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Fracture resistance and histological findings of immature teeth treated with mineral trioxide aggregate

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Abstract – The objective of the present study was to test the hypothesis that the fracture strength of calcium hydroxide and mineral trioxide aggregate (MTA)-filled immature teeth decreased over time. Immature mandibular incisors from sheep were extracted and the pulps were extirpated using an apical approach with a barbed broach, and the teeth were divided into three experimental groups. Group 1: untreated teeth. Group 2: the root canals were filled with calcium hydroxide paste. Group 3: the root canals were filled with MTA. All specimens were kept in saline with 1% antibiotics at 4°C for certain periods of time: 2 weeks, 2 months, and 1 year. Then they were tested for fracture strength in an Instron testing machine. The results were subjected to statistical analysis by the Tukey–Kramer tests. A P-value (< 0.05) was considered statistically significant. One tooth from each group was selected randomly for a histological study, examining matrix metalloproteinases (MMP2 and MMP14) and tissue inhibitor of metalloproteinase (TIMP). The results showed the mean fracture strengths decreased over time for all the three groups. Although the untreated teeth showed the highest value (45.5 MPa) at 2 weeks, the fracture strengths decreased significantly after 2 months ($P < 0.05$). On the other hand, the teeth treated with calcium hydroxide or MTA decreased, but not significantly over time ($P > 0.05$). For the MTA-treated teeth, the fracture strengths were not found significantly different from the untreated or calcium hydroxide-treated teeth at 2 weeks or 2 months ($P > 0.05$). However, the strength was significantly higher in the MTA group compared with the other two groups after 1 year ($P < 0.05$). Immunofluorescence images revealed expression of collagen type 1, MMP-2 and MMP-14 in both untreated and endodontically treated teeth. However, TIMP-2 was only observed in the MTA-treated teeth. In conclusion, the teeth with root treatment with MTA showed the highest fracture resistance at 1 year ($P < 0.05$). An explanation could be that MTA induced the expression of TIMP-2 in the dentin matrix and thereby possibly prevented destruction of the collagen matrix.

One of the challenges in dentistry is the treatment of immature, pulpless, permanent teeth that are candidates for endodontics. The introduction of apexification by the use of calcium hydroxide was pioneered by Heithersay (1) and Frank (2). This treatment gave adequate apical healing because of its antibacterial capability caused by a high pH and the ability to induce remineralization in periapical tissues (3, 4). However, it was reported that the fracture strength of calcium hydroxide-filled immature teeth decreased over time presumably because of changes in the organic matrix of dentin (5).

Mineral trioxide aggregate (MTA), a relatively new material currently being used in pulp therapy (6), is made primarily of fine hydrophilic particles of tricalcium aluminate, tricalcium silicate, silicate oxide, and tricalcium oxide (6–8). The initial pH of MTA when hydrated is 10.2 and the set pH is 12.5, which is comparable with that of calcium hydroxide. The material has been shown to have excellent biocompatibility (6, 9), antimicrobial properties (8), low cytotoxicity (7, 10), and low microleakage (11). However, recent study also indicated that root dentin was weakened after exposure to calcium hydroxide and MTA in 5 weeks (12).

The mechanical properties of dentin are fundamentally determined by dentin matrix, which is mostly composed of collagen type I (13, 14). Matrix metalloproteinase (MMP)-2, 14 and membrane type 1 (MT1) are found to play an important role during the degradation of collagen matrix of dentin (15–17). On the other hand, the tissue inhibitor of metalloproteinase (TIMP) inhibit active forms of MMPs, especially TIMP-2 inhibits MMP-2 (18). It is speculated that both calcium hydroxide and MTA in the root canal of dentin may affect the activities of MMPs or TIMP-2, thus influence the mechanical properties of dentin. As there is limited information in the dental literature concerning the long-term effect of either calcium hydroxide or MTA as root end-filling materials on the fracture strength of immature

teeth, the present study has the aim to study the effect of two endodontic materials mentioned above on the fracture strength of root dentin after apexification treatment for different length of time. Our hypothesis is that the fracture strength of calcium hydroxide and MTA-filled immature teeth will decrease.

Material and methods

This study employed 84 mandibular incisors extracted from young slaughtered sheep, approximately 9 months of age. Care was taken not to damage the teeth during extraction, and they were stored in 1% Chloramin-T until use. The teeth were cut approximately 3 mm from the apex to create access to the filling materials. The pulps were extirpated using an apical approach with a barbed broach, and the teeth were divided into three experimental groups, with 28 in each group:

- group 1: untreated teeth;
- group 2: the root canal was filled with calcium hydroxide paste (Ultradent®–UltraCal™ XS™, South Jordan, UT, USA) using a syringe with a capillary tip, and the paste was carried to the coronal part of the pulp cavity using a Lentulo® spiral (Dentsply, Woodbridge, ON, Canada) at slow speed, and then the canal was sealed with zinc oxide eugenol cement (IRM®, Dentsply, Woodbridge, ON, Canada);
- group 3: the root canal was filled with ProRoot® MTA system (Dentsply, Woodbridge, ON, Canada) using a plugger and further compacted by vertical compacters of various sizes to the apex. The MTA was carried to the coronal part of the pulp cavity using a Lentulo® spiral at slow speed, and then the apex was covered with a wet cotton pellet, and the teeth were covered with a damp gauze. The MTA was left for 12 h to ensure complete setting.

All specimens were radiographed from lateral and facial views of the teeth before and after the root treatment (Figs 1 and 2).

All teeth were kept in saline with 1% antibiotics at 4°C for certain period of time: 2 weeks, 2 months, and 1 year. The saline was exchanged once in a month. One tooth from each group was selected randomly for histological study, and the rest samples were embedded in a block of orthodontic resin (Dentsply), 23 mm × 20 mm × 15 mm, in such a way that the long axis of the tooth was aligned with the central axis of the resin block, and incisal edge of the tooth was positioned 4 mm away from the surface of the resin. The resin was left 1 h for complete setting. The specimens were

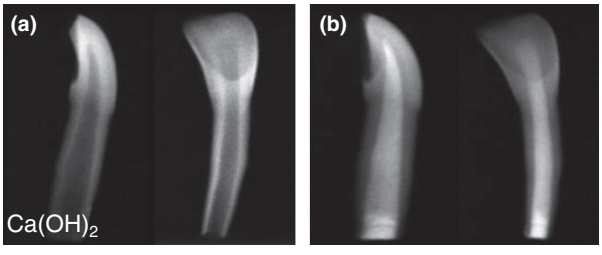

Fig. 1. Lateral and facial views of the mandibular incisors before (a) and after (b) root filling with calcium hydroxide.

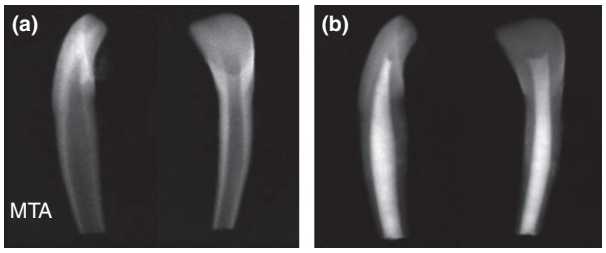

Fig. 2. Lateral and facial views of the mandibular incisors before (a) and after (b) root filling with mineral trioxide aggregate (MTA).

mounted in an Instron universal testing machine (Instron, model 1125, Canton, MA, USA). A spade was placed on the facial surface of the specimen parallel with the incisal edge and close to the block of the resin, 3 mm from the incisal edge. A force was applied with the spade at a speed of 1 mm min^{-1} until fracture, which surfaces were further studied under a light microscope (×30, Nikon, Mississauga, ON, Canada) with their images digitally captured. Finally, the fracture strength (force $area^{-1}$) was calculated in MPa.

Statistical analyses

The results were analyzed using multiple regression with material type as the exposure variable and time as an explanatory variable. If a significant interaction was identified, it was examined to determine whether the modification was quantitative or qualitative. If it was qualitative, separate regression analyses were performed for each level of the factor. The Tukey–Kramer statistic was used to determine statistical significance when multiple pair-wise comparisons were involved. A P-value < 0.05 was considered to be statistically significant.

Histology

Three teeths (one from each group) were decalcified by Decal (Tallman, NY, USA) for 4 days, and embedded in paraffin. Serial sections of 5–10 μm were made by means of a ultra microtome, and were collected on poly-L-lysine-coated glass slides, dried and stored at room temperature until use.

The sections were deparafinized, and then exposed to 3.0% hydrogen peroxide in methanol and phosphate-buffered saline (PBS). The sections were blocked in 10% porcine serum for 30 min at room temperature, and the four primary antibodies were applied at 4°C overnight: collagen type I antibody (AB 7658; Chemicon, Temecula, CA, USA; dilution, 1:500 and 1:250); MMP-2 (AF 1488, R&D Systems, Minneapolis, MN, USA); membrane type MMP-14 (MT-MMP-14; AB8102, Chemicon), and TIMP-2 (MAB3310; Chemicon). After being washed in PBS for 5 min, the sections were detected by incubation with biotinylated secondary antibody (Vectastain; Vector Labs, Burlington, ON, Canada; dilution, 1:200) for 30 min at room temperature. The sections were then incubated for 30 min with the Avidin Biotin Complex reagent (ABC) (Vector Labs). The color was developed

Table 1. Mean fracture strength over time of immature teeth with root canal untreated, or after therapy with either calcium hydroxide or mineral trioxide aggregate (MTA)

Mean fracture strength, mean ± SD (MPa)		Grouped by time		
		Two weeks	Two months	One year
Grouped by materials	None	45.47 ± 13.7 aA	26.60 ± 3.6 bB	25.77 ± 8.4 cB
	Calcium hydroxide	36.82 ± 7.1 aC	33.86 ± 14.9 bC	26.95 ± 7.7 cC
	MTA	36.98 ± 7.8 aD	29.28 ± 6.4 bD	35.93 ± 4.2 dD

Groups identified by different letters are significantly differ ($P < 0.05$).
Groups with same letters are not significantly differed ($P > 0.05$).
Column data are compared by lower case and row data by upper case.

with 3.3'-diaminobenzidine tetrahydrochloride (Sigma, Saint Louis, MO, USA), and the sections were counterstained with hematoxylin.

Results

Table 1 and Fig. 3(a and b) summarize the results of the fracture strength testing. The mean fracture strengths decreased over time for all the three groups (Fig. 3a). After 1 year specimens with no treatment, calcium hydroxide and MTA treatment showed decreased fracture resistance for 47%, 28%, and 2%, respectively. The period of observation was found to significantly influence the force required to fracture dentine ($P < 0.05$). There was a statistically significant material by time interaction and this interaction was qualitative in nature. In other words, the fracture force required to fracture dentine was different for the different materials used depending on time. Therefore, the materials were compared at each of the three time periods: 2 weeks, 2 months, and 12 months.

The mean amount of force required to fracture dentine in untreated specimens at 2 weeks was 23% and 23.3% greater than that required to fracture dentine in the MTA-treated and calcium hydroxide-treated specimens, respectively (Fig. 3b) This difference, however, was not statistically significant ($P > 0.205$). At 2 months the amount of force required to fracture dentine in the calcium hydroxide-treated specimens was 15.6% greater than the MTA-treated specimens and 27.3% greater than the untreated specimens. These differences were not statistically significant ($P > 0.05$). At 12 months the force required to fracture dentine in the MTA-treated specimens was 33.3% greater than calcium hydroxide-treated specimens and 39.4% greater than the untreated specimens. There was no difference between the calcium hydroxide and untreated samples ($P > 0.05$) but there was a statistically significant difference ($P < 0.05$) between the MTA-treated specimens and the other two groups.

The histological analysis was performed at 2 weeks after root canal treatment for the specimens untreated and treated with calcium hydroxide or MTA. The immunofluorescence images revealed expression of collagen type 1, MMP-2 and -14 in both untreated and endodontically treated teeth. TIMP-2, however, was only observed in the MTA-treated teeth (Fig. 4).

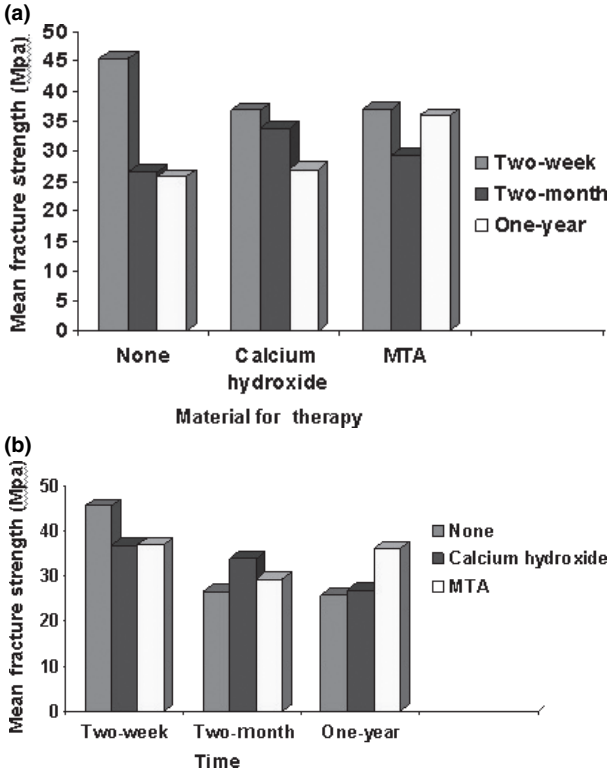

Fig. 3. (a) Mean fracture strength of immature teeth with root canal untreated, or after therapy with either calcium hydroxide or mineral trioxide aggregate (MTA). Mean fracture strengths vs materials for root treatment. (b) Mean fracture strength of immature teeth with root canal untreated, or after therapy with either calcium hydroxide or MTA. Mean fracture strengths vs time.

Discussion

The mechanical properties of dentin are fundamentally determined by dentin matrix, which is largely type I collagen (13, 14). It was recently reported that degradation of dentin organic matrix is mediated mainly by proteases belonging to the family of MMPs instead of bacterial acid (19, 20). MMP-2, -14, and T1-MMP are presented in the human dentin-pulp complex (19, 21, 22), in which MMP-14 is known as an activator of proMMP-2 (17, 23). The activities of MMPs are inhibited by TIMP, especially TIMP-2 inhibits MMP-2 (18).

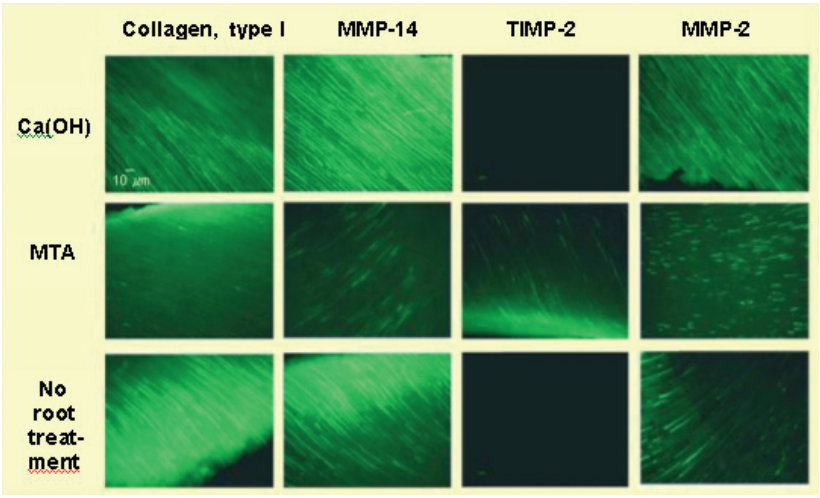

Fig. 4. Immunofluorescence images revealed expression of collagen type 1, matrix metalloproteinase (MMP)-2 and -14 in both untreated and endodontically treated teeth. Tissue inhibitor of metalloproteinase (TIMP)-2 was only observed in mineral trioxide aggregate (MTA)-treated teeth.

The present study showed that the fracture strengths with roots filled with calcium hydroxide decreased, but not significantly over time ($P > 0.05$, Fig. 3a). The strength was measured 36.8 MPa at 2 weeks, then decreased by 8% at 2 months, and further by 20% at 1 year. It was reported that the decrease in fracture strength of the dentin may be related to a change in the dentin matrix (5). According to the histological analysis in this study, except for TIMP-2, MMP-2, and -14 were clearly observed in the dentin matrix (Fig. 4). The activities of these MMPs resulted in the degradation of the organic matrix, thus reduced fracture strengths. Our current results also confirmed with the previous studies that calcium hydroxide had a negative effect on the strength of the dentin (12, 24).

For the MTA-treated teeth, an unexpected finding appeared, the fracture resistance of the specimens at 2 months decrease for 20% than at 12 months increased for 18%, therefore after 1 year teeth from this group showed only 2% decrease in the fracture strength, which is significantly higher ($P < 0.05$, Fig. 3b) than for the other two groups. The highest fracture strength (45.5 MPa) was obtained with untreated teeth at 2 weeks; however, the fracture strengths decreased significantly (44%) ($P < 0.05$) after 2 months and additionally 3% by 1 year.

It is interesting to note that the three different materials tested each were shown (Fig. 3b) to be superior to fracture, at the different periods of observation. Although a statistically difference was shown only at the 12 months, the effect size (% difference) at 2 weeks and 2 months observation periods were large and clinically significant. A clinically significant difference is considered to be >15%.

Immunofluorescence images revealed expression of collagen type I, MMP-2, -14, and TIMP-2 on the dentin (Fig. 4). As an inhibitor of MMPs, TIMP-2 prevented the organic matrix from degradation caused by MMP-2 and -14. Therefore, the reason for high fracture resistance of the dentin at long term might lie in the inhibitor activities of TIMP-2. Reduced expression of MMP-2 and -14 for the MTA-treated teeth (Fig. 4) may also contribute to the high fracture strengths at the end. MTA is a new material currently being used in pulp therapy (6). Because MTA has a pH of 12.5, some of its biological and histological properties can be compared with those of calcium hydroxide. Previous studies has revealed that it has actually induced cementogenesis (25), and bone deposition with minimal or absent inflammatory response (26). Recent studies also show that MTA stimulated the release of production of interleukin and cytokines (27–29), which could induce TIMP expression (30). The four TIMPs, TIMP-1 to -4, are secreted proteins that form complexes with MMPs and inhibit the active forms of all MMPs (20). As the MTA-treated teeth were the only ones that exhibited the expression of TIMP-2 among the three groups, using MTA as a root filling material might prevent the teeth from becoming brittle over time. In contrast, calcium hydroxide reduced to the undetectable level of TIMP-2, thus resulted in the compromised fracture strengths of the dentin over time.

In the clinical situations root canal therapy requires the use of a product that provides a reliable outcome and long-term prognosis. It is believed that current results would shed some light for clinic practitioners in this area.

Conclusions

The present study appears to support the hypothesis that the fracture strength of calcium hydroxide and MTA-filled immature teeth decreased over time. However, the teeth with root treatment with MTA showed the highest fracture resistance at 1 year ($P < 0.05$), as MTA induce the expression of TIMP-2 in the dentin matrix. MTA-treated teeth after the initial decrease in fracture strengths reverse the process, and the strength increased between 2 months and 1 year. This phenomenon can possibly be explained by the fact that MTA induces the expression of TIMP-2 in dentin matrix and suppress the degenerative activities of MMP-2 and -14.

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