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Influence of smear layer removal and application mode of MTA on the marginal adaptation in immature teeth: a SEM analysis

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Abstract – *Objectives*: This study was an *ex vivo* evaluation of the marginal adaptation of mineral trioxide aggregate as an apical barrier using teeth with an open apex and scanning electron microscopy. *Materials and methods*: Eighteen single-rooted human teeth were used. An artificial open apex was created using Gates Glidden drills #6–1 in a crown-down manner until the #1 bur passed through the foramen. A 1.36-mm divergent open apex was created at the foramen by retrograde apical transportation using a #40 Profile 0.6 taper instrument inserted to the length of the cutting blade. The teeth were divided into four groups (n = 4), and two teeth served as controls. The GI = mineral trioxide aggregate (MTA) was placed as a 5-mm-thick apical barrier without removal of the smear layer; GII = MTA was placed with indirect ultrasonic activation; GIII = apical plug was placed after removal of the smear layer without indirect ultrasonic activation; and GIV = MTA was placed with indirect ultrasonic activation, but the smear layer had been previously removed. The root apices were visualized with SEM (1000×), and 12 predetermined material/dentine interface points were measured (gaps). One-way ANOVA and Bonferroni's post hoc tests were used to compare the linear measurements of the gaps between the groups. *Results*: GIV had the lowest gap when compared with other groups, and no statistical differences were found among GI, GII, and GIII. *Conclusions*: The technique of MTA placement using passive ultrasonic vibration in association with smear layer removal improved the marginal adaptation of the material.

When teeth having incomplete root formation suffer pulp necrosis, the processes of dentine-stop formation and root development cease. Consequently, the canal remains large with thin and fragile walls, and the apex remains open. These features make instrumentation of the canal difficult and hinder the formation of an adequate apical stop. In such cases, it is necessary to induce the closure of the apical foramen with mineralized tissue or create an artificial apical barrier to allow for the condensation of the root filling material and to promote an apical seal.

Apexification is a method used to induce apical closure, in which calcified tissue is formed in the apical region of non-vital teeth with incomplete root development. This apical closure is traditionally performed with calcium hydroxide paste and a physiological vehicle (1), requiring successive changes until an apical barrier is formed. When using calcium hydroxide, the total therapy time may vary from 6 months to 2 years depending on the stage of root development and presence or absence of infection (2, 3).

Despite calcium hydroxide's efficacy, this dressing has several disadvantages, such as variability of treatment time, a large number of required appointments and radiographs, difficulty with patient follow up, delayed treatment (4), and the increased possibility of tooth fracture with long-term calcium hydroxide use (5).

An alternative to the multi-appointment $Ca(OH)_2$ apexification procedure is a single-step technique using an apical barrier. This treatment has been described in the literature as the non-surgical compaction of a biocompatible material into the apical end of the root canal. This procedure creates an apical stop that enables the immediate filling of the root canal (1).

The MTA method is currently indicated for apexification in teeth with immature root formation, that is, roots that are <1/3 complete (6). This method provides good sealing, offers biocompatibility, and is able to act in the presence of humidity. Moreover, both the patient and the practitioner benefit as the total treatment time

is reduced, which, for example, facilitates improved cooperation and motivation among children. Furthermore, this method eliminates problems related to a long-term treatment course because it provides adequate provisory sealing, and thus preserves the integrity of the remaining tooth. There are limitations to the application of MTA, however. These limitations are inherent to the absence of an apical barrier, which may result in adaptation failure and accidental extrusion of the material.

The smear layer, which is defined as an amorphous, irregular, and granular layer observed under the scanning electron microscope, results from the cleaning and shaping of root canals (7). Controversy remains regarding the influence of smear layer removal on apical sealing in MTA, and no consensus exists in the literature regarding this issue (8). Because the smear layer consists of organic and inorganic substances, it is necessary to use combined solutions for effective removal. The use of an organic tissue solvent (NaOCl) intercalated with a chelating agent (17% EDTA) allows for removal of both surface debris and the smear layer, which is not successful when one of these irrigation solutions is used alone (9).

Another procedure that may influence the apical adaptation of the MTA barrier is ultrasonic vibration, which would allow this material to flow (10, 11).

Over the years, scanning electron microscopy (SEM) has played an important role in evaluating the marginal adaptation of retrofilling materials (12, 13), the adaptation of apical barriers in cases of an immature root (14), and the microstructure of filling material (15).

Thus, the objective of this study was to assess the influence of both smear layer removal and application of MTA using indirect ultrasonic vibration on the adaptation of the apical plug in teeth with incomplete root formation.

Materials and methods

After approval from the local human ethics committee, a total of 18 extracted, intact, single-rooted human teeth with complete root formation were selected for study. The teeth were stored in 0.5% sodium hypochlorite solution (Lenza Farmacêutica, Divisão Odontológica, Belo Horizonte, Brazil).

Standard access cavities were prepared, and the canal orifices were located. The patency of each canal was confirmed by inserting a size-15 file (Maillefer, Ballaigues, Switzerland) through the apical foramen prior to root canal preparation. The crowns were removed at the amelo-cemental junction, and roots were sectioned close to the cervical portion using a carborundum disk (SS White Artigos Dentários, Rio de Janeiro, Brazil), which yielded a length of 13 mm. Next, a 3-mm portion of the apical third was removed to standardize the root lengths to 10 mm.

An artificial open apex was created in each tooth using the Gates Glidden drills #6–1 (Maillefer) in a crown-down manner until the size-1 bur passed through the foramen. The canals were irrigated with 1.0% sodium hypochlorite solution (Lenza Farmacêutica, Divisão Odontológica) with each change of instrument.

A 1.36-mm divergent open apex was created at the foramen by retrograde apical transportation using a #40 Profile 0.6 taper instrument (Maillefer) inserted to the length of the cutting blade. The root canal was dried with #80 absorbent paper points (Tanari-Tanariman Indústria Ltda., Manacapuru, AM, Brazil), and the integrity and shape of the apical foramen were verified microscopically at 13× magnification (Surgical microscope M-900, DF Vasconcelos, São Paulo, Brazil).

After apical preparation, the teeth were divided into two experimental groups according to the presence or absence of the smear layer ($n = 8$). In the group where the smear layer remained, irrigation was performed with 10 ml of 1.0% NaOCl (Lenza Farmacêutica, Divisão Odontológica). For the group in which the smear layer was removed, final irrigation was performed with 5 ml of 17% EDTA (Lenza Farmacêutica, Divisão Odontológica) for 5 min followed by 5 ml of 1.0% NaOCl (Lenza Farmacêutica, Divisão Odontológica). In both cases, irrigation was performed with a 23-G needle (Injex Indústrias Cirúrgicas Ltda., Ourinhos, SP, Brazil) coupled with a 5-ml syringe. The teeth were dried with #80 absorbent paper points (Tanari-Tanariman Indústria Ltda.).

The specimens were divided again into four experimental groups ($n = 4$), with two teeth serving as controls. The teeth were attached to premoistened foam to simulate the characteristics of periapical tissues and to provide slight resistance to material extrusion during the filling procedure.

Group I

A 1-gram amount of white MTA (WMTA Angelus, Londrina, Brazil) was mixed with 0.35 ml of distilled water according to the manufacturer's instructions. The MTA was placed as close as possible to the apex using MTA-specific points (Ápice Instrumentos Odontológicos, Belo Horizonte, Brazil) under microscopic visualization at 13× magnification (M-900 DF Vasconcelos). The MTA was then compacted with Schilder pluggers (Odous De Deus, Belo Horizonte, Brazil) until a 5-mm thickness was achieved. Size #80 absorbent paper points (Tanari-Tanariman Indústria Ltda.) were moistened in saline solution and inserted into the root canal for 24 h to ensure the cement-curing process. Orthoradial and mesioradial radiographs of the teeth were obtained to assess the thickness and quality of the apical plug.

Group II

Similar to Group I, white MTA was also used for filling the apices of the teeth in Group II. This process was followed by indirect ultrasonic vibration for 5 s at low potency and without irrigation (ENAC Osada, Incorporated, Los Angeles, CA, USA). Moreover, the ST21 point was applied perpendicularly to and along the axis of the endodontic condenser (size

#3), which was in contact with the MTA apical barrier (Fig. 1).

Group III

After removal of the smear layer, apical filling with MTA was performed with similar methods as those used for Group I.

Group IV

The apical filling procedure was performed as described for Group II, but with previous removal of the smear layer.

Control group

The teeth ($n = 2$) were filled with thermoplastified gutta-percha using the Obtura system (SybronEndo, Orange, CA, USA), and 3 mm of the apex was filled with MTA using retrograde instrumentation.

All specimens were stored in the oven at 37°C and 100% humidity for 72 h.

The teeth in each experimental group were analyzed with a scanning electron microscope (JSM-6510LV; JEOL, Tokyo, Japan). Initially, the specimens were dried with air jets and then stored in a desiccator containing gel silica. After the drying period, the samples were sputter-coated with gold for SEM evaluation. The apices of all teeth were analyzed and photographed at 55× magnification with landmarks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 selected (Fig. 2). The teeth were then evaluated at 1000× magnification for possible gaps between the MTA and dentine walls. The Control USER INTERFACE Software, version 2.01 (JEOL Technics Ltd), was used to assess the images and to determine the measurements of the gaps, in micrometers (μm), at the predetermined sites.

One-way ANOVA and Bonferroni's post hoc tests were used to compare the linear measurements of the gaps between the groups. A significance level of 0.005 was used for paired comparisons. The data were analyzed with the BIOESTAT 5.0 software (Optical Digital Technology, Belém, Brazil).

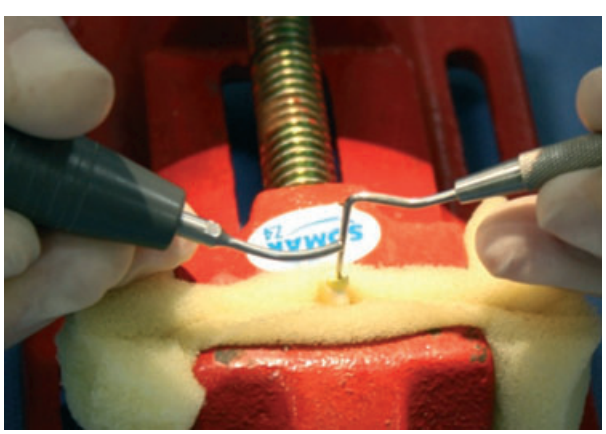

Fig. 1. Indirect ultrasonic vibration in contact with the endodontic condenser for 5 s.

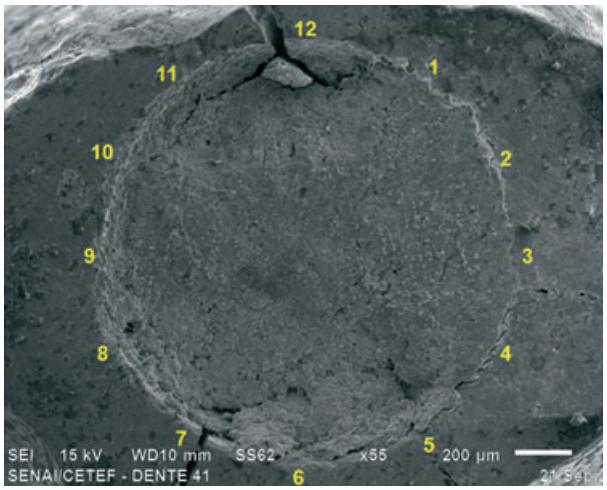

Fig. 2. Microphotograph of an apex filled with MTA (55× magnification). Numbers in yellow represent the sites selected for evaluation at 1000× magnification.

Results

Gaps between the MTA and dentine walls were observed in all groups, which were evaluated at various magnifications (Figs 3–6). Table 1 provides the means and standard deviations for the gap values in the groups.

There was no significant difference in the gap measurements between Groups I, II, and III. However, the technique of MTA application using indirect ultrasonic vibration yielded the lowest gap values when performed in conjunction with smear layer removal (Group IV). Moreover, marginal adaptation of the MTA was significantly improved in this group compared with other treatments, which is reflected by descending gap values among the experimental groups (III > II > I > IV). There was no statistically significant difference between

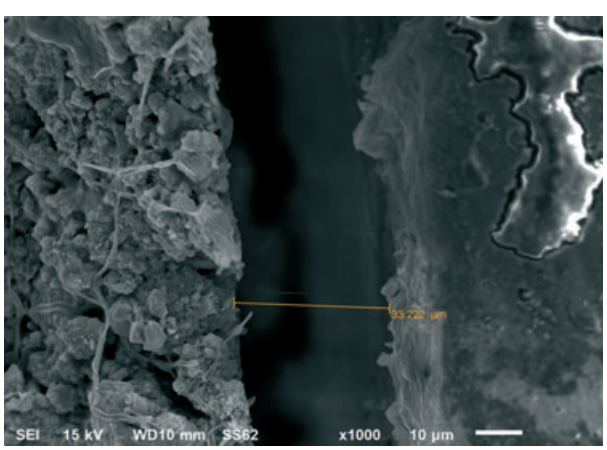

Fig. 3. Microphotograph corresponding to site 3 of a specimen from Group 1 seen at 1000× magnification (presence of smear layer and no ultrasonic vibration), showing a gap measurement of 33.222 μm between the MTA (left) and dentine wall (right).

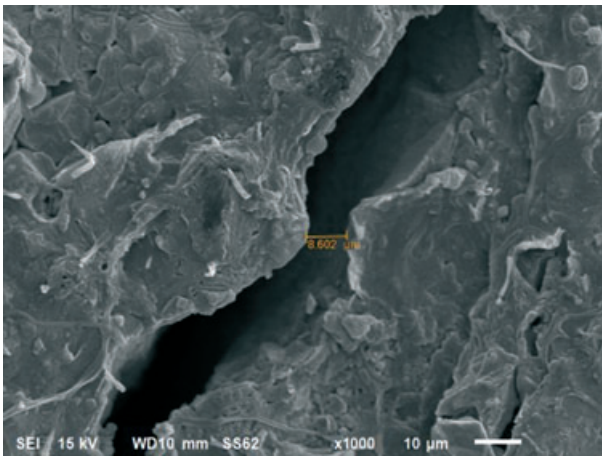

Fig. 4. Microphotograph corresponding to site 4 of a specimen from Group II seen at 1000× magnification (presence of smear layer and ultrasonic vibration), showing a gap measurement of 8.602 µm between the MTA (left) and dentine wall (right).

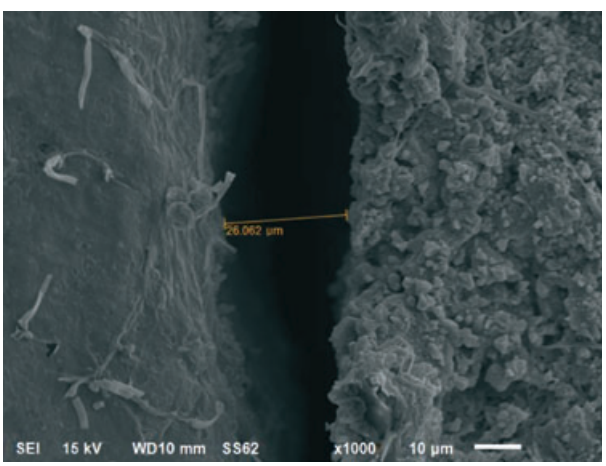

Fig. 5. Microphotograph corresponding to site 9 of a specimen from Group III seen at 1000× magnification (smear layer removal and no ultrasonic vibration), showing a gap measurement of 26.062 µm between the dentine wall (left) and MTA (right).

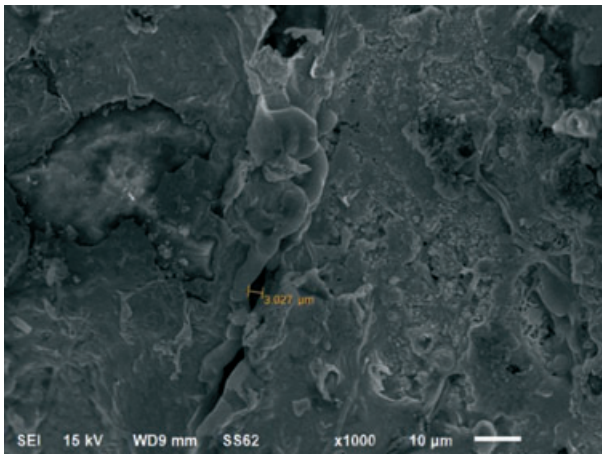

Fig. 6. Microphotograph corresponding to site 9 of a specimen from Group IV seen at 1000× magnification (smear layer removal and ultrasonic vibration), showing a gap measurement of 3.027 µm between the dentine wall (left) and MTA (right).

Table 1. Means and standard deviations of the linear measurements of the gaps

Group	Gaps (µm)	*P* value[1]
(I)	11.97 ± 10.25	<0.005[c d f g h i]
(II)	13.40 ± 7.90	n.s.[a b e j]
(III)	15.47 ± 7.22	
(IV)	3.38 ± 3.54	
(control)	2.44 ± 2.69	

1*P*-values were obtained through one-way ANOVA followed by Bonferroni's post hoc test (level of significance set at 0.005 for paired comparisons).
[a]Group I vs Group II.
[b]Group I vs Group III.
[c]Group I vs Group IV.
[d]Group I vs Control.
[e]Group II vs Group III.
[f]Group II vs Group IV.
[g]Group II vs Control.
[h]Group III vs Group IV.
[i]Group III vs Control.
[j]Group IV vs Control.
n.s., not significant.

Group IV and the controls (application of the material through retrograde instrumentation).

Discussion

Among its various clinical applications, MTA is an alternative to calcium hydroxide in the treatment of immature teeth, in which root formation was interrupted because of either trauma or infection. Recent studies have shown high rates of success for this type of treatment when its use is well indicated, and it is carried out under specific conditions (6, 16, 17).

Artificially open apices were prepared by creating root canals with Gates Glidden drills. Drill #1 was used to pass through the apical foramen as described by De Deus et al. (18). Standardization of the apical foramen and divergent open apex was made by means of retrograde instrumentation using a rotary file #40 with 0.06 conicity inserted into the entire active length. Apical preparation was performed in a way similar to that described by Martin et al. (19), who standardized the apical diameter of the samples. However, Bidar et al. (14) did not consider such a standardization, as their samples were prepared by apical resorption of the teeth, which were attached to foam containing sulfuric acid over a period of 4 days.

There are variations between studies regarding the thickness of the apical barrier (10, 14, 18–21). In this study, the MTA apical plug was 5 mm thick as described by Al-Kahtani et al. (22), who compared the effect of various thicknesses of MTA barriers on bacterial infiltration. Their results showed that 5 mm of the material entirely prevented apical infiltration. Further-

more, other studies also used apical barriers of 5-mm thickness (11, 16).

In an attempt to reproduce the clinical conditions that are present when filling open apices, the teeth were attached to moistened foam to simulate the consistency of periapical tissues and offer a slight resistance to the extrusion of filling material, as described by Martin et al. (19).

Our goal was to test the hypothesis that ultrasonic vibration could promote the adaptation of MTA apical barriers and improve the sealing process. Therefore, we used indirect ultrasonic vibration in association with an endodontic condenser, a procedure also employed by Lawley et al. (10) and Kim et al. (11). According to these authors, orthograde application of ultrasonic vibration was effective in delaying bacterial infiltration. In addition, this procedure also serves as a useful method for MTA condensation because of the greater efficiency of ultrasonic energy compared with manual condensation of the flowing material. The results of this study highlighted that lower gap values were observed following ultrasonic vibration in association with removal of the smear layer. However, indirect ultrasonic vibration alone did not change the marginal adaptation of MTA.

The amount of information available in the literature is not yet enough to establish the effect of the smear layer on the MTA's sealing ability over time. According to Yildirin et al. (8), apical micro-infiltration is lower in the presence of a smear layer when MTA is used for orthograde root canal treatment. According to these authors, removal of the smear layer caused significantly higher apical infiltration compared with samples kept intact for 30 and 180 days. Based on the methodology employed in this study, no significant difference was found between the experimental groups in the presence or absence of the smear layer (Groups I and III). Nevertheless, this difference was significant when results were compared in regard to smear layer removal and the use of indirect ultrasonic vibration (Groups II and IV).

Several *ex vivo* studies on the use of an MTA apical barrier for cases of immature teeth have used methodologies of bacterial infiltration, staining techniques, and fluid filtering (10, 11, 18–22). In this study, we sought to assess MTA adaptation using scanning electron microscopy as described elsewhere (14). A few cracks were observed under SEM, despite a lack of correspondence with the predetermined sites. According to Torabinejad et al. (12), the occurrence of cracks can be related to the process of sample preparation for SEM analysis, wherein the specimens were sputter-coated with gold under critical temperatures.

To perform an evaluation without distorting the results, sites 1, 2, 3, 4, 5, 6, 7, 8, 0, 10, 11, and 12 of all specimens were randomly selected for evaluation and photography at a magnification of 1000×. The microphotographs obtained from each specimen yielded a sampling universe of 12 sites *per* apex, that is, 48 sites for each experimental group. Alternatively, a study by Badr (13) evaluated four sites *per* specimen replica, totaling 40 sites that were randomly determined *per* experimental group (10 specimens *per* group). Bidar et al. (14) evaluated 20 teeth, although they opted for the largest gaps in 4 sites *per* apex for a total of 80 sites per experimental group.

In view of the benefits of using MTA to create an apical barrier in cases of incomplete root formation, additional *ex vivo* and *in vivo* studies should be performed.

Conclusions

Given the limitations of this *ex vivo* study, the following conclusions can be drawn: (i) removal of the smear layer alone did not change the marginal adaptation of MTA; (ii) indirect ultrasonic vibration alone did not change the marginal adaptation of MTA; and (iii) removal of the smear layer in association with indirect ultrasonic vibration promoted marginal adaptation of MTA.

Acknowledgements

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