EFFECT OF OFFICE BLEACHING SYSTEMS ON CHEMICAL COMPOSITIONS OF ENAMEL AND DENTIN: AN IN VITRO STUDY

ABSTRACT

Background and Aim: To determine the change in the mineral component of enamel and dentin as well as to evaluate the differences in surface texture of the same dental hard tissues following three office bleaching procedures in vitro.

Materials and Methods: Sixty extracted human anterior teeth were used. Thirty teeth were used as enamel samples; the buccal surfaces of the remaining 30 teeth were abraded and used as samples for dentin. Prior to bleaching treatments, calcium (Ca), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), fluoride (F), and oxygen (O) levels of each sample were measured using an energy dispersive X-ray spectrometry system (EDS). The teeth were then randomly allocated into three groups; GI: chemically activated office bleaching system (OBS), GII: light activated OBS, GIII: diode laser activated OBS. Following the bleaching treatments, measurements were repeated. Wilcoxon signed rank and Kruskal-Wallis tests were used to analyze the data statistically.

Results: GI decreased the F level of enamel and F, Na, P, K, Ca levels of dentin. GII reduced the F level of enamel and Na levels of dentin. GIII created a reduction in F and K levels on enamel and F, Na, K, Ca levels on dentin. The use of bleaching systems did not make any difference in Na, Mg, P, Ca levels of enamel and Mg levels of dentin. SEM observations revealed no deleterious effect on enamel and dentin.

Conclusion: The use of office bleaching agents and activation mode of the bleaching systems used could affect the chemical composition of dental hard tissues.

Key words: Chemical Compositions, Dentin, Enamel, Office Bleaching Systems.
INTRODUCTION

In recent years the popularity of tooth has grown dramatically, and was estimated that it has been performed on more than one million patients in the dental office. In removing tooth discoloration, bleaching treatment has been recognized as safe, effective, minimally invasive and a non-destructive treatment. There are several methods and approaches in the literature for vital tooth bleaching. The methods comprise several bleaching agents, concentrations, period of application, product format, application mode and light activation. In-office bleaching procedures generally uses relatively high levels of bleaching agents, for example 25–35% hydrogen peroxide (HP) including products, for shorter time periods of application and may be further activated by heat or light. The advantages of office bleaching include minimal dependence on patient adaptation and rapid visible results, which make glad patients who may want to see the quick results. The disadvantages are higher patient cost, the use of chair time, and the necessity of several in-office visits to completed optimal bleaching and retains it.

The mineral content of enamel comprises hydroxyapatite (HAP) crystals, Ca_{10} (PO_{4})_{6} (OH)_{2}. The inorganic component of dental hard tissues has been shown to comprise not only calcium (Ca), phosphorus (P), and oxygen (O), as shown by the formula of calcium HAP, but also carbon (C), magnesium (Mg), sodium (Na), and fluoride (F), as well as numerous trace elements. Hydrogen peroxide (HP) is capable of oxidizing a large variety colored organic and inorganic components of tooth, causing decolorization and hence bleaching of tooth. Even though there are numerous studies in which the potential morphological alterations in enamel caused by the use of high-concentration bleaching agents in the literature have been studied, these adverse effects are still controversial.

Tooth bleaching is not considered as generating macroscopically visible defects. There are many studies that show micro structural changes of dental hard tissue by application of bleaching agents, especially when peroxides are used in high concentrations. However, a range of other studies exhibited little or no topographic changes on bleached dental hard tissues. While the effects of bleaching on morphological changes to tooth tissue are contradictory, it is generally received that peroxides can modify mineral content of enamel and dentin. The combined scanning electron microscopy (SEM) and energy dispersive X-ray spectrometry system (EDS), which enables the examination of organic and inorganic sample surfaces, has capability to conduct an accurate and nondestructive analysis of the samples. The technique of the system is based on strafing the tooth surface with a high-voltage electron beam, giving characteristic wavelength emission for each mineral. The changes in the wavelength of the rays emitted from the surface determine changes in the mineral content on the tooth surface. As the levels of mineral concentration in enamel and dentin are a good indication of the demineralization and remineralization courses, the purpose of this study was to assess the differences in surface structure of tooth as well as to determine the change in the chemical content of dental hard tissues following three office bleaching systems by using an energy dispersive X-ray spectrometry system in vitro.

MATERIALS AND METHODS

Sixty extracted noncarious intact human anterior teeth were stored in distilled water at room temperature until required for the analysis. All calculus or extrinsic stains were cleaned and the roots of the teeth were removed approximately 2mm below the cementoenamel junction. Thirty teeth were used as enamel samples. The buccal surfaces of the remaining thirty teeth were abraded with 600-grit and 1000-grit silicon carbide abrasive paper using a mechanical device (Mecapol P230, Presi, France) to expose the superficial dentin and used as dentin specimens. Prior to bleaching application the buccal surfaces of each tooth were subjected to EDS analyses were performed on the combined SEM and EDS (Bruker Axs XFlash 3001 SDD-EDS, Cambridge, UK).

The teeth were then randomly divided into three groups according to the office bleaching systems used (n= 10). GI: a chemically activated office bleaching system (Opalescence Xtra Boost 38% hydrogen peroxide (HP) / Ultradent) ; GII: a light activated office bleaching agent (Opalescence Xtra 35% HP/Ultradent), in combination with an LED lamp (BioWhite accelerator, BioWhite, Ensodent, Italy). GIII: a diode laser activated office bleaching agent (BiyWhite 38% HP/Ensodent) in conjunction with a diode laser (LaserSmile, Biolase Technology Inc. San Clemente, CA, USA).

Bleaching procedures were performed following the manufacturers’ instructions (Table 1). Bleaching agents were applied onto the buccal surfaces of the samples. All other surfaces were enclosed with plaster. The junction of plaster with bleached surfaces was sealed with wax to prevent the inflow of bleach. The bleaching gel was applied and removed from the tooth surface using a small brush.
for both enamel and dentin samples. After each treatment the samples were rinsed with distilled water for one minute to remove the bleaching agent and then they were stored in distilled water at 37°C. At the end of the bleaching procedures Ca, P, K, Na, Mg, F, and O measurements were repeated, and the changes in mineral levels were recorded in the same manner. The data were analyzed using Wilcoxon signed rank and Kruskal-Wallis tests. P<0.05 values were considered statistically significant.

Two samples from each group were separated for SEM. As the vacuum conditions needed to sputter the enamel and dentin surfaces could possibly result in deterioration of surfaces, the images were obtained using a Zeiss EVO 50 EP SEM (Carl Zeiss, Cambridge, UK) in extended variable pressure mode without coating.

**RESULTS**

No significant differences were found on the element levels among the groups before bleaching (p>0.05). The use of bleaching agents did not make any significant differences in Na, Mg, P, Ca and O levels of enamel (p>0.05). Although a slight decrease was observed on F levels in all groups; only GIII created a significant reduction on the K level of enamel (p<0.05) Results are shown in Table 2. GI significantly decreased the F, P, Na, K and Ca levels; GII reduced only Na level and GIII created a reduction in F, Na, K, and Ca levels of dentin (p<0.05). Results are summarized in Table 3.

SEM observations were recorded to compare the differences among three tested bleaching systems at magnification of 1000x and the results of the correlated SEM analysis showed no relevant alteration on the enamel and dentin surfaces (Figures 1 and 2).

**DISCUSSION**

The use of in vitro models is often important for the initial evaluation of prototypes and the optimization of treatment conditions. In addition in vitro models can be used to gain important information on the safety of the product in terms of its effect on the hard tissues and provide mechanistic understanding of the bleaching process. There have been numerous in vitro models described in the literature which have been used to evaluate the efficacy of bleaching products. In clinical situations, the enamel surfaces are fully exposed to the bleaching agents, whereas dentin exposure occurs via peroxide diffusing through the enamel to reach the enamel-dentin junction before reaching the dentin regions. In cases of defective restorations and nonvital bleaching procedures the dentin may directly be exposed to the bleaching agent. For this reason, it is important to examine the effect of the oxidizing agents on dentin as well as on enamel.

Tooth enamel is the most mineralized tissue of human body. Composition of enamel is 96 weight (wt.) % inorganic material and 4 wt. % organic material and water. In dentin the inorganic material represents 70 wt. %. This inorganic material is mainly composed by a calcium phosphate related to the hexagonal hydroxyapatite, whose chemical formula is \( \text{Ca}_{10}(\text{PO}_4)_{6}\cdot2(\text{OH})_2 \). X-ray energy dispersive spectroscopy (EDS) analysis of enamel and dentin also indicated the presence in small quantities of other elements such as Na, Cl and Mg. In a previous study, the chemical analysis by EDS indicated that the Ca/P relationship was bigger in enamel than dentin. In enamel it was around 1.63, compared with 1.67 in pure hydroxyapatite; that is, more Ca than P. Na, Cl and Mg were also detected and in enamel, Na and Mg indicated a minimum but Ca and P was almost remained constant and Cl was higher. In dentin, the ratio Ca/P was found around 1.5; that was, more P in enamel than in dentin; Cl was not presented, Mg was increased, and Na was decreased. In dentin, the percentage of C and O was higher than in enamel. This is an indication of more weight percentage of organic material in dentin than in enamel, as it is well known.

In the present study, three office bleaching agents with similar HP concentrations (38%, 35%, and 38%) and different activation modes were used. The use of bleaching systems did not make any significant differences in Na, Mg, P, Ca and O levels of enamel (p>0.05). Although a slight decrease

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Table 1. Bleaching Agents Used

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MATERIALS</th>
<th>MANUFACTURER</th>
<th>APPLICATION</th>
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<tbody>
<tr>
<td>I</td>
<td>Opalescence Xtra Boost 38% Hydrogen Peroxide (HP)</td>
<td>Ultradent Dental GmbH, Salt Lake City, USA</td>
<td>2x15 minutes</td>
</tr>
<tr>
<td>II</td>
<td>Opalescence Xtra 35% HP</td>
<td>Ultradent Dental GmbH, Salt Lake City, USA</td>
<td>2 x15 minutes</td>
</tr>
<tr>
<td>III</td>
<td>ByWhite 38% HP</td>
<td>Ensodent, Italy</td>
<td>2 x15 minutes</td>
</tr>
</tbody>
</table>
was observed on F levels in all groups; only GIII created a significant reduction on the K level of enamel (p<0.05). The chemical composition of enamel was not affected by the activation modes and high HP concentrations of the bleaching systems that were used.

At present, the role of K is not known sufficiently. K levels were decreased both in enamel and dentin following bleaching treatments. This result was compatible with the findings of Rotstein et al.\textsuperscript{18} that the bleaching agents caused a reduction in the organic components of dental hard tissues. The loss of Mg was not statistically significant; however, Mg is among the first elements to be dissolved during the demineralization process. Thus, the loss of Mg could be the first sign of demineralization.\textsuperscript{19} In a recent in vitro study, Souza et al.\textsuperscript{20} determined that the percentage of O was comparable between the control and the bleached groups. Contrary to their results, in this study, an increase was observed on O levels in the dentin with the use of all three bleaching systems. Since the amounts of all elements were presented as mass percentage (%) in the study, this could be a relative increase that might be influenced by the decrease of the other elements.

The oral environment provides conditions for enamel remineralization, and demineralized enamel is more susceptible to remineralization.\textsuperscript{21} When a bleaching agent causes demineralization in the enamel, ionic changes are induced, increasing mineral uptake, which replaces the mineral lost during treatment. For this reason, in order to standardize the sole effect of bleaching agents on dental hard tissues, in the present study, the samples were stored in distilled water instead of artificial saliva.\textsuperscript{22} Usually, data are gathered from in vitro studies, and extrapolations to clinical situations are difficult. In addition, in vitro studies do not show results of remineralization that might occur in the dental structure exposed to human saliva. Although few in situ studies have been performed to verify the direct interaction among bleaching agents, saliva, soft tissues and dental structures, further evaluations regarding this matter should be accomplished.\textsuperscript{23} In previous in vitro studies, investigators reported that the use of high-concentration hydrogen peroxide-based agents caused morphological alteration of the enamel surface, characterized by increased porosity of the superficial enamel ultra structure, demineralization and a decrease in protein

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**Table 2.** Mean and Standard Deviation Values of Element Levels of Enamel Before and After Bleaching

<table>
<thead>
<tr>
<th>Elements</th>
<th>Before Bleaching (%)</th>
<th>After Bleaching (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>Ca</td>
<td>42.137±3.934</td>
<td>42.729±2.226</td>
</tr>
<tr>
<td>P</td>
<td>18.002±0.402</td>
<td>18.57±0.385</td>
</tr>
<tr>
<td>K</td>
<td>0.105±0.105</td>
<td>0.065±0.063</td>
</tr>
<tr>
<td>Na</td>
<td>0.227±0.254</td>
<td>0.103±0.157</td>
</tr>
<tr>
<td>Mg</td>
<td>0.164±0.192</td>
<td>0.098±0.116</td>
</tr>
<tr>
<td>F</td>
<td>0.014±0.005</td>
<td>0.016±0.005</td>
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**Table 3.** Mean and Standard Deviation Values of Element Levels of Dentin Before and After Bleaching

<table>
<thead>
<tr>
<th>Elements</th>
<th>Before Bleaching (%)</th>
<th>After Bleaching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>Ca</td>
<td>39.755±2.211</td>
<td>37.802±5.220</td>
</tr>
<tr>
<td>K</td>
<td>0.361±0.239</td>
<td>0.020±0.054</td>
</tr>
<tr>
<td>Na</td>
<td>1.532±0.855</td>
<td>1.575±0.645</td>
</tr>
<tr>
<td>Mg</td>
<td>0.507±0.302</td>
<td>0.599±0.464</td>
</tr>
<tr>
<td>F</td>
<td>0.185±0.494</td>
<td>0.428±1.294</td>
</tr>
<tr>
<td>O</td>
<td>42.316±3.486</td>
<td>44.329±4.938</td>
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</table>
Figure 1. SEM photomicrographs of enamel (a) Before application of bleaching agents. (b) After application of chemically activated office bleaching system (Group I). (c) After application of light activated office bleaching system (Group II). (d) After application of diode laser activated office bleaching system (Group III).

been reported.24-26 Scanning electron microscopy of enamel bleached with carbamide peroxide showed that little or no change in morphology, whereas other studies showed areas of shallow erosions or more substantial changes in enamel surface structure.27 Abrasion, erosion, surface hardness and morphological changes of dental hard tissues following power bleaching using 35% hydrogen peroxide were also investigated. There were no significant differences following the bleaching as the high concentration of hydrogen peroxide did not affect the abrasion, erosion or hardness of both enamel and dentine.28-32

Alterations in tooth surface arising from the normal variation of enamel morphology may be greater than alterations attributed to the effect of peroxides on the teeth.33-36 In addition; the clinical significance of enamel alterations after bleaching is not completely clear.37 In this study, two specimens from each group were examined under SEM and revealed no deleterious effects on enamel and dentin after office bleaching.

In this study there is a lack of follow-up related to the mineral composition of enamel and dentin for extended periods of time after the bleaching procedures.

CONCLUSION

With the limitations of this in vitro study, it may be concluded that the use of office bleaching agents could affect the
chemical composition of enamel and dentin. The change in the chemical composition of dental hard tissues might be affected by the activation modes of the bleaching agents that are used according to manufacturer's instructions.

Figure 2.  SEM photomicrographs of dentin (a) Before application of bleaching agents. (b) After application of chemically activated office bleaching system (Group I). (c) After application of light activated office bleaching system (Group II). (d) After application of diode laser activated office bleaching system (Group III).

REFERENCES


