

Effect of Home-Use and In-Office Bleaching Agents Containing Hydrogen Peroxide Associated with Amorphous Calcium Phosphate on Enamel Microhardness and Surface Roughness

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ABSTRACT

Statement of the Problem: The effects of amorphous calcium phosphate (ACP)-containing bleaching agents on enamel surface have not been clarified yet.

Purpose: The study aims to evaluate the effects of different bleaching agents, either associated with ACP, or not, on enamel Knoop microhardness (KHN) and surface roughness (SR).

Materials and Methods: The home-use hydrogen peroxide (HP) bleaching agents PolaDay 7.5% (HP7.5; SDI Limited, Bayswater, Victoria, Australia), PolaDay 9.5% (HP9.5; SDI Limited); DayWhite ACP-7.5% (ACP7.5; Discus Dental, Culver City, CA, USA) and DayWhite ACP 9.5% (ACP9.5; Discus Dental), and the in-office agents PolaOffice 35% (HP35; SDI Limited) and Opalescence XtraBoost 38% (HP38; Ultradent Products, South Jordan, UT, USA) were applied to polished enamel slabs ($N=10$) for 30 minutes/day for 21 consecutive days (home-use) or in one session a week, for 3 weeks (in-office). KHN and SR were tested before (baseline), during (7, 14, 21 days), and after (7 and 14 days in artificial saliva) the bleaching treatment.

Results: KHN evaluation revealed no significant difference among bleaching agents ($p>0.05$); however, there was a significant decrease during bleaching treatment ($p<0.0001$). KHN values attained in the post-treatment phase were statistically similar to baseline values ($p>0.05$). SR was not altered during and after treatment, with the exception of PH38, which showed an increase in SR during bleaching treatment and a recovery after treatment. The ACP7.5 showed a trend to decreasing SR values during the bleaching treatment, but this decrease was only significant when associated with 14 days of immersion in artificial saliva, when the enamel was less rough than at baseline.

Conclusions: Bleaching agents caused a decrease in enamel KHN, but values were recovered after treatment, showing the importance of saliva in recovering mineral content. SR was altered during or after treatment, depending on HP concentration/association with ACP. The beneficial effects of adding ACP to bleaching formulas on SR may be restricted to lower HP concentrations in association with the remineralizing effect of saliva.

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CLINICAL SIGNIFICANCE

No beneficial effects of adding ACP to bleaching formulas on enamel microhardness were observed, but these observations may be attributable to the lower hydrogen peroxide concentrations in association with the remineralizing effect of saliva, when considering the enamel roughness.

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INTRODUCTION

Over the last few decades, there has been a growing trend toward seeking esthetic improvements, and consequently, an increasing demand for dental bleaching treatments, which have shown effective and safe results.¹

Among dental bleaching systems, the in-office bleaching technique uses bleaching agents containing high concentrations of carbamide peroxide (35–37%) or hydrogen peroxide (HP; 30–40%). The home-use dental bleaching systems contain different concentrations of carbamide peroxide, ranging between 10 and 22%, and HP between 3 and 10%.²

The mechanism of action for dental bleaching occurs by a complex oxidation reaction, in which the HP-containing solution is released on the tooth structure, and during the in-office techniques, this solution can be activated by heat or luminous radiation,³ according to the bleaching system used. The HP is decomposed into water and nascent oxygen, which has a low molecular weight, and penetrates rapidly through enamel and dentin porosities,^{4,5} breaking the weak bonds between chromogenic molecules and organic matrix into smaller, less complex, and lighter molecules.³

The original home-use bleaching protocol, which involves direct contact of the dental structure surface with the bleaching agent for a long period of time (around 8 hours a day for up to 6 weeks), has been replaced by quicker protocols that need to be used for only 30 minutes to 4 hours a day, for 1 to 12 consecutive weeks, depending on the desired bleaching level.⁶ This

diminished application time is justified because a larger amount of active bleaching substance comes into contact with the dental structure during the first hours, resulting in the degradation of approximately 30 to 40% of the carbamide peroxide in the first 4 hours of the agent application;⁷ this also applies to HP,⁸ which releases free radicals that break high-molecular weight carbon rings down into smaller and brighter molecules.⁹

It is known that bleaching treatment may cause morphological alterations in mineralized structures.^{10–12} Such alterations are attributed to a modification in the inorganic composition of dental structures after the treatment with peroxide-based bleaching agents,¹³ which considerably reduce the amount of calcium and phosphorous, in addition to modifying the morphology of a large quantity of crystals in the superficial layer, when compared with non-treated enamel.^{14,15}

Scanning electron microscopy analyses have shown alterations in the enamel surface with the presence of erosions and porosity after being submitted to bleaching treatment.^{11,16,17} Studies performed with HP at concentrations ranging from 30 to 37% have shown that this agent promoted superficial alterations and a reduction in the calcium–phosphorous ratio.^{17–22}

Changes in organic and inorganic contents after bleaching treatment can be observed by means of microhardness tests.²³ Some in vitro studies have shown significant differences in microhardness values of sound enamel and dentin following a bleaching treatment with HP,¹⁰ although the presence of saliva, fluorides, or other remineralizing solutions is able to maintain a balance between the remineralization and demineralization processes.

With the purpose of increasing the mineral deposition on the tooth, amorphous calcium phosphate (ACP) biomaterial has been added to toothpastes,²⁴ mouth rinses,²⁵ chewing gums,²⁶ topical application products,^{25,27} and more recently to tooth bleaching products.²⁸ When ACP, associated with other components or not, is applied, it rapidly precipitates in defects on the tooth surface and undergoes hydrolysis to form apatite, filling these defects. As a result of this process, it is possible for dental tissue remineralization to occur,²⁷ retarding the progression and increasing the regression of caries lesions,²⁶ in addition to enhancing the smoothness and luster of teeth.²⁵ Recently, ACP was added to the composition of bleaching agents containing HP (7.5 and 9.5%) for home-use; however, its effects on enamel microhardness and surface roughness (SR) have not yet been described.

The purpose of this study was to evaluate the influence of amorphous calcium phosphate added to HP at 7.5 and 9.5% and different concentrations of home-use and in-office bleaching agents on microhardness and roughness of enamel.

MATERIALS AND METHODS

Experimental Design

The factors under study were the bleaching agents, at six levels, and times of treatment, at six sublevels:

1. Bleaching agents:
 - HP7.5–7.5% HP (Pola Day, SDI Limited, Bayswater, Victoria, Australia)
 - HP9.5–9.5% HP (Pola Day, SDI Limited)
 - ACP7.5–7.5% HP with ACP (Daywhite ACP, Discus Dental, Culver City, CA, USA)
 - ACP9.5–9.5% HP with ACP (Daywhite ACP, Discus Dental)
 - HP35–35% HP (Pola Office, SDI Limited)
 - HP38–38% HP (Opalescence Xtra Boost, Ultradent Products, South Jordan, UT, USA).
2. Times of evaluation: before (*baseline*), during (7, 14, 21 days), and after (7 and 14 days in artificial saliva) the bleaching treatment

The experimental units were composed of 60 slabs of enamel randomly distributed into the six levels of bleaching agents ($N = 10$). The response variables were Knoop microhardness and mean of SR.

Preparation of Dental Slabs

The research project was approved by the Ethics Committee (Number 2008/0033). In this experiment, 30 unerupted human third molars, extracted for reasons not related to those of the present research, and stored in thymol (0.1%, pH 7.0) after extraction, were used. The teeth were debrided with scalpel blades and periodontal curettes and cross-sectioned with a diamond blade (KG Sorensen, Barueri, São Paulo, Brazil) in a low-speed handpiece (Dabi Atlante, Ribeirão Preto, São Paulo, Brazil), separating the crown from the root. Longitudinal sections were cut to obtain enamel slabs measuring 3 mm × 3 mm, and those with stains or cracks were excluded after visual observation under a stereomicroscope loupe (EK3S3, São Paulo, São Paulo, Brazil) at 30× magnification.

The enamel slabs were embedded in polystyrene resin (Piraglass Com Ind de Art Ornamentais, Piracicaba, São Paulo, Brazil) in 2.0-cm diameter polyvinyl chloride (PVC) molds, leaving the external surface of the enamel uncovered by resin. After 24 hours, the specimens were removed from the molds. Enamel slabs were flattened in a polishing machine (Politriz Aropol 2V, Arotec, São Paulo, São Paulo, Brazil) with decreasing granulations (400, 600, and 1,200) of water abrasive paper under water cooling and polished with diamond pastes of sequentially decreasing granulation (6, 3, 1 and $1/2$ μm) on felt discs under mineral oil cooling by applying gentle manual pressure. Because these procedures were performed by only one researcher, this pressure was standardized for all specimens. The enamel thickness was not measured, but all specimens were visually observed to verify the occurrence of a very thin enamel layer or dentin exposure, and those that were not suitable for microhardness and/or roughness evaluations were discarded. These procedures were necessary to obtain the smooth surfaces required for microhardness and SR tests.

TABLE 1. Description of treatment agents used in this study

Treatment agents	Composition*	Manufacturer	pH [†]
Pola Day 7.5% (PH7.5)	Hydrogen peroxide, additives, glycerol, water, aromatizers	SDI Limited, Bayswater, Victoria, Australia	5.9
Pola Day 9.5% (PH9.5)	Hydrogen peroxide, additives, glycerol, water, aromatizers	SDI Limited	6.1
Day White ACP 7.5% (ACP7.5)	Water, poloxamer 407, glycerin, hydrogen peroxide, propylenoglycol, potassium nitrate, aromatizing, xylitol, methylcellulose hydroxypropyl, eugenol, potassium hydroxide, calcium nitrate, sodium phosphate, mica	Discus Dental, Culver City, CA, USA	7.7
Day White ACP 9.5% (ACP9.5)	Water, poloxamer 407, glycerin, hydrogen peroxide, propylenoglycol, potassium nitrate, aromatizing, xylitol, methylcellulose hydroxypropyl, eugenol, potassium hydroxide, calcium nitrate, sodium phosphate, mica	Discus Dental	7.5
Pola Office 35% (PH35)	Hydrogen peroxide, water, stabilizer, catalyst, pigment and thickener	SDI Limited	4.5
Opalescence Xtra Boost 38% (PH38)	Hydrogen peroxide, chemically activated agent	Ultradent Products, South Jordan, UT, USA	2.2
*According to information provided by the manufacturers.			
[†] Measured in triplicate using a pH meter (MS Tecnopon Equipamentos Especiais LTDA, Piracicaba, São Paulo, Brazil).			

Treatment Agent Specifications

The treatment agents used in this study are described in Table 1, according to their composition, manufacturer, and pH values.

For both home-use and in-office bleaching agents, the manufacturers' instructions as regards application were followed, and the full treatment periods were performed in 21 days.

Before beginning the bleaching treatment, all specimens were submitted to microhardness and SR tests to obtain the *baseline* values.

In-Office Bleaching Application

The bleaching agent Pola Office 35% (SDI Limited) is composed of powder and liquid, which were mixed before use, in the proportion of one spoon of powder to five drops of liquid, obtaining a gel consistency. A thin layer of the gel was applied on the specimen, using a calibrated syringe enabling the placement of 0.02 mL of the gel. After 8 minutes, the gel was removed with gauze. This application protocol was performed three times. After the last application, the specimen was

washed with distilled and deionized water, and subsequently placed in an individual receptacle containing 13.5 mL of artificial saliva solution and stored at 37°C ($\pm 1^\circ\text{C}$).

The bleaching agent Opalescence Xtra Boost 38% (Ultradent Products) is supplied in two syringes containing different gels. The active ingredient in Opalescence Xtra Boost is 38% HP. The product is packaged as two syringes, one containing the liquid HP and the other a chemical activator. To mix the two components, the syringes were joined together and back and forth movements were made 20 times to thoroughly mix them. After mixing, the syringes were separated and the mixture was applied on the human tooth enamel surface, using a calibrated syringe enabling the placement of 0.02 mL of the mixture. After 8 minutes, it was removed with gauze. This application protocol was performed three times. After the last application, the specimen was washed with distilled and deionized water, placed in an individual receptacle containing 13.5 mL of artificial saliva solution and stored at 37°C ($\pm 1^\circ\text{C}$).

The artificial saliva used, a remineralizing solution described by Featherstone and colleagues²³ and

modified by Serra and Cury,²⁹ was changed every two days.

The in-office bleaching treatment protocols were performed for 3 weeks (corresponding to 21 days of treatment), being one session each week.

Home-Use Bleaching Application

Before the application of bleaching agents, an individual mold was made for each specimen, using a 0.4-mm thick flexible polymer in a vacuum plasticizer (Bio-Art Equipamentos Odontológicos Ltda, São Carlos, São Paulo, Brazil).

For the home-use bleaching agent applications, a calibrated syringe was used to place 0.02 mL of each treatment agent on the specimens. The individual mold was placed over the specimen and kept there for 30 minutes a day; after this it was immersed in the container with 13.5 mL of artificial saliva at 37°C ($\pm 1^\circ\text{C}$). Next, the individual mold was removed and washed with distilled and deionized water. The bleaching agents were removed from the specimens with distilled and deionized water.

During the remaining hours of the day, the specimens were kept in 13.5 mL of artificial saliva solution in individual receptacles containing 13.5 mL of artificial saliva solution and stored at 37°C ($\pm 1^\circ\text{C}$). The artificial saliva solution was changed every 2 days. This application protocol was performed daily for 3 weeks (corresponding to the 21 days of treatment).

Post-Treatment Period

After the 21 days of treatment, the fragments were kept in their individual receptacles with 13.5 mL of artificial saliva solution, in a bacteriological stove, at 37°C ($\pm 1^\circ\text{C}$) for 14 days to evaluate the post-treatment period and a possible remineralizing effect of this solution. The solution was changed every 2 days.

Microhardness Tests

At every time period previously described, three microhardness indentations were performed, using a microhardness tester (PanTec Digital microhardness tester HVS—1000/Panambrá, São Paulo, São Paulo, Brazil) with Knoop penetrator and a 25-g load for 5 seconds to make the enamel indentations.

Surface Roughness

The roughness test was performed in three directions (vertical, horizontal, and transversal) with a cutoff of 0.8 mm in each specimen, using a roughness tester (SurfTest Corder SE 1700/Kozaka Corp., Tokyo, Kanto, Japan) at each time period of the treatment.

Statistical Analysis

An exploratory data analysis was assessed using the PROC LAB function of the statistical program SAS (SAS Institute Inc., Cary, NC, USA; Release 8.2, 2001), showing that the data met the prerequisite for a parametric analysis. Data were analyzed by the analysis of variance followed by the Tukey's test. The level of significance adopted was 5%.

RESULTS

Table 2 shows the microhardness test results. The statistical analysis showed no difference among bleaching agents ($p = 0.8180$) and the interaction among the factors was not significant ($p = 0.5027$). However, considering the times of evaluation, there was a significant decrease in enamel microhardness during treatment ($p < 0.05$) for all bleaching agents, but microhardness values attained in the post-treatment phase were statistically similar to baseline values ($p > 0.05$).

Table 3 displays the SR results, showing no difference among the bleaching agents at each time period of the treatment ($p > 0.05$). The statistical analysis revealed that SR was not altered during bleaching treatment, for all agents tested, with exception of the PH38,

TABLE 2. Microhardness means and standard deviations (SD) for each experimental group, and for each evaluation time

Groups		PH 7.5	PH 9.5	ACP 7.5	ACP 9.5	PH 35	PH 38	Evaluation time Mean (SD)	Tukey
Baseline		321.8 (65.6)	300.8 (46.1)	337.7 (24.4)	315.2 (52.1)	333.3 (37.7)	327.5 (27.0)	333.82 (33.81)	A
7 days		311.0 (43.1)	320.3 (38.2)	333.1 (43.5)	323.7 (45.5)	304.4 (27.5)	308.5 (20.1)	315.96 (59.79)	AB
14 days		334.8 (46.4)	285.8 (40.9)	320.7 (28.8)	336.0 (45.2)	312.8 (30.2)	313.6 (22.5)	322.12 (49.15)	AB
21 days		322.2 (63.9)	317.8 (57.4)	343.2 (35.3)	319.3 (43.0)	335.8 (41.7)	335.0 (30.3)	303.18 (45.05)	B
Post-treatment	7 days	309.2 (76.3)	305.4 (53.1)	332.2 (41.7)	312.2 (49.5)	332.6 (31.2)	328.7 (30.4)	322.37 (34.15)	AB
	14 days	296.9 (63.8)	289.0 (26.6)	336.2 (28.6)	326.3 (64.6)	315.4 (28.8)	342.1 (25.4)	325.89 (27.77)	A
Means followed by the same letter (capital in the vertical) are not statistically different ($p>0.05$).									

TABLE 3. Surface roughness means and standard deviations (SD) for each experimental group

	PH 7.5	PH 9.5	ACP 7.5	ACP 9.5	PH 35	PH 38
Baseline	1.2 (0.1)ABa	1.2 (0.1)Aa	1.2 (0.1)Aba	1.2 (0.1)Aa	1.2 (0.0)Aa	1.1 (0.1)Ba
7 days	1.3 (0.1)Aa	1.2 (0.1)Aa	1.3 (0.1)Aa	1.3 (0.0)Aa	1.2 (0.0)Aa	1.3 (0.1)Aa
14 days	1.3 (0.1)Aa	1.3 (0.1)Aa	1.3 (0.1)Aa	1.2 (0.1)Aa	1.3 (0.1)Aa	1.3 (0.1)Aa
21 days	1.3 (0.1)Aa	1.3 (0.0)Aa	1.2 (0.1)ABa	1.2 (0.1)Aa	1.3 (0.0)Aa	1.3 (0.1)Aa
7 days post-treatment	1.2 (0.0)ABa	1.3 (0.0)Aa	1.2 (0.1)ABa	1.2 (0.0)Aa	1.2 (0.1)Aa	1.3 (0.0)Aa
14 days post-treatment	1.1 (0.1)Ba	1.2 (0.1)Aa	1.1 (0.1)Ba	1.3 (0.1)Aa	1.2 (0.1)Aa	1.2 (0.1)ABa

Means followed by the same letter (capital in the vertical, lower case in the horizontal) are not statistically different ($p > 0.05$).

which showed a significant increase in SR during the bleaching treatment, but after 14 days of immersion in saliva the SR values were statistically similar to those at baseline. For the PH7.5 group, data analysis showed that the values were not altered during bleaching treatment, being statistically similar to values obtained at baseline. After 14 days of immersion in artificial saliva (post-treatment), SR values of the PH7.5 group decreased and were statistically different from the bleaching treatment values, but still similar to the baseline and the 7-day post-treatment phase values. For the ACP7.5 group, data revealed that the values were not altered during bleaching treatment, but after 14 days of immersion in artificial saliva (post-treatment phase), there was a significant decrease in SR values, which were statistically different

from the 7- and 14-day bleaching treatment periods and baseline values, but statistically similar to the 21-day treatment and the 7-day post-treatment periods of evaluation.

DISCUSSION

ACP is a substance originally developed by the American Dental Association Foundation (ADAF) for dental remineralization and reversal of caries lesions in enamel.³⁰ Moreover, another study conducted by the ADAF³¹ demonstrated that ACP can make the tooth less sensitive to heat, cold, air pressure, and tactile stimulation when topically applied by professionals or by patients themselves. This is

probably because ACP is capable of obliterating the dentinal tubules³⁰ by rapid precipitation of calcium phosphate ions on the surface and inside the dentinal tubules.^{30,32} Because of its proven remineralizing effect²⁷ and the possibility of reducing dentinal hypersensitivity, the incorporation of ACP into dental bleaching gels became interesting.

In this experiment, the microhardness evaluation results showed that there was no difference among the bleaching agents; however, there was a significant reduction in microhardness values as time went by for all tested materials, irrespective of their concentration, time of application (in-office agents were applied for 24 minutes a week for 3 weeks while the home-use agents were applied for 30 minutes a day for 21 days), or bleaching agent pH.

With regard to the concentration of HP agents, Zantner and colleagues³³ showed that a high concentration leads to a more intense enamel demineralization, which was reflected in the microhardness values. Lewinstein and colleagues¹⁰ performed an experiment to verify the difference in microhardness produced by home-use and in-office bleaching treatments, and observed that the in-office bleaching agents reduced microhardness significantly more than the home-use products, suggesting that the concentration may be an important factor. However, the results of this study showed no statistical difference among the different tested gel concentrations.

It is important to note that the contact time between the gel and the dental structure seems to have a significant effect on its mineral content. With respect to this aspect, Mielczarek and colleagues³⁴ related that both the in-office dental bleaching technique with HP at 38% for 45 minutes and the home-use technique using carbamide peroxide at 10% for 7 days caused no reduction in microhardness and SR of the enamel. But the in-office bleaching time span these authors stipulated is shorter than two sessions of the protocol used in this study (24 minutes—three applications of 8 minutes, in each session). In the case of the home-use bleaching, the time span of 7 days equals 1 week of the protocol used in this study. For these periods, although

the results showed a decrease in microhardness values, these values are still statistically similar to the baseline values, only differing from those obtained in the third week of the bleaching treatment for all gel concentrations. This result differs from those found by Lewinstein and colleagues,¹⁰ who verified a significant decrease in enamel and dentin microhardness when they used a 5-, 15-, and 35-minute application protocol, in a single session.

The literature also diverges with regard to the application time factor for the home-use dental bleaching protocol. Basting and colleagues³⁵ used an application protocol for a home-use bleaching agent of 8 hours *per* day in both enamel and dentin, for 42 days consecutively, whereas Chen and colleagues³⁶ applied the agents 8 hours daily for 14 days. Lewinstein and colleagues¹⁰ used a 14-hour application protocol. In all these experiments, there was a reduction in microhardness values. Interestingly, shortening the application time of the home-use agents to 30 daily minutes (as done in this study) was not enough to avoid the decrease in enamel microhardness, but the baseline microhardness values were reestablished when the specimens were kept in artificial saliva during the post-treatment phase.

It has been suggested that artificial saliva used as storage medium may present a remineralizing effect. Thus, the artificial saliva used in this study had a similar composition as regards mineral content^{23,29} and remineralizing effect,^{36,37} as could be observed in the postbleaching period. In this period, there was an increase in microhardness values for all bleaching agents, showing evidence that the saliva plays an important role in enamel remineralization. Other studies also confirmed that the saliva was able to increase the mineral content of bleached enamel.^{38,39} On the other hand, in a study conducted by Basting and colleagues,³⁵ the immersion of specimens in artificial saliva for 14 days, after a 42-day period of bleaching treatment, promoted an increase in enamel microhardness, but the baseline values were not recovered. This demonstrates that the length of time the gel is applied may exert an influence on the remineralizing capacity of saliva, so that as this time

increases, the saliva may require a longer time to perform its remineralizing action.

With regard to the bleaching agent pH, it must be considered that the more alkaline the agent is, the shorter the exposure time needed, and better bleaching efficiency can be achieved.⁹ Nevertheless, the alkalinity of the material reduces its expiry date, which is the main reason why these materials have an acid pH.⁴⁰ Among the tested agents, the in-office products showed the lowest pH values, and their weekly application could be the reason why they did not differ from the home-use agent (daily application). Despite the buffer action of saliva⁴¹ in increasing the pH values, the in-office agents do not suffer from this effect because the application technique prevents contact with saliva.

It has been verified that the addition of ACP to 7.5 and 9.5% HP gels was unable to prevent enamel mineral loss. Therefore, Costa and Mazur²⁸ evaluated the effect of new bleaching product formulas (among them, 10% carbamide peroxide with ACP) on the microhardness of enamel, during and after bleaching treatment, and verified that the ACP was unable to prevent the decrease in enamel microhardness values. On the other hand, the addition of fluoride^{35,36} or even hydroxyapatite⁴² to the bleaching gels has prevented or minimized mineral content loss from enamel during bleaching treatment.^{35,36} In future studies, the concomitant incorporation of one or more biomaterials into the bleaching agents is suggested in an attempt to potentiate their remineralizing effect.

Bleaching treatment causes changes in the enamel surface, such as erosions and the presence of porosities.^{11,16,22} In the present study, the results showed that there was a significant difference in SR of the enamel for the different evaluation periods. At 7 days of treatment, the 38% HP (PH38) caused an increase in SR after the first application of gel, and this significant rise remained until the test specimen was immersed in artificial saliva during the post-treatment phase. Only after 14 days of immersion in artificial saliva did the specimens show a reduction in SR values, resulting in values statistically similar to those obtained in the baseline period.

It was also verified that the ACP associated with a low concentration of HP (ACP7.5) resulted in a smoother surface even during the bleaching-treatment period; that is, a significant decrease in SR from the 21-day bleaching treatment point in the time period. However, for the same concentration of gel without the ACP (PH7.5), the reduction in roughness values occurred only after the specimens were kept in artificial saliva for 7 days, during the post-treatment phase. A possible explanation for this may be related to the fact that the ACP precipitates into defects on the enamel surface and hydrolyzes to form apatite, filling these defects,²⁷ which may have made the enamel surface smoother when the ACP was associated with the lower concentration of HP tested in the present study. For agents with higher concentrations, such as HP at 9.5%, neither the addition of ACP nor the immersion in saliva had any effects on SR.

In an *in situ* study, Basting and colleagues⁴³ evaluated the micromorphology and SR of both sound and demineralized dentin and enamel, treated with carbamide peroxide at 10% for 3 weeks, and concluded that the bleached enamel (both sound and demineralized) had a rougher surface than specimen surfaces treated with a placebo agent. On the other hand, in a clinical study, Cadenaro and colleagues⁴⁴ demonstrated that both 35 and 38% HP did not change the enamel SR, by performing a profilometric analysis of resin replicas of bleached enamel. This demonstrates that different ways of simulating intraoral conditions may result in different outcomes. Moreover, after a literature review, Attin and colleagues⁴⁵ concluded that studies that simulated intraoral conditions, such as temperature and presence of saliva, obtained lower reductions in microhardness values than those that did not simulate such conditions, supporting the results of the present study. From this aspect, Basting and colleagues⁴⁶ demonstrated alterations in enamel microhardness after the bleaching treatment *in situ* with an agent containing 10% carbamide peroxide, but no alterations in the dentin substrate. On the other hand, in an *in situ* study, Maia and colleagues⁴⁷ demonstrated that home-use bleaching agents, such as the 7.5% HP, did not have any effect on enamel microhardness. Therefore, the influence on human

enamel of bleaching agents associated with ACP biomaterial, as well as different gel concentrations, should be confirmed in further *in situ* and *in vivo* studies.

CONCLUSIONS

1. There were no differences in enamel microhardness of the tested bleaching agents. At the 21-day period of bleaching treatment, bleaching agents promoted a decrease in enamel microhardness when compared with baseline values, which were recovered during the post-treatment phase, when specimens were kept in artificial saliva.
2. Surface roughness was not altered during bleaching treatment for all agents tested, with exception of the 38% HP gel, which showed a significant increase in SR during the bleaching treatment, but after 14 days of immersion in saliva the SR values were statistically similar to those at baseline. Although the 7.5% HP associated with the ACP group showed a trend to decreasing enamel SR values during the bleaching treatment, this decrease was statistically similar to the baseline values. Only when associated with 14 days of immersion in artificial saliva was the enamel treated with this bleaching agent less rough than at baseline.
3. The beneficial effects of adding ACP to bleaching formulas on SR may be restricted to lower HP concentrations in association with the remineralizing effect of saliva.

DISCLOSURE

The authors do not have any financial interest in the companies whose materials are included in this article.

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