

Surface microhardness of three thicknesses of mineral trioxide aggregate in different setting conditions

Noushin Shokouhinejad^{1*},
 Leila Jafargholizadeh²,
 Mehrfam
 Khoshkhounejad¹,
 Mohammad Hossein
 Nekoofar^{1,3}, Maryam
 Raouf⁴

¹Department of Endodontics, School of Dentistry and Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran
²School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
³Endodontology Research Group, School of Dentistry, Cardiff University, Heath Park, Cardiff, UK
⁴Department of Endodontics, School of Dentistry, Oral and Dental Diseases Research Center, Kerman University of Medical Sciences, Kerman, Iran

Received March 29, 2014;
 Accepted May 27, 2014.

¹Shokouhinejad N; Khoshkhounejad M, Department of Endodontics, School of Dentistry and Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran
²Jafargholizadeh L, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
³Nekoofar MH, Department of Endodontics, School of Dentistry and Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran;
 Endodontology Research Group, School of Dentistry, Cardiff University, Heath Park, Cardiff, UK
⁴Raouf M, Department of Endodontics, School of Dentistry, Oral and Dental Diseases Research Center, Kerman University of Medical Sciences, Kerman, Iran
***Correspondence to**
 Noushin Shokouhinejad, DDS, MSc. Associate Professor, Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, North Karegar St., Tehran, Iran 1439955991
 TEL, +98-9123375410; FAX, +98-21-22890433; E-mail, shokouhinejad@yahoo.com

Objectives: This study aimed to compare the surface microhardness of mineral trioxide aggregate (MTA) samples having different thicknesses and exposed to human blood from one side and with or without a moist cotton pellet on the other side. **Materials and Methods:** Ninety cylindrical molds with three heights of 2, 4, and 6 mm were fabricated. In group 1 (dry condition), molds with heights of 2, 4, and 6 mm (10 molds of each) were filled with ProRoot MTA (Dentsply Tulsa Dental), and the upper surface of the material was not exposed to any additional moisture. In groups 2 and 3, a distilled water- or phosphate-buffered saline (PBS)-moistened cotton pellet was placed on the upper side of MTA, respectively. The lower side of the molds in all the groups was in contact with human blood-wetted foams. After 4 day, the Vickers microhardness of the upper surface of MTA was measured. **Results:** In the dry condition, the 4 and 6 mm-thick MTA samples showed significantly lower microhardness than the 2 mm-thick samples ($p = 0.003$ and $p = 0.001$, respectively). However, when a distilled water- or PBS-moistened cotton pellet was placed over the MTA, no significant difference was found between the surface microhardness of samples having the abovementioned three thicknesses of the material ($p = 0.210$ and $p = 0.112$, respectively). **Conclusions:** It could be concluded that a moist cotton pellet must be placed over the 4 to 6 mm-thick MTA for better hydration of the material. However, this might not be necessary when 2 mm-thick MTA is used. (*Restor Dent Endod* 2014;39(4):253-257)

Key words: Microhardness; Mineral trioxide aggregate; Moist; Setting; Thickness

Introduction

Mineral trioxide aggregate (MTA) has been widely used for root-end filling, perforation repair, pulp capping, pulpotomy, and apexification.¹⁻⁵ It is a type of hydraulic cement that sets in an aqueous environment.⁶ According to the manufacturer's instructions, MTA must be allowed to set in the presence of moisture by placing a wet cotton pellet against the intracanal surface of the material for a minimum of 4 hours. Torabinejad and Chivian also recommended covering MTA with a wet cotton pellet when it is used for pulp capping and perforation repair, or as an apical plug.¹

However, there are conflicting results regarding the need for placing a wet cotton pellet over the MTA. In an investigation comparing the effect of the setting condition on the flexural strength of MTA, it has been shown that the flexural strength of MTA was significantly higher in specimens that received moisture from two sides than that in the case of one-sided specimen moisture exposure after a setting time of 24

hours.⁷ In the case of apexification, although a single-visit procedure with MTA has been suggested, the findings of another study support the two-step technique involving the placement of a moist cotton pellet on the MTA.^{8,9} Some authors have shown the positive effect of moisture on the push-out strength of MTA.¹⁰ On the other hand, it has been reported that dry ProRoot MTA powder packed into the root canals could achieve a full set at 72 hours solely because of the moisture absorbed through the root without any extra moisture received from the coronal aspect of the MTA.¹¹ The findings of recent investigations have also indicated that placing a moist cotton pellet on the MTA may be unnecessary to improve the setting of MTA.¹²⁻¹⁴

Besides the controversy over the need to place a wet cotton pellet over the MTA, the definition of wet conditions and the type of moisture are not consistent in the literature. According to the manufacturer's instructions, a wet cotton pellet should be placed on the MTA. However, the type of moisture has not been mentioned. Interaction of MTA with solutions used over the coronal aspect of the material might affect its physicochemical properties depending on the constituents of the moisture. Several studies have shown that the interaction of MTA with a phosphate-containing solution such as phosphate-buffered saline (PBS) results in the formation of apatite crystals.¹⁵⁻¹⁷ Calcium ions released by MTA react with phosphate in PBS, resulting in the formation of hydroxyapatite or carbonated apatite.^{15,16}

In various clinical applications, different thicknesses of MTA are used. Although it has been recommended that 4 to 5 mm-thick MTA be used as an apical barrier in the apexification procedure, there is no recommended thickness for vital pulp therapy, perforation repair, or root canal obturation.^{9,18} Some properties of MTA, such as sealing ability, displacement resistance, and hardness, can be impacted by the material thickness.^{9,19} Microhardness tests can be used for the assessment of the progress and the quality of the hydration process during the setting reaction, as well as the evaluation of the microstructural gradient of MTA materials.^{20,21}

The present study was aimed at comparing the surface microhardness of different thicknesses of ProRoot MTA exposed to human blood from one side and with or without a distilled water- or PBS-moistened cotton pellet on the other side of the material.

Materials and Methods

Polymethyl methacrylate (Plexiglass, Cho Chen Industry Co. Ltd., Tainan City, Taiwan) cylindrical molds with an internal diameter of 4 mm and three heights of 2, 4, and 6 mm (30 molds of each height) were fabricated by computerized numerical control (CNC) laser cutting (LaserProI, GCC, New Taipei City, Taiwan). Floral foams

wetted with whole fresh human blood were placed on glass slabs and then transferred into plastic containers. The human blood collection procedure was approved by the Ethics Committee of the Tehran University of Medical Sciences (No. 19905). Then, the molds were placed on blood-wetted foams. Tooth-colored ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA) was mixed with sterile distilled water according to the manufacturer's instructions and delivered into the molds. The details of the experimental groups are as follows:

Group 1: dry condition ($n = 30$)

This group included three subgroups with the heights of 2, 4, and 6 mm (10 molds of each). After the molds were filled with MTA, the upper surface of the material was not exposed to any additional moisture and was completely covered with a layer of parafilm (Parafilm 'M' Laboratory Film, American Can Company, Greenwich, CT, USA).²²

Group 2: exposed to distilled water ($n = 30$)

Molds with the heights of 2, 4, and 6 mm (10 molds of each) were filled with MTA. A distilled water-moistened cotton pellet was placed on the upper surface of the MTA.

Group 3: exposed to PBS ($n = 30$)

The specimens were prepared in the manner described for the previous groups, but a PBS-moistened cotton pellet was placed on the upper surface of the MTA.

The specimens of each group were stored in separate plastic containers. The closed containers were kept at 37°C and a relative humidity of 100% for 4 days to simulate physiological conditions.

Vickers microhardness test

After 4 days, the upper surface of each sample was wet-polished at room temperature by using silicon carbide sandpapers of 1,000, 1,200, 1,500, and 2,000 grit, respectively. The surface microhardness test was performed by a Vickers tester (Bareiss Prüfgeratebau GmbH, Oberdisingen, Germany) with a pyramid-shaped diamond indenter using a load of 300 g for 10 seconds. Three indentations were made on the polished surface of each specimen at separate locations adjacent to the indentations or from the sample periphery. The Vickers microhardness value (VHN) was calculated using the following formula in which F is the load in kilogram-force and d is the mean of the two diagonals of the impression made by the indenter in millimeters.

$$\text{VHN} = \frac{2 F \sin (136^\circ / 2)}{d^2} = \frac{1.854 F}{d^2}$$

The data were analyzed using one-way analysis of variance followed by the *post hoc* Dunnett T3 test. The significance level was set at $p < 0.05$. All analyses were performed using the Statistical Package for the Social Sciences Version 16 (SPSS Inc., Chicago, IL, USA).

Results

The results of the microhardness tests are shown in Table 1. A significant difference was found between the surface microhardness of the three thicknesses of MTA with no additional moisture on the material ($p < 0.001$). In the dry condition (group 1), the 2 mm-thick MTA samples showed a significantly higher surface microhardness than the 4 and 6 mm-thick samples ($p = 0.003$ and $p = 0.001$, respectively). On the other hand, the surface microhardness of MTA samples having different thicknesses was not significantly different in the presence of distilled water and PBS ($p = 0.210$ and $p = 0.112$, respectively). There was also no significant difference between the surface microhardness of the samples in groups 2 and 3 ($p > 0.05$). Furthermore, the VHN of 2 mm-thick samples set in the dry condition was not significantly different from that obtained for each tested thickness of MTA that received additional moisture from a distilled water- or PBS-wetted cotton pellet ($p = 0.201$).

Discussion

The microhardness of MTA is influenced by many factors such as the thickness of the material, condensation pressure, pH and acid etching of the material.^{9,21,23,24} In this study, the effect of different setting conditions on the surface microhardness of MTA samples having different thicknesses was assessed using a Vickers hardness test. Since some fundamental properties of the material, such as yield strength, tensile strength, modulus of elasticity, and stability of the crystal structure, influence material microhardness, the effect of the setting conditions on the overall strength of the material can be evaluated using

the microhardness test as an indicator of the setting reaction.^{20,23,25}

In the present study, the inferior surface of MTA was in contact with the human blood-wetted foam to partially simulate some clinical situations, such as root perforations, vital pulp therapy, and apexification. However, the inferior surface of the material was not investigated because the effect of blood contamination on the surface microhardness and the compressive strength of MTA had been studied previously.^{26,27} In the dry condition, the upper surface of the MTA was covered with a layer of parafilm to protect it from further moisture exposure. In this study, the specimens were incubated at 37°C and 100% humidity to provide a similar environment to the clinical situation. The assessment of microhardness was performed after incubation for 4 days. It has been suggested that MTA be untouched for at least 72 - 96 hours to decrease the chance of displacement.^{21,24,28}

In the present study, the surface microhardness of the three thicknesses of MTA (2, 4, and 6 mm) was not similar when no moist cotton pellet was placed on the material. In this condition, the 4 and 6 mm-thick samples showed significantly lower surface microhardness than the 2 mm-thick specimens. In samples with a higher thickness, it can be expected that the exposure of the material to moisture only from one side might not be sufficient for the setting of parts that are away from the moisture source. MTA consists of fine hydrophilic particles that set to a hard composition when brought into contact with water through the creation of a colloidal gel.^{6,20,29} In the dry condition, the higher surface microhardness of the thinner samples of MTA might be attributed to the moisture provided from the blood-wetted foam at the bottom of the materials that might act as a moisture source for the setting of the upper

Table 1. Microhardness of tested groups (Mean \pm SD; Unit, Kg/mm²)

Group	Thickness (mm)	Vickers microhardness
Group 1: dry condition	2	71.38 \pm 21.30 ^a
	4	47.01 \pm 11.71 ^b
	6	46.14 \pm 8.15 ^b
Group 2: exposed to distilled water	2	81.52 \pm 18.45 ^a
	4	74.84 \pm 8.14 ^a
	6	72.81 \pm 13.09 ^a
Group 3: exposed to PBS	2	80.68 \pm 7.93 ^a
	4	73.42 \pm 17.63 ^a
	6	69.17 \pm 16.70 ^a

The differences between values with the same superscript letter are not statistically significant at $p < 0.05$. PBS, phosphate-buffered saline; SD, standard deviations.

surface of the MTA. On the other hand, Eid *et al.* showed no significant difference in hardness between the 3.5 mm-thick samples of ProRoot MTA placed in plastic cylinders with or without the application of a wet cotton pellet on the material.¹³ The difference between the results for the 4 mm-thick MTA samples used in the current study and the 3.5 mm-thick MTA samples used in the study conducted by Eid *et al.* could be explained by some differences in the methodologies used.¹³ In the study conducted by Eid *et al.*, the MTA base was in contact with sterile gauze wetted with normal saline and the upper surface of MTA was covered with glass ionomer cement in the dry condition group.¹³

In the current study, there were no significant differences between the surface microhardness of 2, 4, and 6 mm-thick MTA samples exposed to the cotton pellets moistened with either distilled water or PBS at the upper surface of the material. However, the microhardness of 4 and 6 mm-thick MTA samples that received no additional moisture was significantly lower than that of samples with similar thickness but exposed to cotton pellets moistened with distilled water or PBS. In contrast to our results, DeAngelis *et al.* showed no difference between the surface microhardness of 4 mm-thick MTA samples with or without a moist cotton pellet on the material in unvarnished roots.¹² Budig and Eleazer also showed that ProRoot MTA powder packed within the roots could achieve a complete set because of the moisture absorbed through the roots submerged in saline for 72 hours.¹¹ These controversial results might be attributed to the absorption of moisture needed for the hydration of MTA from the intrinsic moisture retained within the dentinal tubules without the need of a wet cotton pellet.^{12,30} Although many studies have confirmed that the interaction between MTA and phosphate-containing solutions results in the formation of apatite crystalline structures over MTA, this study showed no difference between the microhardness of MTA samples having different thicknesses and exposed to PBS, which was comparable to that of the samples exposed to distilled water.^{16,17,31} The precipitation of apatite has been shown to begin within the first few hours of exposure to phosphate-containing fluids, however, the aggregation of greater amounts of apatite crystals occurs with an increase in the exposure time.^{16,32} In this study, the exposure of MTA to PBS for 4 days might be insufficient for altering the properties of the material.

The findings of this study also showed no significant difference between the 2 mm-thick samples of MTA in dry and wet conditions. These results are not in agreement with Walker *et al.*, who showed that 2 mm-thick MTA specimens that received moisture from two sides had a significantly higher flexural strength than those that received moisture only from one side after a setting time of 24 hours.⁷ However, the results for surface microhardness could not be extrapolated to those for flexural strength.

Conclusions

Under the conditions of this study, it could be concluded that placing a moist cotton pellet on a 4 to 6 mm-thick MTA sample was necessary for better hydration of the material. However, this might not be essential when a 2 mm-thick MTA sample is used.

Acknowledgment

This study was supported by a grant from Tehran University of Medical Sciences (grant No: 19905). The authors wish to thank Dr. Ahmad R. Shamshiri for his assistance in the statistical analysis.

Conflict of Interest: No potential conflict of interest relevant to this article was reported.

References

1. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. *J Endod* 1999;25:197-205.
2. Lee SJ, Monsef M, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. *J Endod* 1993;19:541-544.
3. Dominguez MS, Witherspoon DE, Gutmann JL, Opperman LA. Histological and scanning electron microscopy assessment of various vital pulp-therapy materials. *J Endod* 2003;29:324-333.
4. de Souza Costa CA, Duarte PT, de Souza PP, Giro EM, Hebling J. Cytotoxic effects and pulpal response caused by a mineral trioxide aggregate formulation and calcium hydroxide. *Am J Dent* 2008;21:255-261.
5. Camp JH. Diagnosis dilemmas in vital pulp therapy: treatment for the toothache is changing, especially in young, immature teeth. *Pediatr Dent* 2008;30:197-205.
6. Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Ford TR. The constitution of mineral trioxide aggregate. *Dent Mater* 2005;21:297-303.
7. Walker MP, Diliberto A, Lee C. Effect of setting conditions on mineral trioxide aggregate flexural strength. *J Endod* 2006;32:334-336.
8. Witherspoon DE, Ham K. One-visit apexification: technique for inducing root-end barrier formation in apical closures. *Pract Proced Aesthet Dent* 2001;13:455-460.
9. Matt GD, Thorpe JR, Strother JM, McClanahan SB. Comparative study of white and gray mineral trioxide aggregate (MTA) simulating a one- or two-step apical barrier technique. *J Endod* 2004;30:876-879.
10. Gancedo-Caravia L, Garcia-Barbero E. Influence of humidity and setting time on the push-out strength of mineral trioxide aggregate obturations. *J Endod* 2006;

- 32:894-896.
11. Budig CG, Eleazer PD. *In vitro* comparison of the setting of dry ProRoot MTA by moisture absorbed through the root. *J Endod* 2008;34:712-714.
 12. DeAngelis L, Chockalingam R, Hamidi-Ravari A, Hay S, Lum V, Sathorn C, Parashos P. *In vitro* assessment of mineral trioxide aggregate setting in the presence of interstitial fluid alone. *J Endod* 2013;39:402-405.
 13. Eid AA, Komabayashi T, Watanabe E, Shiraishi T, Watanabe I. Characterization of the mineral trioxide aggregate-resin modified glass ionomer cement interface in different setting conditions. *J Endod* 2012;38:1126-1129.
 14. Tsujimoto M, Tsujimoto Y, Ookubo A, Shiraishi T, Watanabe I, Yamada S, Hayashi Y. Timing for composite resin placement on mineral trioxide aggregate. *J Endod* 2013;39:1167-1170.
 15. Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I. Physicochemical basis of the biologic properties of mineral trioxide aggregate. *J Endod* 2005;31:97-100.
 16. Reyes-Carmona JF, Felipe MS, Felipe WT. Biomineralization ability and interaction of mineral trioxide aggregate and white portland cement with dentin in a phosphate-containing fluid. *J Endod* 2009;35:731-736.
 17. Shokouhinejad N, Nekoofar MH, Razmi H, Sajadi S, Davies TE, Saghiri MA, Gorjestani H, Dummer PM. Bioactivity of EndoSequence root repair material and bioaggregate. *Int Endod J* 2012;45:1127-1134.
 18. Giuliani V, Baccetti T, Pace R, Pagavino G. The use of MTA in teeth with necrotic pulps and open apices. *Dental Traumatol* 2002;18:217-221.
 19. Hachmeister DR, Schindler WG, Walker WA 3rd, Thomas DD. The sealing ability and retention characteristics of mineral trioxide aggregate in a model of apexification. *J Endod* 2002;28:386-390.
 20. Lee YL, Lee BS, Lin FH, Yun Lin A, Lan WH, Lin CP. Effects of physiological environments on the hydration behavior of mineral trioxide aggregate. *Biomaterials* 2004;25:787-793.
 21. Nekoofar MH, Adusei G, Sheykhrezae MS, Hayes SJ, Bryant ST, Dummer PM. The effect of condensation pressure on selected physical properties of mineral trioxide aggregate. *Int Endod J* 2007;40:453-461.
 22. Danesh G, Dammaschke T, Gerth HU, Zandbiglari T, Schäfer E. A comparative study of selected properties of ProRoot mineral trioxide aggregate and two Portland cements. *Int Endod J* 2006;39:213-219.
 23. Namazikhah MS, Nekoofar MH, Sheykhrezae MS, Salariyeh S, Hayes SJ, Bryant ST, Mohammadi MM, Dummer PM. The effect of pH on surface hardness and microstructure of mineral trioxide aggregate. *Int Endod J* 2008;41:108-116.
 24. Kayahan MB, Nekoofar MH, Kazandağ M, Canpolat C, Malkondu O, Kaptan F, Dummer PM. Effect of acid-etching procedure on selected physical properties of mineral trioxide aggregate. *Int Endod J* 2009;42:1004-1014.
 25. Chandler H. Hardness testing. 2nd ed. Materials Park, OH: ASM International; 1999. p1-13.
 26. Nekoofar MH, Oloomi K, Sheykhrezae MS, Tabor R, Stone DF, Dummer PM. An evaluation of the effect of blood and human serum on the surface microhardness and surface microstructure of mineral trioxide aggregate. *Int Endod J* 2010;43:849-858.
 27. Oloomi K, Saberi E, Mokhtari H, Mokhtari Zonouzi HR, Nosrat A, Nekoofar MH, Dummer PM. Evaluation of the effect of blood contamination on the compressive strength of MTA modified with hydration accelerators. *Restor Dent Endod* 2013;38:128-133.
 28. Vanderweele RA, Schwartz SA, Beeson TJ. Effect of blood contamination on retention characteristics of MTA when mixed with different liquids. *J Endod* 2006;32:421-424.
 29. Chang SW. Chemical characteristics of mineral trioxide aggregate and its hydration reaction. *Restor Dent Endod* 2012;37:188-193.
 30. Pelliccioni GA, Vellani CP, Gatto MR, Gandolfi MG, Marchetti C, Prati C. Proroot mineral trioxide aggregate cement used as a retrograde filling without addition of water: an *in vitro* evaluation of its microleakage. *J Endod* 2007;33:1082-1085.
 31. Tay FR, Pashley DH, Rueggeberg FA, Loushine RJ, Weller RN. Calcium phosphate phase transformation produced by the interaction of the portland cement component of white mineral trioxide aggregate with a phosphate-containing fluid. *J Endod* 2007;33:1347-1351.
 32. Gandolfi MG, Ciapetti G, Taddei P, Perut F, Tinti A, Cardoso MV, Van Meerbeek B, Prati C. Apatite formation on bioactive calcium-silicate cements for dentistry affects surface topography and human marrow stromal cells proliferation. *Dent Mater* 2010;26:974-992.