Original Article

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Effect of adding zinc oxide and zirconium oxide on decreasing tooth discoloration from mineral trioxide aggregate in a regenerative endodontic model

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Objectives: To investigate the effect of adding zirconium oxide (ZrO) or zinc oxide (ZnO) (5% by weight) on decreasing tooth discoloration from mineral trioxide aggregate in a regenerative endodontic tooth model over 28-day period.
Materials and methods: Thirty human mandibular premolars were prepared to achieve regenerative endodontic models and divided into three groups- (a) original MTA (MTA), (b) MTA+5% ZrO (MTA/ZrO), and (c) MTA+5% ZnO (MTA/ZnO). The powder of MTA was added with ZrO or ZnO in a blending machine until homogeneous. The powder was mixed with the liquid and then placed as a coronal barrier in 3-mm thick at the level of cemento-enamel junction. Each specimen was embedded in a block and placed on a customized platform to control tooth-color measuring area at cervical third on the buccal side. In a light-controlled box, tooth color in CIE L*a*b* values was measured using the spectrophotometer at day 1 (baseline) and day 28. The difference in tooth color was calculated into ΔE value. The specimens at day 28 were selected, horizontally sectioned and photographed to observe discoloration of MTA material and/or dentin.
Results: At day 28, ΔE of MTA (6.83±1.70) was significantly higher than MTA/ZrO (3.70±1.36) and MTA/ZnO (4.20±1.31) ($p<0.01$). In the sectioned specimens, severe discoloration was observed in MTA material and adjacent root dentin. MTA/ZrO and MTA/ZnO showed less discoloration of material and dentin.
Conclusion: Mixing 5% ZrO or ZnO into MTA containing bismuth oxide significantly decreased tooth discoloration in the regenerative endodontic model.

Keywords: mineral trioxide aggregate, regenerative endodontics, tooth discoloration, zinc oxide, zirconium oxide

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Introduction

Mineral trioxide aggregate (MTA) is a calcium silicate-based cement and composed of Portland cement (PC) and bismuth oxide (BO) radiopacifier [1]. The main ingredients of PC are tricalcium silicate, dicalcium silicate, and tricalcium aluminate [1, 2]. MTA possesses excellent sealing ability, bioactive induction of mineralized tissues, and antimicrobial effect [2, 3]. MTA is used in direct pulp capping, pulpotomy, root perforation repair, apexification, endodontic surgery, and regenerative endodontic procedure [4-6].

MTA has a significant disadvantage by induction of tooth discoloration from three possible mechanisms [7-9]. Firstly, BO interacts with remnants of sodium hypochlorite irrigant, a strong oxidizing agent, on root dentin and changed into discolored precipitates [7-9]. Secondly, the discolored precipitation is produced when BO contacts with dentin collagen in the oxygen-free environment, such as in the root canal [8, 10, 11]. Finally, absorption of blood into MTA causes

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discoloration of material [12, 13]. Thus, MTA has a limitation in clinical use with an esthetic concern especially in the anterior region.

Discoloration of MTA is inhibited when BO radiopacifier is substituted by other compound(s) such as zirconium oxide (ZrO) [8]. The other approach to inhibit discoloration by MTA is adding a stabilizing agent to inhibit the discoloration reaction between MTA ingredients and BO. MTA with BO radiopacifier does not present any discoloration if a stabilizing agent, such as zinc oxide (ZnO), is added [8]. In a laboratory study, MTA mixed with 5% ZnO does not induce tooth discoloration while the important physical and chemical properties are not significantly changed [8]. From a preliminary investigation in our previous study [14], it has been noticed that the color of calcium silicate cement mixed with ZrO is stable and not changed over the observation period. In general, ZrO is used to replace BO radiopacifier in MTA. However, ZrO is also possible to be mixed into MTA containing BO as a stabilizing agent to inhibit discoloration.

The objective of this *in vitro* study is to investigate the effect of adding 5% zinc oxide or zirconium oxide on decreasing tooth discoloration from MTA in a regenerative endodontic model over 28-day period.

Materials and Methods

The protocol of this study was approved by the Institutional Ethical Review Committee (MU-DT/PY-IRB 2019/043.3007). The sample size was calculated in the nQuery software (Statistical Solutions Ltd., Cork, Ireland) [8], ten specimens per experimental group.

Preparation of regenerative endodontic model

Thirty intact human mandibular premolars with a tooth color between A2-A3 (Vita Classic shade guide, VITA Zahnfabrik, Bad Säckingen, Germany) extracted for orthodontic reasons were collected. Endodontic access was prepared using a high-speed round and taper diamond bur (Komet, Besigheim, Germany). Next, a model of immature tooth for regenerative endodontic procedure was created (Figure 1), yet the effect of blood contamination was not investigated in this study. The root was sectioned at 6 mm from the cemento-enamel junction (CEJ) using a high-speed fissure diamond bur (Komet) under air-water coolant. The root canal was enlarged and drilled with a peeso-reamer size 5 to simulate a wide root canal with open apices in the immature tooth.

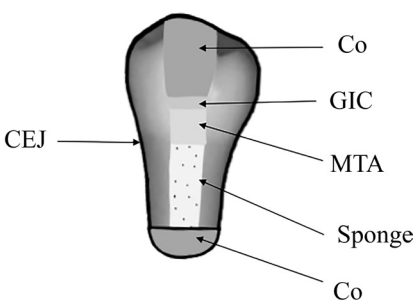

Figure 1 Illustration of a regenerative endodontic model with wide root canal and open apices. MTA was placed with 3-mm thick at the CEJ level (abbreviation: Co- resin composite, GIC- glass-ionomer lining cement, MTA- mineral trioxide aggregate, CEJ- the cemento-enamel junction).

The apical end of root canal was covered with a thin cap of resin composite (Z250, 3M ESPE, St. Paul, MN, USA) bonded with etch-and-rinse adhesive (Single Bond Universal, 3M ESPE) and light cured for 40 s. The root canal was irrigated with 5 ml of 2.5% NaOCl, 3 ml of 17% EDTA, and a final irrigation with 5 ml of 2.5% NaOCl. The root canal was dried with paper points, and moist sponges were packed into the root canal space up to the CEJ to control the level of MTA placement. Thickness of buccal tooth structure at the middle third was radiographically checked and measured from a digital radiograph (DIGORA® Optime, KaVo Kerr, Tuusula, Finland) in the mesio-distal direction.

The regenerative endodontic models were randomly divided, with an equal distribution of the buccal thickness, into three groups (n = 10 of each) according to the material placed for a coronal barrier: (1) ProRoot MTA (MTA, Dentsply Tulsa, Tulsa, OK, USA), (2) MTA+5% ZrO (Riedel-de-Haën™, Loughborough, UK) (MTA/ZrO), and (3) MTA+5% ZnO (Quality Reagent Chemicals, Selangor, Malaysia) (MTA/ZnO). In the groups 2 and 3, ProRoot MTA powder was mixed with 5% (by weight) of ZrO or ZnO by a mixing machine (Tsutsui Ultra-Micro V-Mixer, AAA machine, Tokyo, Japan) for 15 min.

The powder of MTA was mixed with liquid (distilled water) at the powder-liquid ratio of 1:0.3 using a cement spatula. The mixed material was loaded into the root canal using an amalgam carrier and compacted with an endodontic plugger under a dental operating microscope (Carl Zeiss, Oberkochen, Germany) at 10x magnification until 3- mm thick of material was obtained. The density and thickness of MTA were radiographically checked from the digital radiograph in the mesio-distal direction.

A glass-ionomer lining cement (Vitrebond-universal dentin color, 3M ESPE) was placed on MTA at 1-mm thick and light cured for 30 s. The coronal access was filled with the bonded resin composite restoration (color A1). The specimen was mounted in a plastic block using a self-cured acrylic resin. The specimens were kept in 0.1% thymol solution throughout the experimental period to prevent bacterial growth that might affect the tooth color measurement.

Tooth color measurement

Each prepared specimen was rehydrated in the thymol solution for 24 h before the baseline color measurement to prevent the drying effect. The specimen was removed from the storage media and placed on a customized platform with a fixed position to control the area of tooth color measurement. Tooth color at the baseline (day 1) was measured on the buccal surface at the middle third using a spectrophotometer (Vita Easyshade V, VITA North America, Yorba Linda, CA, USA) in a light-controlled box. The specimens were kept in the storage media at the room temperature for 28 days. Tooth color at the same area (controlled by using the customized platform) was re-measured under the same set-up environment. Each measurement was repeated 3 times, and then the average value was calculated. CIE L*a*b* values were obtained from the measurement. The change in tooth color between day 1 (baseline) and day 28 in L* a* and b* (ΔL^*, Δa^*, Δb^*) was then calculated into the value of total color change (ΔE) by the formula:

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

Tooth sectioning analysis

The specimens were randomly selected from each experimental group (n = 3 of each). The specimen was removed from the plastic block and then horizontally sectioned using a high-speed fissure diamond bur (Komet) with air-water coolant at the mid-level of MTA material to obtain the coronal and radicular segments. In the radicular segment, any discoloration of material and/or dentin in the cross-sectional view was observed. In the coronal segment, the specimen was further vertically sectioned in the bucco-lingual direction to obtain the mesial and distal segments for observing the discoloration in the sagittal view. The degree of discoloration was subjectively evaluated.

Statistical analysis

ΔL^*, Δa^* and Δb^* were descriptively analyzed. Normality and homogeneity of variance of ΔE were confirmed using Shapiro-Wilk test and Levene's test. ΔE were analyzed among the three experimental groups using one-way analysis of variance (ANOVA) and multiple comparison using Tukey's test, with a significant level $\alpha = .05$.

Results

The thicknesses of buccal tooth structure at the areas of color measurement were similar among the three experimental groups (MTA 2.41±0.15 mm, MTA/ZrO 2.40±0.16 mm, and MTA/ZnO 2.38±0.19 mm).

Tooth color changes in ΔL, Δa, Δb and ΔE values of MTA, MTA/ZrO, and MTA/ZnO are presented in Table 1. ΔE values (mean ± standard deviation) of MTA, MTA/ZrO, and MTA/ZnO were 6.83±1.70, 3.70±1.36, and 4.20±1.31. ΔE value of MTA was significantly higher than MTA/ZrO and MTA/ZnO (p<0.01). However, ΔE values of MTA/ZrO and MTA/ZnO were not significantly different (p>0.05).

The sectioned specimens of MTA showed the severe discoloration within the bulk of material as well as at the interface between the material and dentin (Figures 2A, B). The sectioned specimens of MTA/ZrO and MTA/ZnO showed the less discoloration within the material, but the discoloration at the interface was still noticeable (Figures 2C-F).

Discussion

In this study, any confounding factors that might affect tooth color measurement were controlled. Firstly, the extracted mandibular premolars with similar tooth color (A2-A3, Vita Shade) were only included. Secondly, the buccal thicknesses at the areas of color measurement, which may be more or less than the thickness in natural immature teeth, were similar among the three experimental groups. Thirdly, the position of measurement and the set-up environment were controlled by using the customized platform and the light-controlled box. The platform was composed of the 'stationary part' for placing a spectrophotometer and the 'mobile part' individually fitted with the block of each specimen. At each measurement, the spectrophotometer was placed on the 'stationary part' to make the probe tip consistently contacted with the buccal tooth surface of each specimen on the 'mobile part'. Finally, the color measurement was performed within 10 seconds to prevent the drying effect on tooth color change [15, 16].

In our study, the severe discoloration within the bulk of material and at the interface of the original ProRoot MTA caused a major tooth color change. The discoloration from MTA is attributed to the destabilization of BO when contacts with sodium hypochlorite irrigant or dentin collagen in the oxygen-free environment [7]. In these circumstances, BO has been changed into the black precipitates that induces discoloration of material and adjacent tooth structure [8].

Mixing 5% ZnO or ZrO into ProRoot MTA achieved approximately 50% reduction of tooth discoloration. Our results are in correspondence with the results from other study that showed 5-15% ZnO inhibited discoloration of MTA (MTA Angelus, Brazil) by stabilizing BO from the oxidizing reaction [8]. Nevertheless, the stabilizing mechanism of ZrO in reducing MTA discoloration should be further investigated and confirmed.

Table 1 Mean tooth color changes in L* a* and b* (ΔL*, Δa*, Δb*) and total color change (ΔE) at day 28 in regenerative endodontic model with MTA, MTA+5% ZrO and MTA+5% ZnO

Group	ΔL*	Δa*	Δb*	*ΔE (mean ± SD)
MTA	-4.86	-0.32	-4.64	6.83 ± 1.70^{A}
MTA/ZrO	-1.60	-0.44	-3.11	3.70 ± 1.36^{B}
MTA/ZnO	-2.87	-0.24	-2.86	4.20 ± 1.31^{B}

*The different superscript letters indicate a significant difference in ΔE between the experimental groups.
Abbreviations: MTA- mineral trioxide aggregate (ProRoot MTA), ZrO- zirconium oxide, ZnO- zinc oxide.

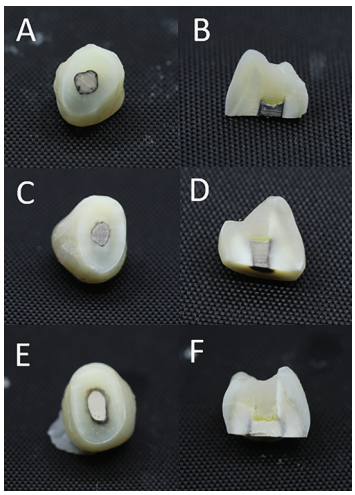

Figure 2 Horizontally (first column) and vertically (second column) sectioned specimens of MTA (A, B), MTA/ZrO (C, D), MTA/ZnO (E, F). The original ProRoot MTA (A, B) showed distinct discoloration within the bulk of material and at the interface between the material and dentin. MTA/ZrO (C, D), and MTA/ZnO (E, F) showed less discoloration especially within the bulk of material, but the discoloration at the interface was still noticeable.

MTA with 5% ZnO or ZrO in our study still presented discoloration at the interface between the material and dentin. Tooth color changes in these experimental groups (ΔE 3.7-4.2) were clinically detectable, in which the perception level by human eyes is ΔE 3.7 [17]. The amount of this additive at 5% (by weight) was not enough to completely inhibit the oxidizing reaction between MTA ingredients and BO. From the previous study, adding ZnO up to 15% was sufficient to completely inhibit discoloration of MTA (MTA Angelus) without a significant change to the other properties [8]. An increase in a ratio of ZnO up to 15% into MTA is possible to improve the inhibition effect on discoloration and should be further evaluated.

Cuttajar, *et al.* [18] studied the modification of MTA by replacement BO with ZrO. MTA required 20-30% ZrO to achieve the radiopacity comparable to MTA with 20% BO, while this amount of ZrO did not significantly change other physical and mechanical properties of MTA [18]. An increase in the ratio of ZrO to inhibit discoloration of MTA is possible up to 20-30%, while the chemical reaction and biocompatibility should be maintained [19].

From a preliminary investigation in our previous study [14], a control group without MTA material was prepared to inspect the storage effect on tooth color change. The change in tooth color was 1.63 in ΔE after 30 days in the storage condition. It has been implied that the tooth color was slightly darker over a period of storage, even without MTA material. Thus, the tooth color changes in our present study were slightly affected by the storage condition.

Clinically, MTA must contact with blood and tissue fluid when used in a regenerative endodontic treatment, which the discolored precipitates could be formed [20]. A contamination with blood tends to increase discoloration of MTA [12, 13]. In our study, the primary effect of a stabilizing agent (ZnO or ZrO) on the inhibition of MTA discoloration was only investigated. The condition of blood contamination should be further studied.

Conclusion

From the limitation in this study, mixing 5% ZrO or ZnO into MTA powder significantly decreased tooth discoloration at 28 days in the regenerative endodontic model.

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

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