Evaluation and Comparison of Occurrence of Tooth Discoloration after the Application of Various Calcium Silicate–based Cements: An Ex Vivo Study

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Abstract

Introduction: Biodentine (Septodont, Saint Maur des Fossés, France), OrthoMTA (BioMTA, Seoul, Korea), and EndoSequence Root Repair Material (ERRM; Brasseler, Savannah, GA) have been developed to overcome the shortcomings of mineral trioxide aggregate (MTA). The purpose of this study was to compare tooth discoloration after the application of ProRoot MTA (Dentsply Tulsa Dental Products, Tulsa, OK) and 3 recently introduced calcium silicate–based cements in the presence and absence of blood. Methods: In total, 104 human anterior teeth were prepared; 96 were randomly divided into 2 groups (blood and saline contamination). Each group was subdivided into 4 experimental subgroups (n = 12) of ProRoot MTA, Biodentine, OrthoMTA, and ERRM that were used to fill the pulp chambers. The remaining 8 teeth served as the saline and blood groups. Color analysis of tooth crowns was performed using a spectroradiometer before the application of materials and at 24 hours, 1 month, and 6 months after application. Repeated measures analysis of variance was used to evaluate the effects of blood, material, and time on color change (ΔE*). Results: Tooth color change in all experimental groups increased over time (P < .05). Blood contamination significantly increased ΔE* (P < .05), but no significant difference occurred between the 4 groups in this respect in the presence of blood. However, in the absence of blood, the ΔE* of Biodentine and ERRM was significantly less than that of OrthoMTA (P < .05). Conclusions: There was no significant difference between tooth discolorations with materials in the presence of blood. However, in the absence of blood, Biodentine and ERRM exhibited less tooth discoloration than OrthoMTA. (J Endod 2016;42:140–144)

Key Words

Biodentine, calcium silicate–based cements, EndoSequence Root Repair Material, mineral trioxide aggregate, tooth discoloration

Several studies have reported undesirable postoperative tooth discoloration after the use of mineral trioxide aggregate (MTA) (1–9). MTA is mainly composed of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, and bismuth oxide (10, 11). Bismuth oxide, the radiopacifier of MTA, has been suggested as the cause of the discoloration (8, 12, 15); however, other metal oxides such as iron, aluminum, and magnesium oxides present in MTA have also been implicated as the source of the discoloration (7). In addition, the interaction of MTA slurry with blood during its hydration may contribute to discoloration (4, 6, 14).

MTA-like materials possess biological properties similar to those of MTA (15–17), such as Biodentine (Septodont, Saint Maur des Fossés, France), OrthoMTA (BioMTA, Seoul, Korea), and EndoSequence Root Repair Material (ERRM) (Brasseler, Savannah, GA). The manufacturers claim that their products overcome the shortcomings of MTA including possible tooth discoloration. The powder of Biodentine contains tricalcium silicate, calcium carbonate, and zirconium oxide as the radiopacifier. The liquid contains calcium chloride to accelerate the setting reaction (16, 17). Tooth discoloration has not been reported after the use of Biodentine.

BioMTA is supplied in 2 forms: OrthoMTA and RetroMTA (BioMTA, Seoul, Korea). The composition of OrthoMTA is similar to that of ProRoot MTA (Dentsply Tulsa Dental Products, Tulsa, OK) except for the exception of the lack of calcium sulfate (18). The discoloration potential of RetroMTA but not OrthoMTA has been assessed (7). ERRM is composed of calcium silicate, zirconium oxide, tantalum oxide, calcium phosphate monobasic, and filler agents (19). It is produced in a premixed state as moldable putty in preloaded syringes (15, 20). There are no data on tooth discoloration produced by ERRM.

The present ex vivo study compared the degree of coronal tooth discoloration of extracted human anterior teeth after the application of tooth-colored ProRoot MTA, OrthoMTA, Biodentine, and ERRM putty in the presence and absence of blood.
Materials and Methods

The study was approved by the ethics committee of Tehran University of Medical Sciences, Tehran, Iran (No. 26197). One hundred four human single-rooted permanent anterior teeth that were extracted because of advanced periodontal disease were selected. The teeth were sound with no coronal restoration, caries, or cracks. For disinfection, they were immersed in 5.25% sodium hypochlorite solution for 1 hour and then stored in normal saline solution until use.

Preparation of Specimens

Extrinsic debris and stains were removed using an ultrasonic scaler (Varios 970; NSK, Kanuma-shi, Tochigi, Japan) followed by polishing with pumice paste and water. For creating standardized specimens, the apical part of each root was removed perpendicular to its long axis with a high-speed diamond fissure bur (#010) (Tizkavan, Tehran, Iran) under a continuous water spray until 5 mm of root remained. Endodontic access cavities were then prepared, and the shortened root canals were cleaned and shaped using #1 to 6 Gates Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland) and irrigated with 5.25% sodium hypochlorite followed by 17% EDTA for 1 minute. In the 96 experimental teeth, a customized cylindrical piece of plastic white foam was inserted into the root canal through the apical opening up to the cementoenamel junction of the labial surface. The apical opening of the root canals and the total surrounding dentin of the cross-sectional surface were treated with 37% phosphoric acid for 15 seconds and then rinsed. The bonding agent (3M ESPE, St Paul, MN) was applied and light cured for 20 seconds. After that, composite resin material (Filtek Z350, 3M ESPE) was placed incrementally and cured using a light-curing instrument (Valo; Ultradent Products Inc, South Jordan, UT) for 40 seconds (Fig. 1A and B).

Blood Collection

Whole fresh human blood was collected from a healthy, consenting volunteer member of the research group by a trained individual in accordance with Helsinki ethical principles for medical research involving human subjects (21) and approved by a panel from the Tehran University of Medical Sciences Ethical Committee (No. 26197).

Experimental Setup

The experimental teeth were randomly divided into 2 groups. The inserted foam in each specimen was then saturated with fresh human blood (group 1) or normal saline solution (group 2), respectively, using an insulin syringe to prevent contamination within the access cavity. The specimens of each experimental group were then randomly assigned to 1 of 4 experimental subgroups (n = 12) and labeled according to the following applied materials: ProRoot MTA, Biodentine, OrthoMTA, and ERRM putty. Each material was prepared according to the manufacturers’ instructions. A 3-mm increment of each material was then placed inside the access cavities using an MTA gun (Medesy, Maniago, Italy) and a flat plastic instrument used with minimal pressure to ensure contact with the coronal surface of the plastic foam insert. A normal saline-wetted cotton pellet was then placed over the materials, and the cavity was temporarily restored with Coltosol (Coltene, Altstätten, Switzerland). The specimens were then incubated at 37°C in fully saturated humidity for 24 hours. Subsequently, the access cavity was then filled with 2 ± 0.2-mm-thick composite resin material, A3 shade (Filtek Z350, 3M ESPE) as described for sealing the apical opening (Fig. 1B).

In the remaining 8 teeth, the customized plastic foam was placed up to beyond the level of the cementoenamel junction of the labial surface and then saturated with normal saline (n = 4) or blood (n = 4), and the access cavity was filled with composite resin material.

Color Assessment

For reproducible tooth positioning, the specimens were mounted in rectangular acrylic resin blocks. A rectangular window measuring 2 × 3 mm was created at the interface of the cervical and middle thirds of the crowns using a needle-shaped diamond bur in such a way that two thirds of its height was in the cervical and one third was in the middle third of the tooth crown (Fig. 1A).

The color of the crowns was measured in a dark room under standard conditions using a Konika CS2000 spectroradiometer (Minolta, Osaka, Japan) with a wavelength range of 380–780 nm and a wavelength accuracy of 1 nm. The light source illuminated the surface of the specimens at an angle of 45° from the vertical axis. The spectroradiometer was positioned at an angle of 0° relative to the vertical axis with an approximately 70-cm distance from the specimen surface. Color measurement was performed in 3 points inside the marked window at 4 time intervals:

1. Before the application of materials
2. 24 hours after the application of materials
3. 1 month after the application of materials
4. 6 months after the application of materials

The mean value of 3 measurements was calculated at each time interval. The color change (∆E) of each specimen was calculated using the following equation (22):

\[ \Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \]

where the L* parameter indicates lightness and ranges in value from 0 (black) to 100 (white), a* indicates greenness/redness (negative...

Figure 1. An illustration of the experimental setup. (A) Coronal view and (B) sagittal view.
values indicate green and positive values indicate red), and b* indicates blueness/yellowness (negative values indicate blue and positive values indicate yellow).

**Statistical Methods**

Repeated measures analysis of variance was used to evaluate the effects of 3 factors (blood contamination, type of material, and incubation time) on \( \Delta L^* \) and \( \Delta E^* \). If there was any significant interaction, appropriate analysis in each group (ie, 1-way analysis of variance followed by post hoc tests or an independent student \( t \) test) was performed. The level of statistical significance was set at 0.20 and 0.05 for the evaluation of interactions and for the main effects, respectively.

**Results**

Means and standard deviations of the color changes (\( \Delta E^* \)) and luminosity (\( \Delta L^* \)) at each time period are shown in Figures 2 and 3.

\( \Delta E^* \)

Blood contamination significantly increased the \( \Delta E^* \) of materials adjusted for the incubation time and type of material (\( P < .05 \)). Irrespective of the type of material and the presence of blood, the correlation of time and \( \Delta E^* \) was significant (\( P < .05 \)).

In the absence of blood, the color change of Biodentine and ERRM putty was significantly less than that of OrthoMTA (\( P < .05 \)). However, in blood-contaminated groups, no significant difference was noted among the 4 materials.

After 6 months, among experimental groups, a significantly greater color change occurred in blood-contaminated OrthoMTA, whereas a significantly smaller color change was noted in Biodentine followed by ERRM in the absence of blood (\( P < .05 \)).

\( \Delta L^* \)

In all experimental groups, \( \Delta L^* \) decreased significantly over time (\( P < .05 \)). Irrespective of the type of material and time, \( \Delta L^* \) significantly decreased in blood-contaminated specimens (\( P < .05 \)).
After adjustment for time and blood contamination, a comparison of materials revealed that the ΔL* was significantly different (P < .05). The ΔL* of Biodentine was significantly lower than that of OrthoMTA and ProRoot MTA (P < .05), but pairwise comparisons of other materials revealed no significant differences.

Discussion

The goal of the present ex vivo study was to compare the degree of coronal tooth discoloration after the application of a currently available materials for vital pulp therapy (i.e., ProRoot MTA, OrthoMTA, Biodentine, and ERRM putty) in the presence and absence of blood.

Extracted human teeth were used, and access cavities were filled with composite resin after the application of the materials after their setting was ensured. In some studies, access cavities were not prepared, and materials were placed in the pulp chamber via the root canal (6, 7). Obviously, not preparing an access cavity is unlike the normal clinical situation and makes it difficult to completely eliminate pulpal remnants from the pulp chamber and pulp horns. Also, Marciano et al (8) reported that composite resin did not play a role in the process of discoloration caused by calcium silicate–based cements.

In the present study, fresh human whole blood was used to assess the effect of blood on tooth discoloration after the use of materials. To assess crown discoloration by materials in the presence of blood, a model was designed that simulated the clinical situation of vital pulp therapy, whereas in several previous studies, MTA-like materials were placed over root canals filled with gutta-percha (5, 9).

In the current study, a spectroradiometer with an external light source was used for color analysis. Spectroradiometers and spectrophotometers measure the reflection coefficient of light within the visible spectrum wavelength. In contrast to a spectroradiometer, a spectrophotometer primarily has a stable light source, and an aperture usually exists between the detector and object (23). It has been shown that color measurement tools that possess an aperture between a translucent object, light source, and receptor result in loss of edges during calculations. This phenomenon must be prevented when accurate color measurement of translucent objects such as teeth and porcelain needs to be performed. This can be achieved by the combined use of an external light source not causing a shadow via a spectroradiometer (23).

The present results revealed that blood contamination significantly increased discoloration associated with all materials. This finding is in accordance with the results reported by Lenherr et al (4). They showed that Portland cement–based materials such as white and gray ProRoot MTA and Portland cement were associated with greater color changes after blood contamination (4). Also, it has been shown that blood contamination of white ProRoot MTA increases its degree of discoloration (6). The exact mechanism for intensified discoloration of calcium silicate–based materials is not fully understood. One possible mechanism is the penetration of erythrocytes into the tooth structure (6). Also, it has been shown that porosities in calcium silicate–based materials may entrap blood components and cause discoloration of the material (4, 24). This has a clinical significance because calcium silicate–based materials are usually in direct contact with living vascular tissue; therefore, these materials must be used only after achieving complete hemostasis to decrease such discoloration.

Aside from blood contamination, the structure and composition of a material are among the important factors determining its discoloration potential. The resultant discoloration depends on metal constituents such as bismuth, iron, aluminum, and magnesium. One possible mechanism of tooth discoloration by white MTA is related to the oxidation of the iron content remaining in the set material, which belongs to the calcium aluminoferrite phase of the powder (6). Regarding the mechanism of discoloration by bismuth oxide, it has been stated that oxidation of this material destabilizes the oxygen in its formulation, which reacts with carbon dioxide and produces bismuth carbonate that causes discoloration (7, 15). Another theory is the interference of bismuth oxide with dentin collagen (8). Yet, another possible interference is between bismuth oxide and sodium hypochlorite if present on dentin (25). This theory is supported by Lenherr et al (4), who reported that the discoloration caused by Portland cement (which is devoid of bismuth oxide) was much lower than that caused by both types of MTA.

Materials in which bismuth oxide is replaced by other constituents such as zirconium oxide and tantalum oxide have been incorporated into the formulations of several new calcium silicate–based materials. Kang et al (7) reported that discs made of ProRoot MTA and MTA Angelus (Angelus, Londrina, PR, Brazil) containing bismuth oxide and bismuth oxide powder caused significant color change, whereas RetroMTA (containing calcium zirconia complex), ENDOCEM Zr (MARUCHI, Wonju, Korea) (containing zirconium oxide), and zirconium oxide powder were not associated with color changes. Also, in their study, tooth discoloration by RetroMTA and ENDOCEM Zr was significantly less than that associated with ProRoot MTA and MTA Angelus, whereas materials containing zirconium oxide caused less discoloration than materials containing bismuth oxide (7). In agreement with the results of Kang et al (7), the current study revealed that in the absence of blood, discoloration caused by OrthoMTA (containing bismuth oxide) over 6 months was significantly greater than that caused by materials containing zirconium oxide (Biodentine) and tantalum oxide (ERRM putty), and no significant difference was noted in this regard between OrthoMTA and ProRoot MTA, which have an almost similar composition. Tetracalcium aluminoferrite and bismuth oxide are present in the composition of OrthoMTA and ProRoot MTA, whereas Biodentine and ERRM putty do not contain bismuth. To date, the science of dental materials has mainly focused on the function and performance of materials, but from now on, emphasis must be placed on the ideal characteristics of materials, particularly their esthetic properties.

In the present study, some degree of tooth color change was seen in the normal saline group in the absence of calcium–silicate based material. We think tooth discoloration in the normal saline group can be attributed to some possible factors rather than contamination. The procedures for filling of the apical opening and access cavity were performed according to the manufacturer’s instruction of composite resin materials. Because of the absence of any experimental calcium–silicate based materials, the access cavities in the blood and normal saline groups were restored with a greater thickness of composite resin material compared with the experimental groups. The presence of composite resin per se, even in the absence of leakage, changes the color parameters recorded by a spectroradiometer. It has been stated that filling the chamber completely with composite resin may cause loss of translucency (26). Furthermore, it should be noted that the normal saline and blood groups in our study did not serve as positive and negative control groups; instead, they were included to assess the degree of tooth color change in the absence of calcium silicate–based materials. However, because this study, similar to the other published articles in the literature, aimed to compare the recorded discoloration caused by different calcium silicate–based materials, it was logical to make a comparison among the experimental groups in the presence and absence of blood. In addition, because of the different setup of the normal saline and blood groups compared with those of experimental ones, the findings obtained for the normal saline and blood groups were not statistically analyzed.
Acknowledgments

Supported by Tehran University of Medical Sciences (grant no. 26197).

The authors deny any conflicts of interest related to this study.

References