

Effect of Synthetic Tissue Fluid on Microleakage of Grey and White Mineral Trioxide Aggregate as Root-End Filling Materials An *in vitro* study

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تأثير سائل النسيج الصناعي في التسرب المجهرى لمجموع الأكاسيد الثلاثية المعدنية الرمادية والبيضاء كمواد تعبئة لنهاية الجذر دراسة مخبرية

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المخلص: الهدف: ظهر أن نجاح الجراحة اللبية يعتمد جزئياً على ختم القمة. ينتج عن مجموع الأكاسيد المعدنية الرمادية ضعف كمية هيدروكسيباتيت الناتجة عن مجموع الأكاسيد المعدنية البيضاء عندما تكون معلقة في محلول الفوسفات الملحي. هدف هذه الدراسة التي أجريت في المختبر يتمحور على مقارنة ظاهرة التسرب المجهرى من مجموع الأكاسيد المعدنية الرمادية والبيضاء كمواد حشو لنهاية الجذر بعد غمرها في سائل النسيج الصناعي. الطريقة: تم تقسيم 55 من أسنان الفك العلوي الأمامية البشرية أحادية الجذور إلى مجموعتين تجريبيتين من 20 سنًا لكل منهما، بالإضافة إلى 3 مجموعات من 5 أسنان لكل منها، كعينات ضابطة، اثنتان منها سلبية وواحدة إيجابية: تم تنظيف قنوات الجذور وترتيب شكلها وتم دمج أطرافها بجوتا-بيرجا. تم ملء نهايات الجذور بالمجاميع التجريبية بمجموع الأكاسيد الثلاثية المعدنية الرمادية والبيضاء. تم تحديد التسرب باستخدام طريقة اختراق الصبغ. تم تحليل البيانات باستخدام تحليل التباين على مستوى دلالة 0.05. النتائج: كان متوسط تسرب الصبغ 0.1 ± 0.40 ملم لمجموع الأكاسيد الثلاثية المعدنية الرمادية و 0.1 ± 0.50 ملم لمجموع الأكاسيد الثلاثية المعدنية البيضاء. لم يكن هناك فرق معتد بين المجموعتين ($P = 0.14$). الخلاصة: على الرغم من اختلاف خصائص وسلوكيات لمجموع الأكاسيد الثلاثية المعدنية الرمادية والبيضاء في سائل النسيج الصناعي، كما لم تكن هناك اختلافات معتد في التسرب المجهرى عند استخدام أيًا من الأكاسيد الثلاثية المعدنية.

مفتاح الكلمات: تسرب الأسنان، مجموع الأكاسيد الثلاثية المعدنية، حشو الأسنان.

ABSTRACT: Objectives: The success of endodontic surgery has been shown to depend partly on the apical seal. Grey mineral trioxide aggregate (GMTA) produces hydroxyapatite twice as often as white mineral trioxide aggregate (WMTA) when suspended in a phosphate buffered saline (PBS) solution. The aim of this *in vitro* study was to compare the microleakage phenomenon of grey and white mineral trioxide aggregates as root-end filling materials after immersion in synthetic tissue fluid (STF). **Methods:** 55 single-rooted extracted maxillary anterior human teeth were divided into two experimental groups of 20 teeth each, plus 3 groups of 5 teeth each as two negative and one positive control groups. The root canals were cleaned, shaped, and laterally compacted with gutta-percha. The root ends were resected and 3 mm deep cavities were prepared. The root-end preparations were filled with GMTA or WMTA in the experimental groups. Leakage was determined using a dye penetration method. Data were analysed using analysis of variance (ANOVA) at the 0.05 level of significance. **Results:** The mean dye leakage was 0.40 ± 0.1 mm for GMTA and 0.50 ± 0.1 mm for WMTA groups, respectively. There was no significant difference between the two experimental groups ($P = 0.14$). **Conclusion:** Despite the different properties and behaviours of GMTA and

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WMTA in STF, there were no significant differences in microleakage when using GMTA or WMTA.

Keywords: Dental leakage; Mineral trioxide aggregate; Endontics.

ADVANCES IN KNOWLEDGE

- Root-end filling materials play a crucial role in the success of periapical surgery.
- Using bioactive materials for root-end filling purposes has demonstrated promising treatment in periapical surgery to increase success rates, as shown in this study.
- On the basis of available information, it appears that mineral trioxide aggregate (MTA) is the material of choice for periapical surgery. However, the difference between using white or grey MTA has not been adequately studied. Therefore, more studies are needed to confirm MTA's efficacy as compared with other materials.
- More studies should be conducted to show the advantages and disadvantages of grey or white MTA.

APPLICATION TO PATIENT CARE

- Inadequate apical sealing is the most common cause of failure in periapical surgery. Therefore, the root-end filling material used should prevent ingress of potential contaminants into periapical tissue.
- MTA was developed because existing materials did not have the ideal characteristics necessary for retrograde root-end fillings. However, MTA has two versions: white and grey, and choosing one over the other to use as retrofill material is still under question.
- This study will help in choosing an appropriate root-end filling material for patients needing periapical surgery.

ENDODONTIC SURGERY MAY BE INDICATED if conventional root canal therapy cannot treat complex anatomy, procedural misadventures, or inflammatory processes.^{1,2} Root-end filling materials are used in periapical surgery to seal the root canal from the periapical tissues.¹ An ideal root-end filling material should be biocompatible and able to seal, insoluble in tissue fluids, non-resorbable, radiopaque, and dimensionally stable.³ Increasing the sealing ability of materials can lead to more successful endodontic treatments.³ Mineral trioxide aggregate (MTA) was first introduced in 1993 as a root repair material and is a mixture of Portland cement, gypsum, bismuth oxide, and trace amounts of metallic oxides.⁴⁻⁷ The first generation of MTA was grey in colour. White MTA (WMTA) was introduced in 2002 as an alternative to grey MTA (GMTA) which does not match with the colour of the anterior teeth.⁸

The measured chemical composition of GMTA and WMTA revealed that Al₂O₃ (122%), MgO (130%), and especially FeO (1000%) are more abundant in GMTA.⁹ WMTA contains smaller and finer particles with a narrower range of size distribution and has less tetracalcium aluminoferrate as compared to GMTA.¹⁰ Regardless of these differences, the success of endodontic materials mainly depends on provision of an acceptable apical seal.¹¹

The sealing abilities of GMTA and WMTA, when applied at a thickness of 3 mm, were similar when

used as a root-end filling material in the presence of water.¹² Set MTA contains many voids in the form of air bubbles, pores, and capillary channels. These voids within MTA and at the interface of MTA and dentin, if sufficiently large, might promote an increased potential for leakage of fluids, bacteria, and endotoxins beyond the MTA.¹³ However, MTA as a bioactive material can exchange calcium ions with an environment that contains phosphate ions; therefore, after placement of MTA in the root canal in the presence of synthetic tissue fluid (STF), and after its gradual dissolution, hydroxyapatite (HA) crystals nucleate and grow, filling the microscopic spaces between MTA and the dentinal wall through a physicochemical reaction.⁵ Initially, this seal is mechanical; however, with time, a diffusion-controlled reaction between the apatite layer and dentin leads to their chemical bonding, creating a seal at the MTA-dentine interface.^{14,15} The reaction between MTA and STF results in the formation of precipitates in the form of HA following the combination of Ca from MTA and phosphates from STF. The chemical aspect is the result of MTA coated with HA, forming a chemical bond to dentin. In fact, STF or any phosphate-containing solution such as phosphate buffered saline (PBS) or Hank's balanced salt solution (HBSS) has the potential to produce HA crystals adjacent to the MTA due to calcium hydroxide production from MTA after mixing with water.¹⁶

On the other hand, GMTA has been shown

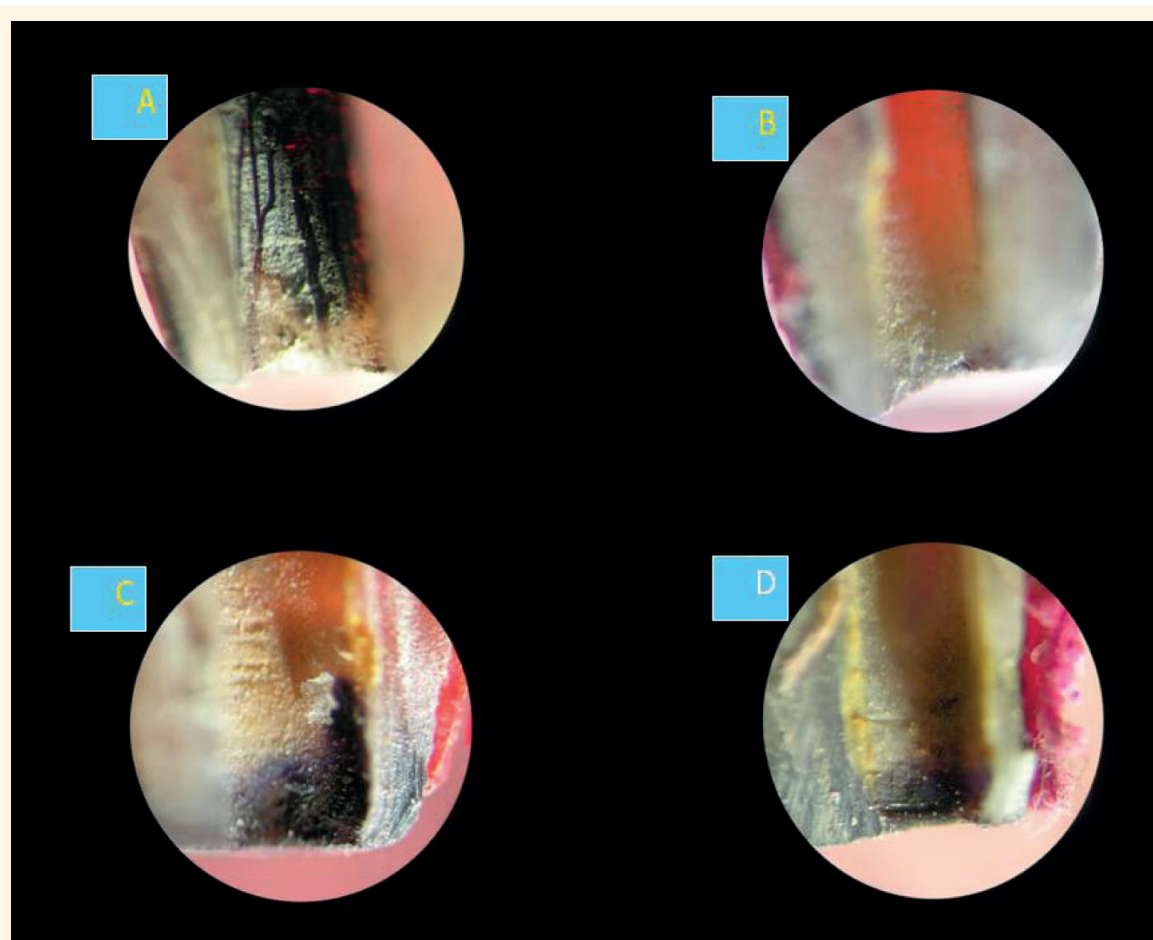


Figure 1: Stereomicroscopic images at $\times 16$ magnification of dye leakage in root-end cavity preparations after immersion in synthetic tissue fluid. (A) positive control; (B) negative control; (C) white mineral trioxide aggregate; (D) grey mineral trioxide aggregate.

to expand significantly more than WMTA when immersed in either water or HBSS.¹⁷ GMTA produced 0.82 g of HA-like crystals as compared with 0.47 g for WMTA (i.e. WMTA produced 43% less HA than GMTA).¹⁷ Therefore, GMTA produces HA almost twice as often as WMTA when suspended in a PBS solution.¹⁸ More HA formation and a greater expansion of GMTA as compared to WMTA has led to the speculation that GMTA and WMTA may not exhibit the same microleakage properties; therefore, the hypothesis is that GMTA and WMTA should not demonstrate the same microleakage phenomenon. The aim of this study was to compare microleakage of GMTA and WMTA when used as root-end filling materials in STF.

Methods

The Ethics Committee of Tabriz University of

Medical Sciences, Iran, approved the proposal of this study. A total of 55 single-rooted human upper anterior teeth (centrals and laterals) with straight canals and mature apices which were extracted for periodontal reasons, and were without cracks or calcified canals, were collected for the experiment and stored in phosphate buffered saline (PBS) solution (Merck KGaA, Darmstadt, Germany) until use. PBS, containing sodium chloride, sodium phosphate, potassium chloride and potassium phosphate was used as a buffer solution as the buffer's phosphate groups help maintain a constant pH and the osmolarity and ion concentrations of the solution mimic those of the human body.

The surface of the roots was cleaned using an ultrasonic device (Joya Electronic, Tehran, Iran). The teeth were randomly divided into 2 experimental groups with 20 teeth each and 2 negative and 1 positive control group, each with 5 teeth. Standard access cavities were prepared with

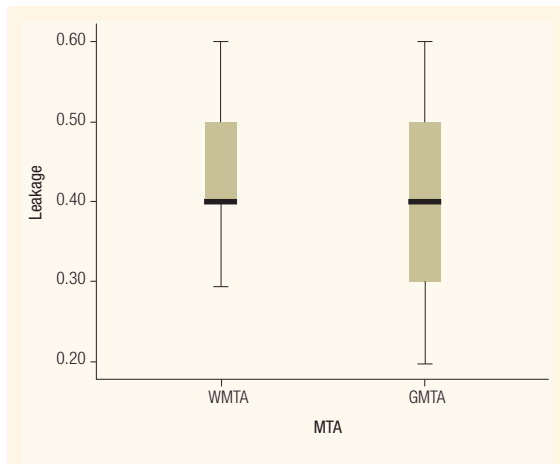


Figure 2: Box plot of dye leakage (in millimetres) of white and grey mineral trioxide aggregate (WMTA and GMTA) after immersion in synthetic tissue fluid, which illustrates the minimum and maximum amount of leakage, as well as the variance in each experimental group.

a round diamond bur under water spray. The root canals were prepared with the conventional step-back technique using K-files (Maillefer, Ballaigues, Switzerland).¹⁹ The master apical file was #35. After each instrument, the root canals were irrigated with 2 mL of 2.5% NaOCl (Golrang, Tehran, Iran) solution. Gates-Glidden drills (Maillefer, Ballaigues, Switzerland) #2 and #3 were used in the coronal third of the canals. The canals were dried with paper points (Apadanatak, Tehran, Iran). The root canals were obturated with gutta-percha (VDW GmbH, Munich, Germany) without sealer, using a cold lateral compaction technique. The apical 3 mm of each root was resected perpendicular to the long axis of the tooth using a fissure bur (Caulk Superbru, LD Caulk Division, Dentsply International Inc., Milford, DE, USA) in a high-speed hand piece under a continuous water spray. The root apices were prepared with a KiS-3D microsurgical ultrasonic instrument (Spartan, Missouri, USA). A circular preparation 3 mm in depth was created in each root. Preparations were checked to ensure that the LC-1 condenser tip (Grand Industries, California, USA) would fit the length and then it was rinsed with saline (Samen, Mashhad, Iran) and dried with paper points. The apical preparations in one experimental group were filled with WMTA (Tooth-Colored Formula, Dentsply, Tulsa Dental, Tulsa, OK, USA) and the other with GMTA (Dentsply, Tulsa Dental, Tulsa, OK, USA). A total of 5 teeth in the negative control groups were filled with WMTA and 5 teeth

with GMTA. The materials were prepared according to the manufacturers' instructions.

All the roots were mounted in red wax with the buccal surfaces facing upward. An X-ray tube was adjusted perpendicular to the buccal surfaces and radiographs were taken. Then the teeth were mounted again in the mesiodistal direction in the red wax and radiographed in the same position to ensure the adequacy of the root-end fillings. Apical preparation in the positive control groups was left unfilled. All the teeth were incubated for 4 hours at 37° C in 100% relative humidity and then immersed in one liter of STF for 3 days at 37° C. The STF had been prepared as follows: 1.7 g of KH_2PO_4 , 11.8 g of Na_2HPO_4 , 80.0 g of NaCl, and 2.0 g of KCl in 10 L of H_2O (pH = 7.2).⁴ The STF was renewed every day. The roots in the experimental and positive control groups were coated with two layers of nail varnish. The resected apical roots were left uncoated. The entire root surfaces of the negative control group samples were covered with 2 layers of nail varnish. Sticky wax was placed over all the surfaces of the teeth that were coated with nail varnish, and allowed to dry. The roots were then submerged in India ink for 48 hours and then rinsed in water. The roots in all the groups were grooved on the buccal and lingual surfaces and split longitudinally into two sections.

Retrofill materials were removed, and the extent of dye penetration was measured separately in mm, using a calibrated stereomicroscope (Carl Zeiss AG, Oberkochen, Germany) at $\times 16$ magnification. Linear dye penetration was measured independently by three observers at three different times under the same conditions; the mean value of the recorded measurements was selected as the extent of dye penetration into each specimen. Statistical analysis was performed by Statistical Package for the Social Sciences software package, Version 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Quantitative values are presented as mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine statistical differences between the groups.

Results

All the preparations in the positive control group demonstrated leakage throughout the entire root lengths [Figure 1A]. The root-end preparations in

the negative control groups, filled with WMTA or GMTA, did not exhibit any leakage [Figure 1B].

The mean dye leakage values for the GMTA and WMTA groups were 0.40 ± 0.1 mm and 0.50 ± 0.1 mm, respectively [Figures 1C & 1D]. There were no significant differences between the two experimental groups ($P = 0.14$) [Figure 2]. Figure 2 illustrates dye leakage in the GMTA and WMTA groups after immersion in STF, which shows the minimum and maximum amounts of leakage, as well as the variance in each experimental group.

Discussion

The ability of dye penetration technique to demonstrate microleakage has been emphasised in different studies.^{20,21} Moreover, many methods such as bacterial penetration, glucose leakage, and fluid filtration have been reported as methods to evaluate microleakage.^{11,22–24} In fact, there is no standard method to investigate microleakage. For instance, the amount of leakage in a bacterial leakage study might be influenced by the kind of bacteria and the culture medium that has been used as an indicator.²⁵ The reliability of any glucose leakage study is questionable because MTA produces calcium hydroxide after hydration, which can react with glucose.²⁵ Fluid filtration using active pressure to measure leakage does not mimic the actual leakage phenomenon.²⁶ In the present study, a dye penetration method was used for assessing the degree of microleakage because it is a common method, and the dye has a molecular weight even less than that of bacterial toxins. However, the limitation of dye leakage studies is that they measure the extent of leakage in only one plane, making it impossible to evaluate the total amount of leakage. Despite its disadvantages, the dye penetration method is popular and has been used in many studies;²⁶ therefore, this method was used in this study. No sealer was used in this study because the aim of this study was to evaluate leakage through retrofilling materials, not through obturating materials within the root canal. Moreover, many studies have demonstrated that sealers can penetrate into the dentinal tubules, occluding them and influencing leakage phenomenon.^{27,28} Therefore, in this study no sealer was used. Other studies have measured microleakage after 24 hours of incubation; in this study, a 72-hour period was used to allow more

time for interaction between the STF and the MTA.

Some previous leakage studies on MTA as a root-end filling material did not mention the storage media, while others used tap water or saline.^{11,29,30} Moreover, some researchers have used phosphate-containing solution as a storage medium before leakage studies to mimic an *in vivo* situation.^{31–33} Parirokh *et al.* showed that placing MTA in a PBS storage medium caused a significant decrease in coronal leakage.³⁴ They postulated that future *in vitro* studies should use PBS as a storage medium to simulate an *in vivo* condition.³⁴ MTA, as a bioactive material, can develop hydroxiapatite on its surface in a synthetic tissue fluid such as PBS.^{5,18} However, long-term studies have been recommended to demonstrate any interaction between PBS or any phosphate-containing medium, and MTA. It might be noteworthy that HBSS, PBS, and STF, as phosphate-containing solutions, have similarities with inorganic components of tissue fluids to some extent. However, STF does not contain organic elements and only maintains the mineral contents of tissue fluid. It is recommended that more studies should be carried out in the presence of blood to closely simulate real conditions.

Lack of dye leakage in the negative control and its presence in the positive control groups showed that the test design was reliable. This study showed that there were no significant differences between the leakage observed with GMTA and WMTA when used as retrofill materials in contact with STF after the initial setting. No study has compared leakage of GMTA and WMTA in the presence of STF. Despite different amounts of HA formation with GMTA and WMTA, the microleakage phenomenon was the same. It seems that more expansion and more HA formation of GMTA, in comparison with WMTA, did not influence leakage.^{16–17} This effect might have two explanations: first, the expansion of GMTA, even if it is greater than that of WMTA, was not sufficient to fill the gaps and create differences in the leakage phenomenon; second, the HA crystals that are created in microscopic gaps between both kinds of MTA and the dentinal wall were sufficient to prevent leakage.

Conclusion

In conclusion, within the limitations of this *in vitro* study on the microleakage phenomenon of

GMTA and WMTA, the results appear to support the use of both of them, although further long-term microleakage studies in the presence of blood should be undertaken.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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