Effect of bleaching agents containing fluoride or calcium on enamel microhardness, roughness and permeability

Charles Alex Rauen1, Júlio Cezar Chidoski Filho1, Bruna Fortes Bittencourt1, Giovana Mongruel Gomes1, João Carlos Gomes1, Osnara Maria Mongruel Gomes1

1Universidade Estadual de Ponta Grossa - UEPG, School of Dentistry, Area of Dental Materials, Ponta Grossa, PR – Brazil

Abstract

Aim: To evaluate the effect of different in-office bleaching agents on the permeability, roughness and surface microhardness of human enamel. Methods: For evaluation of roughness and microhardness, 40 hemi-faces of 20 premolars were subjected to initial roughness (Ra parameter) and microhardness (VHN) measurements. Thirty-two premolar’s crowns were used for permeability test. Then, all specimens were randomly divided into four groups: C - without bleaching (control), HP35 - bleaching with 35% hydrogen peroxide (HP), HPF38 - 38% HP+fluoride, HPC35 - 35% HP+calcium. Final roughness (FR) and microhardness (FM) measurements were evaluated. For permeability, the 32 crowns were immersed in 1% sodium hypochlorite (20 min) and silver nitrate solutions (2 h) and subjected to developing solution under fluorescent light (16 h). Three sections from the crowns were analyzed in light microscope (100x) to evaluate the scores of permeability: Score 0 - no tracer agent penetration; Score 1 - less than half the thickness of enamel penetration; Score 2 - tracer agent reaching half the enamel thickness; Score 3 - entire enamel depth penetration, without reaching dentin and Score 4 - tracer agent reaching dentin. For roughness and microhardness evaluation were used one-way ANOVA and Dunnet post-test for independent samples, and $t$ test for paired samples. For permeability, the data were analyzed by Kruskal Wallis and Dunn tests. Results: A significantly higher permeability and surface roughness were observed in groups HP35, HPF38 and HPC35 compared to the C group, as well as decreased microhardness (p<0.05). Conclusions: All bleaching agents increased permeability and surface roughness, and decreased microhardness of human enamel; thus, the addition of fluoride or calcium was not beneficial.

Keywords: tooth bleaching; dental enamel permeability; hydrogen peroxide; surface properties.

Introduction

Bleaching technique, either with hydrogen peroxide or carbamide peroxide, has become a popular procedure, mainly due to guaranteed benefits such as the ability to promote color change and lighter shades of teeth. The reaction mechanism of hydrogen peroxide is based on the degradation of these substances and release of reactive oxygen species, which diffuse through the enamel surface, acting primarily on dentin, breaking down high-molecular-carbon rings of the chromophore molecules into smaller molecules; which, in turn, diffuse to the surface, providing the desired color change.

There is concern that bleaching agents (hydrogen peroxide and carbamide
peroxide) bring some damage to enamel surface, like increased roughness and permeability. This effect was demonstrated in a previous study where silver nitrate penetration in tooth structure after bleaching was performed and it was found that these agents might diffuse into dentin by opening channels in tooth structure. Two distinct paths were observed when the silver nitrate particles penetrated through enamel and reached dentin: through the prisms and through the microcracks typical of the enamel structure. If nitrate penetrate these channels, oxygen may penetrate more readily due to its atomic weight (seven times less than silver nitrate). Probably, mineral loss may be detected after bleaching procedures and this has been pointed as the cause of microhardness decrease.

It likely that one of the causes of these adverse effects are the free oxygen radicals released during the reaction, which do not act in a specific way and may act on the organic matrix of dental structures. Physiologically, any bleaching therapy has the ability to increase free radical levels in the pulp–dentin complex, which may induce oxidative stress in odontoblasts and tertiary dentin formation. This fact may explain the numerous reports of sensitivity observed during sessions. Also characteristic of these changes are decreases in calcium and phosphate matrices. These changes are detrimental to dental health because along with this process, there is an increased susceptibility to caries formation due to the ease of plaque adherence on the previously demineralized structure.

Fluoride has proven to be effective in promoting remineralization and inhibiting enamel demineralization. Fluoride is not the only way to ensure this remineralization, as calcium compounds also exhibit efficient recovery of lost mineral content. With this objective, fluoride and calcium have been added to bleaching gels as alternatives to reduce these adverse effects caused by enamel bleaching. However, controversial results are found in literature, as authors report that adding fluoride to bleaching gels shows no beneficial results in terms of reduction of enamel demineralization after bleaching.

Therefore, the aim of this study was to evaluate in vitro changes in surface roughness, microhardness and permeability of human enamel when exposed to in-office bleaching agents containing fluoride or calcium in their composition. The null hypothesis tested was that bleaching agents do not influence enamel surface roughness, microhardness and permeability of the specimens.

Material and methods

The research project was approved by the Institutional Review Board - State University of Ponta Grossa: Report Number 25/2011 and Protocol Number 18741/10.

Specimen preparation

Fifty-two human premolars were used in this study. The crowns of the selected teeth were separated from their roots at the cementoenamel junction (CEJ) using a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water cooling at 300 rpm.

Twenty crowns were used for roughness and microhardness evaluation. For permeability, 32 crowns were sealed at the CEJ with adhesive system (Adper Single Bond, 3M ESPE, St. Paul, MN, USA) and resin composite (Z100, 3M ESPE, St. Paul, MN, USA) to prevent further penetration of the tracer agent (nitrate solution; Vetec Química Fina, Xerém, RJ, Brazil) in this area.

For microhardness and roughness tests, the crowns were divided mesiodistally into two portions with a low-speed diamond saw (Isomet 1000, Buehler) at 300 rpm under constant water cooling. Forty enamel specimens (2 mm x 2 mm x 2 mm) were obtained. Study design is displayed in Figure 1.

Surface roughness analysis

After 48 h, the initial roughness (IR) of all specimens was measured with a digital roughness meter (Mitutoyo SurfTest-301; Mitutoyo-Kawasaki, Kanagawa, Japan) in all 40 specimens. The specimens were divided into four quadrants, and one measurement was made in each quadrant. From these values, the arithmetic mean was considered as the IR. The used parameters were Ra, 0.25 mm cutoff and 1.25 mm reading length.

Microhardness analysis

After the roughness analysis, the specimens were flattened with wet #600, 1000 and 1200 grit aluminum oxide abrasive papers and polished with 6, 3, ½ and ¼ µm-grit diamond pastes in a polishing machine (Arotec, Cotia, SP, Brazil) and subjected to Vickers microhardness test (Shimadzu, Kyoto, Japan).
Experimental groups - bleaching treatments

All specimens were assigned as follows: for permeability, 32 crowns were randomly divided into 4 groups (n=8), and for roughness and microhardness, 40 specimens were divided into 4 groups (n=10): Group C - no bleaching (control); Group HP35 - bleaching with 35% hydrogen peroxide (Whiteness HPMaxx, FGM, Joinville, SC, Brazil); Group HPF38 - bleaching with 38% hydrogen peroxide containing fluoride (Opalescence Boost PF, Ultradent, South Jordan, UT, USA) and Group HPC35 - bleaching with 35% hydrogen peroxide containing calcium (HP Blue, FGM, Joinville, SC, Brazil). Bleaching procedures were performed according to manufacturer’s instructions (Table 1). The hemi-teeth were randomized to different experimental groups in a way that specimens from the same hemi-tooth were never assigned to the same experimental group.

The control group (C) was maintained in artificial saliva and was not subjected to any bleaching treatment. All specimens were maintained in artificial saliva during all experiments.

Final roughness and microhardness evaluation

After 48 h of the last bleaching procedure, the specimens (n=40) were subjected to final roughness (FR) and final microhardness (FM) evaluations, in the same way as for the initial evaluations.

Permeability analysis

The methodology of permeability analysis was based on previous studies. After bleaching, all specimens, including the control group (n=32), were stored in a dark environment in artificial saliva for 48 h at 37°C.

The lingual and proximal surfaces and the CEJ were sealed with cyanoacrylate resin (Super Bonder Locitite, Henkel Ltda, São Paulo, SP, Brazil) and a nail varnish to prevent dye penetration of silver nitrate (tracer agent) through this area. The nail varnish was also used to delimit an area of 4 x 4 mm in the middle third of the labial surface. Specimens were then immersed in 50% aqueous silver nitrate solution (Vetec Química Fina, Xerém, RJ, Brazil) for 2 h in a dark and closed environment. Subsequently, the specimens were photodeveloped for 16 h (developing solution, Kodak, Eastman Kodak Company, Rochester, NY, USA). After this, the specimens were washed in tap water and the nail varnish layer was removed with manual cutting instruments.

The specimens were then embedded in a polyvinyl chloride (PVC) tube with acrylic resin (Duralay, Reliance, Dental Mfg. Co., Worth, IL, USA), and three longitudinal slices of each tooth up to approximately 0.4 mm thick were obtained, in a buccolingual direction, with a low-speed diamond saw (Isomet 1000, Buehler) under water cooling.

The slices were then photographed under an optical microscope (Leica®, Olympus BX41-U-CA, Tokyo, Japan) with 100x magnification. The images were made with a digital camera at a 5.1 megapixels resolution. The dye penetration degree was analyzed by three previously calibrated evaluators, using a 0 to 4 score system: score 0 - no dye penetration; score 1 - less than half the enamel thickness; score 2 - half the enamel thickness; score 3 - full extent of enamel without reaching the dentin and score 4 - tracer agent reaching dentin. This evaluation was made visualizing the middle third of the dental crowns. If there was any disagreement between the evaluators, the sample under discussion was jointly analyzed until a consensus was reached. The median scores of the images from the same tooth were considered for statistical analysis using the Kruskal Wallis and Dunn tests (α = 0.05).

Statistical analysis

Roughness and microhardness data were analyzed by one-way ANOVA and Dunnet post-test (α = 0.05) for independent samples, and t test for paired samples (initial
and final values from each group). For permeability, qualitative data were analyzed by non-parametric Kruskall Wallis test and Dunn post-test ($\alpha =0.05$ and confidence interval of 95%).

**Results**

Mean values and standard deviations of initial and final roughness (µm) for each experimental group are in Table 2. ANOVA showed no significant differences between IR of all groups ($p=0.9312$). For FR values, significant differences (Dunnet, $p=0.0383$) were demonstrated between Group C and the other groups. The t test verified that all initial values were statistically different from final values, except for Group C.

Means and standard deviations of initial and final microhardness values are in Table 3. One-way ANOVA showed no statistical differences among the initial values of all groups ($p=0.2849$). However, the FM of Group C and Group HP35 were statistically different from HPF38 and HPC35 ($p<0.05$), and t test demonstrated significant differences between IM and FM ($p<0.05$) for all groups except Group C.

Medians (1st/3rd interquartiles) for permeability obtained from each experimental group are in Table 4. Group C was statistically different from the other groups ($p<0.05$), although different medians were found in each group. The predominant score in each group is shown in Figure 2.

**Discussion**

The main focus of this study was to evaluate the effect of calcium and fluoride addition to bleaching agents as an alternative to reduce the adverse effects on human enamel. Changes in roughness, microhardness and permeability were similar, with variations occurring between bleached samples and control groups (without bleaching), regardless of calcium or fluoride content in their composition.

Similar results were found by Horning et al. (2013), who compared human enamel permeability after exposure to at-home bleaching agent (6% hydrogen peroxide containing calcium) and in-office bleaching (35% hydrogen peroxide). No significant differences between bleached groups were found, but all these groups showed a significantly increased permeability, resulting in no benefit of calcium addition to the bleaching agents. Otherwise, better results with calcium- and fluoride-containing carbamide peroxide gels were found in another study that demonstrated that these gels promoted mineral loss minimization. One hypothesis that may explain this result is related to the lower concentrations of carbamide peroxide in relation to hydrogen peroxide used in this study.

Several factors such as concentration, application time and pH of the bleaching agent may predict the influence of these substances on dental structures. According to Bistey et al. (2007), higher concentration and longer treatment result in more severe changes on human enamel. Another study...

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**Table 2** – Mean values (µm) and standard deviations of initial (IR) and final roughness (FR) for each experimental group*

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>INITIAL</th>
<th>FINAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.31 ± 0.08 Aa</td>
<td>0.31 ± 0.08 Aa</td>
</tr>
<tr>
<td>HP35</td>
<td>0.29 ± 0.12 Aa</td>
<td>0.40 ± 0.09 Bb</td>
</tr>
<tr>
<td>HPF38</td>
<td>0.29 ± 0.08 Aa</td>
<td>0.41 ± 0.05 Bb</td>
</tr>
<tr>
<td>HPC35</td>
<td>0.31 ± 0.09 Aa</td>
<td>0.40 ± 0.08 Bb</td>
</tr>
</tbody>
</table>

* Lowercase letters establish relationship between columns and capital letters establish relationship between rows. Different letters show statistically significant differences ($p<0.05$).

**Table 3** - Mean values (VHN) and standard deviations of initial (IM) and final microhardness (FM) for each experimental group*

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>INITIAL</th>
<th>FINAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>376.1 ± 78.6 Aa</td>
<td>370.2 ± 78.6 Aa</td>
</tr>
<tr>
<td>HP35</td>
<td>413.1 ± 74.6 Aa</td>
<td>369.7 ± 47.1 Ab</td>
</tr>
<tr>
<td>HPF38</td>
<td>406.1 ± 80.3 Aa</td>
<td>284.4 ± 22.2 Bb</td>
</tr>
<tr>
<td>HPC35</td>
<td>342.1 ± 34.1 Aa</td>
<td>258.1 ± 34.6 Bb</td>
</tr>
</tbody>
</table>

* Lowercase letters establish relationship between columns and capital letters establish relationship between rows. Different letters show statistically significant differences ($p<0.05$).

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**Table 4** – Median (1st/3rd interquartiles) for permeability degree and significance for each experimental group*

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>MEDIAN (1st/3rd interquartiles)</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1 (1/1)</td>
<td>A</td>
</tr>
<tr>
<td>HP35</td>
<td>3 (2/3)</td>
<td>B</td>
</tr>
<tr>
<td>HPF38</td>
<td>2 (1.75/2.25)</td>
<td>B</td>
</tr>
<tr>
<td>HPC35</td>
<td>1.5 (1/3)</td>
<td>B</td>
</tr>
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</table>

* Different letters show statistically significant differences ($p<0.05$).
showed a proportional relationship between mineral loss and bleaching agent concentration, pointing out that bleaching agents based on 35-38% hydrogen peroxide showed significantly higher values of calcium loss compared to at-home gels (10% carbamide peroxide). The mineral loss also provides decreased microhardness values, which was found in this study and corroborated by other authors. 

The reasons for these discrepancies between the results of this study with those already published on this issue may be the above-mentioned variants. Not only the concentration and application time, but also the pH of the bleaching agents, may have an important role in mineral loss. A similar study investigated the effect of the bleaching agents’ pH on enamel surface. There was no morphological or chemical change of the enamel surface in neutral or alkaline bleaching solutions. In the present study, care was taken to select products with similar concentrations (35 and 38%), and the total time that the gels remained in contact with enamel was the same (45 min), varying only the instructions proposed by each manufacturer, as shown in Table 1. The pH of each bleaching gel had a slight variation (HPMaxx ~ 6; Opalescence Boost PF ~ 7; HPBlue ~ 8), without significance with respect to our results.

Artificial saliva was used for specimen storage. A recent study demonstrated that artificial saliva is suitable to simulate the oral conditions, being closest to natural saliva. However, as this is a laboratory study; further in vivo studies are required to confirm this hypothesis, since in the oral cavity there is dential fluid movement coming from the pulp chamber of the tooth, which may turn enamel less permeable in the in vitro conditions.

This study showed that all bleaching agents decreased microhardness, and increased permeability and surface roughness of human enamel; thus, the addition of fluoride or calcium was not beneficial. It is worth mentioning that clinicians should be aware of the indiscriminate use these agents, as the remineralizing compounds were not able to reverse the adverse effects in enamel tissue.

References