Original Article

pISSN, eISSN 0125-5614
M Dent J 2022; 42 (2) : 191-198

# Bismuth oxide radiopacifier in non-mineral trioxide aggregate calcium silicate cement: The effect on tooth discoloration in a regenerative endodontic tooth model

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**Objective:** This study aimed to evaluate tooth discoloration from two experimental materials— non-mineral trioxide aggregate calcium silicate cement (non-MTA CSC) containing bismuth oxide (BiO) or zirconium (ZrO) radiopacifier, and two commercial materials— Bio-MA (composing of MTA and BiO), and Biodentine (composing of non-MTA CSC and ZrO).

**Materials and Methods:** The 75 regenerative endodontic tooth models were prepared from human mandibular premolars. The mixed CSC was placed as a coronal barrier in 3-mm thick before the coronal access was restored. The tooth color was measured at the buccal surface using a spectrophotometer *(Vita Easyshade V)* on days 1, 7, 30, and 90. The total color change (ΔE) was statistically analyzed and compared to the clinically detectable level at ΔE 3.7. The samples were sectioned to observe any discoloration of either material or dentine.

**Results:** Over the 90-day period, the non-MTA CSC containing BiO or ZrO and Biodentine groups showed ΔE ≤3.7. The Bio-MA group presented the highest ΔE (>3.7), which was significantly higher than the others ($p$<.05). The discoloration was observed in the Bio-MA material. Non-MTA CSC with BiO or ZrO radiopacifier and Biodentine did not induce the discoloration.

**Conclusion:** Non-MTA CSC with BiO or ZrO radiopacifier and Biodentine did not induce tooth discoloration. However, Bio-MA, which was MTA with BiO radiopacifier, particularly induced the tooth color change (ΔE >3.7) from the discoloration of the material.

**Keywords:** bismuth oxide, calcium silicate cement, spectrophotometer, tooth discoloration, zirconium oxide

**How to cite:** Osiri S, Vibulcharoenkitja P, Banomyong D, **Chotvorrarak K**, Sutimuntanakul S. Bismuth oxide radiopacifier in non-mineral trioxide aggregate calcium silicate cement: The effect on tooth discoloration in a regenerative endodontic tooth model. M Dent J 2022; 42: 191-198.

## Introduction

Discoloration of mineral trioxide aggregate (MTA), such as ProRoot MTA *(Dentsply Tulsa, Tulsa, OK, USA)* or Bio-MA *(M-Dent/SCG, Bangkok, Thailand)*, which is a MTA-based calcium silicate cement (CSC), has been reported [1]. MTA induces tooth discoloration, particularly in cases of vital pulp therapy or regenerative endodontic procedures (REPs) [2, 3]. Tooth discoloration caused by the different calcium silicate-based cements has been described [4]. The discoloration is caused by the staining of substances in the dentinal tubules and/or reflecting of the discolored material through enamel/dentine structure. Hence, the material selection considering discoloration potential is clinical challenging.

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Received: 9 May 2022 Revised: 12 July 2022 Accepted: 12 July 2022

Bismuth oxide (BiO) radiopacifier in MTA is proposed to be the cause of discoloration. The possible mechanisms of discoloration from BiO in MTA are (a) oxidation reaction with sodium hypochlorite [5], (b) destabilizing by amino acid in dentine collagen [6], and (c) dissociation from heat/light in the oxygen-free environment [7]. However, the interaction between the MTA ingredient and BiO may play a key role in the discoloration reaction [8]. BiO is one of the highest radiopaque substances that may not induce discoloration in the CSC without the MTA ingredient, which has not been previously investigated.

Non-MTA calcium silicate cement has been developed to replace MTA to improve material purity and prevent discoloration. For example, Biodentine *(Septodont, Saint Maur des Fosses, France)* or RetroMTA (*BioMTA, Seoul, Korea*) uses di- and/or tri-calcium silicate or calcium carbonate as the main ingredient. The radiopacifier has also been changed from BiO to zirconium oxide (ZrO), and the material does not induce discoloration [9]. However, CSC containing ZrO provides low radiopacity, which is difficult to distinguish from dentine in the radiograph [10, 11].

Therefore, the objective of this study was to evaluate tooth discoloration from two experimental materials— non-MTA CSC containing BiO or ZrO radiopacifier, in comparison to the two commercial CSCs— Bio-MA (MTA/BiO) and Biodentine (non-MTA/ZrO), in the regenerative endodontic tooth model over a 90-day observation period.

## Materials and Methods

The study protocol was approved by the Institutional Review Board (MU-DT/PY-IRB 2018/018.2803). The 80 intact, single-root human mandibular premolars, in tooth shade A2—A3 (*Vita Classic shade guide, VITA Zahnfabrik, Bad Säckingen, Germany)*, extracted for orthodontic reasons were collected. The teeth were radiographed (*Digora™ Optime, Soredex Kavo Imaging, Hatfield, PA, USA*) in the proximal view to check the thickness of the buccal tooth structure, which was approximately 3 mm. All included teeth were cleaned and polished to remove any surface deposits affecting tooth color measurement, then stored in 0.1 % thymol solution at room temperature before being used.

### Regenerative endodontic tooth model preparation

Each tooth was prepared to simulate the immature tooth with the thin root canal wall and the open apex in the regenerative endodontic treatment (Figure 1A). The tooth was sectioned at 6 mm below the cemento-enamel junction (CEJ), and the apical root segment was discarded. After coronal access opening, the root canal was sequentially enlarged using the Gate Glidden drill size 2–5. After preparation, the thickness of the buccal tooth structure was controlled to approximately 3 mm by radiographic measurements. The root end of the crown segment was capped with resin composite *(Single Bond Universal and Filtek Z250 shade A1, 3M ESPE, St Paul, MN, USA)*. A moist sponge (soaked with distilled water) was filled into the prepared canal up to the CEJ level.

The high-purity CSC powder *(Alfa Aesar, Thermo Fisher Scientific, Ward Hill, MA, USA)* was mixed with one of the radiopacifiers, zirconium oxide *(Riedel-de-Haën™, Loughborough, United Kingdom; CSC/ZrO group)* or bismuth oxide *(Scharlau, Scharlab, Barcelona, Spain; CSC/BiO group*), in the ratio of 80:20 (by weight); the CSC powder without a radiopacifier was served as the control (*CSC group)*. The CSC powder was homogeneously mixed with distilled water in the powder-liquid ratio of 1 g to 0.36 ml. For Biodentine,

the powder in a mixing capsule was added with five droplets of the liquid and then mixed in a trituration machine *(Septodont, Hangzhou Sifang Medical Apparatus Co. Ltd., Zhejiang, China)* at 4,000 rpm for 30 s. For Bio-MA, the powder was mixed with the liquid in the powder-liquid ratio of 1 g to 0.35 ml until homogeneous. In summary, the experimental groups (n = 15/group) were 1) CSC/ZrO, 2) CSC/BiO, 3) CSC (control), 4) Biodentine, and 5) Bio-MA (Table 1).

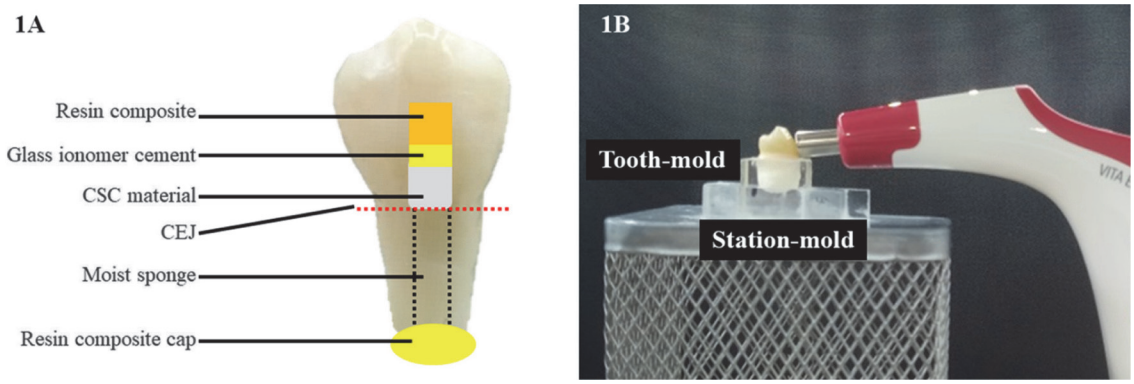


Figure 1 A: The regenerative endodontic tooth model. The calcium silicate cement (CSC) was placed at the cemento-enamel junction (CEJ) level in 3-mm thick. Glass-ionomer cement lining and resin composite were placed as the coronal restoration.
B: The custom-made platform was used to control the area of color measurement. The spectrophotometer *Vita Easyshade V* (ES-V) was fixed on the basement while the *'tooth-mold'* was placed on the *'station-mold'*. The tip of ES-V contacted the buccal tooth surface for measuring the tooth color.

Table 1 Means ± standard deviations of $\Delta E_7$, $\Delta E_{30}$ and $\Delta E_{90}$ of the CSC/ZrO, CSC/BiO, CSC (control), Biodentine, and Bio-MA groups in the regenerative endodontic tooth model

| Group | $\Delta E_7$ | $\Delta E_{30}$ | $\Delta E_{90}$ |
|---|---|---|---|
| CSC/ZrO | $1.2\pm0.7^{Aa\dagger}$ | $1.7\pm0.7^{Aa}$ | $2.0\pm1.0^{Aa}$ |
| CSC/BiO | $2.0\pm1.2^{Aa}$ | $3.4\pm2.0^{ABb}$ | $3.7\pm2.2^{ABb}$ |
| CSC | $3.6\pm1.3^{Ba}$ | $3.6\pm1.4^{Ba}$ | $5.0\pm1.5^{Bb}$ |
| Biodentine | $2.2\pm1.6^{ABa}$ | $3.3\pm2.4^{ABb}$ | $3.6\pm2.4^{ABb}$ |
| Bio-MA | $3.6\pm1.4^{Ba}$ | $7.0\pm1.8^{Cb}$ | $8.7\pm1.9^{Cc}$ |

† The different upper-case letters (column) and the lower-case letters (row) indicated a significant difference between the groups at each time point and between the observation periods in each group, respectively ($p<.05$).

Under a dental operating microscope *(OPMI Pico®, Carl Zeiss, Oberkochen, Germany)* at the 6x magnification, the mixed CSC was placed into the pulp chamber on the sponge and plugged until 3-mm thick was obtained in the radiograph. To create a sealed, oxygen-free environment, 1-mm thick glass ionomer cement *(Vitrebond, 3M ESPE)* was lined over the CSC material. The coronal access was filled with resin composite.

The prepared tooth was fixed in a plastic block with acrylic resin, namely the *'tooth-mold'* (Figure 1B), which was stored in 0.1 % thymol solution at room temperature and kept in a black box between each color measurement. Additionally, another 5 teeth were only filled in the pulp chamber with resin composite (no CSC) to observe the storage effect on the color change.

### Color measurement using a spectrophotometer

The tooth color was measured in the light-controlled box on day 1 (D1, baseline), 7 (D7), 30 (D30), and 90 (D90) using a spectrophotometer *(Vita Easyshade V (ES-V), VITA Zahnfabrik)* using the 'base shade determination' mode. A custom-made platform, namely the *'station mold'*, was created to control the position and the angle of ES-V in each measurement (Figure 1B). The remaining of the storage media on the tooth surface was removed by a moist gauze. The *'tooth-mold'* was placed in the *'station-mold'*, and the ES-V was fixed on the basement, where the probe tip contacted the buccal tooth surface.

To avoid the effect of drying on tooth color, each color measurement was performed within 10s before the specimen was re-immersed in the storage media. The tooth color was measured and recorded as L*, a*, and b*, according to the International Commission on Illumination (CIE). Total color change (ΔE) at each time interval (T) was calculated using the formula:

$$\Delta E_T = [(L^*_T - L^*_1)^2 + (a^*_T - a^*_1)^2 + (b^*_T - b^*_1)^2]^{1/2}$$

T = measuring period at day 7, 30, or 90

Theoretically, ΔE greater than 3.7 would be visibly detected by human eyes, which was considered the clinically acceptable level [12].

To evaluate the discoloration characteristics (if any), two specimens per group were additionally prepared to observe the discoloration pattern at each time interval (D7, D30, and D90). The samples were horizontally sectioned at 1 mm above the CEJ into the coronal and the apical segments. The coronal segment was further vertically sectioned in the bucco-lingual direction. All of the sectioned specimens were photographed using a digital camera *(Canon EOS60D, Canon Inc., Tokyo, Japan)* and observed any discoloration of the material and/or dentine.

### Statistical analysis

The data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA) with a significance at $p<.05$. The Shapiro-Wilk test was used to confirm the normal distribution of ΔE data. The two-way Mixed Analysis of Variance and Bonferroni's test were used to analyze ΔE between the CSC materials at the same period as well as between the different periods of each material. In addition, ΔE was compared whether the value was higher than the clinically acceptable level at ΔE 3.7.

## Results

The tooth color change in $\Delta E_7$, $\Delta E_{30}$, and $\Delta E_{90}$ of the experimental groups are presented in Table 1. Over the 90-day period, the color parameters L*, a*, and b* in all groups were darker (L* closer to 0), more greenish (-a*), and more bluish (-b*) when compared to the baseline (D1).

From D7 to D90, the Bio-MA group showed the highest ΔE while the CSC/ZrO group had the lowest ΔE. For the specimens only filled with resin composite (no CSC) for observing the storage effect, $\Delta E_7$, $\Delta E_{30}$, and $\Delta E_{90}$ were 1.5 ± 0.4, 1.6 ± 0.4, and 2.4 ± 0.7.

In comparison between the observation periods at the short-term ($\Delta E_7 - \Delta E_{30}$) or the long-term ($\Delta E_{30} - \Delta E_{90}$) interval, the ΔE of all groups significantly increased over the periods ($p<.05$) except the CSC/ZrO group (Table 1). The ΔEs of the CSC/ZrO group were not significantly changed throughout the experimental periods ($p\geq.05$). In contrast, the ΔE of the Bio-MA group were significantly changed at both D30 and D90 ($p<.05$). The CSC/BiO group and the Biodentine group showed the significant short-term change of ΔEs at D30 ($p<.05$), but their long-term ΔEs at D90 were not significantly changed ($p\geq.05$). On the contrary, the CSC group (control) showed the significantly long-term ΔE change at D90 ($p<.05$) whereas the short-term ΔE at D30 was not significantly changed ($p\geq.05$).

Compared to the clinically acceptable level (ΔE≤3.7), the CSC/BiO, CSC/ZrO, and Biodentine groups showed ΔE ≤3.7 throughout the observation periods. In contrast, the Bio-MA group had ΔE >3.7 at D30 and D90, while the CSC (control) group presented ΔE >3.7 at D90.

The sectioned specimens of Bio-MA showed distinct black discoloration starting at the border of the material close to the dentinal wall (D7) and extending into the center of the material (D30 and D90) (Figure 2). However, discoloration was not observed in the dentine (Figure 2). The discoloration was not visibly detected in the other groups over the experimental periods (Figure 3). The CSC without or with radiopacifier (ZrO or BiO) was whitish, while Biodentine was yellowish.

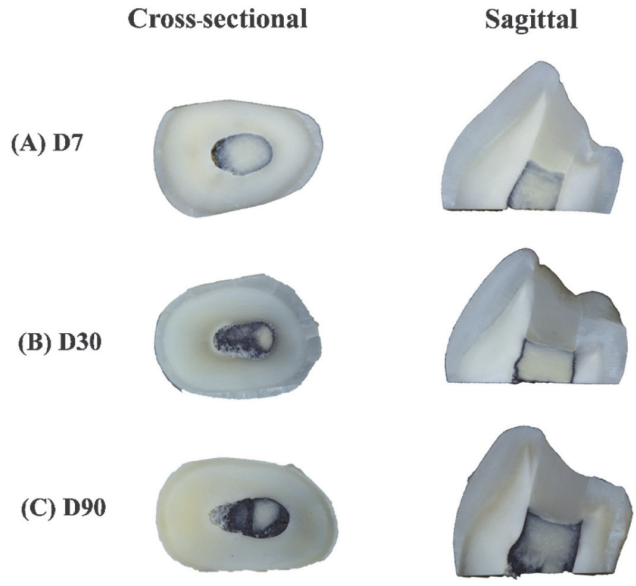


Figure 2 The sectioned samples of Bio-MA, which was an MTA-based calcium silicate cement with bismuth oxide radiopacifier, showed the discolored area around the border of the material at day 7 (D7) (A) and into the center of the material at day 30 (D30) and 90 (D90) (B, C).

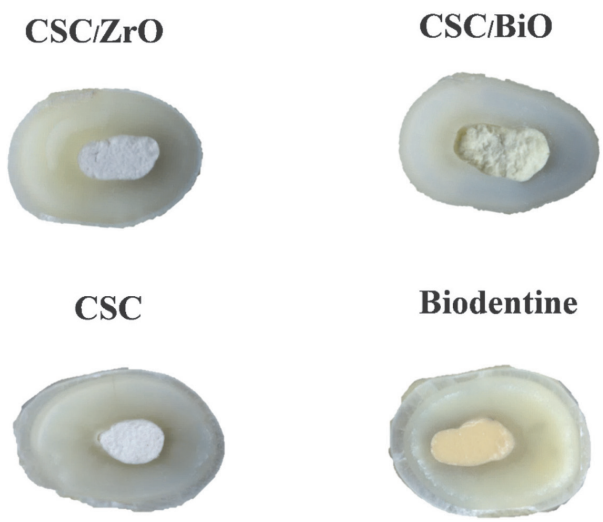


Figure 3 The cross-sectioned samples at day 90 of the calcium silicate cements with zirconium oxide (CSC/ZrO), with bismuth oxide (CSC/BiO), without radiopacifier (CSC), and Biodentine. No discoloration in the material or the dentine was observed.

## Discussion

Tooth color measurement is a sensitive procedure wherein any confounding factors should be controlled [13]. In this study, only mandibular premolars in shades A2—A3 were only selected and then randomly assigned to each experimental group. At the different observation periods, the measuring area at the buccal side was exactly the same that was controlled by the custom-made station. The environment in the color measurement was standardized in the light-controlled box. The tooth color was measured within 10 s to avoid tooth dehydration that could possibly affect tooth color [14]. In addition, the thickness of the buccal tooth structure was measured in the radiograph and controlled at approximately 3 mm. Furthermore, the total color change (ΔE) from the storage effect in this study was minor, thus it would not significantly affect the result of the experiment.

Bio-MA and CSC/BiO are similarly composed of 20% BiO, yet only MTA-based Bio-MA was severely discolored. Therefore, BiO is not the main cause of discoloration and can be used as a radiopacifier in non-MTA CSC. BiO possibly interacts with other ingredient(s) in MTA that is not calcium silicate. For example, calcium sulfate or sulfur trioxide ($SO_3$) in MTA [15, 16] may react with BiO to produce a grey bismuthinite ($Bi_2S_3$) [17]. However, another MTA with BiO (*MTA Angelus, Londrina, PR, Brazil*) did not contain calcium sulfate, but the discoloration of the material still occurred [6, 18]. Thus, the discoloration mechanism from BiO in MTA is still unclear and should be further investigated.

BiO is higher radiopaque than ZrO and, from the results of our study, can be used safely in non-MTA CSC without the discoloration induction. The current CSC with ZrO (e.g. Biodentine) has clinically unsatisfied radiopacity [10]. Therefore, a newly developed CSC may contain BiO to achieve high radiopacity. However, the blood contamination during the material setting should be further investigated since the blood has the potential to induce more discoloration [19].

The tooth color of the CSC/ZrO group was stable and has not significantly changed over the 90 days, while Biodentine (containing ZrO) showed an initial minor color change that was stable after that. This result corresponded to the CSC containing ZrO reported in the other studies [20, 21]. Zirconium oxide is an inert, white radiopacifier that does not interact with any ingredients in MTA or CSC and, therefore, did not induce any tooth discoloration. However, the tooth color change of the Biodentine group was less stable than the CSC with ZrO group since Biodentine contains the polymer in the liquid that may cause a yellowish appearance. The CSC without a radiopacifier showed the long-term tooth color change detectable by the spectrophotometer; however, the material in the sectioned specimen was not noticeably discolored.

The pattern of the tooth discoloration from the Bio-MA group in this study was the reflection of the discolored material through the tooth structure, without any color change in dentine. The result contradicted other studies that found discoloration in both MTA material and dentine [22, 23]. However, in those studies, the bovine teeth were used and immersed in 1–2 % sodium hypochlorite solution for 30 min before in contact with MTA [22, 23]. The large diameter of dentinal tubules in the bovine teeth and the long exposure time to sodium hypochlorite solution may enhance the discoloration distributed into the dentine.

## Conclusion

From the limitation of this study, using the regenerative endodontic tooth model, CSC/BiO, CSC/ZrO, and Biodentine (non-MTA CSC with ZrO) did not induce tooth discoloration. Bio-MA (MTA with BiO) induced severe tooth color change from the discoloration of the material. The bismuth oxide radiopacifier in the calcium silicate cement did not induce discoloration if the mineral trioxide aggregate was not presented in the composition.

## Financial disclosure

This study was supported by a postgraduate research grant from the Faculty of Dentistry, Mahidol University, Bangkok, Thailand.

## Conflict of interest

Prof. Supachai Sutimuntanakul is the inventor of Bio-MA. The other authors deny any conflicts of interest.

## Ethics committee approval

The protocol was approved by the Institutional Review Board, the Faculty of Dentistry and Faculty of Pharmacy, Mahidol University Bangkok, Thailand (MU-DT/PY-IRB 2018/018.2803), in accordance with the Declaration of Helsinki.

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