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Original Article

In vitro evaluation of coronal discoloration following the application of calcium-enriched mixture cement, Biodentine, and mineral trioxide aggregate in endodontically treated teeth

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ABSTRACT

Background: This study sought to assess and compare coronal discoloration following the application of white mineral trioxide aggregate, Biodentine, and calcium-enriched mixture cement in endodontically treated teeth.

Materials and Methods: In this *in vitro* experimental study, 64 freshly extracted sound human premolars were selected, cleaned, and stored in saline. After cleaning, shaping, and obturation the root canal of the teeth, the teeth were randomly assigned to one control (n = 4) and three experimental (n = 20) groups. In the experimental groups, the cement were applied over the canal orifices in 3-mm thickness. All teeth were then restored with composite resin. Color parameters, according to the CIE L*a*b* system, were measured using Vita Easyshade spectrophotometer before application of cement and at 1 week, 1 month, 2 months, and 3 months after the application of cement. The recorded values were statistically analyzed using descriptive and analytical statistics. For analytical statistics, Kolmogorov–Smirnov test was applied to assess normal distribution of data. ANOVA was used to compare the results at baseline and repeated measures. $P < 0.05$ was considered statistically significant

Results: Significant differences were noted in color change (ΔE) between all time points except between $\Delta E4$ (2 months) and $\Delta E5$ (3 months) ($P < 0.01$). However, tooth discoloration caused by the three cement was not significantly different ($P = 0.343$).

Conclusion: The three tested cement were not significantly different in terms of causing coronal discoloration in endodontically treated teeth.

Key Words: Mineral trioxide aggregate, root canal filling materials, spectrophotometry, tooth discoloration

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INTRODUCTION

Dental pulp is composed of ectomesenchymal connective tissue with unique properties. If for any reason the pulp tissue loses its vitality, new dentin formation is impaired and the tooth becomes highly susceptible to infection.[1] In case of dental pulp injury, depending on the extent of injury and pulp

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vitality status, some measures may need to be taken. Correct clinical judgment and proper treatment planning play a significant role in prognosis and outcome of treatment.[2] If patients are not satisfied with the outcome of treatment, they need to invest more time and money to obtain the desired result.

Several materials have been introduced to dental market to improve the quality of endodontic treatments such as calcium hydroxide,[3] zinc oxide eugenol,[4] different types of mineral trioxide aggregate (MTA) cement,[5] Endocem, Biodentine,[6,7] glass ionomer, and calcium-enriched mixture (CEM) cement.[8] These products can use as orifice obturator and repair the perforation. An ideal endodontic cement must be able to seal root canal communications with the surrounding tissue and should be nontoxic, noncarcinogenic, biocompatible, and insoluble in tissue fluids.[9]

Discoloration of tooth structure secondary to the use of endodontic cement is currently the most common concern with regard to endodontic treatment with a frequency of 31.6%–57%.[10,11] This complication has been documented in many previous studies.[4,5,12] Thus, in selection of an endodontic cement, esthetic considerations must be taken into account in addition to biological and functional properties. This issue is particularly important because tooth discoloration, especially in the esthetic zone, causes patient dissatisfaction and necessitates corrective treatments such as prosthetic restorations or tooth bleaching.[13,14] Thus, clinicians should preferably use endodontic cement with minimal discoloration potential.

MTA is derived from Portland cement and contains small hydrophilic particles, tricalcium silicate, tricalcium aluminate, tricalcium oxide, and some other mineral oxides. It sets in the presence of water, and after setting, it converts to a crystalline gel of calcium silicate hydrate and calcium hydroxide.[6] Furthermore, it contains bismuth in its formulation for radiopacity.[3] MTA is available in two forms of gray and white and was first introduced as a root-end filling material; however, over time, it was used for pulp capping, pulpotomy, apexogenesis, and apical barrier formation in open-apex teeth and root perforations.[9,15]

Some studies have reported shortcomings of MTA to be solubility in the saliva, difficult handling, long setting time, absence of a solvent for it, and causing tooth discoloration.[4,16-19] Discoloration of tooth structure following the application of MTA is a common occurrence.[20-22] Bismuth present in the formulation of white and gray MTA is now believed to be responsible for color change in tooth structure.[16,21,23]

Considering the unfavorable properties of MTA, some other calcium silicate-based cement were introduced. Biodentine is a newly introduced calcium silicate-based cement,[24] which according to the manufacturer, has applications similar to those of MTA and is composed of powder and liquid phases. Biodentine powder is composed of tricalcium silicate, calcium carbonate, and zirconium oxide for radiopacity. Its liquid contains calcium chloride as an accelerator to enhance setting of material and decrease its water content. Biodentine is a fast-setting cement, and it is claimed that it can replace the lost dentin.[20,25] In acidic environments, it has a superior function compared to that of MTA.[20] Moreover, it has easier handling than MTA. However, Biodentine can also cause tooth discoloration.[26] However, some studies have reported less discoloration following the application of Biodentine compared to MTA.[25,27]

CEM cement, recently introduced by Asgary *et al.*,[8] has clinical applications similar to those of MTA but with a different formulation.[28-30] It is claimed that CEM cement is more alkaline than white MTA and thus has superior antimicrobial properties, biocompatibility and sealing ability compared to white MTA. Moreover, it has a shorter setting time and higher flowability compared to MTA.[28,29]

Inspection by the naked eye,[6] photography,[5,12] spectrophotometry,[5,22] and X-ray diffraction analysis[5] have been used for colorimetry in the previous studies. In the current study, color parameters were measured by spectrophotometry since this technique has been approved by the American Dental Association and is the most commonly used technique for this purpose.[10]

Results of previous studies on tooth discoloration caused by CEM cement have been controversial.[8] Previous studies showed that MTA can cause tooth discoloration;[25-27] however, the results of studies on tooth discoloration caused by Biodentine have been controversial. Furthermore, limited studies have evaluated tooth discoloration caused by CEM cement,[28-30] and to the best of authors' knowledge, comparison of CEM cement, Biodentine, and MTA in terms of tooth discoloration has not been previously performed. Thus, it is important to find out which one of these cement causes the least discoloration in endodontically treated teeth.

This study aimed to compare coronal discoloration at varying time points following the application of MTA, CEM cement, and Biodentine in endodontically treated teeth.

MATERIALS AND METHODS

In this *in vitro* study, 64 freshly extracted human premolars with intact crowns were selected. The teeth had been extracted for orthodontic or periodontal reasons, the teeth without any crack, detectable discoloration caries, and restoration were selected and randomly assigned to one control (n = 4) and three experimental (n = 20) groups. Sample size was calculated using PASS 11 software (NCSS LLC Company) according to a previous study by Ioannidis *et al*.[22]

Preparation of teeth

All teeth were inspected to ensure they had sound crowns and were then subjected to scaling and root planning. In the first step, access cavity preparation and working length determination were done. Next, #1, 2, and 3 Gates Glidden (Mani Inc., Japan) drills were passively introduced into the canals. Filing was performed by hand K-files (Mani Inc., Japan) and Mtwo rotary files (VDW, Germany) according to the manufacturer's instructions, and root canals were cleaned and shaped as such. It should be noted that during filing, root canals were frequently irrigated with saline and sodium hypochlorite 0.5%. Prepared root canals were then dried by paper points (Gapadent, Germany) and filled with gutta-percha (Gapadent, Germany) and AH26 sealer (DeTrey, Dentsply, Germany) using lateral compaction technique. Gutta-percha was cut at the orifice level and the teeth were randomly assigned to one control (n = 4) and three experimental (n = 20) groups for application of white MTA (Angelus™, Londrina, Brazil), Biodentine (Septodont, Saint-Maur-des-Fossés, France) and CEM cement (Bionique, Tehran, Iran).

In order to prevent discoloration due to gutta-percha and AH26 sealer, gutta-percha was cut at the level of orifice and pulp chamber was thoroughly cleaned from gutta-percha and sealer residues using a cotton pellet dipped in alcohol.

In all teeth except for the control group, 3 mm of the respective cement were directly applied over the orifice on top of gutta-percha [Figure 1]. Control group did not receive any cement after root canal therapy. After the application of bonding agent (3M ESPE, St. Paul, MN, USA), all teeth were restored with Z250 composite (3M ESPE, St. Paul, MN, USA) with a color shade matching the tooth color [Figure 2]. The teeth were then immersed in saline and stored in a glass container at room temperature under natural lighting. Saline solution was refreshed every 3 days.

Colorimetry

Color parameters were measured with a spectrophotometer (Vita EasyShade® compact, VITA Zahnfabrik, Germany) at baseline (before application of cement) and at 1 week, 1 month, 2 months, and 3 months after the application of cement according to the CIE L*a*b* color system. Before each color measurement, the teeth were cleaned and the device was calibrated according to instruction manuals. Tooth color was measured at the mid-buccal area and in room with same light in different times. A mounting jig was used for reproducible position [Figure 3]. According to the manufacturer, the L*, a*, and b* parameters were measured for each tooth by the same observer 3 times and the mean of each value was used to calculate the overall color change using the formula below:

$$\Delta E = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}$$

Where L* indicates lightness, Δa* indicates changes in a* parameter in red-green axis, and Δb* indicates changes in b* parameter in yellow–blue axis.

Statistical analysis

The data were analyzed using SPSS version 18 (SPSS Inc., Chicago, IL, USA). ΔE1 was defined as

Figure 1: The samples after placing the endodontic material.

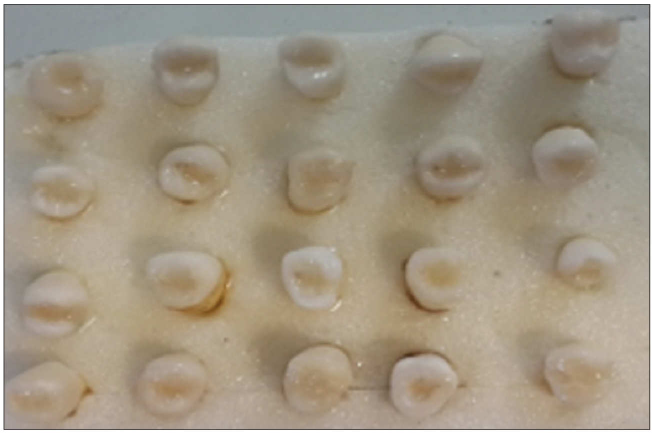

Figure 2: The samples after placing the composite restoration.

Figure 3: Spectrophotometer device (Vita Easyshade).

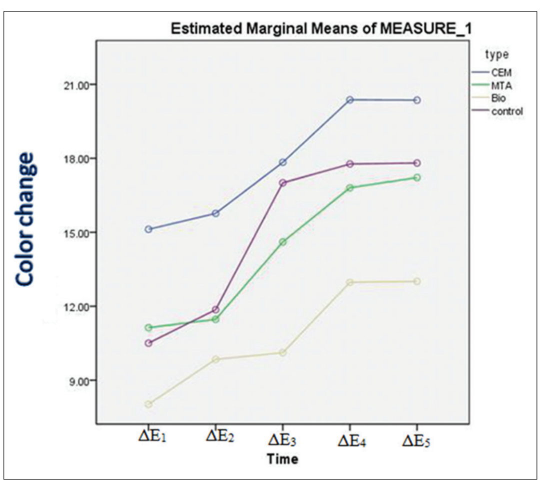

Figure 4: Color change over time in the four groups.

coronal color change in each group immediately after application of cement compared to baseline (before application of cement). ΔE2, ΔE3, ΔE4, and ΔE5 were defined as coronal color change at 1 week, 1 month, 2 months, and 3 months after the application of cement compared to baseline, respectively [Figure 4].

The data were analyzed using descriptive and analytical statistics. The number, mean, and standard deviation (SD) were reported for descriptive statistics of data. For analytical statistics, Kolmogorov–Smirnov test was applied to assess normal distribution of data. ANOVA was used to compare the results at baseline and repeated-measures ANOVA was applied to compare the four groups and different time points. Pairwise comparisons of the groups and time points were carried out using Bonferroni test.

RESULTS

Kolmogorov–Smirnov test was applied to assess the normality of data. The results of Kolmogorov–Smirnov test showed that color change data in all groups had normal distribution.

Levene's test was used to assess equality of variances, which showed equality of variances for baseline color change in the groups ($P = 0.063$). ANOVA was applied to assess the differences in ΔE1 (color change immediately after placement of materials) among the groups, which showed a significant difference in this regard ($P < 0.001$). Thus, repeated-measures ANOVA was applied with ΔE1 as the reaped measure, which showed that the result of Mauchly's sphericity test was significant ($P < 0.001$).

Repeated-measures ANOVA showed that time had a significant effect on color change ($P = 0.001$). Furthermore, this test showed that the interaction effect of time and type of cement on color change was not statistically significant ($P = 0.308$). Color change over time was not dependent on the type of cement used in any group [Table 1].

Tests of between-subjects effects among the three groups showed no significant difference ($P = 0.343$). Bonferroni test was applied for pairwise comparison of time points, which showed significant differences between each 2 time points ($P < 0.01$) except for ΔE4 and ΔE5 ($P > 0.05$).

DISCUSSION

This *in vitro* study compared the coronal discoloration of white MTA, CEM cement, and Biodentine within 3 months in human teeth. Although some previous studies compared discoloration of each of these cement alone or in comparison with other materials, comparison of all three together has not been previously performed.

Table 1: The mean and standard deviation of color change in the four groups

Group	ΔE1		ΔE2		ΔE3		ΔE4		ΔE5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CEM	15.12	3.09	15.77	4.08	17.84	4.38	20.37	2.25	20.36	4.39
MTA	11.13	4.92	11.47	4.89	14.61	5.82	16.80	5.81	17.22	6.50
Biodentine	8.03	2.82	9.85	3.35	10.12	3.98	12.97	4.95	13.01	5.35
Control	10.51	2.98	11.86	0.73	17.01	2.12	17.77	1.85	17.81	2.21

ΔE1: Coronal color change in each group immediately after application of cement compared to baseline; ΔE2: Color change at 1 week after the application of cement compared to baseline; ΔE3: Color change at 1 month after the application of cement compared to baseline; ΔE4: Color change at 2 months after the application of cement compared to baseline; ΔE5: Color change at 3 months after the application of cement compared to baseline. CEM: Calcium enriched mixture; MTA: Mineral trioxide aggregate; SD: Standard deviation

Only premolar teeth were evaluated in this study in order to have approximately the same thickness of dentin in all teeth. Furthermore, considering the greater clinical significance of discoloration in the esthetic zone, extracted human premolars were chosen since they are located in the esthetic zone.

In order to prevent discoloration due to gutta-percha and AH26 sealer, gutta-percha was cut at the level of orifice and pulp chamber was thoroughly cleaned from gutta-percha and sealer residues using a cotton pellet dipped in alcohol. Next, the respective cement were directly placed on gutta-percha in 3 mm thickness. Although in vital pulp therapy, the cement are directly placed on pulp tissue and exert their specific clinical effects, it has been documented that discoloration caused *in vitro* by application of these cement in endodontically treated teeth is the same as discoloration in teeth with vital pulp.[6]

The CIE L*a*b* color system was used in this study for color assessment, which has been approved by the American Dental Association and is the most accurate and most commonly used system for colorimetry.[10] Spectrophotometric measurements were made under standard lighting conditions as recommended to benefit from maximum precision provided by this system.

Evidence shows that time is an important factor affecting discoloration caused by different materials; this statement was also confirmed in the current study. Our results showed significant discoloration in all groups over time compared to baseline ($P < 0.001$). However, color change was not significantly different at 2 and 3 months ($P > 0.05$). Vallés *et al*.[25] evaluated the effect of light irradiation and presence of oxygen on color stability of five calcium silicate-based cement during 5 days and Camilleri[5] compared discoloration of MTA and Biodentine (not applied on tooth structure) during 28 days. Both studies reported optimal color stability of Biodentine, which may be attributed to short course of the two studies because a previous study by Shokouhinejad *et al*.[27] reported coronal discoloration at 6 months following the application of Biodentine. On the other hand, Marconyak *et al*.,[31] and Beatty and Svec[26] in their 2-month study reported less discoloration caused by Biodentine compared to white MTA. However, in our study, tooth discoloration caused by Biodentine was not significantly different from that of White MTA at 3 months.

Arman *et al*.[10] evaluated coronal discoloration caused by CEM cement and MTA after 6 months and reported that discoloration caused by the two cement was equal. Eghbal *et al*.[32] compared CEM cement and MTA in terms of tooth discoloration after 28 days and reported that CEM cement caused less discoloration than MTA. Findings of the current study revealed that discoloration caused by CEM cement and MTA was the same, which is in contrast to the results of Eghbal *et al*.[32] This controversy in the results may be due to time of assessment (28 days in their study versus 3 months in ours).

Vallés *et al*.[24] stated that reaction of bismuth with oxygen in the presence of light is responsible for tooth discoloration caused by white MTA, which results in formation of bismuth oxide crystals. These crystals darken the tooth color and their formation in the presence of visible and ultraviolet light has been documented.[33] Considering the fact that, in our study, the teeth were exposed to natural light during the storage phase, conditions required for formation of the above-mentioned crystals and subsequent discoloration of tooth structure were met. Our results with regard to discoloration caused by white MTA were in line with those of previous studies.[6,12,22,34]

Over time, a significant discoloration occurred in CEM cement group in our study ($P < 0.001$), which was not significantly different from discoloration in white MTA and Biodentine groups ($P = 0.343$). This finding was in line with the results of Arman *et al.*,[10] in their study on 32 dentin-enamel blocks of maxillary incisors. However, Eghbal *et al*.[32] reported that CEM cement had higher color stability than white MTA. Such a controversy in the results of studies may be attributed not only to the effect of time but also to the effect of environment on degree of coronal discoloration.

Nosrat *et al*.[35] claimed that application of CEM cement at the orifice does not cause tooth discoloration (in contrast to MTA). Madani *et al*.[30] reported that tooth discoloration caused by CEM cement was minimal. However, the two aforementioned studies had not been designed to assess tooth discoloration.

Ioannidis *et al*.[22] reported that coronal discoloration in gray MTA and white MTA. Mean (SD) ΔE values after 3rd month were 7.47 (2.22) and 4.26 (1.90) for gray MTA and white MTA, respectively. This value in our study was higher which can be due to the different method of work.

Nosrat *et al*.[35] reported treatment of two cases with revascularization and Madani *et al*.[30] evaluated histopathological pulp responses following the application of CEM cement and MTA in diabetic rats.

In our study, teeth in Biodentine group showed significant discoloration during 3 months ($P < 0.001$), which was not significantly different from discoloration in MTA and CEM cement groups ($P > 0.01$). Shokouhinejad *et al*.[27] evaluated tooth discoloration following the application of different calcium silicate-based cement and reported that in the presence of blood, all cement caused similar tooth discoloration while in the absence of blood, tooth discoloration caused by Biodentine and Endosequence was less than that caused by OrthoMTA. These findings were in contrast to our results, which may probably be due to the different types of MTA cement evaluated in the two studies. Kohli *et al*.[36] reported no discoloration due to the application of Biodentine but showed that white MTA caused discoloration in tooth structure, which was different from our results. This difference between the results of the two studies may be explained by different methodologies and tools used for colorimetry since they stored teeth at 37°C and used ocean optics device for spectrophotometry.

CONCLUSION

All three endodontic cement evaluated in this study can cause coronal discoloration. The three tested cement are not significantly different in terms of the degree of color change caused in tooth structure following their application. Significant differences were noted in color change (ΔE) between all time points except between ΔE4 (2 months) and ΔE5 (3 months).

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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 nonfinancial in this article.

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