COMPARATIVE EVALUATION OF EFFECT OF A REMINERALIZING AGENT ON BLEACHED TOOTH SURFACE: AN IN-VITRO STUDY

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ABSTRACT
The aim of this study is to compare the effect of a new remineralizing agent on tooth surface before and after bleaching procedure. Freshly extracted intact human premolar teeth from patients extracted for orthodontic purposes were collected. Enamel sections were prepared from surfaces of teeth using diamond cutting disc and slow speed hand piece under water cooling. Specimens were divided into 4 groups of 8 specimens each and subjected to surface treatment as follows: Group-1 (Control - Intact tooth surface), Group-2 (Bleached tooth surface), Group-3 (Re mineralization before bleaching), Group-4 (Bleaching followed by remineralization). The re mineralizing agent used was 0.21 % Sodium fluoride- tricalcium phosphate based cream (Clinpro tooth cream, 3M ESPE) for 4 minutes. The bleaching agent used was 10 % Carbamide peroxide gel (Opalescence) for a period of 8-10 hours. Enamel Specimens were examined under scanning electron microscopy-Energy Dispersive X-ray Analysis and data obtained was statistically analyzed. A significant difference was seen in both calcium and phosphate levels of all the four groups mainly between the samples of Group 2 [bleached] and Group 4 [bleaching + re mineralization]. The de mineralizing effect of bleaching on enamel surface can be reduced by surface treatment using a re mineralizing agent as toothpaste containing Beta tricalcium phosphate. The re mineralization effect is relatively greater when the re mineralizing agent is used after the bleaching procedure rather than the procedure.

Keywords: Beta tricalcium phosphate, Calcium, Phosphate, re mineralization, De mineralization, Carbamide peroxide, bleaching.

INTRODUCTION
Teeth play a major role in the beauty of a smile in an individual. Color of the tooth is very important for the patient due to both social and psychological concern. Tooth discoloration shows variation in etiology, appearance, severity, location and adherence to tooth structure. Noticeable discoloration of teeth should not be regarded only as a condition of cosmetic importance, but also it can affect a person’s self-image, self confidence, attractiveness etc. Hence, bleaching has become a highly popular aesthetic dental service offered to patients. Most of the home bleaching agents contain carbamide peroxide at a concentration of 10 % to 15 %. Bleaching using 10 % carbamide peroxide was first reported by Klumser in1960’s. However, usage of these agents on a long term basis can cause dissolution of the surface enamel matrix. Several studies have been reported on use of bleaching agents that cause changes in level of calcium, phosphorous and potassium in dental tissues. Sensitivity following the treatment can occur due to possible removal of mineral content from enamel and dentin by demineralization, however, loss of mineral content can be enhanced by re mineralization. Although, traditionally Fluoride has been extensively used as a Re mineralizing agent; various newer agents like Casein Phosphopeptide-Amorphous calcium phosphate [CPP-ACP] and Fluoride-enriched Casein phosphopeptide-Amorphous calcium phosphate [CPP-ACPF] have been introduced in the last two decades for the re mineralization of teeth. In the recent past, a new fluoride containing Beta- tricalcium phosphate based Clinpro crème (3M ESPE) has been introduced which interacts with demineralized enamel to help boost re mineralization benefits of fluoride. However, the comparative effect of Beta tricalcium phosphate on bleached tooth surface when used before or after have not been reported. Hence, the aim of this study was to compare and evaluate the effect of Beta tricalcium phosphate on bleached tooth surface before and after bleaching with 15 % carbamide peroxide.

MATERIALS AND METHODS

Source of Data
Intact human premolars freshly extracted for orthodontic purposes were collected from patients of age group 14-18 years. Teeth with white spot lesions, caries, restorations, erosions, surface discolorations and cracks were excluded. The collected teeth were disinfected and stored as per the recommendations and guidelines laid by OSHA (Occupational Safety and Health Administration). The Bleaching agent used was 15 % Carbamide Peroxide [Opalescence] and a Beta-tricalcium phosphate based paste [Clinpro] used as a remineralizing agent. A total of 32 enamel sections of size measuring 4 x 4 x 2 mm were prepared from the surfaces of teeth using diamond cutting disc and slow speed hand piece under water cooling. Except the front surface all the other surfaces were covered using an impermeable surface coating material like nail varnish. All the specimens were then divided into 4 groups of 8 specimens each and subjected to surface treatment as follows:
• Group 1 (Control, Intact tooth surface) - No surface treatment [stored in artificial saliva]
• Group II - Bleaching with 15% carbamide peroxide for 8-10 hours per day for 14 days
• Group III - Surface pre-treatment with Beta tricalcium phosphate for 4 min per day for 14 days and then with 15% carbamide peroxide for 8-10 h per day for 14 days
• Group IV - Surface treatment with 15% carbamide peroxide for 8-10 h per day for 14 days and then treatment with beta tricalcium phosphate for 4 minutes per day for 14 days

The treated tooth specimens were examined under Scanning Electron Microscopy [SEM]-Energy Dispersive X-ray Analysis [EDAX] (Figure: 1, 2, 3, 4). Data obtained was compared and statistically analyzed using One-way ANOVA and Tukey’s test.

RESULTS
A significant difference was found between the calcium and phosphate levels in all the four groups.

Group 1 (control) - Ca: 67.76, P: 46.91
Group II (bleached) - Ca: 56.26, P: 34.34
Group III (R+B) - Ca: 60.11, P: 38.6
Group IV (B+R) - Ca: 63.9, P: 42.06

Statistically significant results (P < 0.005) were seen between:
• Both calcium and phosphate levels as shown in Graph 1.
• Both Bleached and Bleaching + Re mineralization group in both calcium and phosphate levels as shown in Table 1.

DISCUSSION
Although bleaching can decrease the discoloration in teeth, several studies have reported on its potential deleterious effect on the outer enamel surface of teeth. In a study Rotstein et al reported a loss of strength and solubility of enamel, dentin and cementum after bleaching. This was attributed to the effect of bleaching agents which changed the ratio of organic and inorganic components of the tissues. In a study by Davari et al the use of suitable therapeutic agents whose presence leads to the recovery of the surface hardness by acceleration of the re-mineralization process has been recommended both during bleaching appointments and also after completion of the bleaching regimen. These therapeutic agents generally include the use of re-mineralizing agents to reduce and repair the potential deleterious effects of bleaching. In this study, a significant difference was seen in both calcium and phosphate levels of all the four groups mainly between the samples of Group 2 [bleached] and Group 4 [bleaching + re-mineralization]. A significant difference was found between the calcium and phosphate levels in all the four groups.

Group 1 (control) - Ca: 67.76, P: 46.91
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Group IV (B+R) - Ca: 63.9, P: 42.06

Group II (Bleached) showed the lowest values due to loss of mineral content upon bleaching while comparatively both Group III (R+B) and Group IV (B+R) showed a higher mineral content. A study reported by Gladwell et al showed that using re-mineralizing agents after bleaching reduced the enamel solubility and sensitivity due to mineral deposition in enamel crystallites. This is in agreement with results of this study where re-mineralization after bleaching showed an increase in the mineral content than if done before bleaching. When comparing Group I (Intact) and Group II (Bleached) a decrease in the mineral content was seen in the latter which can be attributed to the decrease in mineral content of tooth surface. In a study, Tezel et al reported a loss of calcium, phosphate and fluoride elements from the tooth surface after bleaching with carbamide peroxide. In the present study, both Group III (R+B) and Group IV (B+R) showed a higher mineral content; however, Group IV (B+R) where re-mineralization was done using Beta tricalcium Phosphate after bleaching with 15% carbamide peroxide showed the highest increase in the mineral content on tooth surface compared to all the other groups. When statistically compared to all the other groups too it showed a significant difference in both calcium (0.021) and phosphate (0.002) levels. This is in agreement with a study by E. Jorgievskia et al which showed similar results; where re-mineralizing tooth pastes containing Bioactive glass was able to bring about re-mineralization of damaged enamel surfaces caused by 16% carbamide peroxide. The results of this study have shown that the newer re-mineralizing agent containing Beta tricalcium Phosphate [Clinpro tooth crème] when used after bleaching procedures is capable of repairing the damaging effects on enamel surface caused by the bleaching.

<table>
<thead>
<tr>
<th>Tukey’s HSD</th>
<th>(I) group</th>
<th>(J) group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calcium</strong></td>
<td>Group 1 (Control)</td>
<td>Group 2 (Bleached)</td>
<td>11.50</td>
<td>3.69</td>
<td>.021</td>
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<tr>
<td></td>
<td>Group 3 (R+B)</td>
<td>Group 4 (B+R)</td>
<td>7.65</td>
<td>3.69</td>
<td>.187</td>
</tr>
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<td></td>
<td>Group 3 (R+B)</td>
<td>Group 4 (B+R)</td>
<td>3.86</td>
<td>3.69</td>
<td>.725</td>
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<td></td>
<td>Group 2 (Bleached)</td>
<td>Group 3 (R+B)</td>
<td>-3.84</td>
<td>3.69</td>
<td>.728</td>
</tr>
<tr>
<td></td>
<td>Group 2 (Bleached)</td>
<td>Group 4 (B+R)</td>
<td>-7.63</td>
<td>3.69</td>
<td>.189</td>
</tr>
<tr>
<td></td>
<td>Group 3 (R+B)</td>
<td>Group 4 (B+R)</td>
<td>3.79</td>
<td>3.69</td>
<td>.736</td>
</tr>
<tr>
<td><strong>Phosphate</strong></td>
<td>Group 1 (Control)</td>
<td>Group 2 (Bleached)</td>
<td>12.57</td>
<td>3.11</td>
<td>.002</td>
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<tr>
<td></td>
<td>Group 3 (R+B)</td>
<td>Group 4 (B+R)</td>
<td>8.26</td>
<td>3.11</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Group 2 (Bleached)</td>
<td>Group 3 (R+B)</td>
<td>-4.30</td>
<td>3.11</td>
<td>.418</td>
</tr>
<tr>
<td></td>
<td>Group 2 (Bleached)</td>
<td>Group 4 (B+R)</td>
<td>-7.71</td>
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<tr>
<td></td>
<td>Group 3 (R+B)</td>
<td>Group 4 (B+R)</td>
<td>-3.40</td>
<td>3.11</td>
<td>.697</td>
</tr>
</tbody>
</table>
Graph 1: There is significant difference (P < 0.005) found in mean values of both calcium and phosphate

SEM images of all the Four Groups

Figure 1: Group 1 (control group)

Figure 2: Group 2 - Bleaching with 15% carbamide peroxide

Figure 3: Group 3 - Re mineralization + Bleaching

Figure 4: Group 4 - Bleaching + Re mineralization
CONCLUSION
Within the limitations of this in vitro study, we conclude that:

- Although bleaching agents are clinically effective, its potential effects on enamel surface leads to both qualitative and quantitative effect on the mineral content of enamel surface.
- The de mineralizing effect on bleached enamel surface can be reduced by the use of re mineralizing tooth pastes containing Beta tricalcium phosphate.
- The re mineralization effect is greater when the re mineralizing agent Beta tricalcium Phosphate is used after rather than before the bleaching procedure.

REFERENCES

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