Brushing Effect of Abrasive Dentifrices during At-home Bleaching with 10% Carbamide Peroxide on Enamel Surface Roughness

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Abstract

During tooth bleaching abrasive dentifrices might change the outer superficial enamel. The aim of this in vitro study was to evaluate the roughness of human enamel exposed to a 10% carbamide peroxide bleaching agent at different times and submitted to different superficial cleaning treatments. The study consisted of 60 sound human enamel slabs, randomly assigned to different treatment groups: G1 - not brushed; G2 - brushed with a fluoride abrasive dentifrice; G3 - brushed with a non-fluoride abrasive dentifrice; and G4 - brushed without a dentifrice. There were 15 enamel slabs per group. Slabs of molar teeth were obtained and sequentially polished with sandpaper and abrasive pastes. A profilometer was used to obtain the mean of Ra value on the surface of each specimen to initial and experimental times. Bleaching was performed on the enamel surface for six hours daily. After that, each slab received a cleaning surface treatment and was stored in artificial saliva. Analysis of variance (ANOVA) and Tukey’s HSD hoc analysis (α =0.05) revealed significant differences in roughness values over time for enamel bleached and treated with different superficial cleaning methods. G1 and G4 showed no significant differences in roughness over time, G2 and G3 showed a significant increase in the surface roughness values. This in vitro investigation showed the sole use of 10% carbamide peroxide did not alter the enamel surface roughness, but the cleaning treatments that employed the use of brushing with abrasive dentifrices resulted in a significant increase of enamel surface roughness.
Introduction
Home-applied carbamide peroxide bleaching agents are generally indicated for nighttime use for 6-8 hours. A typical pattern of oral hygiene upon rising is to remove the tray and brush the teeth with a dentifrice that may be abrasive. During brushing, the bleaching agent is washed away. Some concern exists regarding possible enamel abrasion due to toothbrushing on recently bleached enamel.

Penetration of bleaching agents into the hard tissues of teeth can result in various changes to these vital teeth. However, the influence of carbamide peroxide containing bleaching agents on enamel surface with respect to microhardness or texture is controversial. Some authors have revealed surface degradation, defects, and porosities using Scanning Electron Microscopy (SEM) evaluation and loss of enamel with microhardness test.

Attin et al. showed application of a fluoride varnish or mouthrinse is recommended after exposing teeth to carbamide peroxide bleaching agents in order to improve remineralization of the enamel.

The use of powders, gels, or pastes in oral hygiene dates back thousands of years. Dentifrices have been refined into complex formulations providing various therapeutic and cosmetic benefits. They contain chemotherapeutic ingredients such as fluoride, antimicrobials, desensitizing agents, tartar control agents, and abrasives. While abrasives in dentifrices play an important role in the cleaning process, cleaning effectiveness may not be solely related to abrasiveness. The presence of anticalculus agents like pyrophosphate and peroxides which are designed to prevent or remove extrinsic and intrinsic stain also play an important role. However, there are concerns that some abrasives may contribute to excessive tooth wear.

Alterations of externally bleached enamel structure after cleaning methods or tooth brushing with abrasive dentifrices, with or without fluoride, have not been evaluated yet. But, if teeth are brushed daily for maintenance of oral health and brushing is generally performed with a dentifrice which contains abrasives, this tooth brushing could cause stronger abrasion on sound bleached enamel.

The lack of evidence of the effects of bleaching agents associated with tooth brushing on the enamel surface, potential to remineralization, and the influence of saliva to remineralize this surface determined the issue of this study, which investigated the effects induced by brushing with abrasive dentifrice, with and without fluoride, after bleaching treatment on the enamel surface roughness.

Method and Materials

Experimental Design
The factors under study included the following:

1. **Cleaning surface treatment in four levels:** Group 1 - not brushed, Group 2 - brushed with an abrasive fluoride...
dentifrice (Proderma Pharmacyle, Piracicaba, São Paulo, Brazil), Group 3 - brushed with an abrasive dentifrice without fluoride (Proderma Pharmacyle, Piracicaba, São Paulo Brazil), and Group 4 - brushed without any dentifrice.

2. **Time in nine levels:** Baseline, 7, 14, 21, 28 days of treatment and 7, 14, 21, and 28 days after the beginning of the treatment, corresponding at post treatment period.

The experimental units consisted of 60 sound human enamel slabs, randomly assigned to different treatment groups (15 enamel slabs per group). Before bleaching treatments, a profilometer (Surf-Corder 1700, Kosaka Lab, Japan) was used to measure the initial surface roughness to establish a baseline. Three repeated measurements were recorded on the surface of each specimen at each seven-day interval (Figure 1).

**Enamel Slab Preparation**
Thirty freshly extracted human, non-erupted third molars were used. Immediately after extraction, the teeth were stored in a solution of 1% thymol (pH=7). The crowns were removed from the roots at the cement enamel junction. The roots were discarded and the crowns were longitudinally sectioned with double-faced diamond disks (model 7020, KG Sorensen, Barueri, Sao Paulo, Brazil) using a low-speed motor driven handpiece (Kavo do Brasil – Joinvile., Brazil). The gingival and occlusal thirds of the crowns were discarded, and only the middle vestibular or lingual sites were used to provide the 60 slabs with a dimension of 4 x 4 mm without stains or cracks. Care was taken to leave the enamel slabs hydrated during this period of preparation. After sectioning was completed, specimens were soaked in distilled and deionized water at 37º C for a week.

The slabs were embedded individually in a polystyrene resin (BL 41110, Cromex, São Paulo, São Paulo, Brazil) in a polyvinyl chloride ring mold 2.0 cm in diameter to expose the external surface of the enamel. The molds were removed and the external surface of the slabs were leveled with a water-cooled mechanical grinder (Maxgrind/Solotest, São Paulo, São Paulo, Brazil). Aluminum oxide disks were used in sequential grit sizes of 400, 600, and 1,000, respectively (Carburundum/3M do Brasil Ltda, São Paulo, São Paulo Brazil), and then polished with a felt cloth in conjunction with abrasive pastes 6, 3, 1, and 1/2 µm along with a mineral oil coolant (Top, Gold, and Ram, Arotec Ind e Com Ltda, São Paulo, São Paulo, Brazil). These procedures were conducted to create parallel surfaces for the roughness tester.

![Figure 1. Study diagram.](image-url)
A standardized circular area of 13 mm² exposed enamel was created on the specimens using an adhesive paper by covering the remaining enamel slab with two coatings of finger nail varnish (Colorama, CEIL, São Paulo, São Paulo, Brazil). The enamel slabs were randomly assigned in four groups and the baseline values of surface roughness were obtained.

**Bleaching Treatment**

An individual tray, similar to the tray used by the patient during bleaching, was fabricated for each specimen using a 0.4 mm-thick flexible ethyl vinyl acetate polymer (Bio-Art Equipment, Ribeirão Preto, São Paulo, Brazil) placed in a vacuum-forming machine (P7, Bio-Art Equipment, Ribeirão Preto, São Paulo, Brazil).

A syringe was used to apply 0.02 ml of bleaching agent to each specimen. A 10% carbamide peroxide bleaching agent (Opalescence 10% Ultradent Co., South Jordan, Utah, USA) was used. The enamel slabs were exposed to the bleaching treatment agents for six hours a day for a period of 28 days.

The specimens were individually closed in containers with 19 ml of remineralization solution similar to artificial saliva (pH = 7.0) at 37°C (Table 1). This solution consisted of a remineralization solution similar to a natural saliva of the calcium and phosphate ion content, proposed by Featherstone et al. 1986 and modified by Serra and Cury in 1992. The specimens were removed after six hours from the storage media, and the bleaching agent was washed away under running distilled and deionized water for five seconds.

During the remaining daily time (18 hours), the slabs were maintained in individual receptacles with 19 ml of remineralization solution at 37°C. The remineralization solution was changed daily.

After the bleaching treatment period (28 days), all groups were kept in the remineralization solution for an additional 28 days post-bleaching period. The cleaning surface treatment was still applied during this post-treatment period.

**Surface Cleaning Treatment**

After the daily bleaching treatment, the samples were submitted to a surface cleaning treatment according to the specifications of each group as follows: Group 1, the control group, was not submitted to a cleaning treatment and the samples remained immersed in the remineralization solution after bleaching treatment. Groups 2 and 3 received a cleaning surface treatment through marketed dentifrice containing calcium carbonate as abrasive with fluoride and without fluoride (Table 2), respectively. Group 4 received the cleaning treatment only with brushing in deionized distilled water.

Brushing procedures were performed once a day for three min during 56 days, with a brushing machine, under a typical brushing force of 200g with 250 cycles per minute, in a freshly prepared slurry with one part of dentifrice to three parts of deionized and distilled water (50 g of toothpaste/150 g deionized and distilled) every day. The brush heads were the same brand of nylon multi-tufted toothbrushes. Each specimen was brushed with the same individual brush head. Sufficient slurry (20 ml) was placed in the reservoir and the specimen was completely covered.

### Table 1. The artificial saliva compounds proposed by Featherstone et al, and modified by Serra and Cury.

<table>
<thead>
<tr>
<th>COMPOUNDS</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ARTIFICIAL SALIVA</strong></td>
<td>pH=7.0</td>
</tr>
<tr>
<td>Ca</td>
<td>1.5 mmol/l</td>
</tr>
<tr>
<td>KCl</td>
<td>50 mmol/l</td>
</tr>
<tr>
<td>PO₄</td>
<td>0.9 mmol/l</td>
</tr>
<tr>
<td>tri-hydroxymethyl-aminomethane</td>
<td>20 mmol/l</td>
</tr>
<tr>
<td>distilled and deionized H₂O</td>
<td>qsp</td>
</tr>
</tbody>
</table>
Table 2. Ingredients used to prepare dentifrices by Proderma Pharmacy (Piracicaba, São Paulo, Brazil).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Abrasive toothpaste with fluoride</th>
<th>Abrasive toothpaste without fluoride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micronized calcium carbonate</td>
<td>52.5 %</td>
<td>52.5 %</td>
</tr>
<tr>
<td>Glycerin</td>
<td>25 %</td>
<td>25 %</td>
</tr>
<tr>
<td>Natrosol Gel</td>
<td>18 %</td>
<td>18 %</td>
</tr>
<tr>
<td>Sodium lauryl sulfate</td>
<td>2 %</td>
<td>2 %</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>0.16 %</td>
<td>-</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>qsp</td>
<td>qsp</td>
</tr>
</tbody>
</table>

The slurries were agitated for 20 min before they were dispensed inside the reservoir. The dentifrices were freshly prepared every two days to keep the neutral pH. At the end of the daily brushing, the samples were removed from the machine receptacles, washed with distilled water, and maintained in individual receptacles at 37ºC until the next bleaching and brushing cycle.

**Surface Roughness Test**
Before and across bleaching treatment, a profilometer was used to measure the initial surface roughness (baseline). Three different directions were used to perform two measurements on the surface of each specimen in a total of six measurements per specimen, with a cut off (λc) of 0.25 mm in a velocity of 0.1 mm/s (ISO 4228). The mean Ra value (μm) was determined for each specimen at each seven day interval.

**Statistical Analysis**
Statistical analysis involved a parametric method using repeated measures analysis of variance (ANOVA) followed by a Tukey's HSD hoc analysis (α = 0.05). Baseline data were performed in order to verify the initial surface roughness and to contrast differences among the groups (P<.05).

**Results**
The mean values of roughness are shown in Table 3, and the behavior of enamel roughness is displayed in Figure 2.

The Tukey test compared differences among time intervals at the 5% level of significance (P<.05). Equal letters indicate mean values that are not significant different, capital letters are considered in horizontal and tiny letters are considered in vertical.

All groups showed similar statistical means of baseline values. However, groups G2 and G3 (brushed with an abrasive dentifrice) had a statistically significant increase in the surface roughness in function of the time compared to the G1 (control group) and G4 (brushed without a dentifrice). Specimens from the control group (G1) stored in artificial saliva presented no differences in surface roughness mean values (P>.05) at the different time intervals.

Group 2 showed a statistically significant increase in the surface roughness from the baseline to the seventh day and to the fourteenth day. This increase was continual and the twentieth day was statistically higher than both baseline and the seventh day. After the twenty-first day, the surface roughness was statistically similar until the end of the post-bleaching period.

Group 3 showed an increase in the surface roughness, but statistical differences may be noticed from the fourteenth day, when roughness surface values were statistically higher than baseline value.

Similar to group 1, group 4 showed no statistical differences among the time intervals.

**Discussion**
Patients who were submitted to tooth bleaching treatment usually brushed their teeth three or four times a day. It is not uncommon for dental professionals to recommend dentifrices for a
Table 3. Surface roughness (Ra) values for each treatment at different time intervals.

<table>
<thead>
<tr>
<th>Period</th>
<th>Not Brushed</th>
<th>Brushing with fluoride</th>
<th>Brushing without fluoride</th>
<th>Brushing without toothpaste</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>means</td>
<td>Standard deviation</td>
<td>means</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.084 A a</td>
<td>0.026</td>
<td>0.039 A c</td>
<td>0.092 A b</td>
</tr>
<tr>
<td>7</td>
<td>0.093 A a</td>
<td>0.026</td>
<td>0.138 A b</td>
<td>0.113 A ab</td>
</tr>
<tr>
<td>14</td>
<td>0.082 B a</td>
<td>0.022</td>
<td>0.156 A ab</td>
<td>0.120 AB a</td>
</tr>
<tr>
<td>21</td>
<td>0.085 B a</td>
<td>0.021</td>
<td>0.164 A a</td>
<td>0.131 AB a</td>
</tr>
<tr>
<td>28</td>
<td>0.085 B a</td>
<td>0.021</td>
<td>0.155 A ab</td>
<td>0.130 AB a</td>
</tr>
<tr>
<td>35</td>
<td>0.089 C a</td>
<td>0.024</td>
<td>0.152 A ab</td>
<td>0.138 AB a</td>
</tr>
<tr>
<td>42</td>
<td>0.087 B a</td>
<td>0.019</td>
<td>0.149 A ab</td>
<td>0.133 AB a</td>
</tr>
<tr>
<td>49</td>
<td>0.087 C a</td>
<td>0.024</td>
<td>0.157 A ab</td>
<td>0.137 AB a</td>
</tr>
<tr>
<td>56</td>
<td>0.079 C a</td>
<td>0.022</td>
<td>0.147 A ab</td>
<td>0.129 AB a</td>
</tr>
</tbody>
</table>

Figure 2. Mean roughness of enamel slabs bleached and submitted to superficial cleaning treatment at different time intervals.
specific purpose like whitening or abrasion in order to improve the bleaching process.\textsuperscript{14} Such a recommendation may be responsible for superficial tooth wear and other complicating factors.\textsuperscript{16}

According to some studies, 10\% carbamide peroxide bleaching agents alter enamel surface\textsuperscript{5,12} as microhardness\textsuperscript{7} or surface roughness.\textsuperscript{7} The oxidative process on enamel surfaces and the pH of tooth-bleaching products have been considered as the primary causes of adverse effects on mineralized tissues after bleaching. Low concentrations of carbamide peroxide supposedly promote various degrees of surface porosity, structural change, and mineral loss depending on the bleaching agent.\textsuperscript{7,9,17,12}

However, in this study no surface roughness alteration was found in G1 or G4 although they were treated with 10\% carbamide peroxide. These results are in conflict with McGuckin et al. who found an increase in the surface roughness of the enamel exposed to 10\% carbamide peroxide.\textsuperscript{7} However, their specimens were stored in an isotonic saline solution, not artificial saliva, after the daily bleaching treatment.\textsuperscript{6}

In this study the specimens were stored in a remineralization solution with Ca and PO\textsubscript{4} in concentrations similar to natural saliva.\textsuperscript{19} The presence of these ions might favor the remineralization process and decrease the risk of superficial erosion.\textsuperscript{19}

In addition the same brand of bleaching agent used in this study was researched in an \textit{in vitro} study by Rodrigues et al. in 2001 who found an increase in the microhardness in contrast to the other bleaching agents that had a low pH and showed a decrease in the microhardness.\textsuperscript{9}

However, the findings obtained show 10\% carbamide peroxide caused some alterations on the surface enamel when associated with abrasive dentifrice. The enamel surface roughness was altered in G2 and G3 after cleaning treatments with abrasive dentifrices. There was an increase in the roughness values between the fourteenth and the twenty-eighth days of bleaching treatment. These values were kept high until the 56th day revealing the surface roughness does not return to values similar to baseline. It is well known abrasive dentifrices cause enamel microwear and slight abrasion may alter the superficial layer of enamel and promote a “new” surface with high roughness values.\textsuperscript{14}

Although group 4, which was brushed with only water, showed no differences in roughness values, it cannot be concluded abrasive dentifrices alone are responsible for the altered bleached enamel surface.

The free radicals and urea released from carbamide peroxide, which are capable of weakening enamel structure, when followed by tooth brushing with an abrasive dentifrice could remove a certain amount of enamel from the surface.\textsuperscript{15} In this circumstance bleaching treatment may have a synergistic effect with the abrasive dentifrices that favors an increase in the surface roughness. Nevertheless, such effect could not be evaluated in the present study.

Though the major therapeutic function of dentifrices are to reduce the incidence of caries by the rational use of fluoride dentifrices can also be an important method to decrease the superficial erosion caused into enamel due to use of low pH agents or bleaching agents\textsuperscript{13}; in the present study the action of fluoride was not relevant. The bleached enamel surfaces brushed with the abrasive fluoride dentifrice have the same surface roughness of those brushed with a non-fluoride dentifrice showing that fluoride had no significant effect in this study.

\textbf{Conclusion}

This \textit{in vitro} investigation showed the sole use of 10\% carbamide peroxide did not alter enamel surface roughness, but cleaning treatments using brushing with abrasive dentifrices resulted in a significant increase of enamel surface roughness. To avoid such an undesirable effect it is prudent to recommend to patients to use a non-abrasive dentifrice.
References
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