Effect of bleaching agents having a neutral pH on the surface of mineral trioxide aggregate using electron microscopy and energy dispersive X-ray microanalysis

Nooh Kazia, Nithin Suvarna, Harish Kumar Shetty, Pradeep Kumar
Department of Conservative Dentistry and Endodontics, Yenepoya Dental College, Mangalore, Karnataka, India

Abstract
Aim: To investigate the effect of bleaching agents having a neutral pH on the surface of mineral trioxide aggregate (MTA) used as a coronal seal material for nonvital bleaching, beneath the bleaching agent, with the help of energy dispersive X-ray microanalysis and scanning electron microscopy (SEM).

Materials and Methods: Six samples of plastic tubes filled with white MTA (Angelus white) were kept in 100% humidity for 21 days. Each sample was divided into 2 and made into 12 samples. These were then divided into three groups. Group A was exposed to Opalescence Boost 40% hydrogen peroxide (HP) (Ultradent). Group B to Opalescence 10% carbamide peroxide (Ultradent) and Group C (control group) not exposed to any bleaching agent. After recommended period of exposure to bleaching agents according to manufacturers’ instructions, the samples were observed under SEM with an energy dispersive X-ray microanalysis system (JSM-6380 LA).

Results: There were no relevant changes in color and no statistically significant surface structure changes of the MTA in both the experimental groups.

Conclusion: The present findings suggest that even high concentration HP containing bleaching agents with neutral pH can be used on the surface of MTA without causing structural changes. The superior sealing ability of MTA and the high alkalinity would prevent cervical resorption postbleaching.

Keywords: Carbamide peroxide; energy-dispersive X-ray; mineral trioxide aggregate; opalescence boost; scanning electron microscopy

INTRODUCTION
A nonvital, discolored tooth is often recommended for bleaching. The risk of cervical resorption postbleaching can be avoided by placing a cervical base before the bleaching procedure.1,2 The walking bleach technique is carried out by reducing 2–3 mm of the root canal filling material after the endodontic therapy, following which an impermeable base such as resin-modified glass ionomer cement is placed on the filling material to prevent cervical root resorption.

Mineral trioxide aggregate (MTA) has been widely used for various endodontic procedures since two decades, such as restoring resorptive defects, horizontal root fractures, sealing perforations, and apexification. MTA has been proved to be biocompatible, stimulate mineralization, and encourage apatite-like crystalline deposits.3

The sealing ability of MTA has a nonstatistically significant difference compared to the cyanoacrylate-sealed control.
as a cervical barrier in the intracoronal bleaching.\textsuperscript{[4,5]} Based on these findings, there appeared the possibility that a bleaching agent may be applied on the MTA in the discolored nonvital tooth after a cervical perforation site has been filled with MTA.

Bleaching agents lower the pH on the root surface, which has been suggested as a mechanism for cervical resorption.\textsuperscript{[6]} Higher pH of the MTA and released calcium hydroxide may further protect the root and prevent cervical resorption. However, an acidic pH in the environment impedes MTA setting and reduces its strength and hardness.\textsuperscript{[7,8]}

The previous study suggested that the acidic condition of bleaching agents attacked the surface of the completely hardened MTA, affecting its surface structure.\textsuperscript{[9]}

However, a case report has shown the successful use of MTA beneath bleaching agents. Here, MTA was used as an obturating material as well as coronal seal beneath the bleaching agent.\textsuperscript{[10]}

This study was done to evaluate the surface and structural changes of MTA after the application of Opalescence Boost (40% hydrogen peroxide [HP]) and Opalescence 10% carbamide peroxide bleaching agent which are known to have neutral pH.

**MATERIALS AND METHODS**

This in vitro study was carried out using, 1 g MTA Angelus (Angelus, Londrina, PR, Brazil), Opalescence Boost (Ultradent Products, Inc., South Jordan) 40% HP, Opalescence 10% carbamide peroxide (Ultradent Products, Inc., South Jordan), 6 plastic tubes (4 mm × 4 mm), disposable scalpel, LED light source, and scanning electron microscopy (SEM) with an energy dispersive X-ray (EDX) system (JSM-6380, LA).

The standard powder/liquid ratio (1 g of white MTA powder to 0.33 mL purified water), according to the manufacturer’s instructions, was used for this study. The mixed MTA was placed in plastic tube (4 mm in length × 4 mm in diameter) and maintained in a container for 21 days at room temperature in which the relative humidity was 100%. In 24 h, MTA has hardened MTA, affecting its surface structure.\textsuperscript{[9]}

MTA samples were exposed to Opalescence 10% carbamide peroxide (pH 6.5). The bleaching agent was mixed according to the manufacturers’ instructions and placed at a 2 mm in thickness on the MTA. It was reacted with the MTA for 5 min and activated with an LED light source for 3 min. This procedure was repeated three times. After that, the surface of the MTA was cleaned with tap water for 1 min, dried with air, and kept in a container at room temperature for 1 week.

**Group B**

MTA samples were exposed to Opalescence 10% carbamide peroxide (pH 6.5). The bleaching agent was placed at a 2 mm in thickness on the MTA. The solutions were activated with an LED light source for 3 min. This procedure was repeated three times. After that, the surface of the MTA was cleaned with water for 1 min, dried with air, and kept in a container at room temperature for 1 week.

**Scanning electron microscopy and energy-dispersive X-ray analysis**

The samples were mounted on aluminum holders with adhesive carbon tape and observed using an operating microscope. Thereafter, the same samples were coated with gold using sputtering (JFC-1600). They were then examined with a SEM; fitted with an EDX system (JSM-6380 LA). SEM observation (magnification: R, the same samples were coated using a secondary electron imaging technique operating at 20 kV and a working distance of 15 mm. In this study, EDX by area mode (analytic area: 0.01 mm\(^2\)) was done on three parts of the MTA surface selected at random. EDX was performed using the following parameters: An X-ray takeoff angle of 35°, a probe current of 1.0 nA, and a counting time of 100 s. Weight percentages of Mg, Si, P, Ca, and bismuth (Bi) were calculated using an ZAF program. Furthermore, a high-speed additional element mapping was performed at 20 kV for 30 s × 10 cycles.

**Statistical analysis**

Statistical analysis was done with the help of Kruskal–Wallis test using SPSS version 13 software (SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Surface color**

There were no relevant changes in color of the MTA surface in both the experimental groups.

**Scanning electron microscopy and energy-dispersive X-ray**

Plate-like and globular structures were observed in all the three groups [Figure 1a-c]. The experimental
groups showed no relevant surface changes that were significantly any different than that in the negative controls, which would have otherwise been expected to have adverse effects on MTA surface such as the presence of woodpecker holes or creases which were seen to be a cause of microleakage in the previous studies.[9] There was no statistically significant difference in calcium and bismuth levels between the experimental groups.

**Group A**
Five MTA samples (2 mm × 2 mm) exposed to Opalescence Boost 40% HP (pH 7).

**Group B**
Five MTA samples (2 mm × 2 mm) exposed to 10% carbamide peroxide (pH 6.4).

**Group C**
Two MTA samples (2 mm × 2 mm) which were left untreated.

A, B, and C: Three different areas of the same sample where EDX readings were taken.

Table 1 and Graph 1 demonstrate that there was no statistically significant difference in levels of Al, Bi, Ca, Mg, and Si between the groups and the levels of Ca is high in all three groups.

**DISCUSSION**
Using a protective barrier before internal bleaching is mandatory to prevent cervical resorption. Various materials have been used as cervical protective barriers. MTA has been used as a successful barrier in cervical perforations.[11] MTA has shown a better intracanal sealing ability over GIC in a previous study.[12]

The effect of bleaching agents on WMTA, when used as a protective barrier over the root canal filling, is disputed. A study done by Tsujimoto et al.[9] suggested that the lower pH derived from the higher concentration of HP in bleaching agents, affected the surface structure of completely hardened MTA. The acidic condition of the bleaching agent

---

**Table 1: Statistical analysis of the energy dispersive x-ray results**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean±SD</th>
<th>Median</th>
<th>95% CI for Kruskal–Wallis test value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
<td>Upper bound</td>
</tr>
<tr>
<td>Al</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% carbamide peroxide</td>
<td>1.52±0.43</td>
<td>1.47</td>
<td>0.98</td>
<td>2.06</td>
</tr>
<tr>
<td>Opalescence Boost 40% HP</td>
<td>1.94±0.62</td>
<td>2.12</td>
<td>1.16</td>
<td>2.71</td>
</tr>
<tr>
<td>Control</td>
<td>2.99±0.58</td>
<td>2.99</td>
<td>−2.24</td>
<td>8.22</td>
</tr>
<tr>
<td>Bi</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% carbamide peroxide</td>
<td>13.12±1.72</td>
<td>12.77</td>
<td>10.98</td>
<td>15.26</td>
</tr>
<tr>
<td>Opalescence Boost 40% HP</td>
<td>10.66±4.52</td>
<td>10.85</td>
<td>5.06</td>
<td>16.27</td>
</tr>
<tr>
<td>Control</td>
<td>14.95±2.07</td>
<td>14.95</td>
<td>−3.67</td>
<td>33.56</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% carbamide peroxide</td>
<td>77.13±2.53</td>
<td>78.383</td>
<td>73.99</td>
<td>80.27</td>
</tr>
<tr>
<td>Cence boost 40% HP</td>
<td>75.32±6.76</td>
<td>72.913</td>
<td>66.92</td>
<td>83.71</td>
</tr>
<tr>
<td>Control</td>
<td>70.40±7.76</td>
<td>70.398</td>
<td>0.70</td>
<td>140.09</td>
</tr>
<tr>
<td>Mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% carbamide peroxide</td>
<td>0.31±0.26</td>
<td>0.35</td>
<td>−0.02</td>
<td>0.63</td>
</tr>
<tr>
<td>Opalescence Boost 40% HP</td>
<td>0.45±0.29</td>
<td>0.54</td>
<td>0.08</td>
<td>0.81</td>
</tr>
<tr>
<td>Control</td>
<td>0.06±0.00</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% carbamide peroxide</td>
<td>0.02±0.05</td>
<td>0</td>
<td>−0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Opalescence Boost 40% HP</td>
<td>0.11±0.13</td>
<td>0.0300</td>
<td>−0.05</td>
<td>0.28</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Si</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% carbamide peroxide</td>
<td>7.90±2.06</td>
<td>8.08</td>
<td>5.34</td>
<td>10.47</td>
</tr>
<tr>
<td>Opalescence Boost 40% HP</td>
<td>11.56±2.97</td>
<td>11.26</td>
<td>7.87</td>
<td>15.25</td>
</tr>
<tr>
<td>Control</td>
<td>11.70±5.21</td>
<td>11.70</td>
<td>−35.12</td>
<td>58.52</td>
</tr>
</tbody>
</table>

attacked the surface of the MTA, which induced the release of Ca ion through these phenomena, and subsequently, the relative concentration of silica increased. SEM images in the previous study showed surface defects such as cracks and woodpecker holes which were suspected to cause microleakage, hence demonstrating that bleaching agents may disturb the surface of even completely hardened MTA, suggesting that acidic attack of bleaching agents makes it prudent to protect the MTA with an intermediate resin before a bleaching procedure.\[9\] Such surface changes were absent in the present study and the levels of calcium remained high in the experimental groups.

In the present study, Opalescence Boost 40% HP and 10% carbamide peroxide bleaching agents were used which are known to have a neutral pH.\[13\]

In this study, MTA sample was mixed according to the manufacturers’ instruction and allowed to set in 100% relative humidity and at room temperature for 21 days until maximum hardness was achieved. The previous studies concerning the structural changes of MTA by acid attack were designed for different setting times after mixture. If the test was performed after insufficient setting time, it is easier to dissolve the components from the surface. Although the compressive strength of MTA at 24 h after mixture was inferior to that of Super EBA cement, there is no statistical difference between two materials after 21 days. This data mean that the MTA hardens completely at 21 days after mixture.

The standard bleaching methods recommended by manufacturers were used in this study. The samples after being exposed to bleaching agents were LED activated. It is believed that most light sources decompose peroxide faster (by increasing the temperature) to form free radicals.\[14\]

Following exposure to the bleaching agents and light activation, the surface of the MTA was observed for color changes. As opposed to the previous study where the color of the bleaching agent had turned gray following exposure to acidic bleaching agents,\[9\] such a surface change was absent in the present study. The surface of MTA was further observed in a SEM, which revealed no relevant structural changes which again was seen in the previous study in the form of creases and woodpecker holes which were believed to be a source of marginal leakage.

EDX analysis of the samples was then done to observe the elemental distribution of the samples and to check for any changes of the bleached groups as compared to the control group. The EDX reading following statistical analysis showed the mean of the elemental distribution in mass% as follows: Calcium levels for Group A were 75.32 ± 6.76, for Group B 77.13 ± 2.53, and for control group 70.40 ± 7.76; the levels of bismuth for Group A were 10.66 ± 4.52, for Group B were 13.12 ± 1.72, and for control group were 13.95 ± 2.7; the silica levels seen in Group A were 11.56 ± 2.97, in Group B were 7.90 ± 2.6, and in control group 11.70 ± 5.21. The magnesium levels

Graph 1: EDX analysis showing the elemental distribution within the MTA samples
were 0.45 ± 0.29 in Group A, in Group B were seen to be 0.31 ± 0.26, and in control group 0.6. The phosphate levels were 0.11 ± 0.13 in group, in Group B 0.02 ± 0.05, and were 0 in control group. Similarly, the levels of aluminum in Group A were 1.94 ± 0.62, for Group B were 1.52 ± 0.43, and for control group were 2.99 ± 0.58.

The statistical analysis was done by Kruskal–Wallis test, and none of the results seen among the groups had statistically significant differences.

In a study by Tsujimoto et al., it was seen that the acidic condition of the bleaching agent attacked the surface of the MTA, which induced the release of calcium ion. The release of calcium ion was due to chemical degeneration of hydrates after the application of bleaching agents. Studies done by Namazikhah et al. concluded that microhardness was significantly affected by MTA that what placed under acidic pH 4.5, and surface microhardness also increased with increase in pH. Microhardness could be enhanced by better hydration during setting process of MTA. However, during the hydration process at lower pH, calcified byproducts dissolve gradually. Reduction in calcium ion was seen with EDAX results of MTA samples hydrated at low pH.

However, in the present study, the level of calcium remained high, which appears to be due to the neutral pH of the bleaching agents used in the study.

MTA after mixing with water is highly alkaline due to the predominant presence of calcium hydroxide in its formulation and has biological properties similar to calcium hydroxide which stops any resorptive process and promotes healing. Calcium hydroxide will halt any resorptive process and promote healing.

Due to superior marginal adaptation of MTA, it is successfully been used to treat invasive cervical resorptions. The present study used MTA-Angelus, which contains 80% portland cement and 20% bismuth oxide, with no addition of calcium sulfate in an attempt to reduce setting time (2 h for ProRoot MTA and 10 min for MTA-Angelus). It has been reported that MTA-Angelus, released calcium ions, and promoted an alkaline pH (Duarte et al. 2003, Santos et al. 2005). Although bismuth oxide is said to improve the radiopacity, MTA-Angelus that contains less bismuth oxide is more radio-opaque than ProRoot MTA.

Placement of intracoronal bleaching pastes is believed to reduce the pH at the root surface, enhancing osteoclastic activity, hence resulting in cervical resorption. Therefore, to prevent leakage of bleaching agents, it is mandatory to use a protective barrier. Glass ionomer is commonly used to provide a coronal seal before nonvital bleaching.

Furthermore, physical properties of MTA mixed with distilled water were significantly affected when compared to MTA kept under pH 5.4 and 6.4. This suggested that higher concentration HP containing bleaching agent with neutral pH may be used on the surface of MTA.

However, a study by Pradeep et al. using walking bleach paste, prepared by mixing sodium perborate with 30% HP, for nonvital bleaching with an MTA coronal barrier showed superior result.

The data obtained using SEM and EDX clearly showed the present hypothesis to be validated because there were no changes in color of MTA in the experimental groups as compared to the control group after application of bleaching agent. There were no statistically significant surface changes among the three groups which suggest that high concentration of HP with a neutral pH did not affect the surface of MTA.

However, further studies are required to evaluate the use of MTA as a cervical barrier post nonvital bleaching.

**CONCLUSION**

Within the limitations of this study, it has been concluded that the use of Opalescence Boost 40% HP and 10% carbamide peroxide bleaching agents in the present study, which are known to have neutral pH, was seen to not only avoid surface changes of MTA which would have otherwise proved to cause microleakage and lead to cervical resorption but also the high calcium levels seen in the study groups would further prevent cervical resorption postbleaching.

**Financial support and sponsorship** Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

Kazia, et al.: Effect of bleaching agents on surface of MTA


