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The Effect of Human Blood and Platelet-rich Fibrin on the Surface Microhardness of Hydraulic Calcium Silicate-based Cements

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A B S T R A C T

Objectives: This study aimed to compare the effect of human blood and platelet-rich fibrin (PRF) on the surface microhardness of hydraulic calcium silicate-based cements (OrthoMTA and RetroMTA).

Materials and Methods: Two types of mineral trioxide aggregate, OrthoMTA and RetroMTA, were mixed and placed into cylindrical molds. The lower surfaces of all cements were exposed to saline. The upper surfaces of cements were exposed to human blood, PRF, or phosphate buffer saline (PBS). After storage for 7 days in fully saturated humidity at 37°C, the microhardness of cement surface exposed to blood, PRF, or PBS was measured using the Vickers microhardness test. The data were analyzed using two-way analysis of variance and post hoc Tamhane's T2 test. The significance level was set at $P<0.05$.

Results: Exposure to blood and PRF significantly decreased the surface microhardness of OrthoMTA and RetroMTA. The microhardness of PBS-contacted cements was significantly higher than that of blood or PRF groups ($P<0.001$). The microhardness values for OrthoMTA exposed to PRF were significantly higher than the blood group ($P=0.020$). There were no significant differences between RetroMTA contacted with blood or PRF groups ($P=0.985$). When exposed to blood or PBS, RetroMTA had a significantly higher microhardness than OrthoMTA ($P<0.001$ for blood, $P=0.002$ for PBS).

Conclusion: Exposure to blood or PRF decreased the surface microhardness of both cements. Blood-contaminated RetroMTA showed significantly higher surface microhardness than OrthoMTA contacted with blood. No significant difference was found between PRF-contacted OrthoMTA and RetroMTA.

Keywords: Blood; Hardness; Mineral trioxide aggregate; Platelet-rich fibrin

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INTRODUCTION

Endodontic management of necrotic immature permanent teeth is a challenging procedure [1]. Regenerative endodontic treatment (RET) has been recently introduced to promote root development [2] and ideally allow for regeneration of dentin-pulp complex [3,4] in necrotic immature teeth. RET involves disinfection of the root canal system, followed by the placement of a scaffold, which is then sealed with a hydraulic calcium silicate-based cement (HCSC), such as mineral trioxide aggregate (MTA) [5,6]. After disinfection of the root canal system bleeding is stimulated periapically to promote the formation of a blood clot as a scaffold within the root canal. Recently, other scaffold alternatives have been proposed and examined in RET. Autologous platelets concentrates such as platelet-rich fibrin (PRF), which contains essential growth

factors that promote the proliferation and differentiation of human dental pulp cells [7] as well as the osteo- and odontogenic differentiation of the stem cells of the apical papilla (SCAPs) [8]. PRF has shown promising outcomes when used in regenerative endodontics (RET) [9,10].

After the scaffold is introduced to disinfected root canal it should be sealed coronally by the placement of a HCSC. Following the introduction of mineral trioxide aggregate (MTA) to the field of endodontics in 1993, the search for materials demonstrating properties similar to MTA but without its disadvantages such as handling difficulties, long setting time, and the potential for tooth discoloration has led to the develop other HCSCs [11]. Several investigations have shown the unfavorable discoloration of tooth structure induced by some types of HCSCs [12,13]. The composition of hydrophilic calcium silicate-based cements (HCSCs) plays a crucial role in their potential for discoloration. Bismuth oxide (BO) has been identified as a significant factor contributing to tooth discoloration in some HCSC formulations. Research has demonstrated that materials that do not contain BO exhibit a significantly lower discoloration potential compared to materials containing BO [14,15]. OrthoMTA and RetroMTA (BioMTA, Seoul, Korea) have been formulated for clinical applications similar to those of ProRoot MTA. OrthoMTA consists of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, free calcium oxide, and bismuth oxide [16]. However, RetroMTA is composed of calcium carbonate, silicon dioxide, aluminum oxide, and calcium zirconia complex. Fewer heavy metal components have been shown in OrthoMTA compared to ProRoot MTA [17]. RetroMTA has the advantage of less tooth discoloration [14]. Bismuth oxide (BO) in the composition of some HCSCs such as OrthoMTA has been recognized as one of the main causative factors for tooth discoloration. Destabilization of BO by strong oxidizing agents, amino acids of the dentin collagen, or exposure to light irradiation in an oxygen-free environment have been suggested as reasons for color instability of HCSCs containing BO [18-20]. Therefore, attempts to develop materials with a lower potential for tooth discoloration and better color stability have led to the introduction of HCSCs with alternative radiopacifiers such as zirconium oxide [13,14]. Furthermore, the zirconia complex in RetroMTA contributes to its shorter setting time [21]. In some randomized controlled trials, it has been shown that OrthoMTA and RetroMTA were comparable to ProRoot MTA and had favorable clinical and radiographic results when used for vital pulp therapy in primary and permanent teeth [22,23].

In RET, the scaffold may interact with HCSC and alter its physicochemical properties [11]. Many in vitro studies have evaluated the effect of blood on HCSCs and shown the detrimental effect of blood on the physical properties of these materials [24-26]. However, it has been shown that the extent and degree of physicochemical and microstructural alteration caused by blood contamination were not similar in HCSCs with different compositions [25-27]. Furthermore, the color stability of HCSCs may be influenced by exposure to blood. Several studies have revealed the exacerbation of tooth discoloration induced by HCSCs when they are applied in the presence of blood [12,13,28].

Microhardness tests can be used for the assessment of the quality and progress of the hydration process during the setting reaction of HCSCs, as well as the evaluation of the microstructural gradient of HCSCs [29].

To the best of our knowledge, there is not enough information available about the possible interaction between HCSCs and autologous PRF. Therefore, this study aimed to compare the effect of human blood and PRF on the surface microhardness of OrthoMTA and RetroMTA which relatively differ in the constitution.

MATERIALS AND METHODS

Preparation of samples

Cylindrical molds made of polymethyl methacrylate (Plexiglass, Cho Chen Industry Co. Ltd., Tainan City, Taiwan), with a height of 4 mm and an internal diameter of 6 mm (as per ASTM E384 standards), were produced using

computerized numerical control laser cutting (Laser ProI, GCC, New Taipei City, Taiwan). The molds were then positioned on a glass slab. OrthoMTA and RetroMTA cements (BioMTA, Seoul. Korea) were prepared according to the manufacturer's instructions and packed into the molds. The upper and lower surfaces of samples were identified by carving letters "U" and "L" on the mold respectively. The MTA was gently packed with an endodontic plugger. All of the procedures including delivering the MTA into the molds and packing them were performed by the same operator. After filling the molds, the lower surfaces of all samples were exposed to saline-soaked cotton gauze. The upper surfaces of OrthoMTA and RetroMTA were exposed to human blood, PRF, or phosphate buffer saline (PBS) as the control group. Therefore, six groups (n=10 for each group) were assessed. Each specimen was exposed to 0.1 mL of blood or PBS. In the PRF group, it was attempted to cover the top surface of each specimen by a piece of PRF (Fig 1).

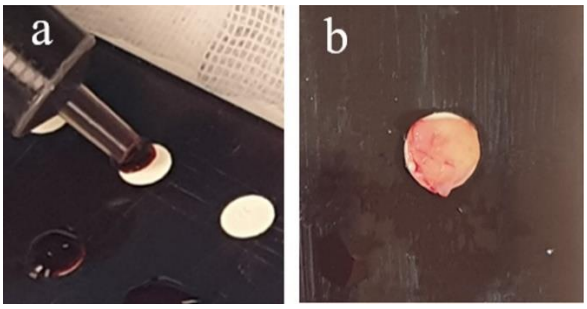

Fig 1. RetroMTA exposed to blood (a) or PRF (b)

The whole fresh human blood was collected from a healthy consented volunteer. This study was approved by the Ethics Committee of Tehran University of Medical Sciences (Ethics code: IR.TUMS.DENTISTRY.REC.1399.180). To prepare PRF, 20 mL of fresh human blood was placed into a test tube without the addition of anticoagulants and immediately centrifuged (PRF Centrifuge DUO Quattro, Nice, France) at 1300 rpm for 8 minutes. To eliminate the exudates, the PRF clot was separated from the layers of platelet-rich plasma and red blood cells and then compressed in a compression box.

The specimens of each group were stored in separate plastic containers. To simulate physiological conditions, the containers were kept in an incubator (Kavooshmega, Iran) in fully saturated humidity at 37°C for 7 days.

Vickers microhardness test

Both upper and lower surfaces of the sample were polished with consecutive use of wet and dry silicon carbide sandpapers (600, 1000, 1500, 2000, 3000, and 5000grit) to obtain a smooth polished surface allowing easy observation of indentation. After that, the microhardness of surfaces exposed to blood, PRF, or PBS was evaluated using a Vickers tester (Bareiss Prüftechnologie GmbH, Oberdischingen, Germany) equipped with a pyramid-shaped diamond indenter. A load of 300g was applied for 10 seconds, with the angle between the opposing faces of the diamond indenter set at 136°. Three indentations were made on the polished surface of each sample at different locations. The microhardness value for each specimen was determined by averaging the results from the three indentations. The Vickers microhardness value was calculated using the following formula: Vickers hardness number = $1.854 \times (F/d^2)$, where F represents the load in kilogram-force and d is the mean of the two diagonals created by the indenter, measured in millimeters using the V-test II basic Vickers (Bareiss, Germany). The V-Test II basic Vickers tester applies a diamond indentor creating a diamond- shape indentation. This system also has a built-in digital microscope capturing high-resolution images of both diagonals of the indentation. Evaluation Software Hardsoft with Dongle receives the images and uses algorithms to extract lengths of the two diagonals. The software finally calculates the microhardness value using the Vickers microhardness formula. The data were analyzed with two-way analysis of variance and post hoc Tamhane's T2 test using SPSS software (SPSS Inc, Chicago, IL). Significance level was set at $P<0.05$.

RESULTS

The mean and standard deviation (SD) of surface microhardness values in the six groups are shown in Figure 2. Two-way analysis of variance revealed that the interaction

between independent variables (type of material: OrthoMTA or RetroMTA; and type of medium: blood, PRF, or PBS) was not statistically significant (P=0.396). Groups showed statistically significant differences regarding the type of material and the type of medium (both P<0.001).

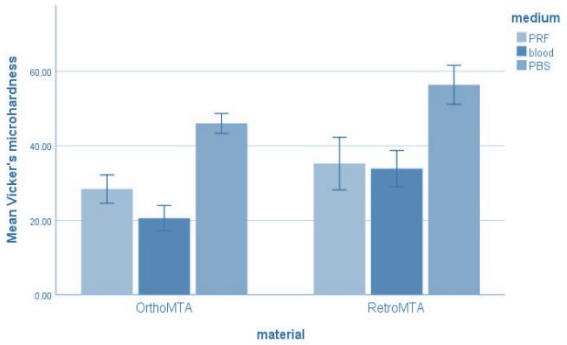

Fig 2. The mean and standard deviation of the surface microhardness of the groups

The results showed that exposure to blood and PRF significantly decreased the surface microhardness of OrthoMTA and RetroMTA. Microhardness of PBS-contacted cements was significantly higher than that of blood or PRF groups (P<0.001). The microhardness values for OrthoMTA exposed to PRF were significantly higher than the blood group (P=0.020). There were no significant differences between RetroMTA contacted with blood or PRF groups (P=0.985).
Regarding the type of material, the findings of this study showed that the surface microhardness of RetroMTA and OrthoMTA did not differ significantly in PRF groups (P=0.106). However, in samples contacting blood or PBS, RetroMTA had a significantly higher microhardness than OrthoMTA (P<0.001 for blood, P=0.002 for PBS).

DISCUSSION

In the present study, the Vickers hardness test was used to assess the effect of exposure to human blood and PRF on the surface microhardness of two types of HCSCs. The microhardness of MTA is affected by various factors, including setting conditions, the thickness of the material, condensation pressure, pH, and acid etching of the material [30].
In the process of RET, one surface of bioceramic material is in contact with blood or platelet concentrates as scaffold, and moist cotton is placed on the other surface to provide the humidity required for the setting of cement. So, we used a two-sided contact system to simulate this clinical condition. Phosphate-buffered saline (PBS) serves as a simulated tissue fluid that replicates clinical conditions in laboratory studies and was considered as the control group in the current research. [31].

In some clinical situations, HCSCs come into contact with blood. This study showed that regardless of the HCSC, blood and PRF groups exhibited reduced microhardness compared to PBS groups. Although no previous study has investigated the effect of PRF on the physical properties of HCSCs, the findings of the current study are in agreement with those of the previous study which showed the detrimental effect of blood on the surface hardness and crystalline structures of RetroMTA and OrthoMTA [25]. Furthermore, it has been revealed that the physical properties of unset HCSCs are compromised when they expose to blood [24,32,33]. Blood contamination adversely alters the microstructure and hydration behavior of HCSCs [24,33] which might be the main reason for the impairment of the physical properties of these materials. In addition, it has been shown that when set in fetal bovine serum (FBS), The surface of MTA exhibited a homogeneous distribution of chemicals and a relatively smooth globular appearance. [34] indicating disruption of the hydration process, which might explain insufficient hardness of MTA when exposed to FBS [35]. Blood plasma contains the cells and proteins that can adsorb to HCSCs [36], especially when the cements are fresh or quite unset, which might retard HCSCs hydration process by blocking the pores within the cements. Reduced water concentration induced by blood contamination during the setting of HCSCs might also prevent the hydration process of HCSCs [25,36]. PRF contains platelets and leukocytes (LPRF) as well as several proteins that might play similar a role to blood in the impairment of hydration behavior of HCSCs resulting in

decreased surface microhardness.

In the present study, RetroMTA exposed to blood showed significantly higher surface microhardness than blood contacted OrthoMTA. This finding might be attributed to the shorter initial setting time of RetroMTA. Previous studies also showed that HCSCs with reduced setting time and those mixed with antiwashout gel were not adversely affected by exposure to FBS compared to cements with long setting time [35,37]. It has been suggested that surface solidification of fast-setting HSCCs might result in a reduced amount of exposure to blood [25]. However, in the present study, there was no significant difference between the microhardness of RetroMTA and OrthoMTA when exposed to PRF.

Considering the higher surface microhardness of RetroMTA in contact with blood, it might be suggested that blood clot within the root canal formed by stimulation of the periapical tissues in RET is better to be sealed with RetroMTA. In this study, the effect of PRF or blood on the setting reaction of MTA was considered. Therefore, the microhardness of the exposed surface of the material to blood or PRF was investigated. Other properties of blood- or PRF-exposed MTA such as dislocation resistance under the possible load applied for filling the access cavity might be impaired. Regarding the probable alterations in the other surface of materials which is not in contact with blood or PRF in endodontic regeneration, it is better to assess the effect of clinical load or pressure on the other surface that is not exposed to PRF or blood using push-out bond strength test.

Furthermore, as the role of HCSCs especially those that contain BO in tooth discoloration has been revealed by several studies, RetroMTA which contains zirconia complex as the radiopacifier in its constitution is a more suitable material compared to BO-containing OrthoMTA, especially in the esthetic area. Although this study showed no significant difference between the surface microhardness of OrthoMTA and RetroMTA when coming into contact with PRF, in order to reduce tooth discoloration, RetroMTA suggests to be applied on the top of PRF. It is worth mentioning that in clinical situations, several factors play important role in the final outcome of RET. Therefore, further clinical trials are needed to confirm the results of this study.

CONCLUSION

Under the conditions of this in vitro study, it could be concluded that exposure to blood or PRF significantly decreased the surface microhardness of both cements. Blood contaminated RetroMTA showed significantly higher surface microhardness than OrthoMTA contacted with blood. No significant difference was found between PRF contacted OrthoMTA and RetroMTA.

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CONFLICT OF INTEREST STATEMENT

None declared.

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