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ABSTRACT: Purpose: To determine the microhardness, color change, surface roughness, and micromorphology of tooth enamel submitted to bleaching treatment with 7.5% hydrogen peroxide (HP) with added calcium, amorphous calcium phosphate, sodium fluoride (NaF), and hydroxyapatite (HA). **Methods:** 80 enamel slabs were used (n= 10). Three commercial agents [Pola Day 7.5%, Day White ACP 7.5% (DW-ACP), and White Class Calcium 7.5% (WC-Calcium)], three experimental (7.5% HP+NaF, 7.5% HP+HA, and 7.5% PH+NaF+HA), a positive control (with HP), and a negative control (without HP) groups were assessed. The commercial products were applied according to manufacturers' recommendations and the experimental ones were applied for 1.5 hours daily. During and after treatment, specimens were stored in artificial saliva. Tests were performed at baseline, 7, 14, 21, 28, and 35 days. Data were analyzed by ANOVA and Tukey's test (α=0.05). **Results:** DW-ACP presented lower microhardness and HP+HA presents the highest values ($P< 0.05$); only the experimental group presented color change similar to the commercial agents ($P> 0.05$). Overall, roughness increased with time ($P< 0.05$) and porosities, and deposition of crystalline structures in groups HP+HA and HP+NaF+HA were noted. The HP+HA agent was capable of reducing the loss of enamel microhardness due to bleaching and also present color change similar to the commercial products. (*Am J Dent* 2015;28:261-267).

CLINICAL SIGNIFICANCE: Home-use bleaching agent with 7.5% hydrogen peroxide containing remineralizing components minimized microhardness decrease during bleaching and increased surface roughness of human enamel, mostly due to deposition of mineral crystals remaining even after bleaching treatment, with whitening effectiveness similar to commercial products.

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Introduction

The bleaching agents used in home-bleaching techniques may lead to morphological changes on mineralized structures, such as tooth enamel.[1,2] These changes are attributed to the modification of organic and inorganic composition of enamel after treatment with bleaching agents based on peroxides.[3] Bleaching treatment reduces substantially the amount of calcium and phosphorus in enamel structure and modifies the morphology of most superficial crystals when compared to unbleached enamel.[4,5]

Some remineralizing components, such as fluoride, calcium, and amorphous calcium phosphate (ACP) are available to minimize the adverse effects on enamel of the bleaching treatment. Recently, these components have been added to bleaching agents in an attempt to reduce the modifications of mineral enamel content. Among these remineralizing agents, ACP was established by the ADA Foundation's Paffenbarger Research Center and added to some tooth bleaching products on the market. However, few studies were conducted to assess the effect of ACP on enamel.[6,7]

Hydroxyapatite (HA) is the main component of hard dental tissues and when artificially synthesized is a biocompatible material. If the fluoride ions replace hydroxyl ions, it gives rise to fluorapatite. The composition of human tooth enamel consists of HA crystals, which correspond to a weight of 17.4% phosphorus[8] and 37.1% calcium.[9] According to a previous study,[10] the synthetic HA is similar in composition and density to tooth enamel. Therefore, this biomineral, which adheres to dental substrate, can be used widely in dentistry for purposes such as protecting enamel caries, recurrent cavities, and restored cavities; sealing grooves, pits, and fissures in primary and permanent teeth; and balancing the mineral loss provided by the bleaching treatments.[11]

Thus, this in vitro study assessed the influence of 7.5% hydrogen peroxide (HP) containing calcium, ACP, fluoride, and hydroxyapatite on microhardness, color change, surface roughness, and micromorphology of sound human enamel. The research hypotheses of the present study were that remineralizing components into bleaching agents (1) would reduce the loss of enamel microhardness due to bleaching, (2) would not affect bleaching effectiveness, (3) the roughness, and (4) the micromorphology of tooth enamel.

Materials and Methods

Preparation of tooth slabs - After approval from the Institutional Review Board (# 059/2009), caries-free human third molars were extracted and stored in a thymol solution (0.1%, pH 7.0). The teeth were debrided with scalpel blades and periodontal curettes and, polished with rubber cups using pumice and distilled water at low speed. A transversal section was made, dividing the root and coronal portions. Longitudinal sections allowed enamel slabs measuring 4 mm × 4 mm. The height of the slab was 3 mm, comprising 1 mm of enamel and 2 mm of dentin. Teeth with cracks or stains were excluded.

The dental slabs were covered with wax except the enamel

Table 1. Treatment agents used in this study, compositions, manufacturers, protocol of treatment, lot numbers, and pH.

Treatment agents	Composition	Protocol of application	pH
Gel without 7.5% hydrogen peroxide (Negative control)	Water, carboxymethylcellulose, and nipagin	1.5 hour, once daily	7.5
7.5% hydrogen peroxide	Water, carboxymethylcellulose, nipagin, and hydrogen peroxide	1.5 hour, once daily	7.2
Pola Day 7.5% (Pola Day)	Hydrogen peroxide, additives, glycerol, water, and flavoring.	30 minutes, twice daily	6.0
Day White ACP 7.5% (DW-ACP)	Water, poloxamer 407, glycerin, hydrogen peroxide, propylene glycol, potassium nitrate, flavoring, xylitol, hydroxypropyl methylcellulose, eugenol, potassium hydroxide, calcium nitrate, sodium phosphate, and mica.	30 minutes, twice daily	7.7
White Class Calcium 7.5% (WC-Calcium)	Neutralized carbopol, potassium nitrate, calcium fluoride, aloe vera, calcium gluconate, stabilizer, deionized water, and surfactant.	1.5 hour, once daily	7.5
7.5% hydrogen peroxide + NaF (HP+NaF)	Hydrogen peroxide, nipagin, carboxymethylcellulose, water, and NaF	1.5 hour, once daily	7.2
7.5% Hydrogen peroxide + HA (HP+HA)	Hydrogen peroxide, nipagin, carboxymethylcellulose, water, and hydroxyapatite	1.5 hour, once daily	7.3
7.5% Hydrogen peroxide + NaF + HA (HP+NaF+HA)	Hydrogen peroxide, nipagin, carboxymethylcellulose, water, hydroxyapatite, and NaF	1.5 hour, once daily	7.5

surface and embedded in polystyrene resin using 2 cm-diameter PVC molds, leaving the enamel surface uncovered by the resin. After 24 hours, the slabs were removed from molds and ground flat to obtain a smooth surface required for microhardness testing.

The enamel specimens were flattened with aluminum oxide abrasive papers[a] of sequentially decreasing grit (400-, 600-, and 1,200), cooled under running water, and polished with diamond pastes of 3, 1, and ¼ µm and felt discs (Top, Ram, and Supra[b]) under mineral oil cooling (Red mineral oil[b]). The specimens were ultrasonically cleaned (USC-2800[c]) with distilled water for a 10-minute period to eliminate residues. Then, the enamel slabs were removed from the polystyrene resin; the specimens were randomly divided into eight groups (n = 10) and kept in a humid environment for 1 day until the start of the bleaching procedures.

Treatment agent specification - Table 1 shows the bleaching agents used in this experiment, according to composition, application protocol, and mean pH value. A pH meter (PG 1400[h]) was used to measure three times; pH mean was obtained for each product.

Bleaching treatment phase - A calibrated syringe was used for the placement of 0.02 ml of each bleaching agent on the dental specimens for negative control without HP, positive control with HP, Pola Day (Pola Day), Day White ACP (DW-ACP), White Class Calcium (WC-Calcium), and HP+NaF. For HP+HA and HA+NaF+HA, 0.01 ml of the HA paste and 0.01 ml of 15% HP gel were used, which were mixed in a dappen dish and then applied on a dental slab. Experimental agents (negative and positive controls, HP+NaF, HP+HA, and HP+NaF+HA) were applied 1.5 hours daily, and commercial products (Pola Day, DW-ACP, and WC-Calcium) were applied according to the manufacturers' instructions. After the application period, the bleaching agent was removed with a soft toothbrush making five back-and-forth movements manually with light pressure over the slabs and then washed with distilled water.[7]

The specimens were stored in 1.5 mL artificial saliva[12] in microtubes at 37ºC (±1ºC) during the remaining hours of the day. Saliva was used as remineralizing solution and changed every 2 days.[12] The dental slabs were stored for a total of 35 days (corresponding to 21 days of bleaching treatment and 14 days post-treatment).

Microhardness test - The microhardness testing (HMV-2[i]) was performed prior to the application of treatment agents to obtain baseline values, after 7, 14, and 21 days of bleaching treatment and, 7 and 14 days post-treatment. The three microhardness indentations were performed by a same operator for 5 seconds at each measurement using a Knoop indenter and a static load of 25 g. The average of the three values was calculated as the Knoop hardness number (KHN) for each specimen.

Color change analysis - The enamel slabs were placed in a white Teflon matrix 5 mm in height and 5 mm in diameter. Color was measured inside a daylight chamber to standardize ambient light for all specimens. The enamel slabs were subjected to an initial color analysis, using a reflectance spectrophotometer CM-700 UV-Visible.[j] The color change was determined by the difference (ΔE) between the coordinates obtained at baseline and those obtained from the timespan study at 7, 14, and 21 days during bleaching treatment and 7 and 14 days post-bleaching, using the CIE*L*a*b system. The total change in color, ΔE, is often used to represent a difference in color and is calculated using the formula $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$. In this study, the comparison of the difference was between the timespan study values and baseline value.

Surface roughness test - The measurement of average surface roughness (Ra, µm) was performed using a mechanical profilometer (Surfcorder SE1700[k]) and accurate to 0.01 µm. The 2-µm radius tip of the device was positioned parallel and with constant pressure of 0.7 mN over the polished surface of enamel slab.

The Ra parameter evaluated represents the arithmetic mean between peaks and valleys recorded. On each specimen, three measurements were performed with the needle passing through the center of the specimen in different positions, turning the specimen 120° at a constant speed of 0.05 mm/seconds and cut-off of 0.25 mm. The average of three readings was considered the average roughness of each surface.

Micromorphology analysis - To perform the micromorphological analysis of human enamel using scanning electron microscopy (SEM), new slabs were prepared. The specimens were dehydrated with ethanol in ascending scales of 70%, 80%, 90%, 95% (5 minutes each, one time) and 100% (5 minutes,

Table 2. Microhardness (KHN) means (S.D.) according to timespan and treatment.

	Timespan study (days)					
Treatment agent	0	7	14	21	28	35
Negative control	321.0 (21.8) ABa	317.3 (24.8) Ba	400.5 (46.8) Aa	369.3 (26.5) ABab	352.1 (33.7) ABa	344.2 (23.0) ABa
Positive control	319.9 (76.4) Aa	292.7 (19.8) Aa	276.1 (54.4) Ab	295.6 (42.3) Abcd	306.5 (15.8) Aab	321.0 (12.2) Aa
Pola Day	279.9 (42.2) ABa	264.5 (28.1) Ba	296.9 (81.9) ABb	288.4 (65.7) ABcd	303.8 (53.9) ABab	334.4 (17.3) Aa
DW-ACP	295.4 (27.1) Aa	258.8 (26.8) ABa	267.1 (36.5) ABb	233.6 (26.6) Bd	276.1 (37.0) ABb	311.2 (22.3) Aa
WC-Calcium	286.4 (50.3) Aa	273.8 (14.7) Aa	282.1 (35.3) Ab	263.8 (34.2) Acd	299.3 (9.9) Aab	315.2 (24.9) Aa
HP+NaF	308.7 (32.7) ABa	261.0 (22.4) Ba	321.5 (40.2) ABab	314.7 (34.3) ABabc	318.9 (40.3) ABab	335.0 (34.9) Aa
HP+HA	304.9 (45.5) Ba	297.0 (36.8) Ba	321.3 (46.8) ABab	384.1 (48.8) Aa	307.5 (56.0) Bab	324.4 (26.9) ABa
HP+NaF+HA	298.2 (35.8) ABa	279.3 (17.5) Ba	320.2 (23.1) ABab	379.9 (94.2) Aab	323.0 (56.4) ABab	348.0 (33.7) ABa

Means followed by different letters (capital case letters on horizontal line and lower case letters on vertical line) differ among them ($P \leq 0.05$).

Table 3. Color change (ΔE)* means (S.D.) according to timespan and treatment.

	Timespan study (days)				
Treatment agents	7	14	21	28	35
Negative control	1.61 (0.97) Ad	0.81 (0.45) Ad	1.92 (0.45) Ad	0.59 (0.22) Ac	0.71 (0.40) Ae
Positive control	7.91 (2.96) Ba	11.04 (2.88) Aa	10.72 (2.12) Aa	9.51 (1.68) ABa	10.85 (2.67) Aa
Pola Day	7.39 (2.30) Ba	10.83 (1.25) Aab	10.55 (2.12) Aab	9.04 (1.82) ABab	9.87 (3.05) Aab
DW-ACP	7.15 (1.80) Aab	8.45 (2.38) Abc	8.03 (1.48) Abc	9.30 (1.73) Aab	9.20 (1.66) Aabc
WC-Calcium	8.16 (2.13) Aa	8.66 (2.50) Aabc	9.01 (1.25) Aabc	9.46 (0.99) Aa	9.17 (1.35) Aabc
HP+NaF	4.33 (1.61) Bc	7.02 (1.81) Ac	7.53 (1.85) Ac	6.93 (1.94) Ab	6.90 (1.74) Acd
HP+HA	6.26 (0.91) Babc	9.51 (1.37) Aabc	8.75 (1.85) Aabc	7.95 (1.94) ABab	7.61 (1.74) ABbcd
HP+NaF+HA	4.83 (1.16) Cbc	8.20 (1.51) ABc	9.41 (1.76) Aabc	7.46 (1.99) ABab	6.33 (1.25) BCd

Means followed by different letters (capital case letters on horizontal line and lower case letters on vertical lines) differ among them ($P \leq 0.05$). *The ΔE values are relative to baseline.

four times). Specimens were fixed in metallic stubs with double-sided carbon tape, and metallization of the specimens was performed by covering with gold/palladium alloy, using a sputter coater (Desk II[l]) with 40 mA for 100 seconds. Then the samples were observed in a SEM (Philips XL30[m]) with acceleration voltage of 20 kV, working distance of 10 mm, and aperture of the objective lenses (spot size) of 5.4 nm for magnifications of ×150, ×1,000, ×2,000, and ×3,500. Analyses of the enamel surfaces were performed at baseline, 7, 14, 21 days of treatment, 7 and 14 days post-treatment for all eight study groups.

Statistical analysis - Data were analyzed by ANOVA with the SAS[n] statistical system using the mixed procedure for repeated measures arrangement. For the covariance matrix, the Akaike information criterion was used, selecting the matrix with the lowest value for this parameter. Means were compared using the Tukey-Kramer test at a pre-set alpha of 0.05.

Results

The results of microhardness and color change tests are presented in Tables 2 and 3, respectively. For the microhardness test, in function of timespan study, no group demonstrated statistically significant differences, except groups DW-ACP and HP+HA at 21 days of treatment ($P< 0.05$); the DW-ACP showed lower KHN values, and the group HP+HA presented higher KHN when compared to baseline values.

Between groups, at 14 and 21 days of treatment, the negative control was similar to groups HP+NaF, HP+HA, and HP+NaF+HA ($P> 0.05$). At 7 days post-treatment, the DW-ACP showed the lowest, and the negative control the highest KHN means ($P< 0.05$). At all other times (baseline, 7 days of treatment, and 14 days post-treatment) the groups were statistically similar to each other ($P> 0.05$).

For the color change test, in function of the timespan study, the negative control group, DW-ACP, and WC-Calcium showed color stability throughout the time, although the negative control group presented low values of ΔE, showing that there was no bleaching ($P> 0.05$). The positive control groups and Pola Day showed higher ΔE at 14 and 21 days during treatment and 14 days post-treatment ($P< 0.05$). The experimental groups containing fluoride and/or hydroxyapatite showed higher ΔE values after 14 and 21 days of treatment, which was maintained at 14 days post-treatment compared at 14 days of bleaching procedure ($P< 0.05$).

Between groups, NC group differed statistically from all groups at all study times ($P< 0.05$), with the lowest ΔE values. Only the group HP+NaF was similar to the experimental groups HP+HA and HP+NaF+HA at 7 days of treatment ($P> 0.05$). At 14 days of bleaching, the commercial groups (Pola Day, DW-ACP, and WC-Calcium) showed no significant difference compared to the experimental group HP+HA, while HP+NaF and HP+NaF+HA were not similar to Pola Day ($P< 0.05$). The positive control differed from DW-ACP and the groups containing fluoride (HP+NaF and HP+NaF+HA) in composition. At 21 days, the HP+NaF group differed statistically from the positive control and Pola Day; but differed only from the positive control group at 7 days post-treatment ($P< 0.05$). At 14 days after bleaching procedure, HP+NaF+HA differed significantly from the other commercial products and the positive control without remineralizing agent ($P< 0.05$).

The surface roughness data are presented in Table 4. The negative control, positive control, and Pola Day groups did not show statistical differences on roughness compared to baseline

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Table 4. Surface roughness (Ra) means (S.D.) according to timespan and treatment agent.

	Timespan study (days)					
Treatment agent	0	7	14	21	28	35
Negative control	0.36 (0.04) Aa	0.30 (0.04) Ac	0.33 (0.04) Ab	0.34 (0.03) Ac	0.35 (0.04) Ac	0.34 (0.03) Ab
Positive control	0.36 (0.03) Aa	0.32 (0.03) Ac	0.32 (0.03) Ab	0.35 (0.06) Ac	0.36 (0.06) Abc	0.36 (0.04) Aab
Pola Day	0.34 (0.05) Aa	0.42 (0.05) Ab	0.35 (0.05) Ab	0.39 (0.04) Abc	0.36 (0.04) Abc	0.36 (0.06) Aab
DW-ACP	0.33 (0.03) Ba	0.45 (0.06) Aab	0.40 (0,03) ABb	0.43 (0.04) Aabc	0.40 (0.03) ABabc	0.39 (0.04) ABab
WC-Calcium	0.38 (0.05) Ba	0.51 (0.06) Aa	0.52 (0.04) Aa	0.51 (0.02) Aa	0.44 (0.05) ABab	0.44 (0.04) ABa
HP+NaF	0.24 (0.02) Db	0.47 (0.05) ABab	0.49 (0.04) Aa	0.41 (0.03) BCbc	0.38 (0.05) Cabc	0.37 (0.03) Cab
HP+HA	0.30 (0.16) Cab	0.50 (0.06) Aab	0.50 (0.06) Aa	0.41 (0.02) Bbc	0.38 (0.03) Babc	0.38 (0.02) Bab
HP+NaF+HA	0.29 (0.02) Cab	0.50 (0.03) ABa	0.55 (0.04) Aa	0.46 (0.05) Bab	0.45 (0.07) Ba	0.33(0.04) Cb

Means followed by different letters (capital case letter on horizontal and lower case letter on vertical) differ among them ($P \leq 0.05$).

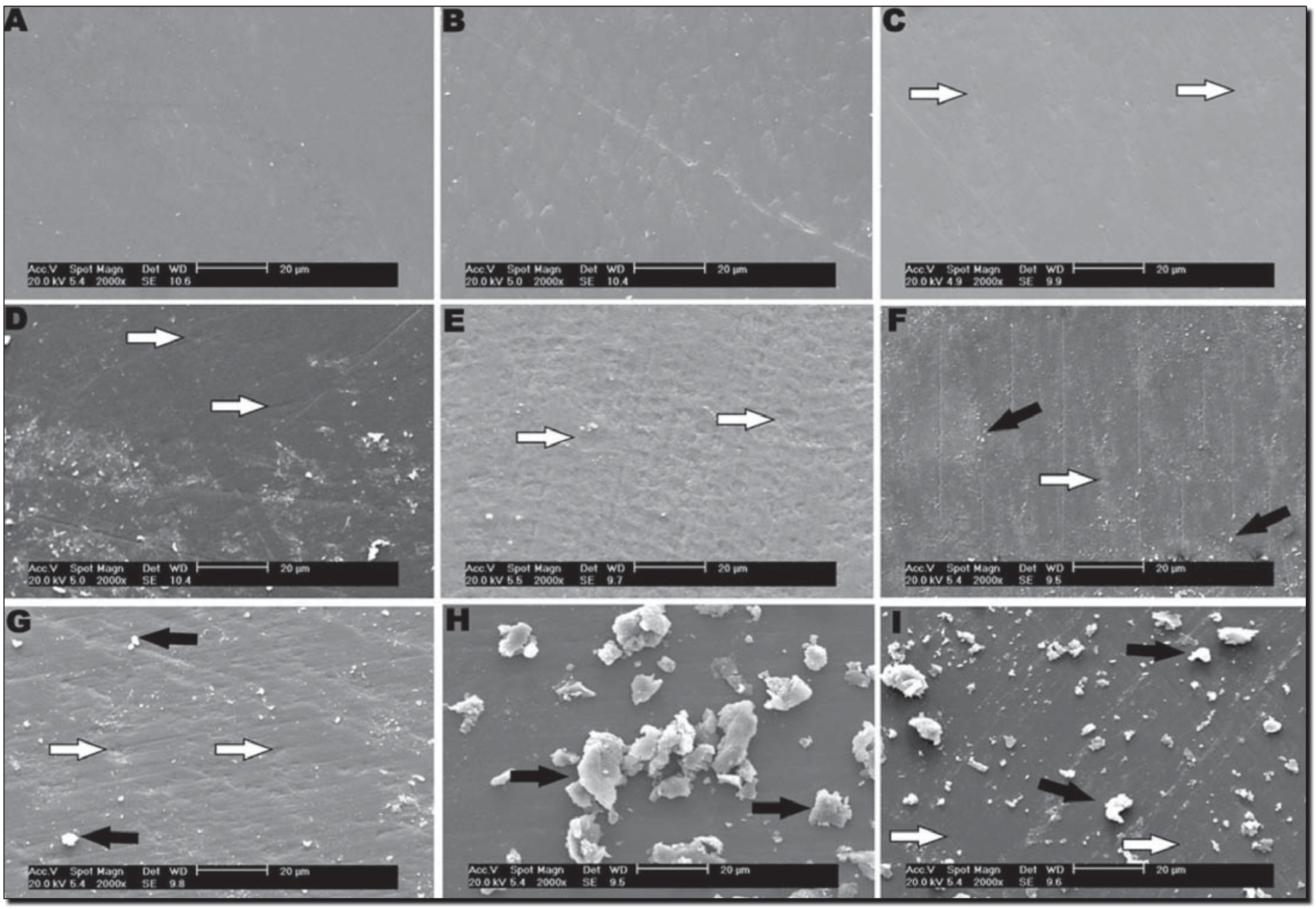

Fig. 1. SEM photomicrographs of: **A.** negative control group at ×2,000 at baseline; **B.** 21 days of bleaching treatment of groups: negative control; **C.** positive control; **D.** Pola Day; **E.** DW-ACP; **F.** WC-Calcium; **G.** HP+NaF; **H.** HP+HA; **I.**, and HP+NaF+HA. Micromorphological changes such as irregularity and porosity presence (white arrows) and deposition of crystalline structures (black arrows) on the enamel surface.

values ($P > 0.05$). All other groups presented statistical differences, represented by an increase on the surface roughness of tooth enamel ($P < 0.05$). Among these groups, at the post-treatment phase, DW-ACP and WC-Calcium presented at 28 and 35 days similar values to the baseline, while HP+NaF+HA did so only at 35 days.

For the SEM evaluation, in most groups there were morphological changes such as presence of porosities and, in some cases, exposure of inter-prismatic enamel matrix, except for the negative control group. The most evident demineralization process was observed for the group DW-ACP at 21 days of treatment. In the groups containing hydroxyapatite (HP+HA and HP+NaF+HA), the deposition of mineral crystals over enamel surface was observed for both treatment and post-treatment phases (Figs. 1, 2).

Discussion

The assumption that remineralizing agents such as HA, fluoride, ACP, and calcium could be beneficial to the microhardness and surface characteristics of tooth enamel in addition to providing a bleaching effect was evaluated. Only HA showed (1) to minimize demineralization; (2) bleaching effectiveness was similar to the commercial bleaching agents at all times during the study, but lower color change at 35 days compared to the positive control; (3) overall, the bleaching treatment increased the roughness; and (4) SEM analysis showed

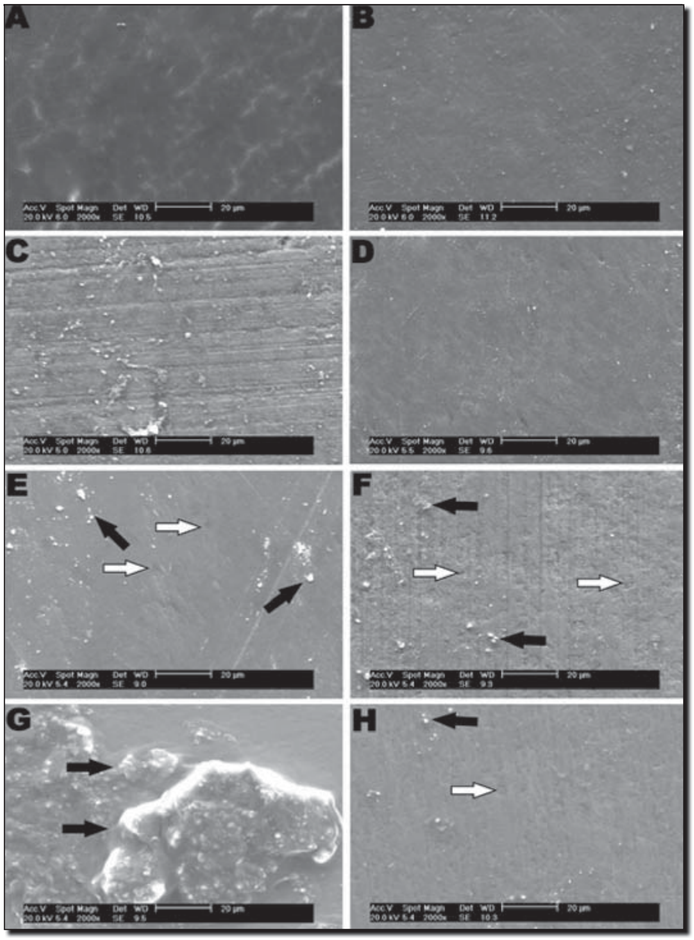

Fig. 2. SEM photomicrographs of experimental groups at 35 days (×2,000): **A.** negative control **B.** positive control; **C.** Pola Day; **D.** DW-ACP; **E.** WC-Calcium; **F.** HP+NaF; **G.** HP+HA; and **H.** HP+NaF+HA. Micromorphological changes such as irregularity and porosity presence (white arrows) and deposition of crystalline structures (black arrows) on the enamel surface.

deposition of crystalline particles on the enamel surface even after the bleaching procedure, which could have contributed to higher Ra values observed for experimental groups containing remineralizing agents; therefore, all hypotheses were rejected. According to the results observed in this study, the addition of remineralizing agents to the bleaching gels showed whitening efficacy comparable to commercial products.[13]

Acidic properties in composition of bleaching agents can affect the physical and chemical structure of the enamel,[14,15] if the pH is below 5.5.[16] In this study, DW-ACP showed the lowest KHN after 21 days of bleaching procedure. Interestingly, the measured pH of this bleaching agent was the highest (7.7) among all agents tested; Pola Day, which had the lowest pH (6.0), showed no statistically significant effects. This result may be due to the presence of glycerin (in DW-ACP), a component that acts as a barrier layer to artificial saliva affecting the remineralizing process.[17] In addition, studies[14,18] showed a reduction on the enamel microhardness depending on the thickening agent used, such as carbopol and glycerin. However, this demineralization effect may not be significant in in vivo conditions, as reported by an in situ study,[19] which observed that home-bleaching agents like 7.5% HP caused no changes on the enamel microhardness.[19]

Previous studies[6,7] evaluating the 10% carbamide peroxide with ACP reported that this remineralizing agent was unable to prevent the enamel microhardness reduction,[6] during the bleaching treatment, concluding that the presence of ACP did not offer significant benefits.[7] However, another study[20] assessed the use of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste mixed with 10% and 16% carbamide peroxide on the microhardness of bleached enamel and found an increase of microhardness as well as whitening efficacy. These varying results may be related to some factors like concentration of ACP, mixture of bleaching agent with ACP, exposure time of bleaching agent with enamel surface, and higher stability of CPP over ACP.

On the other hand, experimental composition containing hydroxyapatite (HP+HA) was the only one with significant increases on enamel microhardness, which was observed at 21 days of treatment; an effect similarly observed using 30% HP combined with hydroxyapatite which can prevent and minimize the loss of enamel mineral content during bleaching.[21] However, experimental compositions containing fluoride (HP+NaF and HP+ NaF+HA) and commercial agents containing calcium (WC-Calcium) did not show a significant difference. Contrarily, the addition of fluoride and calcium to home-applied bleaching agents was an effective method to minimize demineralization.[22-24] All other groups (negative control, positive control, and Pola Day) showed no statistical differences on the KHN values.

The artificial saliva used in this study contained calcium and phosphate ions[12,16] and with remineralizing potential as reported in previous studies,[7,14,15,17,25-27] may have contributed to the post-treatment microhardness values returning to baseline. In addition to this beneficial effect of artificial saliva observed in vitro, replacement of enamel mineral content is also expected in vivo due to important factors such as salivary flow, buffering capacity of saliva, oral hygiene, and topical fluoride,[28] which can increase the enamel remineralization.[25,26] A study[29] that simulated intraoral conditions, such as temperature and presence of saliva, obtained smaller reductions on hardness than those that did not simulate intraoral conditions.

For color change test, the negative control, DW-ACP, and WC-Calcium groups showed color stability across all times. After dental bleaching and immersion in artificial saliva and fluoride solution, a color regression of the bleached teeth was observed, which could be explained by the increased enamel density due to presence of the remineralization processes within the tooth tissue.[30] This could also be justified because the HP+ NaF+HA contents of the two remineralizing components, fluoride and hydroxyapatite, showed the lower color change at 14 days post-bleaching. Moreover, the crystalline structures adhering to enamel surface could diffuse the reflecting light during color measurement. The groups that did not contain remineralizing components, positive control (experimental) and Pola Day (commercial), presented similar values between them at all times.

One can assume that, according to the timespan of the study, all the groups that were submitted to dental bleaching had a color change, except the negative control group. The ΔE values of DW-ACP and WC-Calcium groups remained stable over time and groups that were exposed to HA presented values in the post-treatment period similar to values at 7 days of treatment. All three commercial bleaching agents (Pola Day, DW-ACP, and WC-Calcium) had similar values, when compared to each other, at all times of study. The same was observed

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for the three experimental bleaching agents containing remineralizing components (HP+NaF, HP+HA, and HP+NaF+HA). However, compositions containing fluoride showed slightly lower bleaching effectiveness than commercial products, since the fluoride can react with the bleaching agent, reducing the whitening efficacy.[31]

The color difference of HP+HA resembled the commercial bleaching agents (Pola Day, DW-ACP, and WC-Calcium) at all tested times. A similar result was also reported,[21] which noted that bleaching with HP added to hydroxyapatite was statistically similar to bleaching with HP alone. Nano- and micro-hydroxyapatite materials were applied to the enamel surface, as a suspension or dissolvable polymer films, and whitening effects and durability against hydrodynamic shearing forces with FE-SEM observations was previously reported.[32] Synthetic HA, which has particles with dimensions similar to those found in enamel, could react with natural hydroxyapatite due to its chemical structure similar to the tooth structure,[10,32] creating a thin layer of this material without affecting chemically the deeper tooth tissue.[10]

The negative control, positive control, and Pola Day did not show a statistical difference on surface roughness compared to baseline. All other groups containing components that induce the enamel remineralization, such as ACP (DW-ACP), calcium (WC-Calcium), sodium fluoride (HP+NaF), hydroxyapatite (HP+HA), and fluoride + hydroxyapatite (HP+NaF+HA) showed a significant difference represented by an increase on enamel roughness. This result can be explained by the deposition of these components and their products on the enamel surface.

A previous study[33] reported that fluoride, used separately from the bleaching agent, showed beneficial effect, reducing enamel roughness after bleaching. In this study, the use of fluoride concomitantly with 7.5% HP, formulated in same composition, did not present beneficial results for surface roughness.

The DW-ACP, despite having significantly increased the enamel roughness at 7 and 21 days of treatment, showed values similar to the baseline values at 14, 28, and 35 days. However, one study[7] reported that bleaching with 7.5% and 9.5% Day White ACP did not change the surface roughness of enamel at all tested times, which was possibly due to the presence of artificial saliva and the ACP added to the bleaching agent. These different results may be explained by the treatment protocol used, in which the bleaching agent was applied for 30 minutes once daily,[7] while in the present study was applied twice daily, resulting in contact of product with enamel for a longer period. It is still unclear whether the ACP would have beneficial results on the surface roughness if applied separately from the bleaching agent as a post-treatment therapy. WC-Calcium also returned to baseline values at post-treatment, probably due to the calcium precipitation contained in bleaching agent formulation, as well as the calcium and phosphate ions of the artificial saliva used.

The pH could be related to the increase on surface roughness of enamel, but among the groups in which there were significant increases on values, the pH ranged from 7.2 to 7.7. Although some studies[34,35] indicated that a pH of 7.0 can cause similar effects to those observed with pH below 7.02, which did not seem to be a problem in the present study. A slight increase in roughness values of enamel after use of bleaching agents with pH ranging from 4.9 to 10.8 was reported,[36] and in this study a slight increase (not significant) also was observed for positive control (pH 7.5), negative control (pH 7.2) and Pola Day (pH 6.0). Even bleaching agents with neutral pH produced loss of calcium and phosphate after EDX analysis,[37] which is associated to the oxidation process that teeth are subjected to by bleaching agents and responsible for the changes on the tooth surface.[3,37-39] Furthermore, the free radicals generated during the bleaching treatment are not specific and can react with organic structures of the dental tissues and increase the porosity on the tooth surface.[3]

The SEM images showed that in most groups there were morphological changes, such as presence of porosities for positive control, Pola Day, DW-ACP, WC-Calcium, HP+NaF, and HP+NaF+HA. These changes were more evident in DW-ACP which showed high roughness values, although this not is related to the results for positive control and Pola Day. In HP+HA, no morphological change and deposition of mineral crystals over enamel surface was seen. The same phenomenon would be expected for HP+NaF+HA, but it was noted concurrently with mineral deposition the presence of porosities on enamel, possibly by interaction between HA and fluoride being more advantageous to use the latter element separately. A previous study[10] evaluated a dental paste of synthetic enamel; this paste is exactly like HA crystals of natural enamel and links affected the site within 15 minutes, showing no gap at the interface between repaired layer and natural enamel, with elongated crystals regularly oriented to the tooth surface and developing across the interface.

Once DW-ACP, WC-Calcium, HP+NaF, HP+HA, and HP+NaF+HA showed increase on surface roughness through the timespan study, it was observed that group DW-ACP possibly presented this result due to the reduction of mineral substrate. Groups WC-ACP, HP+HA, and HP+NaF+HA were possibly the opposite, with the increase of mineral substrate due to the deposition of crystalline structures. The group HP+NaF presented both conditions on the superficial layer of enamel.

In the present study, the use of HA together with bleaching agent did not have the beneficial effects on surface roughness, because the deposition of mineral crystals led to an increase on roughness, which may be minimized by polishing. The use of smaller particle (macro or nano-sized) should be investigated in an attempt to reduce the increase on roughness. Thus, although hydroxyapatite is a promising component with many applications in dentistry, its use needs further study to establish criteria for appropriate treatment protocols.

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References

1. Sa Y, Wang Z, Ma X, Lei C, Liang S, Sun L, Jiang T, Wang Y. Investigation of three home-applied bleaching agents on enamel structure and mechanical properties: An in situ study. *J Biomed Opt* 2012; 17:035002.
2. Zalkind M, Arwaz J R, Goldman A, Rotstein I. Surface morphology changes in human enamel, dentin and cementum following bleaching: A scanning electron microscopy study. *Endod Dent Traumatol* 1996;12:82-88.
3. Hegedüs C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. *J Dent* 1999;27:509-515.
4. McCraken MS, Haywood VB. Demineralization effects of 10 percent carbamide peroxide. *J Dent* 1996;24:395-398.
5. Perdigão J, Francci C, Swift EJ, Ambrose WW, Lopes M. Ultra-morphological study of the interaction of dental adhesives with carbamide peroxide bleached enamel. *Am J Dent* 1998;11:291-301.
6. da Costa JB, Mazur RF. Effects of new formulas of bleaching gel and fluoride application on enamel microhardness: An in vitro study. *Oper Dent* 2007;32:589-594.
7. De Abreu DR, Sasaki RT, Amaral FL, Flório FM, Basting RT. Effect of home-use and in-office bleaching agents containing hydrogen peroxide associated with amorphous calcium phosphate on enamel microhardness and surface roughness. *J Esthet Restor Dent* 2011;23:158-168.
8. Lazzari EP. *Dental biochemistry*. 2nd ed., Philadelphia: Lea & Febiger, 1976; 99-111.
9. Spector PC, Curzon ME. Relationship of strontium in drinking water and surface enamel. *J Dent Res* 1978;57:55-58.
10. Yamagishi K, Onuma K, Suzuki T, Okada F, Tagami J, Otsuki M, Senawangse P. Materials chemistry: A synthetic enamel for rapid tooth repair. *Nature* 2005;433:819.
11. Oliveira M, Mansur HS. Synthetic tooth enamel: SEM characterization of a fluoride hydroxyapatite coating for dentistry applications. *Mater Res* 2007; 10: 115-118.
12. Serra MC, Cury JA. The in vitro effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. *Quintessence Int* 1992;23:39-44.
13. Borges AB, Danta RL, Caneppele TM, Borges AL, Rocha Gomes Torres C. Effect of remineralizing agents on the bleaching efficacy of gels. *Gen Dent* 2013;61:67-71.
14. Basting RT, Rodrigues AL Jr, Serra MC. The effects of seven carbamide peroxide bleaching agents on enamel microhardness over time. *J Am Dent Assoc* 2003;134:1335-1342.
15. Basting RT, Rodrigues Júnior AL, Serra MC. The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin in situ. *Oper Dent* 2001;26:531-539.
16. Featherstone JD, Ten Cate JM, Shariati M, Arends J. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. *Caries Res* 1983;17:385-391.
17. Basting RT, Rodrigues AL Jr, Serra MC. The effect of 10% carbamide peroxide, carbopol and/or glycerin on enamel and dentin microhardness. *Oper Dent* 2005;30:608-616.
18. Rodrigues JA, Marchi GM, Ambrosano GM, Heymann HO, Pimenta LA. Microhardness evaluation of in situ vital bleaching on human dental enamel using a novel study design. *Dent Mater* 2005;21:1059-1067.
19. Maia E, Baratieri LN, Caldeira de Andrada MA, Monteiro S Jr, Vieira LC. The influence of two home-applied bleaching agents on enamel microhardness: An in situ study. *J Dent* 2008;36:2-7.
20. Borges BC, Borges JS, de Melo CD, Pinheiro IV, Santos AJ, Braz R, Montes MA. Efficacy of a novel at-home bleaching technique with carbamide peroxides modified by CPP-ACP and its effect on the microhardness of bleached enamel. *Oper Dent* 2011;36:521-528.
21. Jiang T, Ma X, Wang Z, Tong H, Hu J, Wang Y. Beneficial effects of hydroxyapatite on enamel subjected to 30% hydrogen peroxide. *J Dent* 2008;36:907-914.
22. Cavalli V, Rodrigues LK, Paes-Leme AF, Soares LE, Martin AA, Berger SB, Giannini M. Effects of the addition of fluoride and calcium to low-concentrated carbamide peroxide agents on the enamel surface and subsurface. *Photomed Laser Surg* 2011;29:319-325.
23. Chen HP, Chang CH, Liu JK, Chuang SF, Yang JY. Effect of fluoride containing bleaching agents on enamel surface properties. *J Dent* 2008;36:718-725.
24. Chuang SF, Chen HP, Chang CH, Liu JK. Effect of fluoridated carbamide peroxide gels on enamel microtensile bond strength. *Eur J Oral Sci* 2009;117:435-441.
25. de Freitas PM, Basting RT, Rodrigues JA, Serra MC. Effects of two 10% peroxide carbamide bleaching agents on dentin microhardness at different time intervals. *Quintessence Int* 2002;33:370-375.
26. de Oliveira R, Basting RT, Rodrigues JA, Rodrigues AL Jr, Serra MC. Effects of a carbamide peroxide agent and desensitizing dentifrices on enamel microhardness. *Am J Dent* 2003;16:42-46.
27. Sasaki RT, Flório FM, Basting RT. Effect of 10% sodium ascorbate and 10% alpha-tocopherol in different formulations on the shear bond strength of enamel and dentin submitted to a home-use bleaching treatment. *Oper Dent* 2009;34:746-752.
28. Flaitz CM, Hicks MJ. Effects of carbamide peroxide whitening agents on enamel surfaces and caries-like lesion formation: An SEM and polarized light microscopic in vitro study. *J Dent Child* 1996;63:249-256.
29. Attin T, Kielbassa AM, Schwanenberg M, Hellwing E. Effect of fluoride treatment on remineralization of bleached enamel. *J Oral Rehabil* 1997;24:282-286.
30. Li Q, Xu B T, Li R, Yu H and Wang YN. Quantitative evaluation of colour regression and mineral content change of bleached teeth. *J Dent* 2010;38:253-260.
31. Tschoppe P, Neumann K, Mueller J, Kielbassa AM. Effect of fluoridated bleaching gels on the remineralization of predemineralized bovine enamel in vitro. *J Dent* 2009;37:156-162.
32. Dabanoglu A, Wood C, García-Godoy F, Kunzelmann KH. Whitening effect and morphological evaluation of hydroxyapatite materials. *Am J Dent* 2009;22:23-29.
33. Martin JM, de Almeida JB, Rosa EA, Soares P, Torno V, Rached RN, Mazur RF. Effect of fluoride therapies on the surface roughness of human enamel exposed to bleaching agents. *Quintessence Int* 2010;41:71-78.
34. Bitter NC. A scanning electron microscopy study of the effect of bleaching agents on enamel: A preliminary report. *J Prosthet Dent* 1992;67:852-855.
35. Türkun M, Sevgican F, Pehlivan Y, Aktener BO. Effects of 10% carbamide peroxide on the enamel surface morphology: A scanning electron microscopy study. *J Esthet Restor Dent* 2002;14:238-244.
36. Azrak B, Callaway A, Kurth P, Willershausen B. Influence of bleaching agents on surface roughness of sound or eroded dental enamel specimens. *J Esthet Restor Dent* 2010;22:391-399.
37. Paula SS, Soares LE, do Espírito Santo AM, Martin AA, Cavalli V, Liporoni PC. FT-Raman and energy dispersive X-ray fluorescence spectrometric analyses of enamel submitted to 38% hydrogen peroxide bleaching, an acidic beverage, and simulated brushing. *Photomed Laser Surg* 2010;28:391-396.
38. Bitter NC, Sanders JL. The effect of four bleaching agents on the enamel surface: A scanning electron microscopic study. *Quintessence Int* 1993; 24:817-824.
39. Basting RT, Rodrigues AL, Serra MC. Micromorphology and surface roughness of sound and demineralized enamel and dentin bleached with a 10% carbamide peroxide bleaching agent. *Am J Dent* 2007;20:97-102.