In vitro Analysis of Human Enamel Microhardness as Subjected to Prolonged Use of External Bleaching Agents

Análise in vitro da Microdureza do Esmalte Humano Submetido ao uso Prolongado de Agentes Clareadores Externos

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Abstract

The aim of this study was to evaluate the effects of external bleaching agents on the microhardness of human enamel after its prolonged use. Twenty intact human third molars were submitted to mesio-distal crosscut and embedded in polystyrene resin. The specimens were submitted to finish, and half of the enamel surface of each specimen was covered with cosmetic varnish, representing the control group (G0 – did not receive bleaching agents). The sample was divided into four groups (n=10): G1 – one bleaching session with 16% carbamide peroxide; G2 – three bleaching sessions with 16% carbamide peroxide; G3 – one bleaching session with 22% carbamide peroxide; and G4 – three bleaching sessions with 22% carbamide peroxide. Each session lasted 8 hours a day over the course of two weeks, with 45 days interval between G2 and G4 sessions. During this period, the specimens were kept in artificial saliva at 37°C. Then, the Knoop hardness test was done on the middle third of each bleached and non-bleached surface. The data were submitted to analysis of variance (two-way ANOVA), Tukey’s test (p<0.05) and Dunnet test for comparison to G0, which showed the highest superficial hardness averages, differing statistically from the other groups (306.69 KHN). Group 4 showed the lowest average (135.37 KHN). It was concluded that bleaching reduced enamel hardness. Furthermore, increasing of carbamide peroxide (22%) associated with an increased number of sessions (3 sessions) enhanced the decrease in microhardness.

Keywords: Dental Enamel. Peroxides. Tooth Bleaching.

1 Introduction

The relentless pursuit of overly bleached teeth has been making tooth bleaching treatment widely accepted by patients1,2. The aesthetic appeal and ease of obtaining these agents lead many people to use these products inadvertently, ignoring their adverse effects.

The dental market offers bleaching materials with different compositions and concentrations. Carbamide peroxide is often used for homemade dental bleaching, and it can be found in concentrations ranging from 10% to 22%. In contact with saliva and oral tissues, carbamide peroxide decomposes into hydrogen peroxide (active agent) and urea3. The lower molecular weight of hydrogen peroxide allows it to freely transit by interprismatic spaces, through enamel and dentin, causing pigments oxidation of these structures4. Therefore, compounds with pigmented carbon rings are converted in clearer chains, resulting in dental bleaching effect5.

Studies have shown that there is a possibility of subclinical changes in superficial microhardness of the enamel7–11, although this reversing possibility has not been established yet.

Dental bleaching using carbamide peroxide for long periods also destroys different layers of enamel and produces minerals loss7. Changes may occur to the enamel, dentin and cementum after bleaching5. Bleaching agents probably lead to...
cell destruction of the pulp due to enzymatic inactivation and
rupture of normal cellular activity.12,13 Nevertheless, the sodium
ascorbate, a component of these agents, was able to protect
cultured cells against cytotoxic effects of carbamide peroxide.14

The aesthetic appeal of whiter teeth leads people to
pursue it. Ease of access to bleaching agents makes us alert
to the growing use of these materials. Considering these facts,
the aim of this study was to evaluate in vitro the effects of
bleaching agents on enamel microhardness after different
number of sessions and concentrations of homemade dental
bleaching.

2 Material and Methods

2.1 Preparation of specimens

This research project was approved by the Ethics Committee
in Human Research of the University under report n. 113/2008.
Twenty intact human third molars extracted for clinical
reasons were cleaned and frozen in saline solution. After being
defrosted at 25 °C, the roots were removed and the teeth were
submitted to mesio-distal crosscut using diamond disk (Labcut
1010 Low Speed Diamond Saw-EXTEC, Chicago, IL, USA)
under water refrigeration. Vestibular and lingual surfaces
were used in this study. Forty specimens were obtained and
embedded in polystyrene resin. They were submitted to
finishing by using wet sandpaper (Block Stone Waterproof
– Bosch, Campinas, SP, Brazil) with decreasing granulation
(400, 600, 800, 1200, 1500, and 2000) under refrigeration until
flat enamel areas were exposed. Half of the enamel surface
of each specimen was covered with cosmetic varnish and did
d not receive bleaching treatments (Revlon Incorporated, New
York, NY, USA) in order to create the control group (G0). The
other half was later submitted to the bleaching agent, defining
experimental groups described below.

2.2 Experimental groups

The specimens were divided into four groups, as shown
in Table 1. Each session lasted 8 hours a day over the course
of two weeks, as suggested by the manufacturer. During the
intervals between sessions, the specimens were kept at 37 °C
in artificial saliva, switched daily.

Table 1: Experimental groups with respectives used bleaching
agents, number, duration and interval between sessions.

<table>
<thead>
<tr>
<th>Group (N=10)</th>
<th>Bleaching Agent</th>
<th>Number of Sessions</th>
<th>Interval Between Sessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Carbamide Peroxide 16%*</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>G2</td>
<td>Carbamide Peroxide 16%*</td>
<td>3</td>
<td>45 days</td>
</tr>
<tr>
<td>G3</td>
<td>Carbamide Peroxide 22%*</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>G4</td>
<td>Carbamide Peroxide 22%*</td>
<td>3</td>
<td>45 days</td>
</tr>
</tbody>
</table>

*Whiteness Perfect®-FGM Dental Products, Joinville, SC, Brazil.

2.3 Knoop Microhardness Test

After bleaching sessions, specimens were submitted to the
Koop Hardness Test. They were positioned perpendicularly
along the long axis of the edentator (Shimadzu Corporation,
Model HNZ2, Kyoto, Japan). Three edentations were made
(50g for 15 seconds) in the middle third of each surface,
obtaining the average values of Knoop Hardness Number
(KHN). The results were submitted to statistical analysis by
two way-ANOVA and the averages were compared by the
Tukey’s test (5% significance). Dunnet test was applied to
compare the experimental groups with the control group.

3 Results and Discussion

There was a significant reduction (P<.05) of hardness
in all groups when each was compared to non-bleached
specimens (G0: 306.69 KHN; SD = 44.9). The comparisons
between bleached specimens (G1, G2, G3, and G4) are shown in Table 2.

Table 2: Comparison between bleached specimens, varying time and concentration (Values in KHN)

<table>
<thead>
<tr>
<th>Time</th>
<th>16% Concentration</th>
<th>22% Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>202,20 (55,40) Aa</td>
<td>172,10 (35,36) Aa</td>
</tr>
<tr>
<td>3 weeks</td>
<td>201,50 (57,40) Aa</td>
<td>135,10 (36,14) Ab</td>
</tr>
</tbody>
</table>

*Capital letters compare lines (time). Lowercase letters compare columns (concentration), 5% significance level by the Tukey’s test

When 16% carbamide peroxide was used (G1 and G2),
there was no significant hardness reduction between one
and three bleaching sessions. The same occurred with 22%
carbamide peroxide (G3 and G4). However, while comparing
different peroxides concentrations, there was a significant
hardness reduction when 22% carbamide peroxide was used
in three sessions (G4).

The results of this study show that either 16% or 22%
carbamide peroxide used for homemade dental bleaching
reduced human enamel hardness and are in accordance to
those of several authors.4,6,7,15-19

However, some studies have not showed significant
statistical reduction on enamel hardness using low
concentration of bleaching agents (10%). These studies
considered its use safe up to five weeks.20-25 No studies were
found using the same concentration of peroxide (22% for six
weeks) as in our study.

Analyzing the concentrations of bleaching agents used in
the present research, the results showed that 22% carbamide
peroxide significantly impacted the hardness reduction only
when used for prolonged periods (three sessions-G4). When
used for 14 days (G3), there were no statistically significant
changes other than those produced in specimens bleached by
16% carbamide peroxide (G1 and G2). This fact is supported
by the changes on surface morphology, indicated by the loss of the aprismatic layer, depressions, erosion, and increase in the depth of the irregularities and pores as reported by Junqueira et al.26. Such changes, although microscopic, can make the enamel more fragile and the teeth more susceptible to sensitivity27.

When 16% carbamide peroxide was used, there was no significant statistical difference due to the different number of bleaching sessions, since G1 and G2 did not differ. Studies corroborate this fact, showing that at low concentrations (up to 15%) the hardness reduction is not significant, but there is a change in the morphology of enamel15,16,18,19.

Another fact to be considered is the influence of the storage solution. The artificial saliva, in which specimens are kept during the period between the applications of the bleaching agent, leads to remineralization of the bleached enamel surface2, which could increase microhardness. However, this fact was not observed in our study, since there was microhardness reduction compared with the non-bleached enamel, which was also kept in the same condition.

4 Conclusion

Within the limitations of this in vitro study, we concluded that prolonged use of bleaching agents, especially in higher concentration, may reduce microhardness of enamel, thus possible alternatives to reverse this situation should be investigated.

References


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