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# Deleterious Effects over Enamel Surface Bleached with Low and High Concentration Gels Containing Calcium – *in vitro* Evaluation

## Efectos Deletéreos sobre la Superficie del Esmalte Blanqueada con Geles de Alta y Baja Concentración que Contienen Calcio - Evaluación *in vitro*

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**ABSTRACT:** The objective of this study was to evaluate the morphological effects of bleaching with hydrogen peroxide 40 % (HP 40 %) and carbamide peroxide 20 % (CP 20 %), with and without the addition of calcium (2000 ppm), in enamel. Bovine enamel blocks (25 mm2) were randomly divided into 5 groups (n=12) accordingly to the bleaching gel (HP 40 % and CP 20 %) and the presence of calcium (with and without). Control group were immersed in artificial saliva. The treatments were carried out for 14 days. Roughness (Ra) and Knoop microhardness analysis were performed for enamel surface before and after treatment. Data were analyzed by repeated measures ANOVA and Tukey test ($p<0.05$). The bleached group with 40 % HP had the lowest values of microhardness. There was a statistically significant difference between the initial and final readings. For surface roughness were no differences between the tested materials; but there was an increase of roughness for all groups after bleaching. It was concluded that all bleaching agents tested promoted a surface microhardness decrease after treatment, however the calcium addition promoted a slighter surface alteration, and all bleaching agents promoted a surface roughness increase after treatment.

**KEY WORDS: bleaching agents, dental enamel, hydrogen Peroxide, carbamide peroxide.**

## INTRODUCTION

Tooth bleaching is one of the most conservative procedure required for patients desiring to improve the color appearance of their teeth, since it is simple to execute, safe and effective (Omar *et al*., 2019; Zanatta *et al*., 2020). Mainly the mechanism of tooth whitening occurs by the diffusion of hydrogen peroxide (HP) through enamel and dentin, reacting with the organic pigments, known as chromophores, and the main responsible for teeth discoloration (Kwon *et al*., 2015; Correia *et al*., 2017; Joiner & Luo, 2017). The HP releases unstable free radicals that oxidize the organic pigments, decreasing their size and promoting their removal from the dental structure by diffusion, thereby promoting less light absorption by the tooth tissues and consequently a lighter appearance (Joiner, 2010; Alqahtani, 2014; Joiner & Luo). This reaction can be influenced by several factors such as temperature, pH, the whitening gel active ingredient concentration and the contact time between the tooth and the gel (Borges *et al*., 2015; Torres *et al*., 2019; Zanatta *et al*.).

Although considered a conservative treatment, there is a concern regarding adverse effects over the enamel and dentin surface properties caused by the contact with the gel. Alterations such as decreased microhardness, increased roughness and erosive effects are frequently reported, but with controversial results published in literature (Cadenaro *et al*., 2008;

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Cavalli *et al*., 2011; Borges *et al*., 2012; Sa *et al*., 2013; Anaraki *et al*., 2015; Attia *et al*., 2015; Borges *et al*., 2015; Zeczkowski *et al*., 2015; Torres *et al*.). These adverse effects are modulated by the pH of the gels (some present acidic nature), the peroxide concentration, prolonged contact with tooth tissues and presence of calcium and fluoride salts. Therefore, in order to minimize these, elements such as calcium, fluoride, amorphous calcium phosphate (ACP), phosphopeptide casein - amorphous calcium phosphate (CPP-ACP) and hydroxyapatite (HA) have been added in the bleaching agents' composition, and tested regarding their ability to reduce surface alteration without interfering with the whitening effectiveness (Chen *et al*., 2008; Borges *et al*., 2009, 2011; Alexandrino *et al*., 2014; Coceska *et al*., 2016). The availability of high levels of calcium as a soluble salt leads to calcium and phosphate mineral precipitation over the enamel surface. The saliva protective action involves the remineralization potential also providing calcium and phosphate to the dental hard tissues, besides buffering capacity that avoids the pH decrease and further loss of mineral content (Jiang *et al*., 2008; Tschoppe *et al*., 2009; Borges *et al*., 2012, 2014a; Attia *et al*.).

Thus, the aim of the present study was to evaluate if the addition of calcium into high and low concentration of commercial peroxide gels reduces the harmful effects on enamel surface. The null hypothesis tested was that the addition of calcium to bleaching agents would not reduce the enamel microhardness or increase its roughness.

## MATERIAL AND METHOD

**Sample preparation.** This study was approved by the Animal Ethical Research Committee of the University of Taubate (protocol 006/15). Sixty sound bovine incisors were obtained in a slaughterhouse (Frigorífico Mondelli, Bauru, SP, Brazil). The teeth were immersed in a 0.1 % thymol solution (Byoformula Imp Exp, Sao Jose dos Campos, SP, Brazil) for 24 hours at room temperature for disinfection, and afterwards they were cleaned with pumice/water slurry and Robinson brush (Microdont, São Paulo, SP, Brazil).

From the bovine incisors, 60 enamel/dentin blocks with 5 x 5 mm (length x width) were obtained using a diamond disc (Microdont, São Paulo, SP, Brazil) coupled at a low-speed dental hand piece, under irrigation. The blocks were evaluated in a stereomicroscope (x20) and those with cracks or defects in the enamel surface were replaced.

The samples were then embedded in crystal polystyrene resin (Valglass Comercio e Industria Ltda, Sao Jose dos Campos, SP, Brazil) using a circular PVC mold with 20 x 20 mm (diameter x height), leaving the enamel face exposed. Then, the enamel was flattened and polished with sequential water-cooled silicon carbide paper (#320, #600, #1200, #2000 and #2500) (3M ESPE, St Paul, MN, USA) each for 30 s, using a circular polishing machine (Aropol E, Arotec, Cotia, SP, Brazil) with 200 rpm rotation, under constant water irrigation. Between each step, the samples were cleaned with distilled water for 10 min in an ultrasonic bath (Cristofoli, Campo Mourao, PR, Brazil) to remove any debris present over enamel surface.

The samples were stored in artificial saliva for two weeks, with daily changes (1.5mM Ca, 0.9mM P, 0.1 buffer solution of TRIS-Hydroxymethy-laminomethane, pH 7.0) (Byoformula Imp Exp, Sao Jose dos Campos, SP, Brazil), in order to standardize the enamel surface mineral condition before the beginning of the experiment.

**Baseline Knoop Surface Microhardness (KHN) and Roughness (Ra).** The initial Knoop surface microhardness (KHN0) of all samples were obtained using a microhardness tester (Digital Microdurometer HMV-2T, Shimadzu, Tokyo, Japan), with 50 g load for 15 s. Three indentations were made in the central part of the enamel, spaced 100 μm apart, and the KHN of each sample were defined by arithmetic mean. Samples with KHN ranging from 10 % of the mean hardness values of all samples were replaced.

After group division, the enamel roughness (Ra) was measured using a contact profilometer (Surftest SJ 301, Mitutoyo, Tokyo, Japan), and the Ra parameter was obtained with a 2 μm diamond tip and a 0.25mm cutoff. Three readings were made on each enamel sample and values were averaged.

**Group Division.** After the KHN0 measurements the samples were stratified into 5 groups (n = 12), according with the bleaching gel tested: CP20 % - 20 % carbamide peroxide (Opalescence PF, Ultradent Products Inc, South Jordan, UT, USA) or HP 40 % - 40 % hydrogen peroxide (Opalescence Boost, Ultradent Products Inc, South Jordan, UT, USA); and the presence of calcium (with and without). The control group received no treatment and was kept only in artificial saliva. Figure 1 shows the chart of group division.

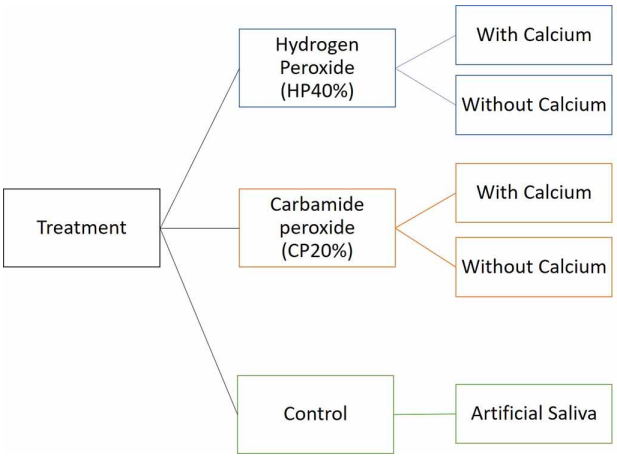


Fig. 1. Flow chart of group division.

**Bleaching treatment.** For the groups bleached, the bleaching gels were applied over the enamel surface (1 mm thickness) for 60 minutes for the groups treated with carbamide peroxide (CP20 %) and for 40 minutes for the groups treated with hydrogen peroxide (HP40 %) at 37º C. After this period, the gel was removed, and the samples were rinsed in running water, and individually stored in 10mL of artificial saliva (37ºC ± 2ºC) until the next bleaching session. The bleaching treatments were performed during 14 days for the CP20 % groups, while for the HP40 %, two applications were made with interval of 7 days within each other.

For the groups treated with calcium, it was added 2000 ppm of calcium chloride ($CaCl_2$) in the gel formulation of the respective group with manual stirring until salt dissolution. The mixture was made every day, immediate before use. At the end of treatments, the samples were kept immersed in artificial saliva for 7 days for color stability, then final microhardness and roughness were obtained as previously described.

**Statistical analysis.** The normal distribution of values was verified by Shapiro-Wilk test, and the microhardness and roughness data were subjected to the analysis of variance test (ANOVA) for repeated measures followed by Tukey test (a = 0.05).

## RESULTS

The microhardness results were presented in Table I. The microhardness means showed that was a significant decrease between the initial (baseline) and final times for all tested materials, except for the control group. The group treated only with HP40 % showed the lowest surface microhardness means, but without difference among HP40 % + Ca and CP20 %. The other groups showed similar microhardness means differing only from the control group.

All experimental groups showed increased enamel roughness (Table II) when compared the initial (baseline) and final times, except control group (artificial saliva). There was no significant difference among bleaching agents tested and all differed from control group after treatment.

Table I. Surface microhardness means. (Standard deviation of bovine enamel.

| Treatments | Baseline ($KHN_0$) | Final (after bleaching) |
|---|---|---|
| CP 20 % | 243.43 (±19.3) aA | 158.97 (± 17.4) bcB |
| CP 20 % + Ca | 243.07 (±23.7) aA | 176.03 (±33.0) bB |
| HP 40 % | 242.73 (±14.4) aA | 146.76 (±19.1) cB |
| HP 40 % + Ca | 243.87 (±19.2) aA | 168.33 (±12.6) bcB |
| Saliva (control) | 242.57 (±26.9) aA | 243.63 (±24.0) aA |

Means followed by different uppercase letters showed significant differences (p<0,001) for time. Means followed by different lowercase letters showed significant differences (p<0,001) for bleaching treatments.

Table II. Surface roughness means (SD) of bovine enamel.

| Treatments | Baseline | Final (after bleaching) |
|---|---|---|
| CP 20 % | 0.063 (±0.017) aB | 0.123 (±0.042) aA |
| CP 20 % +Ca | 0.063 (±0.019) aB | 0.100 (±0.019) aA |
| HP 40 % | 0.064 (±0.014) aB | 0.111 (±0.037) aA |
| HP 40 % +Ca | 0.061 (±0.015) aB | 0.109 (±0.027) aA |
| Saliva (control) | 0.061 (±0.014) aA | 0.060 (±0.012) bA |

Means followed by different uppercase letters showed significant differences (p<0,001) for time. Means followed by different lowercase letters showed significant differences (p<0,001) for bleaching treatments.

## DISCUSSION

The null hypothesis was not accepted since there was alterations at surface microhardness and roughness after bleaching procedures. Although previous studies suggested adverse effects on dental structures, such as decreased wear resistance, increased surface roughness, decreased microhardness values and morphological changes immediately after tooth whitening (Lewinstein *et al*., 2004; Basting *et al*., 2007; Borges *et al*., 2014b) these results are still conflicted.

The enamel microhardness values reduction indicated that bleaching treatments promoted mineral loss content, and this loss was not related to the gel concentration. Our study corroborated with this hypothesis since the bleached enamel with 40 % hydrogen peroxide had the lowest microhardness values, being similar to the gel associated with calcium, and also with the 20 %CP gel without calcium. In all of these groups the reduction of microhardness was higher than 20 %, which is the maximum acceptable by ISO 28399 for safety (Torres *et al*.). The presence of calcium was effective only for the 20 %CP, promoting lower microhardness reduction compared with 40 %HP, however still greater than the control group. The idea of adding calcium to the gels remains over the fact that the hydroxyapatite from enamel and dentin are based on calcium and phosphate, therefore when in contact with an undersaturated gels, its dissolution may occur. However, when in contact with supersaturated solutions, mineral precipitation or remineralization can happen (Borges *et al*., 2014b; Torres *et al*.). The results of our study indicated that the addition of 2000 ppm co calcium chloride was not effective to prevent enamel microhardness reduction, corroborating with previous studies (Rauen *et al*., 2015; Torres *et al*.). The concentration tested might be lower to promote the mineral deposition but was chosen to maintain the gel viscosity.

For the surface roughness results, all tested groups showed an increase surface roughness, regardless calcium was added or not, in bleaching agent composition. Only the control group immersed in artificial saliva showed the same roughness values after final evaluation. The whitening process occurs through an oxidation reaction of its active ingredient, carbamide or hydrogen peroxides, leading to oxygen release which consequently promotes tooth bleaching. In the course of this process, larger molecular organic chains were cleaved into smaller ones, and this process leads to several chemical changes and promotes surface roughness increase. However, this process is temporary and can be reversed by saliva remineralizing action, so it is not considered a harmful enamel effect. Thus, our study corroborated with Attia *et al*., but this condition is considered reversible after the remineralization processes. The effects of calcium may support the remineralization process, reducing bleaching agent deleterious effects on enamel surface.

Based on the methodology employed in this study, it can be concluded that all bleaching agents tested promoted a surface microhardness decrease after treatment, however the calcium addition promoted a slighter surface alteration, and all bleaching agents promoted a surface roughness increase after treatment.

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**RESUMEN:** El objetivo de este trabajo fue evaluar los efectos morfológicos del blanqueamiento con peróxido de hidrógeno 40 % (HP 40 %) y peróxido de carbamida 20 % (CP 20 %), con y sin calcio (2000 ppm), en el esmalte. Para este efecto se dividieron aleatoriamente bloques de esmalte bovino (25 $mm^2$) en 5 grupos (n = 12) de acuerdo con el gel blanqueador (HP 40 % y CP 20 %) y la presencia de calcio (con y sin). El grupo de control se sumergió en saliva artificial. Los tratamientos se llevaron a cabo durante 14 días. Se realizaron análisis de rugosidad (Ra) y microdureza Knoop para la superficie del esmalte antes y después del tratamiento. Los datos fueron analizados mediante ANOVA de medidas repetidas y prueba de Tukey ($p < 0,05$). El grupo blanqueado con 40 % de HP tuvo los valores más bajos de microdureza. Hubo una diferencia estadísticamente significativa entre el resultado inicial y final. Para la rugosidad de la superficie no hubo diferencias entre los materiales probados; pero hubo un aumento de rugosidad en todos los grupos después del blanqueo. Se concluyó que todos los agentes blanqueadores probados promovieron una disminución de la microdureza superficial después del tratamiento, sin embargo, la adición de calcio promovió una alteración superficial más leve y todos los agentes blanqueadores promovieron un aumento de la rugosidad superficial después del tratamiento.

**PALABRAS CLAVE: agentes blanqueadores, esmalte dental, peróxido de hidrógeno, peróxido de carbamida.**

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