

Effects of bleaching using 10% carbamide peroxide with calcium or amorphous calcium phosphate on enamel mineral content and hardness

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ABSTRACT

This study evaluated enamel mineral content and surface microhardness before and after bleaching treatment using 10% carbamide peroxide (CP) containing calcium (Ca) or amorphous calcium phosphate (ACP). Thirty-six bovine slabs were randomly allocated into 3 groups ($n = 12$) according to bleaching treatment: G1 - Opalescence PF 10% (CP), G2 - NiteWhite ACP (CP+ACP), and G3 - Opalescence PF (10%) with calcium (CP+CA). The bleaching agent was applied on enamel surface for 6 h/day over a period of 21 days. Enamel surface was evaluated by Knoop microhardness (KNH) and micro energy-dispersive X-ray fluorescence spectrometry (μ -EDXRF) at baseline and at after bleaching treatment. Data were statistically analyzed by repeated measures ANOVA and Tukey's test ($\alpha = 0.05$). There was a significant decrease in

microhardness after bleaching treatments for all study groups, but no difference between bleaching gels. There was no difference in the Ca/P ratio measured by μ -EDXRF for all groups at the study times, but the mean value was lower in group CP+CA than in group CP+ACP. Group CP was similar to both CP+ACP and CP+CA. It can be concluded that enamel microhardness decreased after the bleaching process, regardless of the presence of calcium or ACP, but there was no significant change in the Ca/P ratio of enamel after bleaching for each tested gel. This indicates that the bleaching gels have erosive potential, causing softening of enamel without promoting surface loss, regardless of the presence of calcium of ACP ions.

Received: September 2019; Accepted: November 2019.

Keywords: Enamel, tooth bleaching, hydrogen peroxide.

Efeitos do tratamento clareador utilizando peróxido de carbamida a 10% com adição de cálcio ou fosfato de cálcio amorfo no conteúdo mineral do esmalte e microdureza

RESUMO

Este estudo avaliou o conteúdo mineral do esmalte e a microdureza superficial antes e após o tratamento clareador, utilizando peróxido de carbamida 10% (PC) contendo cálcio (Ca) ou fosfato de cálcio amorfo (ACP) em sua composição. Trinta e seis espécimes de esmalte bovino foram alocados aleatoriamente em 3 grupos ($n = 12$) de acordo com os tratamentos clareadores: G1 - Opalescence PF 10% (CP), G2 - NiteWhite (CP+ACP); e G3 - Opalescence PF (10%) com cálcio (CP+CA). O agente clareador foi aplicado na superfície do esmalte por 6 h/dia por um período de 21 dias. A superfície do esmalte foi avaliada por microdureza Knoop (KNH) e espectrometria de fluorescência de raios X micro-dispersiva (μ -EDXRF) no início e após o tratamento clareador. Os dados foram analisados estatisticamente pelo teste ANOVA de medidas repetidas e Tukey ($\alpha = 0,05$). Houve uma diminuição

significativa da microdureza após os tratamentos clareadores para todos os grupos estudados, mas não houve diferença entre os diferentes géis. Não houve diferença da relação Ca/P mensurada por μ -EDXRF para todos os grupos nos tempos estudados; no entanto, o grupo CP+CA apresentou menor valor comparado ao grupo CP+ACP. O grupo CP foi similar aos grupos CP+ACP e CP+CA. Portanto, pode-se concluir que houve redução significativa da microdureza do esmalte após o clareamento, independente da presença de cálcio ou APC na composição dos géis, embora não tenha havido alteração significando na relação Ca / P do esmalte após o clareamento. Isto indica um potencial erosivo dos géis clareadores, causando o amolecimento sem perda da estrutura do esmalte, independente da presença dos íons cálcio e ACP.

Palavras-chave: Esmalte, clareamento, peróxido de hidrogênio.

INTRODUCTION

As a result of modern western aesthetic parameters, which consider white teeth to be more attractive, there is current concern regarding oral appearance and tooth discoloration. Dental bleaching – a simple, effective treatment for removing dental pigments – is one of the resources for improving the appearance of discolored teeth¹.

The active agent in dental bleaching is hydrogen peroxide (HP), which penetrates the tooth structure, breaking down the chromophore molecules that cause dental pigmentation into smaller, lighter colored substances, as has been frequently reported over the years¹⁻⁴. However, several studies have also described adverse effects of HP on tooth structure, such as microhardness reduction, mineral content changes and decrease in calcium and phosphate⁴⁻¹⁰, mainly after longer exposure of dental tissues to highly concentrated HP¹¹⁻¹³.

These deleterious effects are attributed to the erosive potential of some bleaching gels, which damage enamel, reducing its integrity and increasing its porosity, mainly due to the loss of inter-rod spaces¹⁴⁻¹⁵. Attempts have therefore been made to develop bleaching gels that are less deleterious to enamel, by incorporating remineralizing agents such as calcium, fluoride, CCP-ACP and others, with contradictory results. The addition of calcium, for example, can increase bleaching gel saturation and decrease mineral loss, overcoming the undesirable effects of the treatment¹⁶. However, a recent report showed that the addition of calcium alone to a 35% HP bleaching gel was not efficient in preventing enamel microhardness reduction¹⁷, and its desirable effects remain uncertain.

Despite microhardness and roughness analyses, the loss of calcium and phosphate on the tooth surface during bleaching are not fully understood, and research has been conducted on the molecular constituents of dental structures. Hydroxyapatite calcium/phosphate quantification is an indicator of the degree of enamel demineralization,⁹ and some studies have reported that the amounts of calcium and phosphate are lower in whitened teeth^{18,19}.

The aim of this study was to evaluate the enamel mineral content after bleaching using carbamide peroxide gels with calcium or amorphous calcium phosphate (ACP) added to their formulation.

MATERIALS AND METHODS

Thirty-six bovine incisors were cleaned with periodontal curettes (HU-Friedy, Chicago, IL, USA), brushed with pumice and stored in 0.1% thymol solution (Byoformula, São José dos Campos, SP, Brazil) until use. Enamel samples were obtained from the coronal portion using a diamond disc (KG Sorensen, Barueri, SP, Brazil) with a low-speed handpiece under constant water irrigation. Four dental blocks (4 mm x 4 mm) 3 mm thick were cut from the buccal surface of each tooth and embedded in crystal polyester resin, keeping the enamel surface exposed. The samples were polished with 600-, 800-, 1500-, and 2500-grit water-cooled aluminum oxide papers (Arotec, Cotia, São Paulo, Brazil). Samples were immersed in distilled water and cleaned in ultrasound for 30 min to remove any debris left by the polishing.

Initial Knoop Microhardness

Initial microhardness was obtained for each sample using a Knoop indenter (Future Tech-FM-1e, Tokyo, Japan). Three indentations were made 100 µm apart with a 50 g load for 15 s, to prevent cracks on the enamel surface during the experiment. The microhardness value of each sample was defined by the arithmetic mean of the three measurements.

Initial micro energy-dispersive X-ray fluorescence spectrometry (µ-EDXRF)

Semi-quantitative elemental analyses of calcium (Ca) and phosphorus (P) levels in the enamel were carried out before treatments, using micro energy-dispersive X-ray fluorescence spectrometry (µ-EDXRF). Ca and P weight percentages (wt%) from enamel samples were evaluated by collecting three spectra from each sample before and after the bleaching treatments. Measurements were performed with a count rate of 100 s per point (live time). Voltage in the tube was set at 15 kV, with automatic current adjustment and a beam diameter of 50 µm. The equipment was adjusted using a certified commercial reagent (SIGMA, 2008) of stoichiometric hydroxyapatite (Sigma-Aldrich, Poole, UK) synthetic $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, grade 99.999%) as a reference¹⁹. The measurements were collected under fundamental parameters of characteristic X-ray emission of the elements Ca and P, and the element oxygen (O) was used as chemical balance.

The samples were previously evaluated by a micro-energy-dispersive X-ray fluorescence spectrometer (m-EDXRF-1300, Shimadzu, Japan).

Groups and treatment

After the initial microhardness and m-EDXRF measurements, samples were divided into 3 groups (n=12) according to bleaching treatment: **CP** – 10% carbamide peroxide (Opalescence PF - Ultradent Inc., South Jordan, UT, USA), **CP+ACP** - 10% carbamide peroxide with amorphous calcium phosphate (NiteWhite ACP - Discus Dental, Culver City, CA, USA), and **CP+CA** – 10% carbamide peroxide with calcium (Opalescence PF + 2000 ppm calcium). For group CP+CA, the gel was obtained by mixing Opalescence PF 10% with 2000 ppm of calcium chloride (CaCl₂). The pH was measured before and after calcium addition and found to remain unchanged (pH = 6.8).

For all experimental groups, the bleaching agent was applied on the enamel surface (1 mm thickness) for 6 h/day at 37°C ± 1°C, after which the samples were rinsed with distilled water and individually stored in artificial saliva (1.5 mM Ca, 0.9 mM P, 0.1 Tris buffering solution, pH 7.0) until the next bleaching session. The bleaching treatments were performed for 21 consecutive days.

Final microhardness and micro energy-dispersive X-ray fluorescence spectrometry

Final microhardness and micro energy-dispersive X-ray fluorescence spectrometry were measured following the same steps as described above, after 7 days.

Statistical analysis

Data were statistically analyzed by repeated measures analysis of variance (RM ANOVA) and Tukey post-hoc test for multiple pairwise comparisons. The main variables were “bleaching agent” and “time”. Statistical analysis was carried out in the statistical software SAS 9.1 (SAS Institute, Cary, NC, USA) with a confidence interval of 95%.

RESULTS

Data showed homogeneity of experimental variances and errors with a normal distribution of both variables studied (Knoop Microhardness and μ-EDXRF). Table 1 shows the microhardness

results. Two-way ANOVA shows a significant interaction only for the time factor (p = 0.0009), with lower values after the treatment for all groups when compared to the initial values. For the bleaching agent, there was no difference between groups.

Table 2 shows the mean values for μ-EDXRF. There was no significant difference between times (p = 0.95), but there were significant differences between bleaching agents (p = 0.04). The Ca/P ratio was higher for group CP+ACP than for group CP+CA. Group CP presented similar results to groups CP+ACP and CP+CA.

DISCUSSION

Changes in the mechanical properties of enamel after bleaching indicate surface alterations due the action of peroxide on hydroxyapatite and its organic components²⁰⁻²³. Several studies have shown that enamel treated with 10% carbamide peroxide might exhibit porosity and morphological surface alterations after a bleaching process^{24, 25}. In the present study, the microhardness analysis (Table 1) showed significant mineral loss for all groups after bleaching treatment, as has been observed previously²⁴. This significant

Table 1: Knoop microhardness means (SD) for experimental groups.

Bleaching agent	Time	
	Baseline	After bleaching
CP	223.0 (8.2) Aa	113.5 (7.7) Ba
CP+ACP	217.6 (7.3) Aa	109.2 (12.5) Ba
CP+CA	221.7 (11.4) Aa	116.6 (6.9) Ba

Mean followed by different letters indicates statistical differences (p < 0.05). Capital letters compare the different times for each bleaching agent and lowercase letters compare bleaching agents at each time.

Table 2: Mean values (SD) of Ca/P ratio for experimental groups.

Bleaching agent	Time	
	Baseline	After bleaching
CP	1.95 (0.10) Aab	1.96 (0.04) Aab
CP+ACP	1.97 (0.11) Aa	1.97 (0.09) Aa
CP+CA	1.92 (0.06) Ab	1.91 (0.06) Ab

Mean followed by different letters indicates statistical differences (p < 0.05). Capital letters compare different times and lowercase letters compare bleaching agents.

decrease in microhardness after bleaching with 10% carbamide peroxide was similar to that found in other studies²⁶⁻²⁹ and may be related to bleaching gel composition, hydrogen peroxide concentration, activators, pH, and bleaching agent thickener^{19,28}.

Carbamide peroxide gels contain urea, which degrades proteins in the organic matrix of the enamel, leading to a structural alteration of its surface³⁰⁻³². The Carbopol and glycerin could also act as demineralizing agents³¹. Such changes in enamel organic and inorganic parts after bleaching can lead to a decrease in microhardness³³⁻³⁴.

The interaction between the bleaching agent and hydroxyapatite results in the following reaction: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 8\text{H}^+ \rightarrow 10 \text{Ca}^{2+} + 6 \text{HPO}_4^{2-} + 2\text{H}_2\text{O}$, where the calcium (Ca) element and phosphate (P) group analysis are good indicators of enamel demineralization³⁵. In the present study, there was no significant difference in the Ca/P ratio before and after bleaching treatments for all groups (Table 2). This is similar to the results reported by Duschner *et al.*³⁶, who found no significant difference in Ca and P components after dental bleaching.

After bleaching, there were significant differences among groups in the Ca/P ratio. Changes in the enamel were probably caused by the other components in the formula, such as fluoride and calcium, which can promote surface remineralization, although we did not observe better behavior in relation to the enamel Ca/P ratio prior to whitening.

For the μ -EDXRF analysis (Table 2), the results showed that there was no statistical difference in the ratio of calcium and phosphate, which are the chemical constituents of enamel mineral content. This is in agreement with other studies that have reported no difference in enamel micro-chemical analysis and no change in calcium and phosphate concentrations³⁶⁻³⁷. However, different studies have reported mineral loss after bleaching treatment, with changes in calcium and phosphate concentrations^{19,26,38}. This can be explained by methodological differences such as the bleaching gel used, the protocol followed, the remineralizing agent in its composition, presence of calcium, and pH; in addition to the byproducts that could affect the oxidation reaction and even the storage solutions. In the present study, the samples were stored in artificial saliva, which is considered a remineralizing agent. In addition, the bleaching gels contained fluoride or calcium, which would make enamel mineral loss less likely.

Cochrane *et al.*³⁹ claim that fluoride ions can promote net remineralization of the dental surface if calcium and phosphate ions are bioavailable. All the bleaching agents used in the present study contain fluoride. In addition, NiteWhite ACP contains calcium and phosphate, and calcium was added to the bleaching agent 10% CP + Ca. The best result for Ca/P ratio observed for 10% CP + Ca, when compared to Nite White ACP, can be explained by the greater amount of calcium (2000 ppm) available for enamel remineralization during the bleaching process. Although the ACP gel was also a source of bioavailable calcium and phosphate for the same purpose, results are better when amorphous calcium phosphate (ACP) is stabilized by casein phosphopeptide (CPP), known as CPP-ACP, which becomes a higher calcium and phosphate reservoir under these conditions⁴⁰.

The microhardness and μ -EDXRF analyses provided different results. We believe that these two tests evaluate different enamel structures. Microhardness indirectly assesses loss of structure, including inorganic and organic contents, due to the possible interactions between the matrix and the oxidation reaction from the bleaching agent and its byproducts, as described previously. The μ -EDXRF test evaluates the quantity of calcium and phosphorus according to the stoichiometric balance of elemental oxygen. Thus, our hypothesis is that the microhardness surface analysis and Energy Dispersive X-Ray Fluorescence Spectrometric evaluate the enamel structure in different ways. Although there is no positive correlation between the tests, they can be complementary, in search of better understanding of the chemical reaction at molecular level. Further studies are required to confirm this hypothesis and correlate new technologies such as μ -EDXRF, FT Raman Spectroscopy and Fourier Transformed Infrared Spectroscopy with current surface analysis to achieve a better evaluation of mineral loss after dental bleaching treatment.

Based on the methodologies employed in this study, we conclude that regardless of the bleaching agents used, enamel microhardness decreased after the treatments. However, there was no difference in the proportion of calcium and phosphorus on the surface. This suggests that the bleaching gels have erosive potential which softens the enamel without promoting surface loss, regardless the presence of calcium of ACP ions.

FUNDING

This study was supported by São Paulo Research Foundation – FAPESP - Grant number 2009/05592-8.

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