Original Article

In-Vitro Evaluation of the Effect of MTA Setting on Apical Microleakage of Open Apex Canals with MTA Apical Plug

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Abstract

Background and Aim: Mineral Trioxide Aggregate (MTA) has a high clinical success rate when used as the apical plug. The conventional method of using MTA as an apical plug is doneduring two treatment sessions. This study aimed to evaluate the possibility of complete filling of root canal with gutta percha and AH26 sealer immediately after the placement of MTA plug.

Materials and Methods: A total of 88 single-rooted teeth were selected for this experimental study. The teeth were prepared and randomly divided into two groups of 40 each. Four teeth were considered as the positive control group and the remaining 4 as the negative controls. In group 1, MTA apical plug was placed, specimens were stored in saline solution for 24h and then filled with gutta percha and AH26 sealer. In the 2nd group, the roots were filled immediately after the placement of MTA. In the positive control group, the root canals were left unfilled and in the negative control group, root canals were filled with gutta percha and sealer. The specimens were then immersed in 1% methylene blue, demineralized in 5% nitric acid and cleared in methyl salicylate. Dye penetration was measured by a stereomicroscope in micrometer. T-test was used for statistical analysis.

Results: The mean dye penetration was 7813 μm in the first and 9152 μm in the second group. According to t-test, the 2nd group had significantly greater microleakage than the first group ($p<0.05$).

Conclusion: MTA needs to be exposed to moisture for final setting and root canal obturation must be delayed until complete setting of MTA.

Key Words: Apexification, MTA, Microleakage

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Introduction

Non-vital open-apex teeth have lost their pulp tissue before their complete development and closure of their apices. As the result, they have open apices. Such cases may also result from periapical trauma or surgery [1]. In such conditions, instrumentation is difficult since there is no sufficient apical barrier stop. In such cases, formation of an apical calcified tissue barrier is necessary for condensation of root canal filling material and achieving an optimal apical seal [2]. Apexification is a treatment method for closure of root canal apices during which, a calcified barrier is formed separating the root canal pulp tissue from the periapical tissue. This barrier is made of dentin, cementum, bone or osteodentin, blunting the root apex and sometimes slightly increasing the root length [3, 4]. Several materials have been successfully used to stimulate barrier formation at the apex. Mixture of water and calcium hydroxide is among the most

favorable materials for this purpose [3]. Its bactericidal and alkaline pH is probably responsible for apical calcification. Despite the popularity of apexification with calcium hydroxide, this treatment has drawbacks including long course of treatment, unpredictable apical constriction, difficult follow up of patients and risk of injury to the respective tooth during treatment [3, 5]. Recently, it has been demonstrated that placement of an MTA apical plug is much more effective than calcium hydroxide [6]. MTA was introduced by Torabinejad et al, in 1993 [7] and has shown numerous clinical and radiographic success as the apical plug materialfor immature teeth ever since [6]. MTA has different clinical applications in dentistry for direct pulp capping, repair of internal resorption, apical plug, apexification, repair of root perforation and pulpotomy [3]. MTA has a seal ability superior tothat of amalgam, super ethoxybenzoic acid (EBA), intermediate restorative material (IRM) and zing oxide eugenol (ZOE) [3, 8, 9]. Also, it has superior properties as the direct pulp capping material compared to calcium hydroxide in animal and human studies [3,10, 11]. Biocompatibility of MTA is also higher than that of amalgam, IRM and ZOE. MTA prevents microleakage and induces regeneration of the main tissue when in contact with pulp tissue and peri-radicular tissues [6].

In contact to tissue, MTA does not cause any chronic inflammatory or foreign body reaction. MTA's biocompatibility is to the extent that it does not cause any chronic inflammatory reaction in case it leaks out from the apex into the periapical tissues [12]. MTA is a hydrophilic cement that sets under wet conditions in less than 4 hours [6] and is able to form a barrier at the apex in open apex canals. Thus, it prevents the extrusion of root canal filling materials [13]. A main advantage of this material is immediate formation of an artificial apical plug enabling final root canal filling after its setting [2]. MTA contains 50-75% calcium hydroxide and 15-25% silicium dioxide. These two components together comprise 70-95% of the cement. When these raw materials are mixed, tricalcium silicate, dicalcium silicate, tricalcium aluminate and tetracalcium aluminoferrite are formed. Addition of water to the hydrated cement converts it to hydrated silicate gel. Two forms of MTA are available in the market: white and grey. The difference between the two is in the concentration of aluminum, magnesium and iron compounds. White MTA lacks aluminoferrite; which causes the grey shade in grey MTA [12]. Chemical characteristics of MTA are similar to those of Portland cement except for bismuth oxide that has been incorporated into MTA as a radiopaque material for radiological purposes [12,14,15].

MTA should be prepared immediately before its use. The powder has to be stored in sealed bottles away from moisture. The powder is mixed with sterile water in a 1:3 ratio on a glass slab or paper pad using a metal or plastic spatula. The mixture can be delivered to the site of application using a plastic or metal carrier. The excess moisture is removed using a gauze or foam. If the mixture is too dry small amount of water can be added because MTA needs moisture for its setting. A moist cotton pellet should then be placed inside the canal and the access cavity has to be sealed with a temporary restoration for at least 3-4 hours [6]. In the conventional technique, MTA is used as the apical plug usually in the first treatment session with at least 5 mm length. The moist cotton pellet is then placed over it, the respective tooth is temporarily restored and the final root canal filling is postponed to the next session to allow complete setting of MTA.

In 2006 Felippe et al. evaluated the effect of Proroot MTA, Dentsply on apexification and periapical healing of teeth with incomplete root formation and concluded that use of calcium hydroxide is not necessary for the apexification to occur [2]. In 2007, Simon et al.assessed the outcome of one-visit apexification treatment with MTA (Angelus) and concluded that one-visit apexification treatment with the use of MTA apical plug may be a predictable treatment and replace the use of calcium hydroxide [1].

Sarris et al, in 2008 evaluated the clinical efficacy of MTA (Pro-root MTA Dentsply) as the apexification material in immature non-vital permanent incisors in children. The results showed successful long-term use of MTA as an apexification material in non-vital teeth [16]. Considering the time-consuming nature of two-visit treatment and its disadvantages such as prolonged treatment course, risk of canal contamination in between sessions and patient's poor compliance, this in-vitro study aimed to evaluate the effect of MTA setting on

apical microleakage in open apex canals with MTA apical plug.

Materials and Methods

In this in-vitro study, a total of 88 freshly extracted single-rooted human teeth were selected and stored in saline solution until reaching the sample size [17]. Specimens all had completed apices and relatively straight roots. After reaching the sample size, debris and soft tissue residues were removed from tooth surfaces using a periodontal curette. For further cleaning and disinfection, specimens were immersed in 0.5% sodium hypochlorite solution for 7 days. Next, the coronal portion of teethwas cut to obtain 15 mm roots. All canals were prepared by introducing #15 K file followed by #20 and #25 up to #40 to the working length. Canals were then flared up to #80 file using the step-back technique. In between files, canals were washed with 2 ml of 2.5% sodium hypochlorite solution and the irrigating solution was allowed to drainthrough the canal apex. Next, 3 mm of the apices were cut using a diamond disc and hand piece along with water and air spray in order to eliminate the apical root canal branches. Afterwards, # 2 and #3 Gates Glidden drills were introduced into the canal to the working length and the apices of samples were prepared up to 0.9 mm as such. In between the use of these drills, canals were irrigated with 2 ml of 2.5% sodium hypochlorite solution. At the end, canals were rinsed with 1 ml of distilled water to wash off the remaining sodium hypochlorite and dried with paper point. A total of 80 teeth were selected among the prepared samples and the terminal 5 mm of the canals were filed with MTA (Pro-root MTA, Dentsply, Maillefer) in a way that after the placement of MTA, the remaining working length was 10 mm. Density of the MTA was radiographically approved. If any void was detected, MTA was again packed into the canal to eliminate the void and by adding MTA, the plug thickness reached 5 mm. Excess or leaked out MTA were cleaned. After confirming the length and density of MTA, 40 teeth that had previously received MTA plugs, were filled with gutta percha and AH26 sealer.

In the second group, immediately after the placement of 5mm apical plug, 40 specimens were selected and filled with gutta percha and AH26 sealer using the lateral condensation technique. Gutta percha excess was removed and sealer excess was cleaned with 99% alcohol. The remaining 8 specimens were divided into 2 groups of 4 as the positive and negative control groups. In the positive control group, root canals were left empty and in the control group, canals were filled with gutta percha and AH26 sealer. Apex was sealed with adhesive wax. All specimens were stored in an incubator at 37°C and 100% humidity for 72 h to allow setting of materials. After removing the excess gutta percha and sealer, the orifice of all specimens was sealed by adhesive wax. In the negative control group, the entire root surface was coated with 2 layers of nail varnish and teeth were immersed in 1% methylene blue solution for one week. After this time period, specimens were removed from the dye and washed with distilled water. To eliminate excess dye, nail varnish coat on the root surfaces was scraped off using #15 scalpel blade. The teeth were then decalcified in 5% nitric acid, dehydrated in ethyl alcohol and immersed in methyl salicylate solution for clearing [18]. All samples were evaluated under stereodissecting microscope at 12X magnification to observe the dye penetration depth. The results were analyzed by t-test.

Results

Both groups showed microleakage (4 specimens were excluded from group 1 due to apex resorption and failure in clearing of the tooth structure, 5 specimens were excluded from group 2 due to apical root resorption, destruction of MTA plug and failure in clearing of teeth). According to Table 1, of 40 understudy teeth in group 2 (immediate filling of canals after the placement of MTA apical plug), 35 teeth were studied and all showed microleakage. Maximum and minimum dye penetration in micrometer in the two test groups have been demonstrated in Table 2. Extent of dye penetration in micrometer and standard deviation for each group are demonstrated in Diagram 1.

In the positive control group, dye penetration was observed in the entire length of canal. In the negative control group, no dye penetration was noted. The mean dye penetration was 7813 micrometer in teeth with 24h final setting and 9152 micrometer in teeth with immediate setting. T-test showed signif-

icant differences in mean dye penetration between the two groups (before and after final MTA setting) (P<0.05).

Table 1. The frequency of microleakage in the two test groups

Group	Number	Leakage	No leakage
1: 24h setting	36	36	0
2: Immediate setting	35	35	0

Table 2. Maximum, minimum, mean, SD and degree of microleakage (dye penetration) in mm in the two test groups

Group	Number	Maximum (µm)	Minimum (µm)	Mean	SD
1	36	11535/00	4988/00	7813/0	125/651
2	35	15205/00	6947/00	9152/1	1552/07

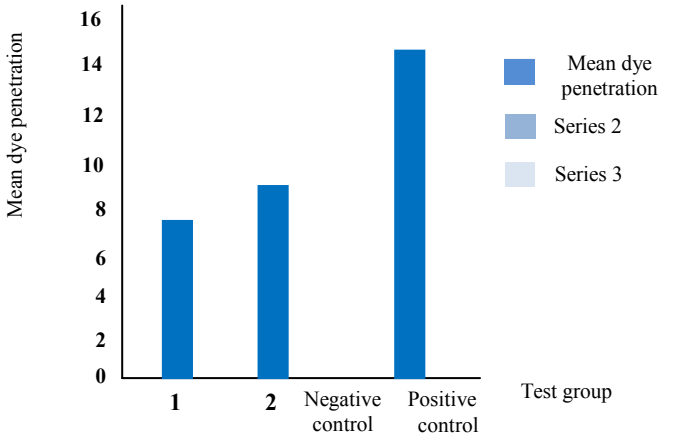

Diagram 1. The mean dye penetration in mm in the test and control groups groups

Discussion

Root canal obturation followed by immediate restoration of the coronal part is the key factor for long-term preservation of treated tooth. To reduce the risk of fracture (calcium hydroxide application increases the risk of fracture), one-visit application of MTA plug in the apical 5 mm of the root was suggested for apexification treatment. According to a study by El Meligy in 2006, MTA (Pro-root, Dentsply Tulsa Dental Specialties, Tulsa, OK) has optimal seal ability, good marginal integrity, high biocompatibility and logical setting time (4h). Although MTA is more expensive and technique-sensitive than calcium-hydroxide, clinical and radiographic follow up of teeth treated with MTA have shown absence of clinical symptoms and formation of a hard tissue barrier at the apical region [3]. These results are similar to those of Torabinejad and Chivian and it appears that MTA (Pro-root MTA, Dentsply) is a suitable choice for apexification [6]. The present study evaluated apical microleakage in open apex canals with MTA apical plug. The degree of microleakage in the two experimental groups was measured by measuring the maximum distance of dye penetration from the restoration (not the apex) in micrometer.

This experimental study was conducted on mature teeth that were prepared with GG drills to simulate immature teeth.

According to the study by Al Kahtani et al, in 2005, 5 mm thickness of MTA plug (Pro-root MTA plug) created excellent seal in open apex teeth and has an adequate depth for resistance against movement [19]. In our study, 5 mm MTA plug was used as well. The seal ability was tested at two different time points because for complete setting, MTA requires humidity for at least 3 to 4 hours [6]. Thus, final restoration of the root canal and coronal part of the tooth has to be postponed to the 2nd visit. The restoration end in our study was within 0 to 0.5 mm distance from the apex. Several methods such as bacterial leakage, dye penetration and fluid filtration have been used for the assessment of the seal ability of MTA. Bacterial leakage and dye penetration are the most commonly used techniques for this purpose. The ability of dye penetration method for detection of microleakage has been demonstrated in several studies [20, 21]. Dye penetration is a relatively quantitative technique. Kazemi et al. demonstrated comparable results of-dye penetration and bacterial leakage techniques for evaluation of MTA seal ability [22]. The dye used in our study was 1% methylene blue. In the pilot study, India ink was used that showed no penetration into the MTA. To ensure dye penetration into the canal, the positive control group was used. The negative control group was used to ensure dye penetration into the canal only through the apex.

Both groups showed microleakage but it was significantly greater in the 2nd group (immediate final restoration before the complete setting of MTA). These results are in agreement with those of Viz-

girda, Frederick and Paul Liewehr in 2004 [23]. They evaluated the apical seal of MTA (Pro-root MTA, Dentsply) as a root canal filling material and compared it to that of laterally condensed gutta-percha and thermoplasticized gutta-percha. They concluded that gutta-percha provided an apical seal superior to that of MTA.

Shabahang and Torabinejad in 1999 stated that the materials used for apical plug such as MTA and calcium hydroxide are applied to the canal walls and therefore can cause microleakage [5].

Our study results were also in accord with those of Hachmeister et al, in 2000. In their study, one and 4 mm thick apical MTA plugs were tested in bacterial leakage model and all barriers showed bacterial penetration by day 70. They mentioned that it was the intracanal delivery technique and not the MTA that led to the leakage [24].

Martin et al. studied the sealing properties of two MTA apexification procedures using an in vitro apexification model. The teeth were divided into two groups with artificially created open apices. The first group received 3- to 5-mm-thick apical plugs and the remaining canal spacewas backfilled withthermoplasticized gutta-percha. In the 2nd group, the entire root length was filled with MTA. Both groups showed microleakage. Although MTA root fillings exhibited a better seal than MTA apical plugs at 48 hours, seals of these two groups were not significantly different after 4 weeks [25].

Our study results are in contrast to those of Al Kahtani et al, who evaluated the seal by varying depths of apical MTA plugs (Pro-root MTA, Dentsply) in a bacterial leakage model and showed that 5 mm thickness of MTA plug had no bacterial leakage [19]. Torabinejad et al, in 1994 evaluated the microleakage by using methylene blue and found that MTA (Pro-root MTA, Dentsply) had lower microleakage compared to the other 4 materials used as apical plug. Orthograde application of MTA has higher technical sensitivity. Placement should be radiographically confirmed and condensation has limitation because open apices have minimum resistance. Furthermore, difficult delivery of the material to the apex, irregularities and diverge anatomy may limit the adaptation to dentinal walls and cause a marginal gap in dentin surfaces [26]. In our study, MTA was used in both groups and microleakage of MTA was not compared with another material but degree of microleakage in both groups was higher than expected. This finding may be attributed to the technical sensitivity of orthograde use of MTA or the penetrating property of methylene blue into the MTA. The two groups showed no dye penetration when India ink was used and even in presence of two coats of nail varnish, it penetrated into the tooth surface. Future studies with the use of other leakage models such as staining withAg radioisotope, electrochemical method and bacterial penetration are required to compare the degree of microleakage of these materials into MTA with the present study results and better results may be obtained. Also, this study may be carried out with orthograde placement of MTA and the obtained results may be compared with the present results. Our study model showed that MTA should be allowed more time in presence of humidity to complete its setting and final root filling and restoration of coronal part after the placement of apical MTA plug should be delayed until complete setting of MTA.

Conclusion

This study showed that MTA required more time in humid condition to complete its setting andrestoration of tooth crown after the placement of apical MTA plug should be delayed until complete setting of MTA.

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