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Original Article

Tooth discoloration effects of calcium silicate based barrier materials used in revascularization and treatment with internal bleaching

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Abstract *Background/purpose:* Usage of barrier materials is an important step in revascularization procedure. One of the undesired properties of these barrier materials is to cause coronal tooth discoloration. The aim of this *in vitro* study was to evaluate the tooth discoloration induced by ProRoot MTA (PMTA), Biodentine, and MM-MTA, as well as the efficacy of internal bleaching on this discoloration.
Materials and methods: Forty-two maxillary incisor teeth were prepared. Triple antibiotic paste (TAP) was placed in the root canals and incubated for 3 weeks. After removing the TAP, blood embedded spongostans were inserted into the root canals, and PMTA, Biodentine, or MM-MTA was placed over them. The teeth were incubated for 4 weeks at 37 °C; then, the internal bleaching agent was sealed for one week. The tooth color was measured throughout the study and the color change values (ΔE) of each specimen were calculated, and the data was statistically analyzed using the one-way ANOVA and Tamhane's T2 tests.
Results: The TAP significantly decreased the luminosity of the teeth ($p < 0.05$); however, no significant differences were observed between the tooth discolorations induced by the PMTA, Biodentine, and MM-MTA ($p > 0.05$). The teeth in the Biodentine group were more whitened than those of the PMTA and MM-MTA groups ($p < 0.05$).

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Conclusion: Although the PMTA, Biodentine, and MM-MTA caused similar color alterations in the teeth, more bleaching was observed on those teeth discolored using TAP + blood + Biodentine.

Introduction

Revascularization is a regenerative procedure which has become an alternative to apexification for the treatment of immature permanent teeth. Basically, revascularization consists of the disinfection of the root canal with antibiotic paste, creating bleeding into the root canal system, and followed by the placement of a barrier material over the blood clot. One of the major drawbacks of this procedure is the tooth discoloration that can be induced by the antibiotic paste,[1] blood,[2] or barrier material.[3,4]

Mineral trioxide aggregate (MTA) has been widely used in revascularization procedures due to its biocompatibility and good sealing properties.[5,6] However, its poor handling characteristics, long setting time, and potential discoloration effects are the major disadvantages of using MTA. The metal oxide content of MTA has been reported to induce discoloration;[7,8] therefore, Biodentine has recently been proposed as an alternative to MTA for clinical applications like vital pulp therapy, perforation repair, root-end filling, apexification, and revascularization. Biodentine has been shown to possess bioactive and biocompatible properties;[9] however, little information is available regarding the discoloration effects of Biodentine.[10–13] This limited amount of data has indicated that Biodentine does not cause meaningful color changes in teeth. Additionally, MM-MTA is a new commercial tricalcium silicate based cement that, according to the manufacturer, has been reported to have a reduced setting time and better physiochemical characteristics. To the best of the authors' knowledge, the literature shows no published studies evaluating the discoloration effects of MM-MTA as a barrier material.

Endodontically treated and discolored teeth can be whitened by using oxidizing agents. Among these agents, hydrogen peroxide which can easily penetrate into the enamel and dentine, has become popular and effective for internal bleaching techniques. The application of internal bleaching to teeth discolored after vital pulp therapy and revascularization has been previously demonstrated.[3,14]

The aim of this *in vitro* study was to evaluate the tooth discoloration induced by ProRoot MTA (PMTA) (Dentsply, Switzerland), Biodentine (Septodont, France), and MM-MTA (Micro-Mega, France) in the presence of blood, and to assess the whitening effects of hydrogen peroxide on these discolored teeth.

Materials and methods

The study protocol of this research was reviewed and approved by the Ethics Committee of the Necmettin Erbakan University, Faculty of Dentistry (Protocol no. 2015/006).

Specimen preparation

Forty-two maxillary central and lateral incisors extracted for periodontal reasons, without caries, cracks, previous endodontic treatment, or coronal restoration, were collected for use in this study. The teeth were stored in a physiological saline solution for disinfection until further processing, the calculus was removed using periodontal cretuars, and each specimen was polished. The study protocol of this research was carried out following the methods of Shokouhinejad et al.,[2] with minor modifications. The apical parts of the roots were removed with a water cooled diamond disc, leaving a root segment with a length of 8 mm, while the endodontic access cavities were prepared and root canals were enlarged with #1 through #5 peeso reamers (Mani Inc., Tochigi, Japan). The root canals were then flushed with 17% EDTA (Imicryl, Konya, Turkey), followed by 2.5% sodium hypochlorite (NaOCl) (Caglayan Kimya, Konya, Turkey), for 5 min each. Finally, the apical openings of the roots were closed using composite resin. The tooth color was measured at the baseline (t_0) by using a spectrophotometer (VITA Easyshade Advance; Vita Zahnfabrik, Bad Sackingen, Germany).

The triple antibiotic paste (TAP) used in this study was prepared by mixing antibiotic powders compounded of equal portions of metronidazole (Nidazol, 500 mg film tablet, Ulagay, Istanbul, Turkey), ciprofloxacin (Cipro, 500 mg film tablet, Biofarma, Istanbul, Turkey), and cefaclor (Sanocef 750 mg film tablet, Actavis, Istanbul, Turkey), with sterile distilled water (1000 mg powder/1 mL distilled water). TAP was applied to the enlarged root canals with a Lentulo spiral. A cotton pellet was placed over the TAP, and the access cavities were restored with Cavit (3M ESPE, St Paul, MN, USA). The specimens were then incubated for 3 weeks at 37 °C in 100% humidity. After the incubation period the temporary restoration was removed, the root canals were irrigated with 5 mL of 2.5% NaOCl for the removal of the TAP,[1] and the tooth color was measured (t_1). The teeth were then randomly divided into 3 experimental groups ($n = 14$).

For this study, fresh human blood was collected from a volunteer member of the research group; then, spongostans were cut into slices, immersed in the blood, placed into the root canals of all of the samples, and condensed up to the cementoenamel junction. PMTA, Biodentine, or MM-MTA was placed over the blood embedded spongostans, moistened cotton pellets were put over the materials, and the teeth were temporarily restored. The specimens were incubated for 4 weeks at 37 °C, and after the completion of the incubation period, the temporary restorations were removed and the tooth colors were recorded (t_2).

Hydrogen peroxide gel (35%) (Opalescence Endo, Ultradent products, USA) was placed over the coronal barrier

materials, temporary restorations were carried out, and the specimens were incubated for 1 week at 37 °C. After the completion of the bleaching treatment, the temporary restorations were removed, and the last color measurements were performed (t_3).

Color assessment

The tooth color was measured from 3 mm above the cementoenamel junction, and the measurements were performed 3 times for each sample. These results were reported using the CIE L*a*b* color system (International Commission on Illumination), where L* indicates the luminosity (0/black—100/white), a* represents the red—green parameter, and b* represents the yellow—blue parameter (Fig. 1). In addition, ΔE_1 describes the color difference between the time points: t_1 and t_2 (after blood + material application), whereas ΔE_2 indicates the color difference between the time points: t_2 and t_3 (after bleaching). The ΔE values were calculated using following formula:

$$\Delta E = \left[(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2\right]^{1/2}$$

Scanning electron microscopy (SEM)

One disc shaped sample (6-mm diameter and 2-mm thickness) from each test material was prepared according to the manufacturer's instructions. The samples were fixed in a 2.5% glutaraldehyde solution, and then evaluated with a scanning electron microscope (SEM) (EVO LS10, Zeiss, Oberkochen, Germany) to illustrate the surface and the structure of the test materials. The SEM micrographs were taken from representative areas at ×5000 magnifications (Fig. 2).

Statistical analysis

The IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used to analyze the results. The normal distribution of the data was confirmed using the Shapiro—Wilks test, and the paired *t*-test was used to determine the differences in the luminosity after the TAP application. The ΔE values were statistically analyzed using the one-way ANOVA and Tamhane's T2 test, with the significance level set at $p < 0.05$.

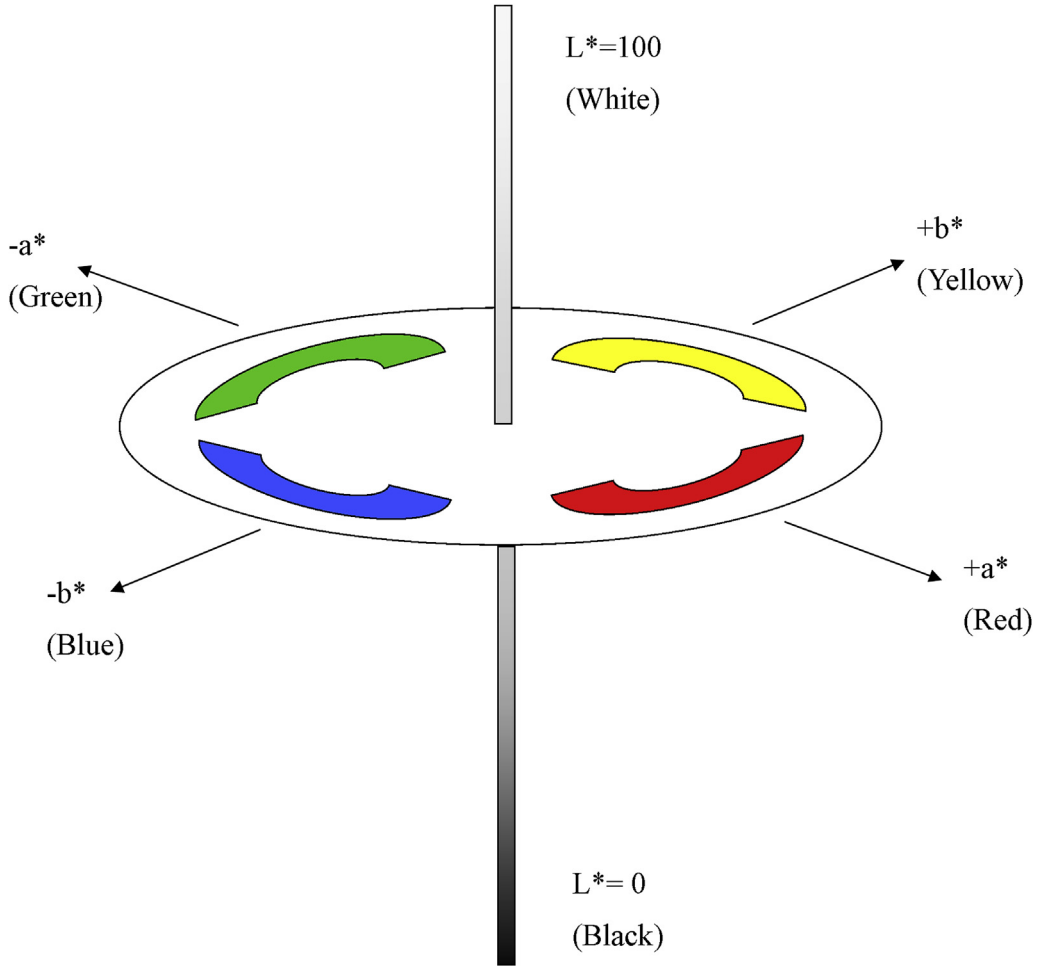

Fig. 1 Illustration of the CIE (International Commission on Illumination) L*a*b* color space.

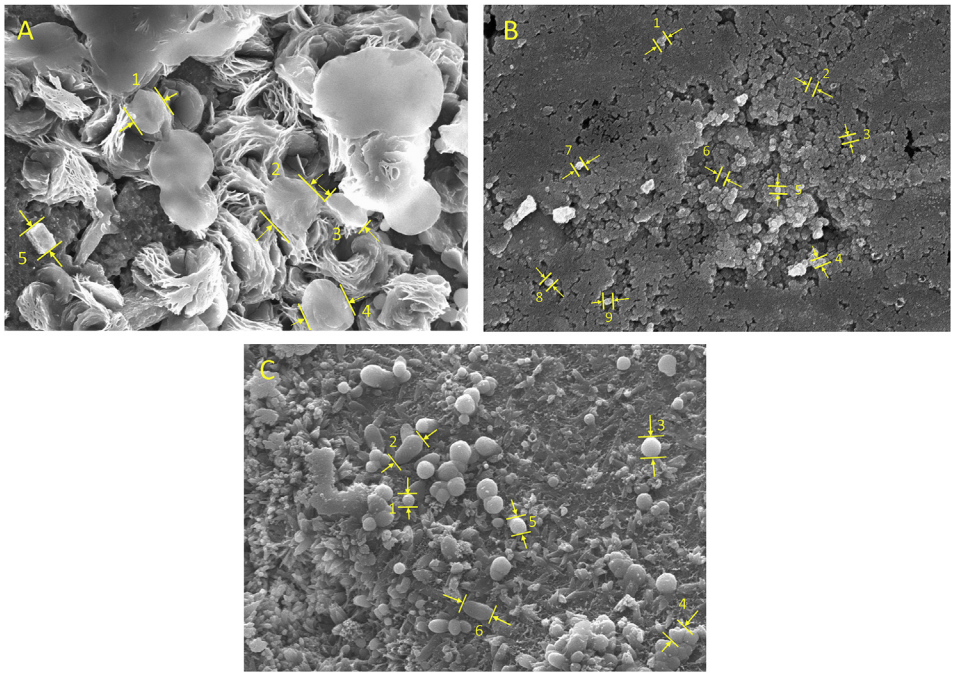

Fig. 2 Representative SEM micrographs of surfaces of PMTA (A), Biodentine (B) and MM-MTA (C) (magnification = 5000×), showing the sizes of randomly selected particles (between yellow arrows) (PMTA, A1: 4.78 μm, A2: 6.83 μm, A3: 5.92 μm, A4: 5.93 μm, A5: 4.14 μm; Biodentine, B1: 559 nm, B2: 377 nm, B3: 289 nm, B4: 401 nm, B5: 378 nm, B6: 323 nm, B7: 562 nm, B8:453 nm, B9: 437 nm; MM-MTA, C1: 2.02 μm, C2: 4.54 μm, C3: 2.54 μm, C4: 2.32 μm, C5: 2.33 μm, C6: 4 μm)

Results

All of the experimental groups indicated discoloration on their buccal surfaces (Fig. 3), and the luminosity values of the test groups at each time period are shown in Fig. 4. The means and standard deviations of the color changes (ΔE_1 and ΔE_2) are shown in Table 1. Overall, the TAP with cefaclor significantly decreased the luminosity of the teeth ($p < 0.05$); however, no significant differences were observed between the tooth discolorations induced by the PMTA, Biodentine, or MM-MTA ($p > 0.05$). The hydrogen peroxide gel more significantly whitened the discoloration caused by the Biodentine than the discoloration caused by the MM-MTA and PMTA ($p < 0.05$), although there was no statistically significant difference between the whitening in the MM-MTA and PMTA groups ($p > 0.05$).

Discussion

The root model used in the present study was modified from the report by Shokouhinejad et al.,[2] in which this model was used for vital pulp therapy. The experiment was conducted by applying TAP, blood, and barrier materials for all of the specimens to simulate the clinical conditions, and each specimen had its own control. Therefore, the discoloration effects of the TAP, blood, and material were not assessed separately. Instead, the discoloration after the combination treatment, and the differences among the barrier materials were evaluated.

This study has confirmed that TAP can discolor *in vitro* teeth. The first TAP introduced for bacterial disinfection contained metronidazole, ciprofloxacin, and minocycline,[15] and the minocycline that binds to the calcium of hydroxyapatite has been reported to be the main cause of that discoloration.[16] In the present study, the minocycline was replaced with cefaclor to diminish the discoloration. However, the results of this research have demonstrated that TAP with cefaclor decreased the luminosity of the teeth, which coincides with the findings of previous studies.[17] The discoloration mechanism of cefaclor is still unknown. Based upon a clinical revascularization scenario without TAP could not be carried out, we decided to place TAP into the canals of all samples despite its possible masking effects for the materials discoloration.

The findings of the current study indicated that the blood contaminated PMTA, Biodentine, and MM-MTA caused similar color changes, which is in agreement with a recent report[2] showing no differences between calcium silicate based cements in the presence of blood. The main difference occurred in the absence of blood, indicating that Biodentine displayed less of a color change.[2] Additionally, Felman and Parashos[7] revealed that blood contact with the mineral trioxide aggregate deepened the discoloration. However, the presence of blood was a constant parameter of the present study, so the effects of the blood contamination alone on the degree of discoloration could not be evaluated. It is worth noting that blood contamination is inevitable in the revascularization procedure.

The lysis of red blood cells results in blood disintegration products, such as iron containing hemoglobin and hematin derivatives, and these products infiltrate into the dentine tubules and lead to discoloration.[18] On the other hand, blood can show its discoloration effects by penetrating into

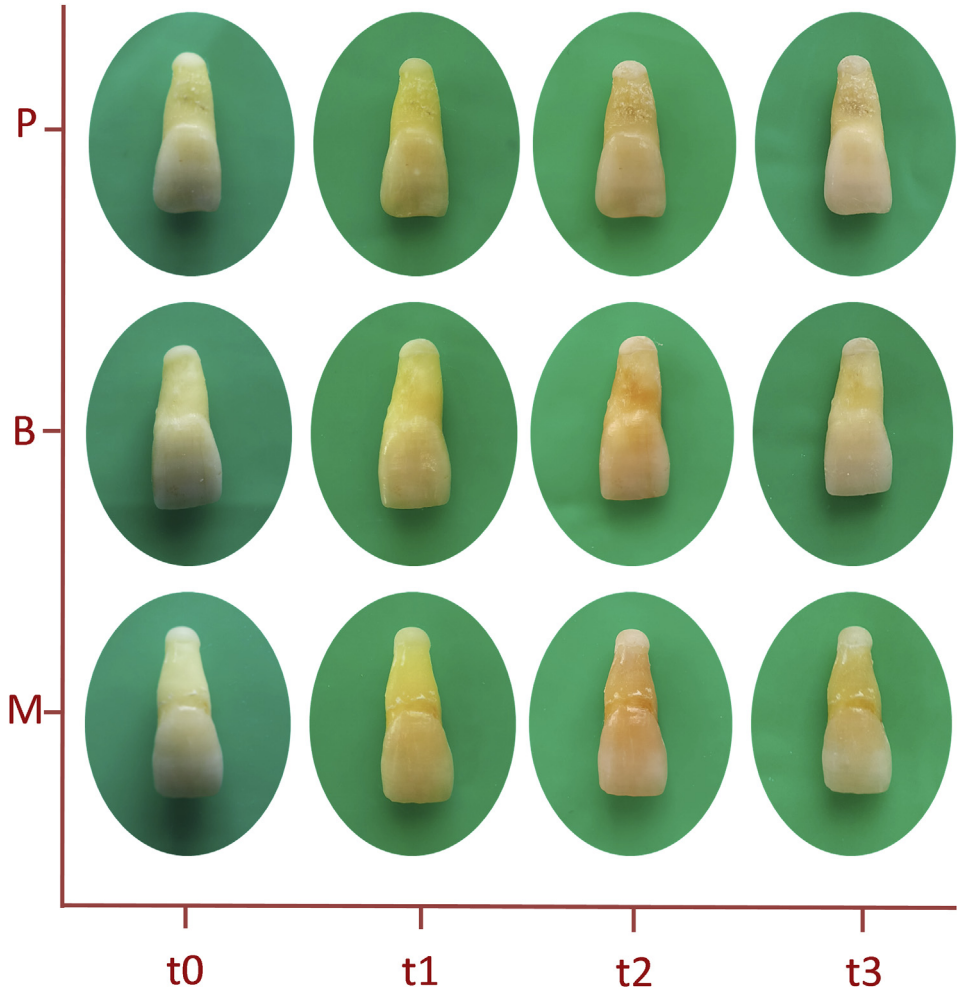

Fig. 3 Representative images of the specimens in experimental groups (P: ProRoot MTA; B: Biodentine; M: MM-MTA) at different time points (t_0, baseline; t_1, three weeks after applying TAP; t_2, four weeks after applying blood + material and t_3, one week after bleaching), showing the pattern of discoloration and bleaching.

the gaps in the cement materials, which have different porosity properties. PMTA and Biodentine have been reported to show similar porosity values,[19] whereas PMTA revealed larger pores than the MM-MTA.[20] However, because all of the tested materials induced equal discoloration of the teeth, it might be speculated that the pore size of the material does not play a significant role in the degree of discoloration.

Tooth discoloration could also be attributed to the components of the barrier material; for example, the metallic contents of calcium silicate based cements, such as bismuth, aluminum, magnesium, and iron, could promote discoloration.[8,21] However, although calcium silicate

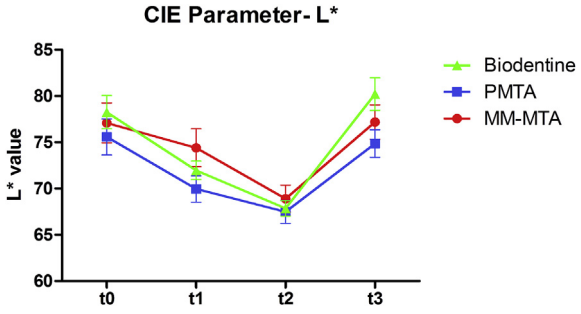

Fig. 4 CIE Parameter-change in the L* value (mean) of experimental groups measured at t_0, t_1, t_2 and t_3. L* value indicates the luminosity (0/black—100/white).

Table 1 Means and standard deviations of color change values after blood + material application (ΔE_1) and after bleaching (ΔE_2).

	PMTA (Mean ± SD)	Biodentine (Mean ± SD)	MM-MTA (Mean ± SD)	p value
ΔE_1	4.98 ± 3.35[a]	6.84 ± 3.34[a]	7.58 ± 2.83[a]	0.096
ΔE_2	8.33 ± 3.89 [b]	14.23 ± 6.88[a]	9.39 ± 3.87 [b]	0.009*

SD, standard deviation. *$p < 0.05$. Different lowercase letters represent statistically significant differences between the test materials (one-way ANOVA and Tamhane's T2 test, $\alpha = 0.05$).

based materials include staining components, blood is the most likely reason for tooth discoloration, despite the barrier material, according to the findings of the current study.

Hydrogen peroxide (35%) was chosen as an internal bleaching agent in the present study due to its previous success in whitening.[17] Overall, the teeth in the Biodentine group were more bleached than the specimens in PMTA and MM-MTA groups, which could be related to the particle size of the material. Different particle sizes affect the diffusion of the calcium silicate based material into the dentinal tubules[22] and a finer particle size improves the manipulation characteristics of the material, and also reduces the setting time.[23] Moreover, a finer particle size provides the material with a greater surface area. One previous study revealed that Biodentine consists of finer particle sized tricalcium silicate, and has a high specific surface area.[24] This increased surface area may contribute to the bleaching agent being more effective, since the activated hydrogen peroxide could more easily oxidize the darkened compounds.

Another explanation for the greater whitening in the Biodentine group could be the composition of the material. Bismuth oxide is the radiopacifying agent used in MM-MTA and PMTA, while Biodentine includes zirconium oxide instead. Previously, it has been shown that calcium silicate based cements containing bismuth oxide demonstrate more discoloration than zirconium oxide containing cements,[21] contributing to more improvement of discoloration of teeth in Biodentine group.

Our *in vitro* results suggest that blood is the main causative factor for tooth discoloration, rather than the barrier material. Unlike vital pulp therapies, hemorrhage control is not performed in the revascularization procedure. Contrarily, the stimulation of bleeding is one of the factors which improve clinical success; therefore, preventing this discoloration is not possible in revascularization. Moreover, although the Biodentine group exhibited more favorable bleaching results, the approximate baseline luminosity values were obtained after the intra-coronal bleaching of all of the specimens. Further studies are required to compare the discoloration effects of these three commercially available calcium silicate based cements with longer time parameters.

In conclusion, TAP with cefaclor has been shown to induce discoloration, and all of the blood contaminated calcium silicate based cements exhibited similar color changes. However, when Biodentine is used as a barrier material, more whitening could be obtained with a bleaching treatment.

Conflicts of interest

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

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