Effects of New Formulas of Bleaching Gel and Fluoride Application on Enamel Microhardness: An In Vitro Study

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Clinical Relevance
The results of this study suggest that tooth bleaching may lead to a reduction in enamel microhardness. Nonetheless, the application of a high concentration fluoride post-bleaching treatment may increase enamel microhardness and may restore enamel hardness to a level similar to non-bleached enamel, if the bleaching formula contains fluoride, potassium or ACP.

SUMMARY
This in vitro study evaluated the new formulas of bleaching products and the effect of subsequent applications of fluoride on the hardness of enamel during and after tooth bleaching. The crowns of 60 extracted intact human molars were sectioned longitudinally; the buccal part was embedded in acrylic resin, the occlusal part was ground flat, exposing enamel and dentin, and then polished. Baseline Knoop microhardness (KHN) of enamel was determined. The specimens were then randomly divided into six groups of 10 specimens, and each group was assigned to a specific 10% carbamide peroxide (CP) bleaching agent. A: Opalescence, B: Opalescence PF (3% potassium nitrate and 0.11% fluoride), C: Nite White Excel 3 (ACP), D: Opalescence + F (acidulated phosphate fluoride 1.23%), E: Opalescence PF + F, F: Nite White Excel 3 + F. The teeth were bleached for eight hours; after each procedure, the specimens were stored in artificial saliva at 37°C. Immediately after day 21 of bleaching, the specimens in groups D, E and F received fluoride 1.23% for five minutes. KHN tests were performed before (baseline=control), during (14, 21) and two weeks (35 days) after the bleaching procedure and were statistically compared using ANOVA/Tukey’s t-test (α<0.05). The statistical analysis revealed no significant difference among the bleaching materials (p=0.123). A significant enamel KHN reduction (p<0.001) was observed for all bleaching materials, with no difference among them. Two weeks after bleaching, all the groups that received fluoride showed a significant increase in microhardness. For the new bleaching formulas, the enamel was restored to a value similar to baseline.
INTRODUCTION

External tooth bleaching is a popular method of whitening teeth. The home vital bleaching technique is the most popular bleaching method and involves the fabrication of a custom bleaching tray in which carbamide peroxide (CP) or hydrogen peroxide (HP) is placed and worn over the teeth, usually overnight, to obtain the desired esthetic effect. Different concentrations of CP gel are used. The best combinations resulting in limited side effects, safety and efficacy are obtained by using products approved by the American Dental Association (ADA), all of which contain a 10% concentration of CP. Although home bleaching has been shown to be a safe and effective procedure to lighten discolored teeth, it presents some side effects. It has been shown that bleaching with 10% CP may result in a change in calcium, phosphate and fluoride content in enamel. Some authors have reported slight surface alterations, as assessed by scanning electron microscopy and a decrease in surface hardness. In order to decrease tooth sensitivity and reestablish surface hardness, some companies have incorporated potassium nitrate and fluoride into whitening gel formulas. More recently, ACP (amorphous calcium phosphate), a compound created at the ADA Foundation’s Paffenbarger Research Center, is available in tooth whitening products. Although this compound is available in tooth whitening products that are currently on the market, its effect on enamel microhardness is not yet known.

The use of fluoride during or after bleaching has been shown to be beneficial. By forming a calcium fluoride layer on enamel, which inhibits demineralization or a decrease in microhardness, it has been proposed that the presence of fluoride may act as a remineralizing agent. In addition, studies have shown that the application of fluoride on softened enamel results in remineralization. It is not yet known whether a small amount of fluoride, 0.11%, is enough to harden dentin enamel or whether a higher concentration of fluoride, such as 1.23% or 2.23% (fluoride varnish), is required for application on the tooth after a bleaching procedure. This study evaluated 1) the new formulas of bleaching products on the hardness of the enamel subsurface during and after tooth bleaching and 2) the effect of subsequent applications of fluoride solution on the hardness of bleached enamel subsurface post-bleaching.

METHODS AND MATERIALS

The roots of 60 extracted intact human molars were sectioned 2 mm from the CEJ (Figure 1) and sectioned longitudinally, cutting the crown of the teeth in half (Figure 2). The buccal half of each tooth was embedded in acrylic resin (Figure 3), the lingual half was saved for another study. The occlusal portion was ground flat, exposing enamel and dentin (Figure 4), then polished with up to 4000 grit water-cooled carborundum paper discs (Buehler, Lake Bluff, IL, USA). Prior to this experiment, the teeth were stored in artificial saliva for 10 days at 37°C. The artificial saliva contained 0.103 g/L of CaCl₂, 0.019 g/L MgCl₂·6H₂O, 0.544 g/L KH₂PO₄, 2.24 g/L KCl, 4.77 g/L HEPES buffer acid and KOH was added to adjust the pH to 7.0. After the storage period, baseline subsurface enamel Knoop microhardness (KHN; kg/mm²) was determined. The specimens were then randomly divided into six groups of 10 specimens, and each group was assigned to a specific bleaching agent containing 10% CP (Table 1). The groups were as follows:

- Group A: Opalescence
- Group B: Opalescence PF
- Group C: Nite White Excel 3 (NWE 3)
- Group D: Opalescence + F (acidulated phosphate fluoride [APF] 1.23%, Dentsply, York, PA, USA)
- Group E: Opalescence PF + F
- Group F: Nite White Excel 3 + F

Prior to bleaching, the buccal enamel surfaces were dried with cotton pellets. The occlusal surface was iso-

Table 1: Bleaching Products Evaluated

<table>
<thead>
<tr>
<th>Bleaching Product</th>
<th>Content</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opalescence</td>
<td>10% CP</td>
<td>Ultradent Products, South Jordan, UT, USA</td>
</tr>
<tr>
<td>Opalescence PF</td>
<td>10% CP, 3%KNO₃, 0.11% F</td>
<td>Ultradent Products, South Jordan, UT, USA</td>
</tr>
<tr>
<td>Nite White Excel 3</td>
<td>10% CP, ACP</td>
<td>Discus Dental, Los Angeles, CA, USA</td>
</tr>
</tbody>
</table>

Figures 1-5: Specimen preparation and bleaching application.
lated with a jig made of vinyl polysiloxane impression material (Exafast Putty, GC Corporation, Itabashi-Ku, Tokyo, Japan) to ensure that the bleaching gel was only applied on the buccal surface. The buccal surfaces of the specimens were then covered with a 1 mm layer of the bleaching gel (Figure 5). The specimens were bleached for eight hours and kept in a humid atmosphere at 37°C. After each bleaching procedure, the gel was removed with a cotton pellet and the specimens were washed and dried with an air/water syringe for five seconds; the specimens were then stored in artificial saliva at 37°C. The teeth were bleached for 21 consecutive days. Immediately after the twenty-first day of treatment, the specimens from Groups D, E and F received the APF 1.23% for five minutes.

Test Method

Knoop microhardness (KHN; kg/mm²), at a load of 100 g, with an indentation of 20 seconds, was determined using a microhardness tester (Kentron Microhardness tester, Torsion Ballance Company, Clifton, NJ, USA). KHN tests were performed on the specimens’ enamel subsurface at baseline (control) and after 14, 21 and 35 days. The thirty-fifth day was 14 days post-bleaching. Four indentations were performed on an area 5 x 3 mm in the central part of the exposed enamel of each specimen (Figure 6) at each time period, keeping the long axis of the diamond parallel to the buccal surface. The first four measurements (baseline) were taken on the most mesial part of the central area (Figure 6), the other measurements were taken at 14, 21 and 35 days on the most distal part. The first measurements were taken at least 300 µm from the edge of the enamel, spaced 100 µm apart.

Statistical Analysis

KHN was compared for the three bleaching agents, along with the subsequent application of fluoride and the different time intervals, using a three-way ANOVA/Tukey’s t-test (p<0.05). In addition, the KHN values of each group were analyzed by one-way ANOVA/Tukey’s t-test (α<0.05) for the bleaching time factor, within the group.

RESULTS

The mean KHN of the bleaching materials with and without the fluoride post-bleaching application at baseline and at 14, 21 and 35 days is given in Table 2 and Figure 7. The statistical analysis revealed no significant difference among the bleaching materials (p=0.123). The three-way ANOVA showed a significant enamel KHN reduction (p<0.001), a main effect of accumulated bleaching time with no differences among the bleaching materials. The one-way ANOVA showed that, at two weeks post-bleaching, the groups that received fluoride had a significant increase in microhardness when compared to the groups that did not receive it (p<0.01). Hence, the application of fluoride solution post-bleaching resulted in KHN values that were not significantly different from baseline for two of the groups: Opalescence PF (p=0.069) and NWE 3 (p=1.894).

DISCUSSION

Despite the widespread use of bleaching, there is no general agreement as to the effect of these agents on enamel. While the recorded data of one study may not resemble others, hardness change trends can be com-
Some studies have reported that bleaching teeth with 10\% CP does not effect the surface hardness of enamel.\textsuperscript{2,20-27} However, this study showed a reduction in enamel microhardness for all bleaching gels. This finding is in agreement with other studies.\textsuperscript{6,14,28-36} Differences in the methodologies employed, such as bleaching time and bleaching formula, could influence the results. More severe changes have been observed in prolonged bleaching treatments,\textsuperscript{29} and different bleaching times can produce an increase or decrease in enamel hardness.\textsuperscript{33} With the increased acidity of bleaching gels, alterations to the enamel structure are more likely to be observed.\textsuperscript{28}

In this study, the presence of fluoride, potassium nitrate or ACP in the bleaching agent did not prevent hardness reduction, nor did it show the KHN of enamel as being restored post-bleaching. This is in agreement with previous studies.\textsuperscript{6,14} Research has shown that adding ACP to whitening gels will reduce tooth sensitivity—via remineralization—after bleaching. It has also been shown to enhance the natural healing remineralization process of saliva.\textsuperscript{39} A clinical study showed that ACP works via a unique method that uses amorphous calcium phosphate compounds in a carbonate solution to crystallize and form hydroxyapatite.\textsuperscript{39}

Although microindentention hardness tests do not provide specific information about the changes within a substance, these tests are commonly used to detect changes in the enamel and dentin surface following demineralization and remineralization experiments.\textsuperscript{16} Hence, it has been shown that loss of mineral content or demineralization alters enamel hardness, even though saliva, fluorides or other remineralizing solutions can maintain the balance between demineralization and remineralization.\textsuperscript{24,40} In vitro studies have demonstrated loss of minerals after the application of 10\% CP on enamel.\textsuperscript{24,41-43} Only an elemental or histochemical analysis is able to identify the specific alteration in enamel after bleaching.

Attin and others,\textsuperscript{14} Featherstone and others\textsuperscript{14} and Ten and others\textsuperscript{9} have shown that the application of fluoride to softened enamel results in remineralization. Different concentrations of fluoride have been used post-bleaching treatment. In this study, 1.23\% APF was used. All the groups that received fluoride showed a significant increase in microhardness when compared to groups that did not receive fluoride. This finding is in agreement with Attin and others\textsuperscript{14} and Lewinstein and others.\textsuperscript{16} Another study showed a reduction in demineralization when fluoride was applied to the enamel post-treatment.\textsuperscript{17} In the current study, the application of fluoride solution restored the KHN of enamel to a value similar to baseline for the two groups that had fluoride/potassium nitrate (Opalescence PF) or ACP (NWE 3) in their formula.

This study tried to simulate clinical conditions. Artificial saliva was used to store the teeth in-between and after bleaching treatments. Although artificial saliva was used in this study, no remineralizing effect was seen either during or after bleaching treatment. A study\textsuperscript{11} compared the effects of bleaching in situ and in vitro. In the in vitro study, the specimens were stored in deionized water. It was concluded that saliva allows the reposition of mineral and the reestablishment of hardness values similar to non-bleached specimens. Although artificial saliva was used in this study, it was not able to reestablish baseline tooth hardness. Also, in this in vitro study, the teeth were not brushed with toothpaste. In vivo, if patients brush with a fluoride toothpaste, it might help with the remineralization process. The oral environment provides conditions for enamel remineralization and demineralization and enamel is more susceptible to remineralization.\textsuperscript{42} Ten percent CP gel was applied for eight hours to simulate overnight home bleaching. Carbamide peroxide is active for up to 10 hours, with about 50\% of the active agent being used in the first two hours.\textsuperscript{43} To achieve the maximum benefit, carbamide peroxide is designed for night application.\textsuperscript{44,46} Many studies bleach teeth for two hours; instead, the authors of this study simulated home bleaching, which is done overnight, while the patient is sleeping. Bleaching specimens for a shorter period of time might have yielded less of a decrease in enamel KHN.

In order to evaluate the enamel KHN of new bleaching formulas that use fluoridated toothpaste on specimens, future research is suggested.

**CONCLUSIONS**

In this in vitro study, although incorporating fluoride/potassium nitrate or ACP did not prevent a

### Table 2: Enamel KHN (kg/mm²) and Standard Deviation (±) of the Bleaching Materials During and After Treatment

<table>
<thead>
<tr>
<th>Material/Days</th>
<th>Baseline</th>
<th>14</th>
<th>21</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opal</td>
<td>391.40 (33.31)</td>
<td>342.81 (24.87)</td>
<td>298.57 (21.68)</td>
<td>294.50 (32.21)</td>
</tr>
<tr>
<td>Opal PF</td>
<td>386.14 (24.26)</td>
<td>312.39 (29.04)</td>
<td>291.67 (21.11)</td>
<td>301.83 (24.96)</td>
</tr>
<tr>
<td>NWE 3</td>
<td>399.28 (18.75)</td>
<td>314.52 (21.47)</td>
<td>283.88 (22.24)</td>
<td>319.17 (11.26)</td>
</tr>
<tr>
<td>Opal + F</td>
<td>404.78 (15.94)</td>
<td>315.06 (17.34)</td>
<td>327.80 (17.39)</td>
<td>362.49 (10.85)</td>
</tr>
<tr>
<td>Opal PF + F</td>
<td>400.85 (14.53)</td>
<td>342.02 (22.25)</td>
<td>302.15 (28.21)</td>
<td>376.41 (18.16)</td>
</tr>
<tr>
<td>NWE 3 + F</td>
<td>388.36 (13.74)</td>
<td>316.36 (17.18)</td>
<td>306.08 (18.87)</td>
<td>368.01 (14.72)</td>
</tr>
</tbody>
</table>
References


37. De Oliveira R, Paes Leme AF & Giannini M (2005) Effect of carbamide peroxide bleaching gel containing calcium or fluoride on human enamel surface microhardness *Brazilian Dental Journal* 18(2) 103-106.


