

# Effect of Different Durations of Home Bleaching on Tooth Discoloration Caused by Mineral Trioxide Aggregate: An In Vitro Study

Keyvan Saati <sup>1</sup>, Ramtin Chitsazha <sup>2</sup>, Negin Firouzi <sup>3</sup> , Sohrab Tour Savadkouhi <sup>4</sup>, Negar Firouzi <sup>5</sup>

<sup>1</sup> Department of Restorative Dentistry, Dental Branch, Islamic Azad University of Medical Sciences, Tehran, Iran

<sup>2</sup> Department of Periodontics, Faculty of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>3</sup> Department of Endodontics, Faculty of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>4</sup> Department of Endodontics, Faculty of Dentistry, Islamic Azad University of Medical Sciences, Tehran, Iran

<sup>5</sup> Faculty of Dentistry, Islamic Azad University of Medical Sciences, Tehran, Iran

## Corresponding author:

Negin Firouzi, Department of Endodontics, Faculty of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

neginfirouzi@gmail.com

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## Abstract

**Background and Aim:** Discoloration is an unfavorable side effect of regenerative endodontic procedures using mineral trioxide aggregate (MTA). The efficacy of home bleaching of discolored teeth with carbamide peroxide has not been well investigated, and the minimum required duration of home bleaching is still unclear. This study aimed to compare the effects of different durations of home bleaching on tooth discoloration caused by MTA.

**Materials and Methods:** This in vitro, experimental study used 16 tooth blocks of bovine central incisors. To cause discoloration, white MTA was applied for 40 days in cavities drilled in blocks. The color parameters were measured at baseline and at 14, 28, and 42 days after the application of 20% carbamide peroxide using a spectrophotometer. Data were analyzed by repeated measures ANOVA and Tukey's post-hoc test.

**Results:** The color change ( $\Delta E$ ) value was  $22.9 \pm 10$ ,  $26.3 \pm 10.9$ , and  $27.03 \pm 11$  at 14, 28, and 42 days after bleaching, respectively. Significant color change occurred at 2, 4, and 6 weeks after the application of carbamide peroxide ( $P < 0.001$ ). The color change increased at 42 days ( $\Delta E$  of 3.1, or 13% compared with baseline), which was the highest amount among all time points. However, pairwise comparisons showed that it was not statistically significant ( $P = 0.4$ ).

**Conclusion:** It appears that 14 days is the required time for bleaching of teeth discolored by MTA. Longer bleaching times showed insignificantly higher efficacy for tooth whitening.

**Key Words:** Tooth Bleaching; Carbamide Peroxide; Tooth Discoloration; Mineral Trioxide Aggregate

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## Introduction

Regenerative endodontics is becoming a trending treatment for necrotic immature permanent teeth. It aims to regenerate the pulp-dentin complex damaged by infection, trauma, or developmental anomalies in

immature permanent teeth with necrotic pulp [1].

Mineral trioxide aggregate (MTA) has been widely used in endodontic procedures including regenerative endodontic treatments. However, one main drawback of MTA is tooth

discoloration due to its clinical application in vital pulp therapy, perforation repair, and revascularization of immature necrotic teeth [2,3].

As a bleaching agent, hydrogen peroxide is directly applied on the tooth or is generated by the activity of carbamide peroxide or sodium perborate. Carbamide peroxide and sodium perborate are efficacious because they gradually release hydrogen peroxide, decreasing the side effects of the release of bleaching molecules in high concentrations [4]. According to the literature, carbamide peroxide, hydrogen peroxide, and sodium perborate are the most commonly used bleaching agents. These agents cause chromatic alterations by oxidative reactions [5]. It should be noted that bleaching of non-vital teeth is associated with risks due to the possibility of change in dentin permeability and structure, penetration of hydrogen peroxide into the dentinal tubules, risk of relapse, overall compromise of some properties of dental hard tissues, dental fractures during treatment, and severe external resorption of the root at the cervical area [6]. Such risks could be reduced by using a lower concentration of hydrogen peroxide. Moreover, products containing 0.1-6% hydrogen peroxide can only be purchased by dental practitioners. External bleaching is a superior technique due to lower rate of discoloration relapse, which is attributed to the extended time of exposure to the bleaching agent without the risks of internal bleaching procedure [4]. External bleaching is preferred due to its much lower concentration of bleaching agent (10% carbamide peroxide), which minimizes the secondary side effects [7].

Due to its favorable biological outcomes, regenerative endodontic procedures might be an alternative to conventional root canal therapy. According to the American Association of Endodontists, regenerative endodontic procedures mainly aims to promote bone healing, increase the root wall thickness

and/or root length, and elicit a proper response to vitality tests [8]. After chemical disinfection, a scaffold is created in the canal space, and the cervical area is sealed with 3-4 mm of a calcium silicate-based cement such as grey or white MTA [9,10]. However, discoloration of the crown is one drawback of this technique, which might be caused by MTA. Ioannidis et al. evaluated discoloration by grey and white MTA by spectrophotometric analysis, and reported that both materials discolored teeth, with grey MTA causing more discoloration [11]. In addition, it has been reported that blood contaminated-MTA, interactions between MTA and NaOCl, and the effects of light and oxygen might give rise to further discoloration [12], causing significant clinical problems because tooth discoloration has adverse effects on the quality of life in children, adolescents, and their families. Therefore, it is considered a "patient-oriented evidence measure" [12,13]. The American Association of Endodontists' recent considerations for regenerative procedures recommend that the risk of discoloration should be included in informed consent forms. Although MTA, as a coronal barrier, might cause tooth discoloration to some extent, no superior alternative has been identified for it [14].

Regenerative endodontic treatment is frequently applied to treat incisor teeth, in which tooth discoloration affects the quality of life as a patient-oriented outcome. Discoloration can be resolved through oxidative reactions of chromogen molecules. Hydrogen peroxide ( $H_2O_2$ ) is the principal active agent applied on the tooth directly or released from carbamide peroxide [ $CO(NH_2)$ ,  $H_2O_2$ ] or sodium perborate ( $NaBO_3 \cdot 4H_2O$ ) [5].

The most serious drawback of non-vital tooth bleaching is external resorption of the cervical area of the root, attributed to acidic pH and massive release of bleaching molecules from a higher concentration of hydrogen peroxide. Therefore, the gradual release of

H<sub>2</sub>O<sub>2</sub> can neutralize pH changes. The home bleaching technique is preferred due to its daily replacement, requiring lower concentrations of the bleaching agent (10% carbamide peroxide); accordingly, the risk of possible side effects can be diminished. However, the efficacy of home bleaching of discolored teeth with carbamide peroxide has not been evaluated [15]. The question is what duration of home bleaching has greater effect on color change caused by MTA. This study compared the effects of different durations of home bleaching on tooth discoloration caused by MTA.

## Materials and Methods

### Sample preparation:

In this *in vitro*, experimental study, 16 bovine upper central incisors were stored in saline solution for further evaluation. The present study was approved ethically by the Research Council, Dental Faculty of Islamic Azad University. The samples were assessed clinically and radiographically to ensure no stains, cracks, caries, or calcification. The teeth were cleaned from debris and calculus using a scaler, followed by cleaning with a rubber cup and pumice paste. The samples were sectioned to obtain enamel-dentin cubic blocks measuring 4×5 mm, with a standardized thickness of 2±0.1 mm from the center of each tooth. A cylindrical cavity measuring 2.5 mm in diameter and 1 mm in depth was prepared on the dentin surface of each cube with a high-speed diamond bur under water coolant, leaving 1 mm of the labial surface intact. The samples were immersed in 5.25% NaOCl (Nikdarman, Iran) for 3 minutes, washed, and immersed in 17% EDTA (Marvabon, Iran) (pH=7.7) for 2 minutes to remove the smear layer, followed by a final irrigation with saline. White MTA (Angelus Solucoes Odontologicas, Londrina, Brazil) was mixed according to the manufacturer's instructions and applied to the cavities by a Dycal applicator. A piece of wet

cotton pellet was placed over the MTA, and the cavities were restored with Cavit (3M ESPE, St Paul, MN, USA). After 24 hours, the cotton pellet was removed, and the cavities were restored with composite resin (Filtek Z250; 3M ESPE, St. Paul, MN, USA). The samples were incubated in saline at 37°C for 40 days. Once the color change ( $\Delta E$ ) was visually noticeable, the temporary restoration was removed, and the tooth  $\Delta E$  was recorded (t<sub>0</sub>).

### Bleaching procedure:

The 20% carbamide peroxide (Opalescence PF, Ultradent Products Inc., South Jordan, Utah, USA) was applied on the enamel surface of each block for 2–4 hours every day according to the manufacturer's instructions and to simulate the clinical situation.

### Color assessment:

Color parameters were measured at 2, 4, and 6-week intervals after external bleaching using a spectrophotometer (Vita Easy Shade 3D Master; Vita Zahnfabrik, Bad Säckingen, Germany). A single operator measured the parameters under constant illumination. The samples were mounted in putty impression material (Perfect F-Putty, Han Dae Chemical, Korea) to ensure a standard position in which the tip of the device was perpendicular to the tooth surface. The spectrophotometer was calibrated optimally using white and green lines and made three measurements; the mean was calculated and used for subsequent analyses. The  $\Delta E$  was determined based on the CIE L\*a\*b\* system. L\* represents lightness ranging from black (0) to white (100), a\* and b\* represent the chromaticity of green (+a\*), red (-a\*), yellow (+b\*), and blue (-b\*). The  $\Delta E$  value was calculated according to the following formula:

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

### Statistical analysis:

Data were analyzed with SPSS version 22 (SPSS Inc., IL, USA). Repeated measures one-way ANOVA was used to compare the  $\Delta E$  difference between each two time points after

external bleaching.

## Results

This study was performed on 16 samples of bovine tooth blocks at three follow-up time points of 28, 14, and 42 days. We also had a control sample tested without the application of carbamide peroxide which was darkened slightly, but not significantly, during the follow-up period.

The magnitude of  $\Delta E$  according to the follow-up time was calculated based on  $L^*$ ,  $a^*$ , and  $b^*$  parameters; the values are presented in Table 1.

The  $\Delta E$  value was  $22.9 \pm 10$ ,  $26.3 \pm 10.9$ , and  $27.03 \pm 11$  at 14, 28, and 42 days after bleaching, respectively (Table 2).

Based on the results of repeated-measures ANOVA, there was no significant difference between the  $\Delta E$  values at 14, 28, and 42 days (Table 2). Also, the rate of  $\Delta E$  in the period of 14 to 42 days was 3.13 (13.1%),

The coefficient of variation (CV) was between 40 and 42, which was a high value (due to the difference in natural colors of bovine teeth), but it did not affect the overall changes.

**Table 1.**  $L^*$ ,  $a^*$ , and  $b^*$  parameters in the groups at different time points

Color parameter	14 days	28 days	42 days
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
$L^*$	70.08 $\pm$ 27.61	78.69 $\pm$ 18.46	78.85 $\pm$ 21.62
$a^*$	-2.11 $\pm$ .98	-2.45 $\pm$ .73	-6.76 $\pm$ 2.41
$b^*$	26.41 $\pm$ 9.34	25.93 $\pm$ 10.68	22.08 $\pm$ 10.65

SD: Standard deviation

**Table 2.** Comparison of  $\Delta E$  at the examined time points (n=16)

Time intervals	$\Delta E$ Mean $\pm$ SD	CV	$\Delta E_{28} - \Delta E_{14}$ (%)	$\Delta E_{42} - \Delta E_{28}$ (%)	$\Delta E_{42} - \Delta E_{14}$ (%)
Day 14	23.9 $\pm$ 10	41.8	2.4 (10.04)	0.73 (3.05)	3.13 (13.1)
Day 28	26.3 $\pm$ 10.9	41			
Day 42	27.03 $\pm$ 11	40.7			
P value	0.403				

CC: coefficient of variation; SD: Standard deviation

P value: Repeated-measures one-way ANOVA

## Discussion

This study revealed that carbamide peroxide, as an external bleaching agent, can effectively resolve the discoloration induced by MTA. This study used the Vita 3D Master spectrophotometer to obtain optimal accuracy and high reliability [15]. Tooth discoloration was evident after 40 days of MTA application [16,17]. The  $\Delta E$  at 14, 28, and 42 days showed significant effect of bleaching on discoloration ( $P < 0.001$ ). Different factors such as the concentration of bleaching agent and application time determine the effectiveness of bleaching agents. In addition, according to previous studies [18-20], 10% carbamide peroxide yields optimal results in both vital and non-vital teeth; therefore, the present study was designed to determine the effect of different durations of home bleaching on tooth discoloration caused by MTA.

Bernardon et al. investigated the effect of home bleaching on 50 individuals in two groups using 10% and 16% carbamide peroxide. The treatment was applied daily for 2 hours for 45 days, and color recording was performed with a spectrophotometer on days 15, 30, 45, and 180 after bleaching. Although they found no significant difference between different concentrations, 10% of patients achieved the desired color after 30 and 45 days [18]. Aka and Celik evaluated the efficacy of 10% carbamide peroxide and a pre-loaded tray with 6% hydrogen peroxide. The results showed that both products had a bleaching effect, but 10% carbamide peroxide was more effective [19]. Burrows evaluated different bleaching methods and verified that 10% carbamide peroxide had the best efficacy with lower risks and side effects. Notably, 10% carbamide peroxide yielded optimal outcomes in both vital and non-vital teeth. However, the study was not

conducted on discoloration induced by MTA [20].

Tooth discoloration is particularly problematic in the esthetic zone. Some endodontic materials result in discoloration of dental structures. Some recent in vitro studies showed the accumulation of MTA by products at the MTA-dentin interface and intratubular dentin or surface of the material [21,22]. Furthermore, evidence shows that the calcium released from the MTA's interaction with phosphate ions in the tissue fluids leads to deposition of carbonate apatite [23]. The pigmented by products are produced by the binding of MTA constituents to plasma proteins or phosphate ions in the dentinal fluid and oxidation and conversion to colored products [24].

Previous studies demonstrated no significant micro-surface changes by the bleaching process with 10% carbamide peroxide, 20% carbamide peroxide, and 13% or even 35% hydrogen peroxide [25]. It has been confirmed that 35% hydrogen peroxide causes morphological alterations in enamel with substantial reductions in calcium and phosphorus contents; while carbamide peroxide does not have such side effects [26, 27]. However, morphological alterations in the enamel surface have been reported after tooth bleaching with 10% carbamide peroxide, including increased surface roughness and reduced surface hardness, with adverse changes in the enamel elastic modulus, increasing the surface corrosiveness and fragmentation of enamel protein matrix [28]. Therefore, increased roughness of teeth due to bleaching procedures might cause more susceptibility to discoloration and can be affected by concentration of bleaching agent and bleaching time [29].

Carbamide peroxide also releases urea

and hydrogen peroxide at low concentrations and is used to bleach vital teeth, and more recently non-vital teeth. Considering the efficacy of carbamide peroxide with a low concentration for bleaching with minimal damage, it was selected and used as a gel in the present study. To achieve the desired result with carbamide peroxide, we increased the time duration and examined its effect, and found that 14 days was sufficient for carbamide peroxide to correct the color of teeth discolored by MTA. Although carbamide peroxide improved the color after 14 days and until the end of the study, i.e. 42 days, there was no statistically significant change after 14 days.

Carbamide peroxide significantly whitened the teeth for 14 days. Subsequent follow-ups on days 28 and 42 still showed changes in tooth color, but these changes were not statistically significant.

Future in vivo studies are required to better elucidate this topic. Moreover, the effect of bleaching agents should be evaluated in longer follow-ups.

## Conclusion

The use of carbamide peroxide for 14 days seems to be effective and sufficient to whiten teeth discoloration by MTA.

## Conflict of Interest

None.

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