**EFFECT OF DENTAL BLEACHING ON CARIES RESISTANCE OF HUMAN ENAMEL (IN VITRO STUDY)**

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**ABSTRACT**

**Objective:** To evaluate the effect of two different bleaching agents on surface microhardness of dental enamel and its influence on the susceptibility of the bleached enamel to acidic attack and hence the possibility of development of dental caries or not.

**METHODS:** A total of 80 freshly extracted sound human incisors were used. The labial surfaces were ground flat and then divided into two halves in which the right half was bleached and the left half was unbleached (Control side). The specimens were divided into two equal groups according to the bleaching agent used, group A1: bleached with Opalescence PF 10% carbamide peroxide bleaching agent applied for thirty minutes /day for four days, group A2: bleached with Opalescence–Boost PF 38% hydrogen peroxide applied for fifteen minutes /day for only one application. Following bleaching, the specimens were stored for fourteen days in the storage medium whether distilled water (B1) or artificial saliva (B2), then half of the specimens was subjected to pH cycling (C1) and the other was not subjected (C2). Microhardness was measured on both halves using a Vicker’s microhardness tester. Three indentations were made on each half and the mean of the three readings was calculated as the mean microhardness value for each side.

**Results:** Groups treated with Opalescence-Boost PF 38% HP showed lower microhardness values than that treated with Opalescence PF 10% CP in all groups. Storage in artificial saliva without subjection to pH cycling has resulted in increased microhardness values of bleached enamel treated either with Opalescence PF 10% carbamide peroxide or with Opalescence-Boost PF 38% hydrogen peroxide. Subjection to pH cycling has resulted in decreased micro hardness of bleached enamel relative to unbleached one in all groups, even in groups stored in artificial saliva.

**Conclusions:**

1. In-office bleaching agents with high concentrations of hydrogen peroxide are capable of causing reduction in surface microhardness relative to at-home bleaching agents with low concentrations.
2. Artificial saliva is capable of minimizing the possible adverse effects of bleaching agents.
3. Bleaching did not improve enamel resistance to demineralization.
INTRODUCTION

Management of discolored teeth is considered a major demand by society especially young women. Staining or discoloration might be extrinsic due to excessive consumption of tea or tobacco or intrinsic (internalized discoloration) caused by chemicals or drugs such as tetracycline or fluoride that affect teeth during amelogenesis (Watts et al., 2001). Discolorations could be managed through microabrasion, operative intervention or by dental bleaching which is considered more conservative. Bleaching is an accepted technique because of its simplicity, relative low cost and conservation of tooth structure (Matis et al., 2002). The tooth whitening process involves the direct contact of the whitening product on the surface of teeth for an extended period. However, this direct contact with enamel for prolonged times has increased concerns about the potential adverse effects of these agents on enamel (Efeoglu et al., 2005). Previous studies have investigated the possible adverse effects of hydrogen peroxide and carbamide peroxide bleaching agents on the physical, mechanical, chemical properties of enamel with a variety of methods: Scanning Electron Microscopy analysis (SEM), profilometry analysis, microhardness testing, fracture toughness testing, measuring the amount of calcium loss and infrared absorption spectroscopy. Although there are many different techniques available to assess the mineral content of enamel, microhardness tests have been the test of choice of many researches to evaluate the possible demineralization effect of the bleaching agents (Efeoglu et al., 2005). Further investigations were needed to examine the bleached enamel surface considering its resistance to acidic attack and consequently the risk of development of new carious lesions.

MATERIALS AND METHODS

1- Selection of teeth

Eighty freshly extracted sound human maxillary incisors were used for this study.

2- Grouping of the teeth:

Teeth were divided into two equal groups of forty teeth each according to the bleaching agent used (A1, A2). Each group was subdivided into two equal subgroups of twenty teeth each according to the storage medium (B1 distilled water, B2 artificial). Each subgroup was further divided into two equal divisions of ten teeth each according to whether teeth were subjected to pH cycling (C1) or not (C2)

3- Preparation of the specimens

a- Mold fabrication

A mold with internal dimensions of 1mm X 4mm X 6mm was fabricated from steel.

b- Fixation of teeth

The mold was placed on a clean glass slab and the inner walls of the mold were painted with separating medium then self-cure acrylic resin (Dentsply) was mixed and poured into the mold. Teeth were embedded into the acrylic resin horizontally before it reached the dough stage in which the labial surfaces of the teeth were perpendicular to the long axis of the mold. After complete setting of acrylic resin, the block was then removed from the mold by pushing it from the under surface.

C- Preparation of the labial surface of teeth

The labial surfaces of teeth was ground with an abrasive disk. It was divided into two halves by a longitudinal groove using a carbide fissure bur size 0.8 and a high speed hand piece. One half was assigned for bleaching [right side (R)] and the other half was covered with several layers of nail polish (dark red) act as control [left side (L)], the nail polish was removed after bleaching using acetone.
4- Bleaching procedure

One half of the specimens (group A1) was bleached with Opalescence PF 10% carbamide peroxide bleaching agent which was injected on the right half of the specimen and left for thirty minutes /day for four days. The other half of the specimens (group A2) was bleached with Opalescence-Boost PF 38% hydrogen peroxide bleaching agent which was injected on the right half of the specimen for fifteen minutes /day for only one application.

5- Storage of the specimens

One half of specimens was stored in distilled water (subgroups B1) and the other half was stored in artificial saliva (subgroup B2) for fourteen days.

6- PH cycling

After the fourteen days immersion in the storage medium, specimens in division (C1) were subjected to pH cycling by immersion of specimens in a container containing 50ml of acetate demineralizing solution (pH= 4.3) for six hours /day at room temperature. Specimens were then removed from the demineralizing solution, rinsed with distilled water, and immersed in another container containing 50ml of remineralizing solution (pH=7.0) at room temperature for eighteen hours /day. This cycle of demineralization-remineralization was repeated daily for a total of fourteen days during which solutions were changed every day (Alves et al., 2007).

7- Microhardness measurement

Enamel surface microhardness was measured using a Vicker’s hardness tester. A load of 100 gm was applied for 15 seconds. Three measurements were taken for each half of the specimen. The mean of the three readings was recorded as the enamel surface microhardness value for each half.

8- Statistical analysis

Data were presented as mean and standard deviation (SD) values. One-way Analysis of Variance (ANOVA) was used for comparison between more than two groups. Tukey’s post-hoc test was used for pair-wise comparison between the means when ANOVA test is significant. Paired t-test was used to compare between bleached and unbleached halves. The significance level was set at P≤0.05.

RESULTS

Table 1 showed that group treated with Opalescence PF 10% CP, stored in artificial saliva and not subjected to pH cycling (A1 B2 C2) showed the statistically significantly highest microhardness. This was followed by group treated with Opalescence PF 38% HP, stored in artificial saliva and not subjected to pH cycling A2 B2 C2, followed by group treated with Opalescence PF 10% CP, stored in distilled water and not subjected to pH cycling A1 B1 C2 then group treated with Opalescence PF 38% HP, stored in distilled water and not subjected to pH cycling A2 B1 C2. There was no statistically significant difference between group treated with Opalescence PF 10% CP, stored in artificial saliva and subjected to pH cycling A1 B2 C1 and A2 group treated with Opalescence PF 38% HP, stored in artificial saliva and subjected to pH cycling B1 C1, which showed lower values. There was no statistically significant difference between group treated with Opalescence PF 10% CP, stored in artificial saliva and subjected to pH cycling A1 B1 C1 and group treated with Opalescence PF 38% HP, stored in distilled water and subjected to pH cycling B1 C1 which showed the statistically significantly lowest microhardness values.
TABLE (1) The means, standard deviation (SD) values, results of ANOVA and Tukey’s tests for comparison between the groups (Bleached half).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 B1 C1</td>
<td>267.9</td>
<td>15.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>A1 B1 C2</td>
<td>384.1</td>
<td>19.6</td>
<td></td>
</tr>
<tr>
<td>A1 B2 C1</td>
<td>336.1</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>A1 B2 C2</td>
<td>500.6</td>
<td>18.3</td>
<td></td>
</tr>
<tr>
<td>A2 B1 C1</td>
<td>283.4</td>
<td>23.9</td>
<td></td>
</tr>
<tr>
<td>A2 B1 C2</td>
<td>357.3</td>
<td>26.4</td>
<td></td>
</tr>
<tr>
<td>A2 B2 C1</td>
<td>329.1</td>
<td>33.1</td>
<td></td>
</tr>
<tr>
<td>A2 B2 C2</td>
<td>430.2</td>
<td>33.2</td>
<td></td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05, A1: Opalescence PF10% Carbamide, A2: Opalescence Xtra Boost 38%HP, B1: Distilled water, B2: Artificial saliva, C1: pH cycling and C2: No pH cycling

Means with different letters are statistically significantly different according to Tukey’s test

Table 2 and graph 1 showed that microhardness of enamel treated with Opalescence-Boost 38% hydrogen peroxide is lower than that treated with Opalescence PF 10% carbamide peroxide after immersion in distilled water and subjection to pH cycling. The difference was found to be statistically significant at P 0.004. However, both bleached enamel samples have shown lower microhardness values than unbleached enamel and the difference was statistically significant at p-value of 0.001 for Opalescence PF 10% CP.

It was also found that microhardness of enamel treated with Opalescence-Boost 38% HP is lower than that treated with Opalescence PF 10% CP after storage in artificial saliva but without pH cycling and the difference is statistically significant at p 0.001. For the same groups, bleached enamel showed higher microhardness values than unbleached enamel and the difference was statistically significant at P-value of 0.001.

TABLE (2) Showing means, standard deviation (SD) of microhardness values of bleached and unbleached enamel halves.

<table>
<thead>
<tr>
<th>Group</th>
<th>Bleached Mean</th>
<th>Bleached SD</th>
<th>Unbleached Mean</th>
<th>Unbleached SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 B1 C1</td>
<td>267.9</td>
<td>15.4</td>
<td>385.6</td>
<td>25</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>A1 B1 C2</td>
<td>384.1</td>
<td>19.6</td>
<td>388.8</td>
<td>17.5</td>
<td>0.061</td>
</tr>
<tr>
<td>A1 B2 C1</td>
<td>336.1</td>
<td>26</td>
<td>381</td>
<td>26.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>A1 B2 C2</td>
<td>500.6</td>
<td>18.3</td>
<td>378.6</td>
<td>29.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>A2 B1 C1</td>
<td>283.4</td>
<td>23.9</td>
<td>376.6</td>
<td>24.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>A2 B1 C2</td>
<td>337.3</td>
<td>26.4</td>
<td>371</td>
<td>33.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>A2 B2 C1</td>
<td>329.1</td>
<td>33.1</td>
<td>372.2</td>
<td>30.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>A2 B2 C2</td>
<td>430.2</td>
<td>25</td>
<td>366.7</td>
<td>26.5</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05, A1: Opalescence PF10% Carbamide, A2: Opalescence Xtra Boost 38%HP, B1: Deionized water, B2: Artificial saliva, C1: pH cycling and C2: No pH cycling
Groups stored in artificial saliva and not subjected to pH cycling showed statistically significant higher enamel microhardness values relative to unbleached enamel irrespective of bleaching agent used. **Graph(2)**

In all groups irrespective of bleaching agent used, pH cycling decreased microhardness values statistically significantly compared to no pH cycling. **Graph(3)**

**DISCUSSION**

Exposure of the tooth enamel to bleaching agents either in-office or at-home may alter its surface properties; such as surface microhardness, surface roughness, surface texture, refractive index and color *(Attin et al., 2009)*. It was thought that low surface microhardness is responsible for easier penetration of cariogenic microorganisms and dissolution of the inorganic components of the dental tissues increasing the risk of dental caries. The labial surfaces of teeth were ground flat in order to make the indenter of the microhardness tester perpendicular to the long axis of the teeth *(Chen et al., 2008)*. In order to simulate the intra-oral conditions, artificial saliva was used as a storage medium to evaluate the remineralization potential of artificial saliva and whether it can restore microhardness of bleached enamel to normal or not *(Efeoglu et al., 2007)*. In this vitro study, pH cycling was used to reproduce a dynamic process with alternate demineralization and remineralization that represents the beginning and progression of dental caries *(Alves et al., 2007)*. Surface microhardness in this study was measured with Vicker’s microhardness tester, which has been used to qualify surface mineral changes in dental enamel, as
there is a direct relation between the mineral content of enamel and its microhardness, therefore, microhardness profile can be used not only as a comparative measure of hardness changes, but also as a direct measure of mineral gain and loss as a consequence of demineralization and remineralization (Mielczarek et al., 2008). Considering the effect of bleaching agent on enamel microhardness, both bleaching agents (Opalescence PF 10% carbamide peroxide and Opalescence-Boost 38% hydrogen peroxide) was found to reduce the microhardness of the enamel surface. However, this reduction in enamel microhardness is statistically insignificant in group A1 bleached with Opalescence PF 10% carbamide peroxide, this is in accordance to Lopes et al., 2002, Bastin et al., 2003, Leurinstien et al., 2004, Alves et al., 2007, Ulukapi et al., 2007, Rodrigues et al., 2007, Delfino et al., 2009, Pinto et al., 2009. But it was significant in group A2 bleached with Opalescence-Boost 38% hydrogen peroxide. These results are in agreement with Attin et al., 2005, Oliveria et al., 2005, Costa and Mazur, 2007, Bastin et al, 2001, Rodrigues et al, 2004, Park et al, 2004, Leurinstien et al., 2004, Lopes et al., 2002, Al-Salehi et al., 2007 who found that enamel microhardness was noticeably affected immediately after in-office bleaching using high concentration of hydrogen peroxide (35%) more than at-home bleaching with 10% carbamide peroxide. This may be due to the higher concentration of hydrogen peroxide in Opalescence-Boost and the absence of urea in the composition of Opalescence-Boost which kept the pH close to the critical level for enamel demineralization. This was in accordance to Lopes et al., 2002. Chen et al in 2008 reported that even concentration as low as 10% carbamide peroxide reduce the enamel microhardness at a level comparable to that produced by higher concentration. In contrary, a study by Potocnik et al in 2000 concluded that 10% carbamide peroxide bleaching agent did not affect interior enamel microhardness but only caused local microstructural changes similar to initial caries in enamel.

Considering the effect of the storage media on enamel microhardness, it was found that microhardness of bleached enamel increased significantly after the fourteen days immersion in artificial saliva, especially in groups treated with Opalescence PF 10% carbamide peroxide. This was in accordance with Toteda et al., 2008, Bastin et al., 2003, Lopes et al., 2002, Zaintiner et al., 2007, who revealed that saliva can reverse some mineral loss caused by bleaching treatment and the reduction in bleached enamel microhardness may be reverted by a period of remineralization following the whitening procedure and any microstructural defects promoted by bleaching agents may be repaired by the absorption and precipitation of the salivary components present in artificial saliva (Alves et al., 2007).

Considering the effect of pH cycling on bleached enamel, it was found that a statistically significant decrease in enamel microhardness was observed in groups subjected to pH cycling even in groups stored in artificial saliva. This might occurred because enamel become not able to inhibit the demineralization because it was exposed to a combination effect of both bleaching agent which already increased the mineral loss, and pH cycling (Pinto et al., 2009).

**CONCLUSIONS**

1. In-office bleaching agents with high concentrations of hydrogen peroxide are capable of causing reduction in surface microhardness relative to at-home bleaching agents with low concentrations.
2. Artificial saliva is capable of minimizing the possible adverse effects of bleaching agents.
3. Bleaching did not improve enamel resistance to demineralization.
REFERENCES


