Remineralization of bleached enamel with novel nutraceutical agents: An in vitro study

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Abstract

Background/Purpose: The effect of bleaching agents on dental hard tissues was uncertain. Moreover, reverting the side effects caused by bleaching using nutraceuticals as remineralizing agents remained uninvestigated. Hence, the aim of this study was to evaluate the effect of 22% carbamide peroxide (CP) on the mineral content of enamel and to evaluate the remineralization of bleached enamel after treatment with three different remineralizing agents.

Materials and Methods: A total of 30 extracted human maxillary incisors were selected, and their initial mineral content was analyzed using scanning electron microscopy with energy dispersive X-ray spectroscopy (EDX). All the specimens were bleached with 22% CP and their mineral content was reanalyzed. They were then randomly divided into three groups. Group A: Remineralization with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), Group B: Remineralization with a 6.5% grape seed extract (GSE) solution prepared in phosphate buffer, and Group C: Remineralization with 6.5% wheat grass solution in phosphate buffer. Remineralization was carried out for 10 min, and EDX analysis was carried out again. Statistical analysis was done using one-way ANOVA and Student’s t-test.

Results: Bleaching with 22% CP significantly decreased the mineral content of the enamel. All the three remineralizing agents significantly increased the mineral content. Group A (CPP-ACP) showed the highest remineralization than Group B and C but was not statistically significant.

Conclusion: Within the limitations of the present study, it can be concluded that bleaching with CP decreases the mineral content of enamel and agents such as CPP-ACP, GSE, and wheat grass help in remineralizing the bleached surface.

Keywords
Energy dispersive X-ray spectroscopy, nutraceutical, remineralization, vital bleaching

Introduction

Vital bleaching is considered as an easy, effective, and a conservative way to lighten the discolored teeth. The etiology of tooth discoloration is complicated and is broadly classified as intrinsic, extrinsic, or internalized in nature.[1]

Bleaching agents such as hydrogen peroxide or carbamide peroxide (CP), undergo ionic dissociation when applied to the tooth structure and give rise to the formation of free radicals such as nascent oxygen, hydroxyl radical, per hydroxyl, and superoxide anions, which are unstable free radicals. These highly reactive free radicals reach-out for electron rich regions of pigment inside the dental structure and cause breakdown of the large pigmented molecules into smaller, less pigmented ones.[2]

However, the side effects of bleaching on enamel such as decrease in mineral content and hardness, increased roughness and morphological alterations remain quite a debatable topic in the literature due to conflicting results from previous studies.[3]

One of the most common clinical complaints associated with bleaching is the sensitivity of teeth. The incidence of hypersensitivity was reported to be 10-90% with the in-office bleaching procedure. Although it could be mostly mild or moderate, it is at times so severe that the treatment is discontinued.[4] Enamel is generally considered to be impermeable. Nevertheless, the oxidative free radicals generated from hydrogen peroxide have low molecular weight and are able to denaturize proteins. These free radicals create porosities on the enamel structure which may thus permit diffusion through
Remineralization of bleached enamel with novel nutraceutical agents

Sajjan, et al.

the organic matrix of dentin. From dentine hydrogen peroxide reaches the pulp, where it directly activates A - δ nerves which thereby results in pain.[5] Thus, the surface morphological changes may contribute to the sensitivity associated with bleaching.

Along with surface remineralization, the surface requires to be free of oxygen free radicals as the residual free radicals can cause pulpal inflammation,[6] compromised bond strength for immediate composite restoration[3] and altered optical properties of bleached enamel. So, it would be beneficiary if remineralizing agent also has antioxidant property. In this view, grape seed extract (GSE) and wheat grass, which are known antioxidants, were tried as surface remineralizing and desensitizing agents.

GSE, a nutraceutical, is a rich source of proanthocyanidin (PA), mainly composed of monomeric catechin and epicatechin, gallic acid, and polymeric and oligomeric procyanidins.[7]

GSE as a remineralizing agent was evaluated by confocal laser scanning microscopy. It was concluded in this morphological analysis that GSE has a positive effect on remineralization process of the artificial carious lesion.[8] However, chemical analysis of the bleached surface, regarding changes in the chemical composition of the surface has not been studied in any of the previous studies.

Wheatgrass is another nutraceutical with many health benefits. It is a good source of Vitamin A, Vitamin C, Vitamin E, Vitamin K, thiamin, riboflavin, niacin, Vitamin B6, pantothenic acid, calcium, phosphorus, potassium, iron, zinc, copper, manganese, and selenium.[9] Wheat grass has anti-inflammatory, anticancer, antiaging, and antioxidant property.[10] However, no studies regarding the remineralization potential of these novel nutraceutical agents on bleached enamel can be found in the literature. Hence, the aims of the study were:

1. To evaluate the effect of 22% CP on the mineral content of enamel
2. To evaluate the remineralizing potential of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), GSE, and wheat grass on the bleached enamel.

The mineral content was assessed using energy dispersive X-ray spectroscopy (EDX) (ZEISS, EVO18, Special Edition, Department of Physics, Osmania University, Hyderabad.)

Materials and Methods

About 30 extracted human maxillary incisors were cleaned using ultrasonic scaler and analyzed using EDX (ZEISS, EVO18, Special edition. Department of Physics, Osmania University, Hyderabad) to determine the initial mineral content. 22% CP (Philips Zoom, M & M Dental Associates, Mumbai, India) was applied to the labial surfaces 8 h/day for 2-week. Then, samples were washed and stored in artificial saliva. Post-bleaching EDX analysis was done.

Preparation of 6.5 % w/v of GSE solution in phosphate buffer (pH 7.4)

About 6.5 g of GSE powder was dissolved in 100 ml of phosphate buffer solution.

Preparation of 6.5% w/v of wheat grass solution in phosphate buffer (pH 7.4)

About 6.5 g of wheat grass powder was dissolved in 100 ml of phosphate buffer solution.

Results

EDX was used to determine the calcium and phosphorus content in % weight of sound, bleached and remineralized enamel in each group. The calcium (Ca) and phosphorus (P) content was converted into Ca/P ratios. The EDX graphs of the highest Ca/P ratio attained among sound enamel samples, bleached samples, and Group A, B, and C samples are shown in Graphs 1-5, respectively. Table 1 shows a comparison of the mean Ca/P ratios of sound and bleached enamel using t-test. There was a statistically significant decrease in the mineral content after bleaching (P = 0.021). Table 2 represents the mean Ca/P ratio of bleached and remineralized samples analyzed by t-test. There was a significant increase in the mineral content after surface treatment with remineralizing agents in all the groups (P = 0.00). One-way ANOVA was applied to compare the mean Ca/P ratios of the study groups after remineralization shown in Table 3 which showed no statistically significant difference between the three study groups (P = 0.633). Graph 6 represents relationship between the remineralization potential of study groups.

Discussion

Bleaching agents produce free radicals which cause fragmentation of pigmented molecules into smaller species and alter the light absorption and thereby reduce or eliminate the stain.[10] It was stated in a review that \( \text{H}_2\text{O}_2 \) induces alteration of the chemical composition of enamel by reducing the Ca/P

Table 1: Comparison of mean Ca/P ratios of sound and bleached enamel using t-test

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sound enamel</td>
<td>30</td>
<td>1.8973</td>
<td>0.17924</td>
<td>5.203</td>
<td>0.021*</td>
</tr>
<tr>
<td>After bleaching</td>
<td>30</td>
<td>1.7284</td>
<td>0.14705</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The mean difference is significant at the 0.05 level. Ca/P: Calcium/ phosphorus
Raman spectroscopic study showed that exposure to 10% CP for more than 7 days resulted in a significant decrease in phosphate concentration in the bleached enamel. On the contrary, a study did not report any mechanical, morphologic, or chemical changes following vital bleaching with CP. Hence, the effect of bleaching on dental enamel is not yet clear and is still a controversial subject.

Table 2: t-test values to analyze the mean Ca/P ratio of bleached and remineralized samples in study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedure</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (CPP-ACP)</td>
<td>Bleached enamel</td>
<td>1.6999</td>
<td>0.07893</td>
<td>68.105</td>
<td>0.00*</td>
</tr>
<tr>
<td></td>
<td>Remineralized enamel</td>
<td>1.8550</td>
<td>0.12169</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (GSE)</td>
<td>Bleached enamel</td>
<td>1.7286</td>
<td>0.20867</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Remineralized enamel</td>
<td>1.8474</td>
<td>0.30024</td>
<td>26.195</td>
<td></td>
</tr>
<tr>
<td>C (wheat grass powder)</td>
<td>Bleached enamel</td>
<td>1.7567</td>
<td>0.13456</td>
<td></td>
<td>0.00*</td>
</tr>
<tr>
<td></td>
<td>Remineralized enamel</td>
<td>1.7767</td>
<td>0.12429</td>
<td>41.287</td>
<td></td>
</tr>
</tbody>
</table>

*The mean difference is significant at the 0.05 level. CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate, Ca/P: Calcium/phosphorus. GSE: Grape seed extract

Table 3: Comparison of mean Ca/P ratios of study groups using one-way ANOVA

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedure</th>
<th>Number of mean samples</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (CPP-ACP)</td>
<td>Remineralized enamel</td>
<td>10</td>
<td>1.8550</td>
<td>0.12169</td>
<td>0.464</td>
<td>0.633</td>
</tr>
<tr>
<td>B (GSE)</td>
<td>10</td>
<td></td>
<td>1.8474</td>
<td>0.12429</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (wheat grass powder)</td>
<td>10</td>
<td></td>
<td>1.7767</td>
<td>0.30024</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate, GSE: Grape seed extract, Ca/P: Calcium/phosphorus

Graph 1: Elemental analysis of sound enamel by energy dispersive X-ray spectroscopy. Calcium/phosphorus ratio: 2.2185

Graph 2: Elemental analysis of bleached enamel by energy dispersive X-ray spectroscopy. Calcium/phosphorus ratio: 1.3185

Graph 3: Elemental analysis of enamel sample by energy dispersive X-ray spectroscopy after remineralization with casein phosphopeptide amorphous calcium phosphate. Calcium/phosphorus ratio: 2.7313

Graph 4: Elemental analysis of enamel sample by energy dispersive X-ray spectroscopy after remineralization with grape seed extract. Calcium/phosphorus ratio: 1.9859

EDX is used for elemental characterization. The ratio of Ca and P is also indicative of chemical changes since change can suggest that the mineral phase was altered or that a significant substitution of ions may have occurred.
In the current study, the elemental analysis revealed a statistically significant decrease in the mean Ca/P ratio from 1.8973 to 1.7284 after bleaching.

CP breaks down to produce water, oxygen, carbon dioxide, and ammonia, which would result in a slight lowering of pH of the bleaching agent. This reduction in pH might have an effect on the dissolution of the mineral content of enamel.\(^\text{[13]}\)

Bleaching procedure may have potential side effects such as sensitivity which could be due to surface enamel loss and decrease in the mineral content. There is decrease in the hardness of teeth and increase in roughness. It was also reported that the residual peroxide on the surface interferes with resin attachment, inhibits resin polymerization, and the bond strength of composite resin to the tooth is compromised if bonding is performed immediately.\(^\text{[13]}\) This could also be because of decreased mineral content of the surface.

So, an attempt to remineralize the enamel surface may be useful in the prevention of sensitivity to the patient and also increase the success of adhesive restorations if required to be done immediately after the bleaching.

A newer concept for remineralization is the use of milk and milk products, which appear to have a protective effect against the mineral loss.\(^\text{[14]}\) Results of the current study showed that there was a significant increase in the mean Ca/P ratio from 1.6999 to 1.8550 after remineralization with CPP-ACP.

CPP binds to pellicle and plaque at the tooth surface and stabilizes high concentrations of calcium, phosphate, and fluoride ions. This nano complex attaches to dental plaque and tooth surfaces and acts as a calcium and phosphate reservoir. The attached CPP-ACP maintains a supersaturated mineral environment by releasing calcium and phosphate ions, and thereby helps in enhancing remineralization of enamel.\(^\text{[15]}\) The results obtained also correlated with a morphological study in which a combination of CPP-ACP and bleaching agent prevented negative changes of hardness, roughness, and morphology.

In the case of immediate bonded restorations, the oxygen free radicals need to be taken care of as they can cause compromised bond strength. Thus, antioxidants may have a beneficiary effect. The present study tested the benefits of GSE and wheat grass for their remineralizing potential, both of which are basically very good antioxidants.\(^\text{[3,16]}\) A very few studies were reported on the effects of natural products or nutraceuticals on the remineralization processes of dental hard tissues.

GSE has been recently advocated for its beneficial antioxidant, antibacterial, and free radical scavenging properties. The biologically active constituents of GSE are polyphenols, mainly PAs.

The results of the current study show a significant increase in the mean Ca/P ratio from 1.7286 after bleaching to 1.8474 after remineralization with GSE. The results of the present study are in accordance with a scanning electron microscopic study that showed scaffolding deposits on the enamel surface with cluster-like structures resembling remineralization process initiation.\(^\text{[7]}\) The remineralization potential of GSE on artificial root caries was also evaluated using a polarized light microscope and confocal laser scanning microscope.\(^\text{[8]}\)

Gallic acid, one of the major constituents of GSE and Galla Chinensis, were assumed to facilitate mineral deposition, predominately on the surface layer.\(^\text{[7]}\)

Remineralization with wheat grass solution showed a statistically significant increase in the mean Ca/P ratio from 1.7567 to 1.7767. The oxygen radical absorption capacity of wheatgrass was found to be very satisfactory in phytotherapy research studies.\(^\text{[16]}\)

The major clinical utility of wheat grass in diseased conditions might be due to the presence of biologically active compounds and minerals in it and due to its antioxidant potential. The antioxidant potential of wheat grass could be by virtue of the high content of bioflavonoids in it such as apigenin, quercetin, and luteolin. Furthermore, its therapeutic potential may also be related to the presence of indole compounds namely choline and laetrile.\(^\text{[9]}\) However, the exact mechanism by which wheat grass solution increased the mineral content of enamel is not well understood.

According to the results obtained in the present study, all the three remineralizing agents significantly increased the mineral content of the bleached surface, with no statistically significant difference between the groups.

To our best knowledge, this is the first attempt to evaluate the remineralizing capacity of GSE and wheat grass on bleached enamel. These two novel nutraceutical agents were able to significantly contribute to the remineralization process.
Although CPP-ACP will help in remineralization, it does not have the antioxidant property. The presence and diffusion of oxygen free radicals might lead to pulpal inflammation and decreased resin bond strength. Nutraceutical agents tested in the present study also possess antioxidants which might help in neutralizing the oxygen free radicals. Hence, these products can be considered as surface remineralizing agents post-bleaching. Although bleaching is an effective procedure, it has its own limitations like alteration in the mineral contents. Clinicians should be aware of the role of remineralizing to overcome the side effects of bleaching.

Further, there is a need for long-term studies as well as ex vivo and in vivo studies in future to evaluate the efficiency of these remineralizing agents in a clinical scenario. Future studies are required to elaborate the mechanism of action of these newer agents and the clinical protocol of their application. Spectroscopic analysis of the color change followed by application these novel remineralizing agents to be done.

**Conclusion**

Within the limitations of the present study, the following conclusions were drawn:

1. There was a significant decrease in the mineral content after bleaching with 22% CP
2. CPP-ACP, GSE, and wheat grass significantly increased the remineralization of bleached enamel with no statistically significant difference between the three groups
3. The added advantage of the anti-oxidant property of GSE and wheat grass powder can be utilized along with remineralizing potential.

**References**
