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Surface microhardness of different thicknesses of a premixed bioceramic material with or without the application of a moist cotton pellet

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ABSTRACT

Background: This study was conducted to assess the effect of thickness and hydration condition on the surface microhardness of Endosequence Root Repair Material putty (ERRM; Brasseler USA, Savannah, GA), a premixed bioceramic material.

Materials and Methods: Polymethyl methacrylate cylindrical molds with an internal diameter of 4 mm and three heights of 2, 4, and 6 mm were fabricated. In Group 1 (dry condition), the molds with heights of 2, 4, and 6 mm (10 molds of each) were filled with ERRM. In Groups 2 and 3 (wet condition), a distilled water- or phosphate-buffered saline (PBS)-moistened cotton pellet was placed directly on the upper surface of ERRM, respectively. The lower surface of ERRM was in contact with floral foams soaked with human blood. After 4 days, Vickers microhardness of the upper surface of ERRM was tested. The data were analyzed using two-way analysis of variance. Significance level was set at $P < 0.05$.

Results: No significant difference was found between the microhardness of three thicknesses of ERRM (2, 4, and 6 mm) with or without placing a distilled water- or PBS-moistened cotton pellet over the material ($P > 0.05$).

Conclusion: Based on the results of this study, it could be concluded that placing a moistened cotton pellet on ERRM putty up to 6 mm thick might be unnecessary to improve its surface microhardness and hydration characteristics.

Key Words: Dry, hardness, pellet, phosphate-buffered saline, root repair, silicates, wet condition

INTRODUCTION

Mineral trioxide aggregate (MTA) exhibits many of ideal properties of an endodontic material.[1,2] The search for biomaterials demonstrating properties similar to MTA but with improved working time and handling characteristics has led to the introduction of Endosequence Root Repair Material (ERRM). It has been recommended for repair of perforations, apical plug creation, root-end filling, pulp capping, and pulpotomy.[3] ERRM is an aluminum-free material

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primarily composed of calcium silicate, monobasic calcium phosphate, zirconium oxide, tantalum oxide, and filler agents with a working time of 30+ min. It is available as a premixed product in two specifically formulated consistencies: A syringable paste and thick condensable putty. ERRM has been shown to be antibacterial,[4] biocompatible,[3,5,6] bioactive,[7] and of high pH.[8] Previous studies reported that it was equivalent to ProRoot MTA in sealing ability[9] marginal adaptation,[10] and compressive strength.[11]

According to the manufacturer, setting reaction of ERRM initiates and completes in the presence of moisture naturally in the root canal and dentinal tubules. No clear recommendation is found regarding the need of placing a wet cotton pellet over the intradental surface of ERRM. However, Caronna *et al*.[12] showed that the surface hardness of a 4 mm thick ERRM paste that was exposed to phosphate-buffered saline (PBS) at one surface was not improved by placing a sterile water-wetted cotton pellet on the other surface.

Interaction of calcium silicate-based materials with different solutions could affect their physicochemical properties.[13-16] Formation of apatite crystals as a result of interaction of calcium silicate-based materials with a phosphate-containing solution such asPBS has been shown by several studies.[7,17-21] The bioactivity of ERRM after exposure to PBS has been revealed by the precipitation of apatite crystalline structures over the material.[7]

In various clinical applications, different thicknesses of endodontic materials are used. It is of interest to know whether thicker ERRM needsmoremoisture from the intradental side of the material to improve the material hardening. Little information is available regarding the physical properties of ERRM in different setting conditions. The aim of this study was to assess the surface microhardness of different thicknesses of ERRM putty(2, 4, and 6 mm) exposed to human blood from one side and with or without placing a distilled water-or PBS-moistened cotton pellet over the other side of the material.

MATERIALS AND METHODS

Specimen preparation

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 laser cutting (Laser ProI, GCC, New Taipei City, Taiwan). The molds were placed on the floral foams soaked with whole fresh human blood that was obtained from a healthy volunteer. The blood collection tubes were spray coated with the anticoagulant K2EDTA to prevent clotting. The human blood collection procedure was approved by the Ethics Committee of the Tehran University of Medical Sciences (No. 21244). In Group 1 (dry condition), cylindrical molds with heights of 2, 4, and 6 mm (10 molds of each) were filled with premixed ERRM putty. The upper surface of ERRM was not exposed to any additional moisture and was covered with a layer of parafilm (Parafilm 'M' Laboratory Film, American Can Company, Greenwich, CT, USA).[22] In Groups 2 and 3 (wet condition), a distilled water- or PBS-moistened cotton pellet was placed directly on the upper surface of ERRM, respectively.

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

Vickers microhardnesstest

The surface of ERRM was tested using an endodontic explorer to check the setting of the material. After that, the upper surface of ERRM was polished at room temperature using silicon carbide sandpapers of 1000, 1200, 1500, 2000, and 3000-grit under constant water irrigation. The surface microhardness test was performed using a Vickers tester (Bareiss Prufgeratebau GmbH, Oberdischingen, Germany) with a pyramid-shaped diamond indenter using a load of 300 g for 10 s. The angle between the opposite faces of the diamond indenter was 136°.Three indentations were made on the polished surface of each specimen at separate locations. Thus, in total, 30 indentations were obtained for each thickness per group. The microhardness value of each specimen was calculated as the average of three indentations.

The Vickers microhardness value was calculated based on the following formula: Vickers hardness number = $1.854 \times (F/d^2)$, where F is the load in kilogram-force and d is the mean of the two diagonals produced by the indenter in millimeters.[23] The mean and standard deviation of microhardness values werecalculated.

The data were normally distributed. Therefore, two-way analysis of variance was used in testing the effect of thickness and setting condition on microhardness. Significance level was set at $P < 0.05$.

RESULTS

The results of the microhardness tests are presented in Table 1. Statistical analysis showed no significant effects for "thickness of material" and "setting condition" ($P = 0.13$ and $P = 0.55$, respectively). There was no significant difference between the microhardness of three thicknesses of ERRM (2, 4, and 6 mm) with or without placing a distilled water- or PBS-moistened cotton pellet over the material ($P > 0.05$).

DISCUSSION

In the present study, microhardness of ERRM was assessed in dry and wet conditions using a Vickers hardness tester. Previous studies showed the usefulness of Vickers hardness as an indicator of the progress and quality of the hydration process during setting reaction and the strength of calcium silicate-based materials.[23,24] The inferior surface of ERRM was exposed to human blood-soaked foam to partially simulate some clinical situations such as root perforations, vital pulp therapy, and creation of apical plug. Since this study was not designed to assess the effect of blood contamination on physical properties of ERRM, the microhardness of the lower surface of the material was not tested. In dry condition, the upper surface of MTA was covered with a layer of parafilm to protect it from further moisturizing.

It has been suggested that MTA be untouched for at least 72-96 h to prevent the chance of materials displacement.[23,25] According to the manufacturer, the setting time of material is 4 h under normal condition. However, Charland *et al*.[16] contradicted the reported setting time by manufacturer as they showed that ERRM was not completely set in 48-h. In the study on cytotoxicity comparison of mineral trioxide aggregates and ERRM putty and paste, Damas *et al*.[6] also found that the complete set of ERRM paste and putty samples was not obtained within the 72-hour and 120-hour time period. However, it is worth to mention that the design of Damas *et al*.'s [6] study is completely different from that of the present study. In that study, the upper and lower surface of the materials within the molds was not in contact with moistened foam and specimens were just kept in 100% humidity. But in the present study, in order to simulate the clinical conditions, the lower surface of the ERRM in all groups was in contact with blood and the upper surface of ERRM with distilled water or PBS in groups of wet conditions; then, specimens were kept in 100% relative humidity at 37°C. Therefore, it is expected that the accessory findings of Damas *et al*.[6] which was about the setting of the materials could not be extrapolated to that of this study. Furthermore, additional research would be necessary to determine the time needed for ERRM in the situations similar to clinical conditions to be completely set.Therefore, according to the previous studies on MTA and Charland *et al*.'s study,[16] the present study allowed ERRM to set for 4 days. Furthermore, before making microhardness measurement, the surface of the material was tested using an endodontic explorer and all the samples showed to be clinically "set."

There are few studies to evaluate the microhardness of ERRM using a wide variety of loads (50-1000 g).[12,26] The load in this study was selected based on a pilot test that showed that the load of 300 g could create a clear indentation.

In this study, the microhardness of the upper surface of 2, 4, and 6 mm thick ERRM was not significantly different with or without placing wet cotton pellets over the material. Although some authors have shown the positive effect of moisture on the flexural and push-out strength of MTA,[27,28] the results of a number of studies indicated that placing a moist cotton pellet on the calcium silicate-based materials might be unnecessary to improve their setting.[14,29,30] The results of this study are also in accordance with Caronna *et al*.[12] who showed that placement of adistilled water-moistened cotton pellet over the surface of a 4 mm thick ERRM paste that was in contact with PBS from the other side did not improve its surface microhardness. The results group 1 (ERRM in dry

Table 1: Mean ± SD, minimum, and maximum microhardness values of test groups

Condition	Group	Thickness (mm)	Vickers microhardness (kg/mm^2)		
			Mean ± SD	Minimum	Maximum
Dry	Group 1	2	69.23±20.95^a	48.28	90.18
		4	57.81±17.21^a	40.6	75.02
		6	58.14±17.53^a	40.61	75.67
Wet	Group 2: Exposed to distilled water	2	70.20±16.65^a	53.55	86.85
		4	71.62±16.13^a	55.49	87.75
		6	71.55±12.67^a	58.88	84.22
	Group 3: Exposed to PBS	2	70.99±5.50^a	65.49	76.49
		4	69.19±15.37^a	53.82	84.56
		6	70.03±14.49^a	55.54	84.52

Mean values with the same superscript letter are not statistically different at $P < 0.05$; SD: Standard deviation; PBS: Phosphate-buffered saline

condition) might be explained by the absorption of moisture from the blood-soaked foam at the bottom of the material that might act as a moisture source for the setting of internal parts and upper surface of the ERRM.

In the present study, the surface microhardness values following placement of PBS-moistened cotton pellet over the material was not significantly different from those specimens that received moisture from distilled water-moistened cotton pellet. This result is in agreement with the finding of a study that showed no positive effect of PBS on surface microhardness of MTA when compared with distilled water.[31] Although several studies revealed an increase in the bioactivity of calcium-silicate materials over time,[7,18,19,32] in this study, the 4 days interval might be insufficient for altering the microstructure and properties of the ERRM that was exposed to PBS. On the other hand,it should not be out of sight that placement of a PBS-wetted cotton pellet on the intradental side of calcium-silicate cements for a long period of time, in order to obtain the materials' bioactivity, may have the disadvantage of losing the coronal seal.

The results of the present study showed no difference between the surface microhardnesse of the different thicknesses of ERRM. Although it has been stated that the hardness of MTA was affected by thickness,[31,33] this study revealed that thickness is not an influencing factor on microhardness of ERRM in either dry or wet condition. The results of this *in vitro* study which was performed in polymethyl methacrylate molds without any dentinal structure being in contact with the material showed that placinga wet cotton pellet on the other surface of 2, 4, and 6 mm thick ERRM samples was not necessarily improve the surface microhardness. Also, in clinical situations, the material is in contact with dentin that might provide enough moisture to initiate and complete the setting reaction in the absence of additional moisture. Therefore, further in vivo research is recommended to confirm the results of this study.

CONCLUSION

Under the conditions of this *in vitro* study, it could be concluded that placing a distilled water- or PBS-moistened cotton pellet on ERRM up to 6 mm thickwas unnecessary to improve its surface microhardness.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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