm/min in different studies.[21,45] In the studies with the aim of evaluating the resistance in compression, lower speeds have been used; however, higher speeds are recommended to simulate the impact trauma.[45] Like similar studies which used linguolabial force, we used a force at a crosshead speed of 5 mm/min; however, it is recommended that this study be also performed at higher velocities and in labiolingual direction to evaluate the fracture strength in impact trauma situations.

In our study, the force was applied at lingual CEJ because this position results in a consistent fracture only through the root and root canal filling.[42] Although the force applied in *ex vivo* studies cannot completely simulate the clinical situations, standardizing the force in all of the study groups makes it possible to compare the strengthening effect of materials tested.

The results of the present study revealed the capacity of MTA and CEM cement to reinforce the immature teeth similar to intra-canal bonding techniques. This result is consistent with the findings of other studies.[21,22,46] The exact mechanism of this phenomenon is still unknown. In the study by Hatibovic-Kofman *et al.*,[22] MTA-treated teeth showed an initial decrease in fracture strength; however, after 2 months, the process was reversed, and the strength increased until one year. The authors attributed this phenomenon to the induction of tissue inhibitor of metalloproteinase (TIMP-2) and suppression of matrix metalloproteinase (MMP-2 and -14) by MTA. Because this study was carried out on non-vital extracted teeth, the induction of TIMP-2 is a matter of question.

A finite element analysis study[47] showed that the materials with similar elastic modulus to dentin could reinforce the weak roots. This hypothesis can explain the failure of gutta-percha or Resilon to reinforce immature roots.[19,37] It also explains the capability of intra-canal bonding techniques to strengthen the weakened teeth.[2,17] The elastic modulus of MTA is not available; however, the elastic modulus of Portland cements is around 1.7 GPa during early setting which further increases to 15-30 GPa after two weeks.[48] Considering the elastic modulus of dentin which is about 14-18.6 GPa,[23,47] the reinforcing effect of MTA may be explained by its similar elastic modulus to dentin. This hypothesis also explains the gradual increase in the fracture resistance of MTA-filled teeth found by Hatibovic-Kofman *et al*.[22]

In contrast to the aforementioned studies, other investigators believe that the alkalinity of MTA can theoretically weaken root dentin similar to the findings on calcium hydroxide.[5-8,20] Another hypothesis is that a combination of little tensile strength of MTA and lack of bonding to dentin can weaken the dentin.[23] An *ex vivo* study showed decreased fracture resistance in calcium hydroxide-filled teeth.[20] In this study, MTA-filled teeth had greater strength *vs* control although the small sample size of the study caused the differences statistically insignificant. With sufficient sample size, MTA might show reinforcing effect on weakened roots. Therefore, the results of this study should be interpreted with caution.

In the present study, CEM cement showed a distinct strengthening effect on immature teeth. The mechanism of this effect may be similar to the one described for MTA; however, as a result of different chemical composition[25,49] and lack of data on modulus elasticity of CEM cement, the mechanism of reinforcing effect of CEM remains to be elucidated.

One concern regarding the obturation of the entire canal with MTA or CEM cement is the setting of the materials. Moisture is required for complete setting of MTA.[49] However, uncertainty remains about the amount of moisture necessary to obtain the maximum beneficial properties of MTA.[50] An *ex vivo* study on mature teeth showed that even dry MTA powder packed into the canal can be adequately hydrated only by the moisture absorbed through the root.[50] No coronal or apical moisture was used in that study. Consequently, setting of MTA in immature teeth with thin dentinal walls and open dentinal tubules is more predictable. Furthermore, wide open apex provides better contact with apical tissue fluid. In the present study, the moisture from the apical, coronal, and even through the thin root walls was adequate for complete setting of the materials.

As mentioned earlier, the fracture resistance of MTA-filled teeth is time-dependant.[22] It is recommended that this study be repeated in different time intervals to better reveal the pattern of alteration in fracture strength of MTA-filled teeth.

An important issue neglected in the studies on fracture strength of MTA-filled teeth is the role of fatigue. None of these studies applied cyclic loads prior to fracture testing. We also had no suitable equipment for cyclic loading; however, it is recommended to consider this issue in future studies on fracture strength of immature teeth.

CONCLUSION

Within limitations of this study, we conclude that after 6 months, MTA and CEM cement exhibit distinct reinforcing effect on immature teeth.

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