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Comparative *in vitro* study of the sealing efficiency of white *vs* grey ProRoot mineral trioxide aggregate formulas as apical barriers

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Abstract – Mineral trioxide aggregate (MTA) has been proposed as one of the materials which can be used in a one-visit apexification technique. Recently conventional grey MTA has been replaced by a new white MTA formula. The aim of this study was to compare the root canal adaptation of white MTA to that of grey MTA when used as an apical barrier in teeth with open apices. We also examined whether a previous calcium hydroxide intracanal medication affects MTA's sealing ability and investigated the ability to remove calcium hydroxide from the root canal walls. Forty-nine teeth were prepared in a manner to simulate a divergent open apex of immature teeth. Four teeth were used in a preliminary experiment to demonstrate the inefficacy of calcium hydroxide removal from the canal walls in teeth with open apices. Four groups of 10 teeth each were created: groups A and B were treated with calcium hydroxide intracanal medication and then received an apical plug of grey and white MTA respectively. Groups C and D received an apical plug of grey and white MTA respectively without previous intracanal medication. Four teeth served as negative and one as a positive control. The marginal adaptation and sealing ability of the apical barrier were tested by means of a dye tracer (basic fuchsine) after longitudinal sectioning. It was found that MTA apical barrier resisted displacement during gutta-percha condensation. Calcium hydroxide pretreatment, adversely affected white MTA sealing ability ($P < 0.05$).

In several cases an endodontic treatment must be performed in teeth with blunderbuss apices as a result of either pulpal necrosis in teeth with incompletely formed roots or of root apex resorption. Similar problems are faced in severely overinstrumented root canals and failed apicectomies. Endodontic treatment of permanent teeth with open apices has always been a challenge to endodontists, since an apical stop against which gutta-percha can be condensed is missing in these cases. The usual treatment procedure proposed for such cases is the repeated intracanal placement of $Ca(OH)_2$ in order to induce an apical hard tissue barrier formation (1).

However, the calcium hydroxide apexification has some inherent disadvantages such as prolonged treatment time, unpredictability of apical closure, difficulty in patient follow up, susceptibility to coronal microleakage and financial concerns (2, 3). Moreover, the long-term use of calcium hydroxide root canal dressing appears to weaken the root structure possibly by neutralizing, denaturating or dissolving the acidic components of dentin (4). Since these components are considered to act as bonding agents between the collagen network and the hydroxyapatite crystals, their destruction may render the tooth more prone to fracture (4). To overcome all these limitations a single-visit apexification technique using an apical barrier has been alternatively advocated (5). Over the years, many materials have been proposed as potential apical barriers like tricalcium phosphate, calcium hydroxide, dentin plugs, calcium phosphate cement and more recently mineral trioxide aggregate (MTA) (ProRoot MTA; Dentsply, Tulsa, OK, USA) (2). Recently, conventional grey MTA has been replaced by a new white formula, possibly to overwhelm occasional tooth discolouration problems observed with the former material (6). The grey MTA formula has already been used as an apical barrier in teeth with open apices, providing very good clinical results (7, 8). However, there is still no evidence that the white MTA formula is equally effective. On the other hand an in vitro study concerning repair of furcal perforations with MTA concluded that the two MTA formulas showed no significant difference in preventing bacterial leakage (9).

Complete chemomechanical preparation and obturation of the root canals system is a quite difficult task. This is especially true in necrotic teeth with open apices where peripheral filing of the canal walls is impossible. Taking this into account, a calcium hydroxide intracanal medication has become an essential step in root canal disinfection. However, complete removal of the calcium hydroxide dressing from the root canal wall of a completely formed tooth is not feasible (10, 11) and this may be more pronounced in the apical portion of an incompletely formed root since the canal walls diverge from each other. Whether residual calcium hydroxide in the apical area may affect MTA's adaptation to the canal walls remains to be seen.

The aim of this study was: (i) to comparatively evaluate the root canal sealing efficiency of white vs grey MTA formulas when used as apical barriers in teeth with open apices and (ii) to examine whether a previous calcium hydroxide intracanal medication affects MTA's sealing capacity. The null hypothesis tested was that white and grey formulas do not differ in terms of root canal adaptation when used as apical barriers and that a previous calcium hydroxide intracanal dressing does not affect MTA's sealing ability.

Materials and methods

Forty-nine freshly extracted single-rooted, single-canal human teeth were collected and stored in 10% formalin solution. The teeth were X-rayed and inspected under an operational microscope (×10) to exclude the possibility of root fractures. After preparation of straight-line access cavities, the apical 2 mm of the roots were resected with a diamond disc so as to centralize canal exit. The root canals were instrumented with K-files, under copious irrigation with 2% NaOCl, up to #90, until 1 mm of the last file protruded the apex. Then, a divergent open apex was prepared via a retrograde instrumentation by means of a ProTaper F3 rotary instrument (Dentsply, Maillefer, Ballaigues, Switzerland). The rotary instrument was inserted into the prepared canal until the last cutting blade reached the resected apex and the preparation of a divergent apex was confirmed radiographically. Four of these prepared teeth were used in a preliminary study in order to examine the efficacy of calcium hydroxide removal from the canal walls. The rest of the teeth were randomly divided in four groups (A–D) consisting of 10 teeth each, four teeth served as negative controls (one for each of the groups A–D) and one tooth as a positive control. All the teeth were inserted up to the cemento–enamel junction into a distilled-water moistened flower arrangement foam. Prior to insertion a cotton pellet was fitted into the divergent apex so as to act as a barrier, preventing foam penetration into the root canal while the direction of insertion was noted with a permanent marker in the crown. The cotton pellet was removed with a barbed broach after the tooth reached its final position. All the experimental procedures were done while the teeth were in this arrangement.

In the preliminary experiment mentioned above, a calcium hydroxide dressing (pure calcium hydroxide mixed with distilled water), was placed into the root canal with a lentulo spiral for a period of 1 week, after which the medicament was removed by filing the canal walls with a K-file binding to canal at the working length. The removal procedures were accompanied with irrigation by 10 ml of 2% NaOCl and the root canal was dried using paper points. Two techniques were employed to confirm the presence of calcium hydroxide in the root canal walls. First, the apical 6 mm of each of the four specimens were examined by computerized X-ray microtomography (Model 1072, SkyScan, Aartselaar, Belgium). The scanning procedure was completed using 100 kV and 98 μA at ×35 magnification resulting in a pixel size 8.09 × 8.09 μm. Second, the teeth were split longitudinally by means of Macrotome 2 (Metals Research Ltd, Cambridge, England, UK) and photographed with a video-microscope (×35 and ×100) (MS500C, Micro-Scopeman Moritex Europe Ltd, Oxford, UK).

The teeth of groups A and B were treated with intracanal calcium hydroxide, delivered with a lentulo spiral whereas groups C and D did not receive premedication. After 1 week, calcium hydroxide was removed as previously described. Then groups A, C received grey MTA as an apical barrier and groups B, D received white MTA. Each of the negative controls was treated as the rest of the teeth of the corresponding group whereas the positive control was left completely empty.

MTA was placed in an orthograde approach by means of Micro-Apical Placement apparatus (MAP, Produits Dentaires, Vevey, Switzerland) and was condensed to a thickness of 4 mm using the thick end of a paper point #80 applying very mild pressure. A moistened paper point was left in the canal to facilitate proper setting of the material and the cavities were sealed with cotton pellet and cavit (Cavit G; 3M ESPE AG, Seefeld, Germany). The MTA was left for 48 h to set and then a finger plugger was used to test proper setting and resistance to displacement. After that, teeth were obturated with thermoplasticized gutta-percha (Obtura II; Obtura Spartan, Fenton, MO, USA) and Roth Root Canal Cement Type 601 (Roth International LTD, Chicago, IL, USA) and the access cavities were sealed with IRM (Dentsply DeTrey, Konstanz, Germany). The whole system was stored in 100% relative humidity at 37°C.

Two weeks later the teeth were removed from the foam, X-rayed and photographed at the apex under the video-microscope at ×100 magnification for estimation of plug adaptation to the canal walls. The crowns of the teeth were resected with a diamond disc so as to obtain a common root length and were covered with two layers of nail varnish except the apical 2 mm of the roots. The negative controls were covered completely in contrast to the positive control which was not covered at all. Then, all teeth were immersed in basic fuchsine 1% solution buffered to neutral pH (7, 4) for 48 h. After washing in tap water, teeth were longitudinally sectioned by means of Macrotome 2, inspected and photographed under the video-microscope at ×35 and ×100 magnification and the maximum linear dye penetration was calculated in a personal computer with a proper software (Adobe Photoshop 7.0, Adobe Systems Inc., San Jose, CA, USA). For each specimen maximum linear penetration was calculated as the mean of five repeated measurements.

The results of the dye leakage study were analyzed by two-way ANOVA which was followed by a Kruskall–Wallis ANOVA on Ranks and an all pairwise multiple comparison procedure (Dunn's method) while the relation of the plug to the apex was analyzed with the Friedman's analysis of variance.

Results

Removal of $Ca(OH)_2$ from root canal

The micro-CT examination (Fig. 1a,b) and the photographs from the video-microscope (Fig. 2a,b) confirmed the presence of adequate residual calcium hydroxide in the apical area in all specimens.

Adaptation of MTA apical plug at the apex.

Observation of MTA's adaptation at the apex revealed unpredictable behaviour. In all four groups, there were teeth in which the material showed signs of underfilling, slight overfilling and excessive overfilling (Table 1) (Figs 3–5). Nevertheless, gutta-percha penetrated the MTA barrier in only one tooth (Fig. 6). White MTA when used without previous intracanal medication (group D) overfilled the apex more often than any other group. This difference was the only statistically significant ($P < 0.01$).

Dye leakage study

The negative controls did not show any dye penetration whereas the positive control was completely penetrated by the dye. In 36 out of the 40 specimens the basic fuchsine did not reach the MTA/gutta-percha interface (Figs 7–10). The linear dye penetration and mean thickness barrier are presented in Table 2. Statistical analysis of linear dye penetration revealed that white MTA sealed significantly better when used without previous intracanal medication. Although no other significant differences were found between the groups teeth which received calcium hydroxide intracanal dressing had a tendency to leak more (Figs 7, 8).

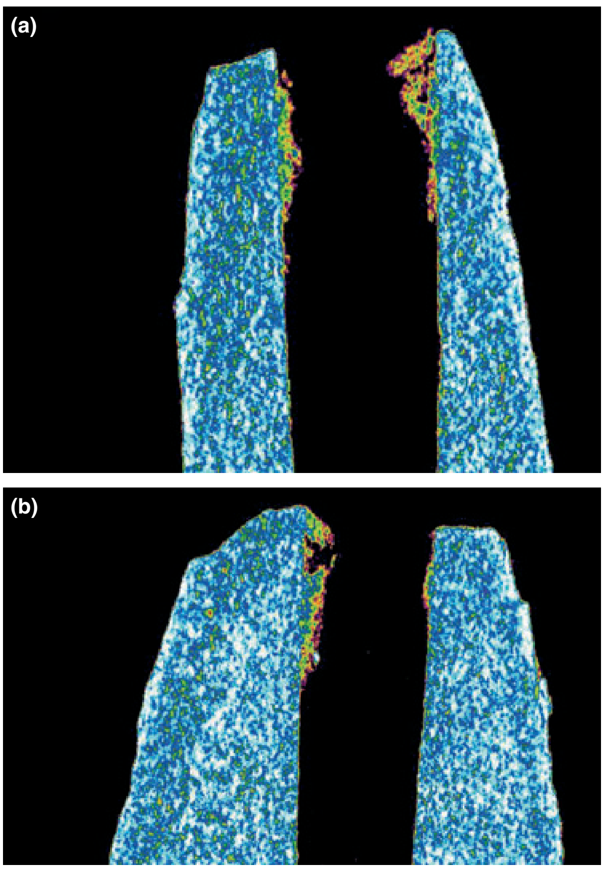

Fig. 1. (a, b) Residual calcium hydroxide as illustrated by Micro-computed tomography.

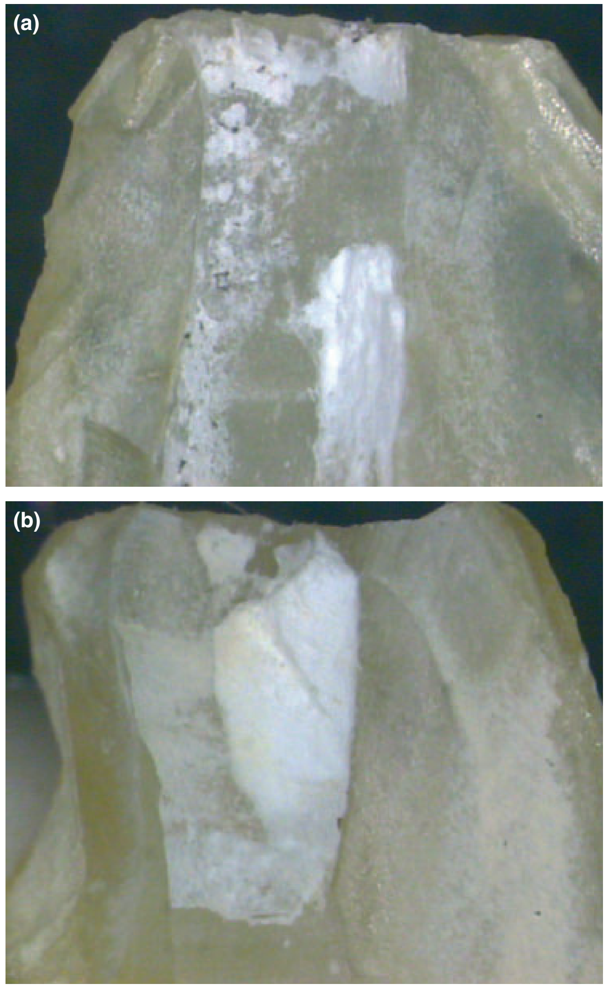

Fig. 2. (a, b) Longitudinal sections of teeth revealing the presence of substantial amounts of residual calcium hydroxide at the divergent apex area (magnification ×100).

Table 1. Relationship of mineral trioxide aggregate (MTA) apical plug to the root apex

	Underfilled	Slightly overfilled	Excessively overfilled
Grey MTA + $Ca(OH)_2$	4	4	2[a]
White MTA + $Ca(OH)_2$	3	6	1[a]
Grey MTA without $Ca(OH)_2$	3	4	3[a]
White MTA without $Ca(OH)_2$	1	3	6[b]
Total	11	17	12

Same letter reveals no significant difference whereas different letter reveals statistically significant difference at $P < 0.01$.

Fig. 3. Photograph of the open apex of a tooth filled with grey mineral trioxide aggregate (MTA). An area of underfilling is seen at one side of the MTA barrier (magnification ×100).

Fig. 4. Photograph of the open apex of a tooth filled with white mineral trioxide aggregate (MTA). A slight overfilling of MTA barrier is evident (magnification ×100).

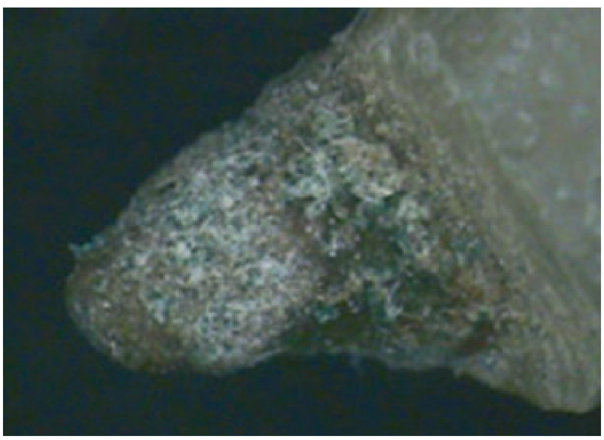

Fig. 5. Excessive protrusion through the open apex of grey mineral trioxide aggregate barrier (magnification ×100).

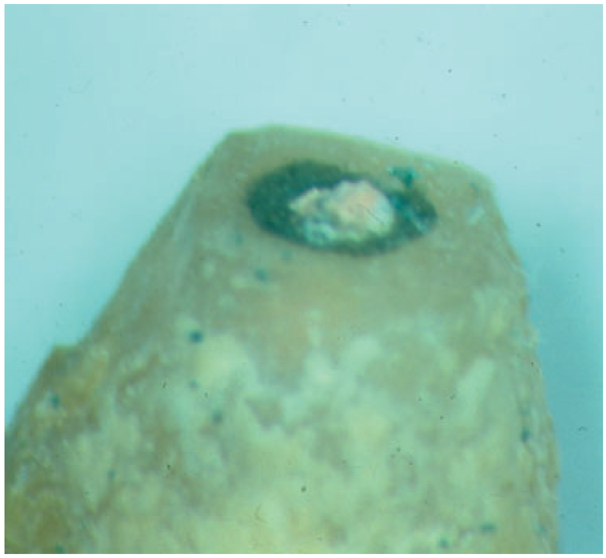

Fig. 6. Gutta-percha penetration through mineral trioxide aggregate barrier (magnification ×16).

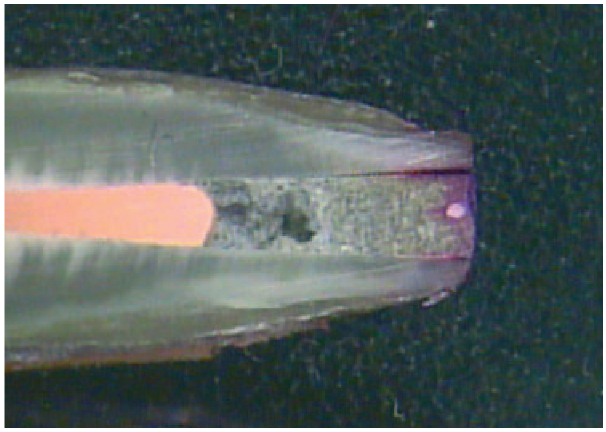

Fig. 7. Longitudinal tooth section revealing basic fuchsine penetration in a tooth filled with grey mineral trioxide aggregate barrier and gutta percha. Pretreatment with $Ca(OH)_2$.

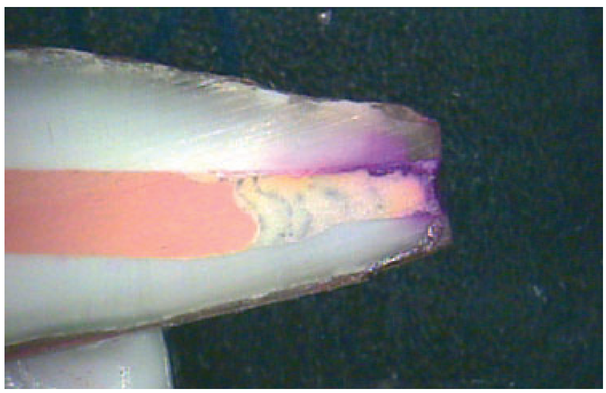

Fig. 8. Longitudinal tooth section revealing basic fuchsine penetration in a tooth filled with white mineral trioxide aggregate barrier and gutta percha. Pretreatment with $Ca(OH)_2$.

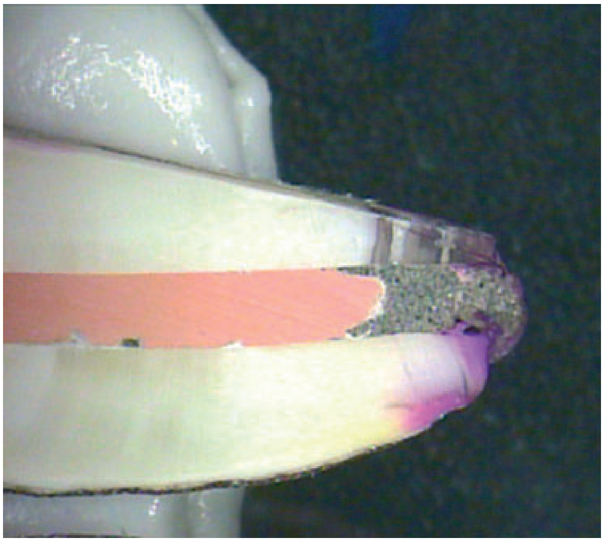

Fig. 9. Longitudinal tooth section revealing basic fuchsine penetration in a tooth filled with grey mineral trioxide aggregate barrier and gutta percha. Pretreatment without $Ca(OH)_2$.

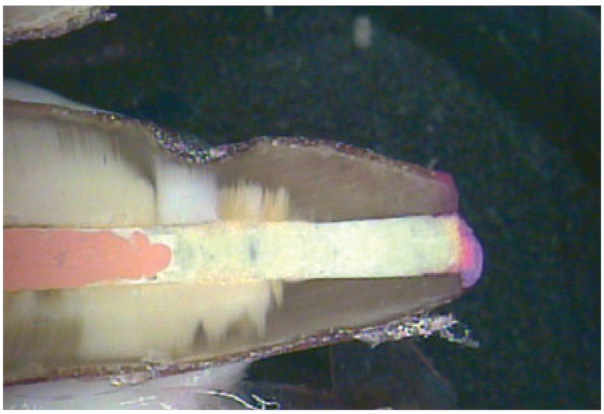

Fig. 10. Longitudinal tooth section revealing basic fuchsine penetration in a tooth filled with white mineral trioxide aggregate barrier and gutta percha. Pretreatment without $Ca(OH)_2$.

Discussion

Our preliminary study verified the presence of a substantial amount of residual calcium hydroxide at the apical 6 mm of the divergent canal walls in all four specimens, as shown by means of micro-computed tomography and video-microscope. Only four teeth were used in this study, as the inefficacy of calcium hydroxide removal has already been demonstrated in mature teeth (10, 11) and it was our intention to show that this finding is equal or even more pronounced in teeth with open apices. This is easily explained by the fact, that the divergent part of the canal cannot be mechanically prepared and calcium hydroxide is removed in this area only by NaOCl irrigation, resulting in more residues.

The present study evaluated MTA's effectiveness as an apical barrier in a one visit apexification technique in terms of gutta-percha extrusion and marginal adaptation. The possible effect of a calcium hydroxide dressing on MTA marginal adaptation was also examined.

The apical barrier is used mainly to produce an apical stop against which gutta-percha can be adequately condensed. MTA served efficiently as an apical barrier as there was only one tooth in which gutta-percha protruded through the barrier and this probably was attributed to failure of the investigator to properly condense MTA. In all other specimens, MTA formed a plug that resisted displacement during condensation of thermoplasticized gutta-percha. However the plug's features were unpredictable and ranged from an underfilled to an excessively overfilled open apex. Twelve out of forty experimental teeth were excessively overfilled. Extrusion of MTA at the periapical tissues, although not desirable, is not considered a clinical problem, as MTA's biocompatibility has been well established (12, 13). Moreover MTA has been proved to consistently induce apical hard tissue formation (2, 13). Underfilling, on the other hand could be a potential pathway for apical microleakage since in this situation considerable gaps in the mass of the plug may be seen during micro-computed tomography of the apical plug (Fig. 11).

The goal of a 4-mm thickness barrier was not easily achieved because of the difficulties of handling the material at the apex. The use of an operating microscope

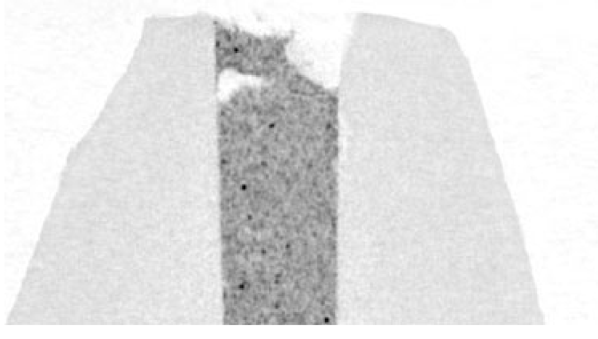

Fig. 11. Image of grey mineral trioxide aggregate apical plug as illustrated by Micro-computed tomography.

Table 2. Dye penetration and thickness of the mineral trioxide aggregate (MTA) barrier

	Grey MTA + $Ca(OH)_2$	White MTA + $Ca(OH)_2$	Grey MTA without $Ca(OH)_2$	White MTA without $Ca(OH)_2$
Mean linear penetration	2.08 ± 0.43 SE[a,b]	2.57 ± 0.40 SE[a]	1.66 ± 0.32 SE[a,b]	1.16 ± 0.22 SE[b]
Mean thickness of barrier	4.33 ± 0.48 SE	4.56 ± 0.20 SE	3.97 ± 0.36 SE	4.61 ± 0.20 SE

Same letter reveals no significant difference whereas different letter reveals statistically significant difference at $P < 0.05$.

may facilitate placement and handling at the apex. To address the problem an ultrasonic condensation of MTA's plug has been advocated (14). Lawley et al. (15) found that ultrasonic condensation led to significantly less bacterial penetration compared to hand condensation, after 45 days. This difference however was not observed at 90 days. On the contrary, Aminoshariae et al. (16) found better adaptation and less radiographic voids with hand condensation than with ultrasonics. More research is required to find the ideal placement technique which seems to be the major problem of the procedure. Furthermore, during placement and condensation an amount of MTA is inevitably left on the canal wall irregularities and its removal is almost impossible without weakening the already thin root canal walls. Thus, the adherence of gutta-percha to dentinal walls is compromised and this may adversely affect it's sealing ability.

Group D (white MTA without previous calcium hydroxide dressing) showed a significantly higher number of excessively overfilled specimens than any other group. This may be attributed to a difference in consistency of white MTA, immediately after mixing, which leads to different handling characteristics compared to grey MTA. The significant difference from group B [white MTA previously medicated with $Ca(OH)_2$] could be explained by the residual calcium hydroxide in the apical region in group B which could mechanically prevent the white MTA from overfilling the open apex. However, our findings are in direct contrast to the findings of Felippe et al. (17) who found that a premedication with $Ca(OH)_2$ favours MTA extrusion in an *in vivo* histological study in immature dog teeth.

The dye leakage study revealed that in only four teeth the fuchsine penetrated the full length of the barrier and reached the gutta-percha interface, a finding that confirms MTA's sealing ability in apical microleakage. Comparing white to grey MTA, there were no statistically significant differences, so white MTA can achieve a comparable apical seal to that of grey MTA. This is in direct contrast with the findings of Matt et al. (18), who found in a similar study that grey MTA demonstrated significantly less leakage than white MTA. Nevertheless, their study was conducted in teeth with convergent apices and methylene blue was used as dye tracer although the problem of decolouration of methylene blue in contact with MTA has been demonstrated (19).

The use of a calcium hydroxide dressing prior to MTA placement as an apical plug has not been well documented. Hachmeister et al. (3) found that medication with calcium hydroxide had no significant effect on grey MTA leakage. This finding is similar to the findings of this study concerning grey MTA. Other studies dealing with the use of MTA as apical barrier (18, 20, 21) have not addressed that problem since they did not consider a calcium hydroxide medication. In this study, statistical analysis revealed only one significant difference ($P < 0.05$) within the white MTA groups, namely, white MTA sealed better when used without previous intracanal medication with calcium hydroxide. This led us to the assumption that calcium hydroxide interferes with white MTA at the apical region. Residual calcium hydroxide may merely be a mechanical obstacle to MTA's adaptation to the root canal walls or chemically react with MTA, thus influencing the surface characteristics of the cement (22). Such a chemical reaction may be more pronounced in white formula since its composition is different from that of grey MTA, e.g. absence of Fe_2O_3 (23).

Since we found an inferior apical seal of white formula in the presence of calcium hydroxide residues, one may consider disinfecting the root canal by other intracanal medicaments (e.g. chlorexidine) in cases of teeth with open apices. However, this proposal must be confirmed by clinical studies. The *in vivo* findings of Felippe et al. (17) suggest that the $Ca(OH)_2$ premedication is not a prerequisite, as the mere presence of MTA as an apical plug led to favourable results in terms of periapical inflammation and hard tissue formation in dogs teeth.

Researchers have tested the MTA apical plug in convergent canal walls (18, 20). However, in such cases an MTA plug is not a prerequisite, as there are other obturation techniques that can also provide an acceptable obturation of the canal, e.g. the 'dip' techniques (24). In this study, a main concern was to reproducibly simulate the divergent open apex of immature teeth and to achieve that, we used the open apex model as described by Hachmeister et al. (3) since the main aim of the study was to examine the marginal adaptation of the material to the divergent canal walls. Equally important was the use of the flower arrangement foam in which the teeth were inserted. The foam was used to mimic the slight resistance of periapical tissues to material extrusion and also to provide a humid ambient for the teeth. Since there is no standard method of evaluating adaptation of MTA in a divergent apex, a combination of methods were used: X-rays, the videomicroscope, a micro-computed tomographer and a dye-penetration study. In the dye-penetration study, we chose to use basic fuchsine 1% buffered solution because of it's neutral pH (about 7.4), that does not interfere with MTA's properties, and it's low molecular weight. In a preliminary study, we found that this solution cannot be decolourated by materials that exhibit an alkaline pH such as MTA.

In a one-visit apexification technique, we must keep in mind that an apical barrier is used mainly to produce an apical stop against which gutta-percha will be adequately condensed. The obturation of the canal with gutta-percha and sealer will play the key role for a bacteria-tight apical seal as in every non-surgical endodontic treatment. Taking into consideration the excellent biocompatibility of MTA, its use as an apical barrier stands as a realistic alternative to multi-appointment calcium hydroxide apexification procedure.

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Supplementary material

The following supplementary material is available for this article:

Video clip S1. 3-dimensional reconstruction of MTA apical plug by micro-computed tomography

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1600-9657.2007.00516.x (This link will take you to the article abstract).

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