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Research Report

The apical leakage of mineral trioxide aggregate as the retrograde filling material with various mixing agents

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ABSTRACT

Background: *Mineral trioxide aggregate (MTA) is relatively considered as a new material in endodontic. It even has been used as retrograde filling material due to its biocompatibility, antibacterial effect, sealing ability and anti-moist effect. Some materials have been used as mixing agent to achieve an appropiate setting of MTA.* ***Purpose:*** *The aim of this study is to investigate the effect of the mixing agents of MTA towards the apical leakage when they are used together as retrograde filling materials.* ***Method:*** *The samples of this research consist of 30 human extracted upper central incisors. First, the crown of each tooth is sectioned. The root canals are prepared by using the conventional technique and then are obturated with gutta percha. After cutting the root apex, 2 mm from apical, class 1 cavities are prepared by using fissure bur with the depth of 3 mm. The samples then are divided into 3 groups with 10 teeth for each. Group I uses aquabidest as mixing agent of MTA (MTA-aquabidest), group II uses saline (MTA-saline), while group III uses 0.12% chlorhexidine (MTA-chlorhexidine). The apex of each group then is filled with the mixing MTA determined already. Afterwards, clearing method is used to evaluate the apical leakage. The apical leakage actually is determined by measuring the depth of methylene blue penetration with stereomicroscope. The statictical analyses of the linear dye penetration then are performed with analysis of varians ANOVA.* ***Result:*** *The dye penetration for both MTA-aquadest and MTA-saline groups indicates the lowest penetration, and there is even a significant difference compared with MTA-0.12% chlorhexidine group (p<0.005).* ***Conclusion:*** *It can be concluded that aquabidest and saline as mixing agents of MTA produce less apical leakage compared with 0.12% chlorhexidine.*

Key words: *Apical leakage, retrograde filling, MTA, saline, chlorhexidine*

ABSTRAK

Latar belakang: *Mineral trioxide aggregate (MTA) merupakan bahan yang relatif baru dalam bidang endodontik. Bahan tersebut diindikasikan sebagai bahan pengisi retrograd karena bersifat biokompatibel, antibakteri, kerapatannya bagus dan tidak terpengaruh kelembaban.Untuk mendapatkan settingnya, beberapa bahan telah digunakan sebagai bahan pencampur MTA.* ***Tujuan:*** *Penelitian ini bertujuan untuk mengetahui pengaruh bahan pencampur MTA sebagai bahan retrograd terhadap kebocoran apikal.* ***Metode:*** *Bahan penelitian berupa 30 gigi insisivus sentral atas bekas cabutan. Mahkota gigi dipotong dan saluran akar dipreparasi menggunakan teknik konvensional dan diobturasi dengan guta perca. Akar dipotong dengan jarak 2 mm dari apeks dan dibuat preparasi kavitas kelas I menggunakan bur fisura dengan kedalaman 3 mm pada ujung akar tersebut. Akar gigi tersebut dibagi dalam 3 kelompok masing-masing 10. Kelompok I menggunakan akuabides sebagai bahan pencampur MTA (MTA-akuades), kelompok II menggunakan salin (MTA-salin) dan kelompok II menggunakan Chlorhexidine 0,12% (MTA-chlorhexidine). Ujung akar kemudian diisi campuran MTA sesuai kelompoknya. Evaluasi kebocoran apikal menggunakan teknik clearing. Kebocoran apikal ditentukan dengan mengukur kedalaman penetrasi larutan biru metilen menggunakan mikroskopstereo. Hasil pengukuran dianalisis menggunakan analisis varian (ANOVA).* ***Hasil:*** *Penetrasi warna pada kelompok MTA-akuades maupun MTA-salin menunjukkan hasil yang paling kecil dan kedua kelompok tersebut berbeda secara signifikan dengan kelompok MTA-chlorhexidine 0,12% (p<0,005).* ***Kesimpulan:*** *Bahan pencampur akuades dan salin menghasilkan MTA dengan kebocoran apikal yang lebih kecil dibandingkan chlorhexidine.*

Kata kunci: *Kebocoran apikal, pengisian retrograd, MTA, salin, chlorhexidine*

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INTRODUCTION

One way to overcome the failure of root canal treatment is by conducting endodontic surgery, which is by cutting the apex of the tooth and then by putting the retrograde filling materials at the tip of the apex.[1,2] Mineral trioxide aggregate (MTA) is a new retrograde filling material developed for a variety of endodontic treatments, such as for pulp capping, root perforation, apexification, and retrograde filling materials.[3-6] Nowadays, MTA is widely used as a retrograde filling material due to its biocompatibility, antibacterial effect, sealing ability, and anti-moist effect. Though MTA still has some weaknesses one of which is that MTA needs moisture to reach its settings, this condition is precisely appropriate to make MTA to be used as retrograde filler since it is always in a moist environment, in the periapical area.[1,2,7-9] Nevertheless, MTA setting time will expire after 28 days.[10]

MTA, moreover, consists of hydrophilic particles composed of tricalcium silicates, tricalcium aluminates, tricalcium oxide, and silicate oxide as well as its additional material, bismuth oxide, used to add its radiopacity. The nature of the hydrophilic particles is what causes the MTA requires moisture to its setting.[11] As a result, the commonly mixing agent of MTA powder is aquabidest with the ratio 3: 1. After being mixed with aquabidest, MTA then will form a mixture that looks like wet sand, and later it will form an amorphous specific crystal structure with granules-shaped appearance.[12] It means that the nature of MTA are influenced by particle size, powder-liquid ratio, temperature, and humidity.[11]

The bonding of MTA on dentin is possibly derived from its natural hydrophilic properties and its small expansion when located in moist environments. To obtain MTA mixed with better properties, some researchers even use other materials besides aquabidest, such as saline and chorhexidine. In the reaction between the MTA and saline, MTA actually react with water molecules only, whereas NaCl contained in saline does not come to react.[13] The reason is because of the differences in the nature electronegativity among negative ions contained in the MTA powder. [12] Therefore, the result of the reaction between MTA and water will form hydrated colloidal gel. Since saline is considered as a hypertonic solution, the reaction between saline and colloidal will cause the water molecules that exist in the colloidal attracted to the saline solution, as a consequence, the colloidal gel becomes harder.[13] In other words, the less water molecules contained in a material, the less porosity will be created.[14] It may be concluded that MTA mixed with saline has greater compressive strength than MTA mixed with aquabidest. [15]

In addition, chlorhexidine actually is considered as disinfectant that is widely used in endodontic treatment, such as being used as root canal irrigation and sterilization materials.[1] In a research, Stowe even has already concluded that MTA mixed with chlorhexidine can not only cause the increasing of its compressive strength, but can also cause the increasing of its antibacterial effect. The concentration of chlorhexidine used as MTA mixing agent is 0.12%.[1]

The sealing ability of MTA, furthermore, is likely derived from its natural hydrofilic and small expansion when located in a moist environment.[11] The reason is because in moist environments, the further hydration of MTA powder will increase its compressive strength and make the apical leakage lower.[17]

The success of root canal treatment either filled in orthograde or in retrograde is mainly determined by the sealing ability of apical filling quality. The easiest way to evaluate the apical sealing ability is by measuring the leakage rate. The smaller the apical leakage happens, the better sealing ability is. Therefore, this study aims to determine the effect of mixing agents of MTA as retrograde filling materials towards the apical leakage.

MATERIAL AND METHOD

In this research, the samples are 30 human upper central incisors maxillary which have been extracted . However, the teeth should have the following criteria, such as the roots of those teeth are not curved, the roots are fully developed, and the root canal can be accessed by the maximum files until the number 25.

Firstly, the roots of teeth are cut with a distance of 14 mm from the apex of the teeth, and then are prepared by using conventional techniques with files up to number 40 with the working length 13 mm. Every turn of the file, the root canals are irrigated by 1 cc of 2.5% NaOCl and saline, and then they are dried with paper points. Before filling the root canals, they must be coated by paste (Endomethasone) using lentulo which means that the root canals are filled with lateral condensation of gutta-percha point. The gutta-percha number 40 (master cone) which has been marked based on the working length is coated with root canal paste on one third of the apical, and then it is immediately inserted into the root canal. Insert the main spreader between the gutta-percha point and the root canal wall, and then press into the apical direction until it reaches 2 mm from the apex. The remained space then is filled with the additional gutta-percha point until the root canal is full. The gutta-percha point is then cut based on the length work by using hot plastic instrument, while its corona is covered with zinc phosphate cement. Those roots of the teeth are then stored in the 37^{o} C incubator for 24 hours.

Later, the apex in the apical area is cut horizontally perpendicular to the axis of the tooth by using a fissure bur at high speed with a distance of 2 mm from the apical tip. The cut apex is then prepared with retrograde cavity type class I by using a fissure bur with 1.5 mm in diameter and 3 mm in depth. Then, those 30 teeth are randomly divided into 3 groups, each of which consists of 10 teeth. Group I is filled with MTA and aqubidest, Group II is filled with MTA and saline, while Group III is filled with MTA and chlorhexidine. In Group I, for instance, MTA powder is mixed with aquabidest with a ratio of powder: aquabidest 3: 1 (1 gram of powder MTA: 0.33 grams aquabidest). Next, stir it on the cup by using MTA mixer until it becomes homogeneous. After that, it is inserted into the tip of the root which has been prepared in group I by using the micro apical placement (MAP) until it is full. Then, after it is flattened, the cotton that has already been soaked in a solution of phosphate-buffered saline (PBS) is immediately used to cover the entire surface of MTA as well as the tip of the tooth root. The reason is because PBS solution can be used to simulate the moisture in the periapical area.[18]

Like in group I, in group II, in which MTA is mixed with saline, and in group III, in which MTA is mixed with chlorhexidine, moreover, the similar procedures is also conducted. To guarantee the condition is always moist, the application of wet cotton wool soaked in PBS solution on the surface of the tooth root tip must be conducted every day. Besides that, the cotton wool must always be replaced with the new one every day for 28 days. After 28 days, the cotton that covers the tip of the tooth root is removed, and followed by apical leakage testing.

The apical leakage testing actually uses the method of clearing. The entire of the outer surface of the teeth is coated with adhesive wax and closed again with nail polish, except in the surface area of retrograde. The whole of the subjects of this research is then soaked in 2% methylene blue for 48 hours. Afterwards, they are washed with running water for 15 minutes, and the adhesive wax and the nail polish covering them are then removed by using Le crown mess. Furthermore, those teeth are demineralized by soaking them in 5% HNO_3 for 72 hours which should be replaced every 24 hours. The next stage, those teeth are dehydrated in 96% alcohol for 48 hours which also must be replaced every 24 hours. The last stage is conducting clearing in methyl salicylate until the penetration of the color of ink can be observed visually.[19] The observation is conducted with a stereo microscope for measuring the most long penetration of the ink color (in units of mm), from the cut surface of the root tip to the coronal tooth, in order to obtain the number of apical leakage. The result data are finally analyzed by using One-Way ANOVA test followed by t-test.

RESULT

The mean of the apical leakage in each treatment groups can be seen in Table 1. To know the significance of differences among the three treatment groups, One-Way ANOVA test is used with the significance level of 95%. Through ANOVA tests, it is known that $p<0.005$, which means that there are differences in apical leakage among those three treatment groups using MTA with three different mixing agents, aquabidest, saline, and chlorhexidine.

Moreover, to know which pair of those three treatment groups is really different, t-test is then conducted. The result of t-test among those three groups can be seen in Table 2. Based on the result, it is known that the apical leakage of the retrograde filling by using MTA, as raw material, mixed with chlorhexidine has the largest apical leakage compared with the other two treatment groups using saline or aquabidest as the mixing agents of MTA. Meanwhile, the retrograde filling by using MTA mixed with saline had the same apical leakage as that by using MTA mixed with aquabidest.

DISCUSSION

MTA is a relatively new material developed in the field of endodontics, especially as a retrograde filling material. As a retrograde filling material, MTA has the best sealing ability compared with other retrograde materials previously used, such as amalgam, composite resin, reinforced eugenol oxide zinc cement, and glass ionomer cement. However, some mixing material agents, aquabidest and chlorhexidine, are still used to improve the nature of the MTA.[1–4]

In this research, during the hardening phase MTA materials are always applied with cotton soaked in PBS. This condition is the the simulation of periapical tissue condition.[18] In wet condition those MTA materials will continually release calcium and hydroxyl ions. As a result, calcium that released will react with phosphate contained in PBS solution, and then will form amorphous calcium phosphate. Next, the formed calcium phosphate will be hydrolyzed in order to form carbonate apatite type B which is considered as hydroxyapatite. The hydroxyapatite then will fill the gap between those retrograde filling materials and the root canal wall.[18] Nevertheless, all of the samples in this research have apical leakages shown by the methylene blue dye penetration. No retrograde filling material can perfectly adapt to the tooth structure so that there will always be a gap between the materials of retrograde and tooth structure.[20] The gap occurred is mainly caused by the condition in which the coefficient of thermal expansion of those tooth retrograde filling materials do not fit with the coefficient of thermal expansion of the tooth structure.[21]

Based on the result of t-test it is known that there is no difference in the apical leakage of the retrograde filling process using MTA mixed with saline and that mixed with aquabidest, meanwhile the apical leakage of the retrograde filling process using MTA mixed with 0.12% chlorhexidine is known as the largest apical leakage compared with the other two mixing material agents. Actually, the reaction of MTA with aquabidest is almost the same as the reaction of aquabidest with portland cement in which the hardening process occurs through three stages.[10] In the first stage, the reaction of hydration derived from tricalcium aluminate forms hydrated colloidal gel occurred after 24 hours. In the

Table 1. The mean of the apical leakage in the retrograde filling by using MTA together with other mixing agents, aquabidest, saline, and 0.12% chlorhexidine (mm)

The mixing agents of MTA	Number of samples	$\overline{X}$	Standard Deviation
Aquabidest	10	1.200	0.133
Salin	10	1.040	0.217
Chlorhexidine	10	2.560	0.241

Table 2. The result of t-test on the apical leakage of the retrograde filling by using MTA with three different mixing agents, aquabidest, saline, and 0.12% chlorhexidine

Mixing Agents	Aquabidest	Saline	Chlorhexidine
Aquabidest	-	1.766>0.005	-
Saline	-	-	16.778<0.005
Chlorhexidine	15.021<0.005	-	-

p<0.005: Significance

second stage, between the first day and the seventh day, the tricalcium silicate and the tricalcium aluminate reacting with water form $Ca(OH)_2$ form aluminum hydroxide and amorphous calcium silicate. In the third stage, between the seventh day and the twenty eighth day, the reaction is getting slow in which hydrated calcium silicate progressively forms hydrated silicate gel, and then $Ca(OH)_2$ will be spread on the gel giving strength to the cement that has already hardened, as a result, it makes the cement is getting harder.[12] However, the hardening reaction of MTA with saline is actually equal to that with aquabidest. When MTA is mixed with saline, only water molecules reacts with MTA powders, whereas NaCl contained in saline solution does not participate in the reaction. Saline is actually considered as a hypertonic solution, thus, the mixing of MTA powders and saline can be affected by the hypertonic nature of saline. As a result, the hypertonic nature of saline causes water molecules contained in the powder mixture of MTA-saline pulled outside.[12,13]

In other words, the less water molecules contained in a material, the less the resulting porosity. The hypertonic nature of saline actually causes the mixture of MTA-saline less centered than the mixture of MTA-aquabidest.[15] Thus, the less porosity occurs in the materials, the smaller the leaks occur.[14] In this research it is known that the numbers of the apical leakages occurred in the treatment group with the mixture of MTA-aquabidest is the same as those occurred in the treatment group with the mixture of MTA-saline. The reason could be more because of the role of closing the gap between the retrograde filling materials and the dentin of the root canal dentin than because of the difference of the internal nature between MTA materials and the mixing agents, either aquabidest or saline. Moreover, the hydroxyapatite is actually formed from the hydrolysis process of calcium phosphate derived from the reaction of calcium released by MTA with phosphate contained in the PBS solution. This hydroxyapatite even is proven to be able to close the gap of MTA-aquabidest as good as the one of MTA-saline. The group with the mixture of MTA-0.12 chlorhexidine still has the greater apical leakage than the ones with the mixture of MTA and the mixing material agents, either aquabidest or saline, although like in those two groups with the mixture of MTA and either aquabidest or saline, the hydroxyapatite occurs in the gap between teeth in the group with the mixture of MTA-0.12% chlorhexidine.

Chlorhexidine is classified into an inorganic compound. Thus, if it is combined with the organic compounds of MTA (calcium, aluminum, silicate), it will form a complex bond.[23] The complex bond is among aluminum elements of tricalcium silicate, tricalcium aluminate and tricalcium oxide with chlorhexidine, and among silicate elements of tricalcium silicate and silicate oxide. Silicate oxide actually can not only make the hardening process uneasily occur, but can also make a bad adhesion between the mixture of MTA-chlorhexidine and those teeth. Calcium binding to chlorhexidine makes the mixture of MTA-chlorhexidine more fragile since the calcium in MTA is continually taken by chlorhexidine. The fragility of this material affects the apical leakage occurred. Thus, the numbers of the apical leakage occurred on the mixture of MTA-chlorhexide is considered as the highest one compared with that occurred on the mixture of MTA-saline-aquabidest. Although hydroxyapatite which will enhance the sealing ability of MTA is formed in the gap between all of those three different mixing agents of MTA and those teeth, but the factor of the MTA-chlorhexidine mixture play bigger role.

Based on the result of this research, it can be concluded that among three types of the MTA mixing material agents, saline and aquabidest is considered as the best MTA mixing materials.

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