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Original Article

Effects of a bleaching agent with calcium on bovine enamel

Larissa Alexandrino[1], Yasmin Gomes[1], Eliane Alves[1], Hilton Costi[2], Hervé Rogez[3], Cecy Silva[1]

[1]Department of Restorative Dentistry, Federal University of Pará, Belem, Para, Brazil,
[2]Laboratory of Electron Microscopy, Museum Emilio Goeldi, Belem, Para, Brazil,
[3]Department of Food Engineering, Federal University of Pará, and Centre for Agro-food Valorization of Amazonian Bioactive Compounds, Belem, Para, Brazil

Correspondence: Dr. Cecy Silva
Email: cecymsilva@gmail.com

ABSTRACT

Objective: This *in vitro* study analyzed the effects of a bleaching treatment containing 35% hydrogen peroxide (HP) with or without calcium on bovine enamel, using the Knoop hardness number (KHN), tristimulus colorimetry (TC), and scanning electron microscopy. **Materials and Methods:** Forty-five specimens were randomly divided into groups ($n = 5$), which included artificial saliva (negative control [NC]), 35% HP (positive control [PC]), and 35% HP Blue Calcium (HP Blue). The specimens were subjected to three bleaching sessions. During the sessions, the specimens were immersed in artificial saliva at 37°C. Before and after bleaching, KHN tests were conducted using a force of 25 gf for 5 s. TC was performed using the CIE-L*a*b* system and readouts were obtained at the following 4 time points: Before the bleaching treatment; after the first session, the second session, and the third session. The specimens were dehydrated and coated with gold, and the photomicrographs were analyzed in a double-blind manner with a LEO microscope. **Results:** Using one-way analysis of variance and Tukey's test ($P < 0.05$), a statistically significant difference was identified between the initial and final mean KHNs of the NC and PC groups, while the initial and final mean KHNs were not significantly different in the HP Blue group. The final mean values of ΔE, ΔL, and Δb of the PC and HP Blue groups were significantly higher than the initial values ($P < 0.01$ for both). The photomicrographs revealed no differences among the groups. **Conclusions:** Therefore, treatment with HP Blue prevented changes in the KHN without reducing the efficacy of bleaching.

Key words: Color, hydrogen peroxide, microhardness, scanning electron microscopy

INTRODUCTION

The evolutions of bleaching products and improved techniques have made dental whitening the most sought-after esthetic treatment because it is effective, simple, and minimally invasive when compared with other restorative treatments.[1,2]

Bleaching agents containing peroxide have been widely used for the whitening of stained and discolored teeth, both home treatment and in office bleaching and both techniques appear to be effective in treating of the dark teeth.[3,4] Bleaching occurs through an oxidation reaction that results in the dissociation of carbamide peroxide and hydrogen peroxide (HP) molecules, which penetrate the enamel, and consequently the dentine, by diffusion. Complex organic pigment molecules are cleaved into simpler and more hydrophilic molecules through oxidation-reduction reactions mediated by free radicals such as perhydroxyl, which originates from the degradation of HP. Small molecules can then leave the dental structure easily upon contact with water.[5]

Reactive oxygen species are highly reactive, with exceedingly short half-lives and the ability to target a wide range of molecules. Therefore, over a long period of time, the intimate contact

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between bleaching agents and dental tissues can cause adverse effects to the tissue.[6,7] For example, bleaching treatments have been reported to cause a loss of tissue components,[4,8] leading to surface changes associated with decreased calcium and fluoride content in the enamel.[9,10] Dental enamel is evaluated by the Knoop hardness number (KHN) test, which provides data regarding the loss or gain of components in the enamel. The KHN test is nondestructive in nature, as several indentations can be made in the same specimen without causing changes in the enamel during bleaching. The loss of enamel components is indicated by an increase in the size of the diagonal dental impressions produced by a diamond indenter, whilst those diagonals become smaller upon the gain of components, as it is more difficult for the indenter to penetrate the surface. The manufacturers of bleaching products add different concentrations of fluoride ions (F^-) and amorphous calcium phosphate ($Ca_3(PO_4)_2$) to the composition of their products to minimize the loss of surface hardness and possible morphological changes caused by the bleaching treatment.[8,9] Theoretically, the addition of fluoride or calcium ions could saturate the bleaching agent and enable the replenishment of these ions through ion exchange, thereby restoring the acid resistance of the enamel while overcoming the adverse effects of bleaching.[11]

Several methods of analysis and quantification have been developed to compare the changes in tooth color before and after bleaching. Colorimetric analysis is an effective method for the evaluation of color changes because it measures the wavelength of the light beam reflected by an object and allows quantification of a parameter that was previously subjective.[8] The evaluation of specimen color is usually performed using the International Commission on Illumination (*Commission Internationale de l'Éclairage*) standard, wherein the values of the L*, a* and b* coordinates, which refer to the variation in the black and white (luminosity), red-green and blue-yellow scales, respectively, are assessed, thus enabling calculation of the total colorimetric variation (ΔE).[12,13]

The aim of this *in vitro* study was to determine if the presence of 2% calcium in 35% HP solution prevents a loss in dental hardness integrity and morphology during bleaching without affecting the efficacy of the treatment. The KHN, tristimulus colorimetry (TC) and scanning electron microscopy (SEM) methods were used to fully answer this question.

MATERIALS AND METHODS

Specimen preparation

The study protocol was approved by the Ethical Committee for Animal Trials (Belem, Brazil) (No. OD059-12). Bovine incisors (obtained from the Maguari Slaughterhouse, Belem, Brazil) were selected. Each tooth was cross-sectioned at the crown-root boundary along the cementoenamel junction. The crowns were separated and the roots discarded.

The buccal surfaces were sectioned using a double-sided steel disk driven by a low-speed micromotor under water cooling and were smoothed using a diamond tip to form fragments of 9 mm^2 in the area and 2 mm in thickness. Fragment sizes were standardized and measured using a digital caliper. The sectioned portion always corresponded to the central area of the coronary buccal surface to ensure enamel prisms with the same inclination.

The specimen surfaces were flattened in an APL-4 polishing machine (AROTEC Ltda, São Paulo, SP, Brazil) equipped with 400-, 600-, 1200- and 2000-grit wet sandpaper. Polishing was subsequently performed using a felt disk associated with a micromotor and diamond polishing slurry.

The specimens were randomly divided into groups (n = 5) and were subjected to different bleaching treatments and experimental tests [Table 1].

Experimental procedures

The bleaching agents were used according to the manufacturers' recommendations. Bleaching treatments were performed in three sessions, with an interval of 7 days between each session. The Whiteness HP 35% (HP concentrate, thickener, glycol, and water)

Table 1: Summary of experimental conditions

Treatment	Experimental tests
Without whitening (negative control)	Knoop microhardness
	TC
	SEM
HP (positive control)[a]	Knoop microhardness
	TC
	SEM
HP Blue[b]	Knoop microhardness
	TC
	SEM

[a]Whiteness HP 35%, FGM dental products, Joinville, SC, Brazil, [b]Whiteness HP 35% blue calcium 2%, FGM dental products, Joinville, SC, Brazil. TC: Tristimulus colorimetry, SEM: Scanning electron microscopy, HP: Hydrogen peroxide

was applied 3 times for 15 min/session. The Whiteness Blue Calcium 2% (2% calcium gluconate, HP, thickener, glycol, water, and dye) was applied for 40 min/session. The specimens were stored in artificial saliva (2190 mg sodium bicarbonate, 125 mg magnesium chloride, 820 mg potassium chloride, 10 mg nipagin, 24 mg sorbitol, 1,270 mg potassium phosphate, 441 mg calcium chloride, 4.5 mg sodium fluoride, 100 mg nipasol, 8 mg carboxymethylcellulose, and 1 L distilled water)[14] at 37°C between sessions and 24 h after the last session of the bleaching treatment, and this solution was replaced daily. Specimens from the negative control group were not submitted to any whitening treatment, and the artificial saliva was replaced daily.

Knoop microhardness

Before and after the bleaching treatments, five indentations were made on each specimen with a load of 25 gf for 15 s using a Future Tech Microdurometer (Shimadzu Corporation, Kyoto, Japan). The locations of the indentations were chosen to enable the hypothetical mapping of the entire area of the test specimens, including the lateral left and right ends as well as the top, bottom, and center.

Tristimulus colorimetry

Colorimetric readouts were obtained with a Chroma Meter CR-400 Colorimetry (Tristimulus Colorimeter CR-400, Konica Minolta, NJ) and CIE L*a*b* system. These values enable an object to be located in a three-dimensional color space. The L* axis represents the degree of brightness within a sample and ranges from 0 (black) to 100 (white). The a* plane represents the degree of green/red color (−a* = green and +*a = red), while the b* plane represents the level of blue/yellow (−b* = blue and + b* = yellow).

To avoid interference, the specimens were placed on a black background that did not reflect light. Readings were taken before and after each of the three bleaching sessions, with the mean L*, a* and b* values assessed. The values of the color change (ΔE) in the CIE L*a*b* system were calculated using the following formula: $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$, where in $\Delta L = L - L_0$; $\Delta a = a - a_0$; e $\Delta b = b - b_0$

Scanning electron microscopy

For SEM, the specimens were dried, fixed to aluminum stubs and then sputter-coated with gold-palladium for 120 s. The surface morphology of the enamel was examined with a scanning electron microscope (LEO 1430, Carl Zeiss SMT, Oberkochen, Germany) at a magnification of ×500. Each photomicrograph was evaluated in a double-blind manner by three previously calibrated evaluators.

Statistical analysis

The KHN and colorimetry results were tabulated and statistically analyzed using BioEstat 5.0 software. One-way analysis of variance and Tukey's test ($P < 0.05$) were applied.

RESULTS

Table 2 shows the initial and final mean KHNs. The without whitening and HP groups showed statistically significant differences between the initial and final mean KHNs ($P < 0.05$ and $P < 0.01$, respectively). The initial and final mean KHNs were not significantly different in the HP Blue group.

The mean ΔE, ΔL, Δa, and Δb values were also assessed for the groups [Table 3]. The mean Δa was not statistically significant between the initial and final readouts. The final mean ΔE, ΔL, and Δb values were significantly higher than the initial values for the HP ($P < 0.01$) and HP Blue ($P < 0.01$) groups.

The photomicrographs (×500) of the groups demonstrate intact enamel without morphological changes [Figures 1-3].

Table 2: Means, SD and *P* values

Groups (*n*=5)	Mean and SD		*P* value
	Initial KHN	Final KHN	
Without whitening (negative control)	338.80±13.42	358.03±5.42	<0.05
HP (positive control)	355.04±13.62	328.83±7.43	<0.01
HP Blue	344.58±10.18	333.82±3.68	NS

NS: Non-significant, SD: Standard deviation, HP: Hydrogen peroxide, KHN: Knoop hardness number

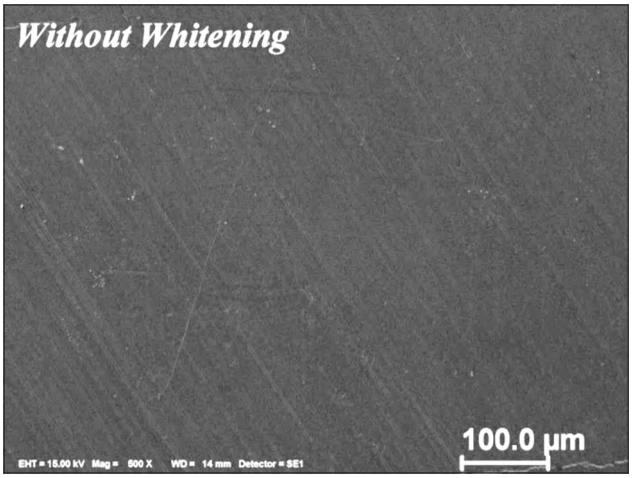

Figure 1: Photomicrograph of unbleached specimen (control group)

Table 3: Color change (ΔE*, ΔL*, Δa* and Δb*) (mean±SD) resulting from the bleaching protocols after each session (T)

Color parameters	Treatment groups	Evaluation periods		
		T1 (first session)	T2 (second session)	T3 (third session)
ΔL*	Without whitening (negative control)	1.90±4.56[a]	−0.93±2.80[a]	−2.01±2.49[a]
	HP (positive control)	−0.31±2.48[a]	3.00±3.34[ab]	7.71±3.48[b]
	HP blue	−0.90±2.47[a]	3.30±1.49[b]	10.24±2.25[c]
Δa*	Without whitening (negative control)	0.36±0.17[a]	0.61±0.67[a]	0.67±0.26[a]
	HP (positive control)	0.32±0.44[a]	0.31±0.18[a]	0.81±0.27[a]
	HP blue	0.15±1.70[a]	0.12±0.12[a]	0.10±0.10[a]
Δb*	Without whitening (negative control)	0.87±1.58[a]	−0.23±2.00[a]	0.05±1.09[a]
	HP (positive control)	0.15±0.57[a]	−4.89±0.99[b]	−4.91±0.35[b]
	HP blue	−2.64±1.44[a]	−3.99±0.59[b]	−4.84±0.384[c]
ΔE*	Without whitening (negative control)	4.30±2.41[a]	3.09±1.41[a]	2.58±2.18[a]
	HP (positive control)	2.15±1.07[a]	6.20±2.33[b]	9.30±2.96[b]
	HP blue	7.00±1.85[a]	5.63±0.98[ac]	11.37±2.06[bc]

Means followed by the same letters are not significantly different from one another by ANOVA and Tukey's test (P<0.05). SD: Standard deviation, HP: Hydrogen peroxide, ANOVA: Analysis of variance

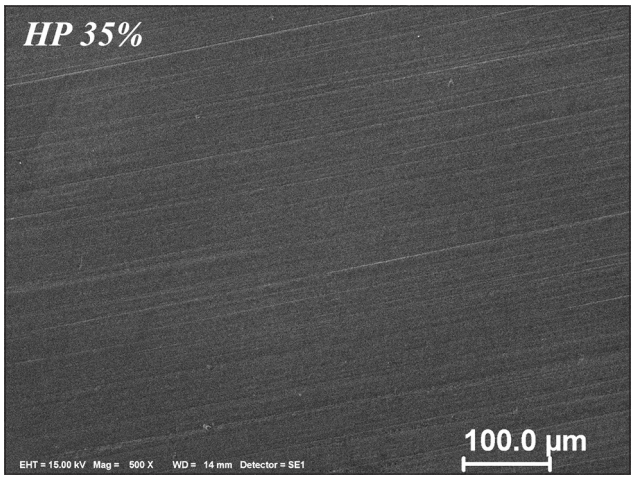

Figure 2: Photomicrograph of bleached specimen with 35% hydrogen peroxide (Whiteness HP 35%)

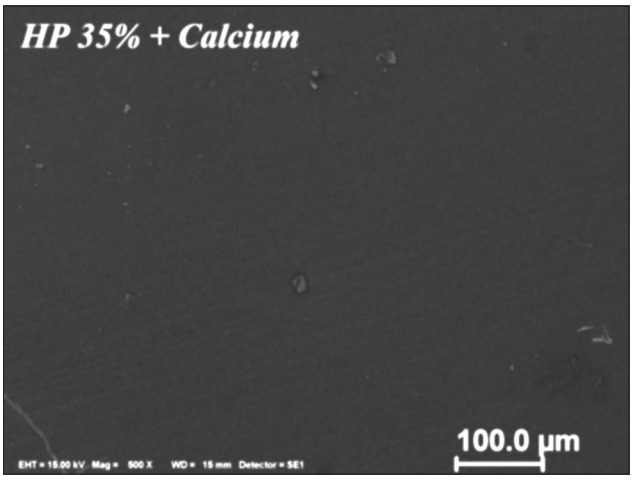

Figure 3: Photomicrograph of bleached specimen with 35% hydrogen peroxide (Whiteness HP Blue Calcium 35%)

DISCUSSION

In laboratory studies, bovine teeth are accepted as surrogates for human teeth because the teeth of both species have similar physical and chemical characteristics.[15,16] The use of bovine teeth for research provides some advantages, including the high availability and the ease with which the specimen surface size can be standardized. In addition, euthanizing the animals at a certain time enables control of age and storage length of the teeth.[15]

The analysis of hardness has been the most widely used method to evaluate the effects of HP on dental enamel.[4] A decrease in hardness is typically related to the loss of mineral content caused by the clearing process.[17] Mineralization agents have been incorporated into bleaching gels to reduce or avoid adverse effects, such as morphological changes, on dental[17,18] and to provide decreased enamel solubility and sensitivity through the mineral deposition of crystals in the enamel.[19]

Hydrogen peroxide in high concentration causes demineralization of enamel, although this change is not considered clinically significant.[20,21] Previous studies have reported that the addition of calcium ions and fluoride promoted the saturation of the bleaching gel, allowing its incorporation in apatite enamel, increasing resistance to demineralization, reducing the adverse effects caused by HP.[8,22,23]

The pH of bleaching agents may cause deleterious effects on tooth structure.[24] Both bleaching materials used in this study have an initial pH of 7.0. According to the manufacturer, the Whiteness HP Blue Calcium

maintains this pH during the period of stay of the product on the tooth enamel, however, the pH Whiteness HP 35% decreases to 5.5, may have favored the reduction KHN of specimens. Sun *et al*.[24] in their study have reported that high concentration of bleaching agents to acidic pH were effective in whitening, however, caused a decrease of KHN and morphological changes.

During bleaching, enamel demineralization is associated with the mechanism of oxidation, the pH of the peroxides and with some components of the bleaching agents.[23] The formation of free radicals occurs during the oxidation of peroxide.[25] These free radicals act nonspecifically and are able to degrade both the organic and inorganic matrix of the substrate, causing enamel loss and affecting the ionic balance that would enable the deposition of calcium on the enamel surface.[23]

CIE L*a*b* is a three-dimensional color system and the most commonly-used index for examining the efficacy of bleaching agents.[26] Some studies have reported that the color change is most affected by the parameters of L* and b*, where L* values increase and b* values tend to decrease.[18] Conversely, the values of the a* coordinate are considered to affect the bleaching process the least.[26,27] The results obtained here for ΔE, ΔL, and Δ b demonstrated that both bleaching treatments were effective, and that the addition of calcium to the bleaching agent didn't affect the effectiveness of the whitening. The changes in these parameters most likely resulted from the removal of pigments through the activity of the bleaching agents.[28]

The morphological analysis by SEM identified no differences between the bleached and control groups. The preservation of surface morphology could be explained by the presence of artificial saliva, which prevented the enamel demineralization during bleaching.[29] The effect of saliva on the loss of calcium and phosphorus (Ca-P) elemental contents after bleaching with 35% HP and reported that saliva increased the Ca-P ratio. Owing to its remineralization and buffering activities, saliva played a key role in the recovery of the tooth enamel structure and promoted the dilution of substances deleterious to tooth structure.[29]

New clinical studies should be developed *in situ* and *in vivo* to evaluate the buffering action of human saliva, due to bicarbonate and phosphate systems, containing inorganic electrolytes, such as phosphorus, calcium and fluoride, as well as enzymes and bacteria, which are unlikely to be reproduced in the laboratory.[30]

CONCLUSIONS

According to the methodology used, this study found that adding 2% calcium to 35% HP was able to prevent changes in enamel hardness and morphology without reducing bleaching efficacy.

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