10.5005/jp-journals-10024-2164

ORIGINAL RESEARCH

In situ Effect of Nanohydroxyapatite Paste in Enamel Teeth Bleaching

[1]Yasmin SB de Lima Gomes, [2]Larissa D Alexandrino, [3]Cristiane de M Alencar, [4]Eliane B Alves, [5]Kelson CF Faial [6]Cecy M Silva

ABSTRACT

Aim: Evaluate *in situ* the effect of nanohydroxyapatite paste (nano-HAP) before bleaching with hydrogen peroxide 35% (HP35%) by ion chromatography (IC) Knoop hardness number (KHN) and tristimulus colorimetry (TC).

Materials and methods: A total of 60 fragments were obtained from third molars included (3 mm × 3 mm × 3 mm) and the specimens were divided into three groups (n = 20): Gas chromatography (CG) (negative control group) = no bleaching; HP35% (positive control group) = HP35% whitening (whiteness HP35%); nano-HAP = application for 10 minutes before bleaching treatment + HP35%. The specimens were fixed to the volunteers' molars. The KHN and TC were measured before and after bleaching. For IC, the dentin layer was removed, leaving the enamel that was crushed, and autoclaved for chemical quantification (calcium, fluorine, and phosphorus). The results of KHN and TC were analyzed statistically by analysis of variance (ANOVA) followed by Tukey test ($p < 0.05$).

Results: The HP35% group showed reduction of the Ca, F, and P ions. The initial and final KHN mean of the CG and nano-HAP did not differ statistically; however, the group of HP35% did differ statistically. The mean ΔE of the HP35% and nano-HAP groups did not differ statistically from each other. However, they differed from the CG.

Conclusion: The nano-HAP paste preserved the KHN, promoted the lower loss of Ca and P ions and an increase of F ions when compared with the CG, but did not influence the effectiveness of the bleaching treatment.

Clinical significance: Nano-HA is a biomaterial that has shown positive results in the prevention of deleterious effects on the enamel by the action of the office bleaching treatment.

[1-4,6]Department of Restorative Dentistry, Federal University of Pará, Belém, Pará, Brazil

[5]Department of Toxicology, Environment Section (SAMAM) Evandro Chagas Institute, Ananindeua, Pará, Brazil

Corresponding Author: Cecy M Silva, Augusto Correa Street No. 1, Guamá, Belém, Pará, Brazil, Phone: +559132521269 e-mail: cecymsilva@gmail.com

Keywords: Dental enamel, Hydrogen peroxide, Hydroxyapatite, Tooth bleaching.

How to cite this article: Gomes YSBL, Alexandrino LD, Alencar CDM, Alves EB, Faial KCF, Silva CM. *In situ* Effect of Nanohydroxyapatite Paste in Enamel Teeth Bleaching. J Contemp Dent Pract 2017;18(11):996-1003.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Dental whitening is indicated for darkened teeth[1,2] by trauma, smoking, endodontic procedures, aging, and for those who have become naturally discolored.[3,4] Peroxide bleaching agents have the ability to penetrate into the dental structure[5] and produce free radicals[6] that oxidize colored organic molecules.[7] Hydrogen peroxide has a low molecular weight chain and, in contact with saliva, it decomposes into water and oxygen.[8] Oxygen, which is the free radical molecule, acts on the long chains of macromolecules that make up the pigments and degrade into smaller molecules, favoring their removal, thus promoting tooth whitening.[9] When they penetrate the enamel interprismatic structure, the free radical molecule can react with the present organic macromolecules, as well as the organic matrix of the enamel.[10] With the removal of the organic part of the enamel and dentin,[11] the roughness increases and the enamel and dentin mineral content is lost. The morphological changes caused by the peroxides of high concentration are not evidenced macroscopically; however, microscopic changes, such as increased porosity, depressions, and superficial irregularities have been reported.[12] Studies have revealed mineral loss, increased susceptibility to erosion or caries, increased surface roughness; reduced enamel tensile strength, reduced fracture stability or a decrease in abrasion resistance of bleached enamel tissues.[13]

The bleaching procedure has shown good results clinically, but *in vitro* studies have shown that the use of high concentrations of bleaching agents has been associated with deleterious effects of tooth structure, as well as increased surface roughness,[9] decreased KHN[10-12] and morphological alterations.[13]

However, laboratory conditions do not reflect clinical conditions. The oral cavity presents several factors that may minimize the deleterious effects caused by the bleaching treatment,[14,15] including the presence of saliva,[14] which suggests reversing the changes related to whitening.[16] Saliva has the ability to buffer, due to bicarbonate, and the phosphate system of inorganic electrolytes, such as phosphorus, calcium, and fluoride and the presence of enzymes and bacteria.[17]

A qualitative analytical study indicated a significant reduction in the calcium content, phosphate, and fluoride in the enamel after bleaching with HP in both low and high concentrations.[14] To recover tooth structure, different peroxide formulations have been developed along with the addition of calcium fluoride.[15]

Biomaterials are materials designed to replace parts of the body and allow the recovery of biological functions affected by diseases or accidents.[18] Hydroxyapatite is a biomaterial[19], i.e., found as a natural mineral constituent found in bone, representing 30 to 70% of the mass of bones and teeth.[18]

In situ studies of tooth whitening allow the interaction evaluation between the association of bleaching agents, saliva,[20] soft tissues, and tooth structure.[21,22] The majority of studies in the literature were developed *in vitro*, making it difficult to compare results obtained on clinical conditions, since teeth are continuously subjected to demineralization and remineralization cycles.[23] *In situ* studies represent an intermediate phase between laboratory and clinical experiments, with the objective of reproducing the process, i.e., studied under the influence of biological factors.[24]

The aim of this work is to evaluate *in situ* the effect of the application of nano-HAP before 35% HP during the bleaching treatment using IC, KHN and TC. The null hypothesis (H_0) was that there was no difference in the application of the nano-HAP paste in the evaluated methods (IC, KHN, and TC) between the groups tested.

MATERIALS AND METHODS

The protocol of this study was analyzed and approved by the Ethics Committee of the Federal University of Para (case No. 188681). Fifteen individuals (8 males and 7 females, from 19 to 34 years old) were selected, who met the inclusion and exclusion criteria described in Table 1. They were included in the study after signing a free informed consent term, as volunteers.

Table 1: Inclusion and exclusion criteria in the selection of patients for the study

Inclusion Criteria	*Exclusion Criteria*
Clinical crown height of upper molars above 5 mm	Presenting dentin sensitivity
Absence of caries	Carriers of gastroesophageal disorders
Absence of periodontal disease	Pregnant or lactating patients
Neutral oral pH (6.5–7.0)	Holders of fixed prostheses or removable orthodontic appliances
Normal salivary flow (0.1–0.5 mL)	Drug users or smokers
	Water sports enthusiasts

Samples of saliva were obtained by collecting saliva mechanically stimulated by the chewing of a piece of club. Patients were instructed not to brush their teeth, eat, or drink for at least 1 hour before collection.[25] The patient was positioned comfortably with the head slightly tilted forward and was instructed to chew 2 cm of rubber that was tied to a 25 cm piece of dental floss to prevent accidental swallowing. The saliva produced in the 1st minute of mastication was discarded before the collection of the saliva produced in the following 5 minutes, which was stored in a graduated vessel at each interval of 1 minute.[26] The volume of stimulated saliva was measured and the volume of the salivary flow was calculated in mL/minute.[27]

Synthesis of Nano-HAP

The nano-HAP was prepared in the laboratory of Biomaterials, UNICAMP. Nano-HAP was synthesized in a wet condition according to the method of Boanini et al,[28] with some modifications.[29] Twenty mL of 1.08 M $Ca(NO_3)2.4H_2O$ solution at pH adjusted to 10 with NH_4OH. The solution was heated at 90°C and 20 mL of 0.65 M $(NH_4)2HPO_4$ solution, pH 10 adjusted with NH_4OH, was added drop-wise under stirring. The precipitate was maintained in contact with the reaction solution for 5 hours at 90°C under stirring, and then, centrifuged at 1800 g for 10 minutes. The precipitate was repeatedly washed with distilled water and centrifuged for six times, and then dried at 37°C overnight.

Preparation of Specimens

For this study, 33rd molar teeth were used, which presented no morphological or structural damage, defects, such as cracks, fractures, and caries. The roots of the teeth were separated from their crowns at the cement enamel junction using a diamond point number 4138 (KG Sorensen, Cotia, São Paulo, Brazil) coupled to a high-speed hand piece (KAVO, Berlin, Steglitz, Germany) with cooling. The teeth were cleaned and stored in distilled

water in the refrigerator until specimen preparation. To obtain 60 specimens (3 × 3 × 3 mm), the buccal and lingual surfaces of the crowns were cut with a double-faced steel disk (KG Sorensen, Cotia, Sao Paulo, Brazil) activated by a low-speed micromotor (KAVO, Berlin, Steglitz, Germany) and regularized with diamond tips number 4138 (KG Sorensen, Cotia, São Paulo, Brazil). In the specimens used for the Ra, KHN, and TC readings, the polishing of the surface was carried out in a polylinker (APL4/AROTEC São Paulo, Brazil) with the use of 600-, 1200-, and 2000-SiC grit granulators with refrigerators to obtain flat, standardized enamel surfaces. Subsequently, they were cleaned with distilled water in an ultrasonic tub (BioWash TD 30 Plus—Bioart, São Carlos/São Paulo/Brazil) for 5 minutes.

Preparation of the Volunteers for the Clinical Phase

Prophylaxis and scraping were performed on patients for at least 2 weeks and no more than 1 month before the start of the experimental phase with fluoride prophylactic paste (NuPro Supremo, Dentsply Int., New York, PA, USA) for the removal of external patches and bacterial plaque. All participants received a kit containing a fluoride-free paste, a soft-bristled toothbrush (Soft ORAL-B, São Paulo, Brazil), dental floss, and written instructions on the use of the material.

Fixation of Specimens

For the fixation of the specimens, the absolute insulation with rubber dam was performed on the upper and lower first molars (Fig. 1). The specimens were fixed to the buccal surface of the selected teeth with adhesive cementing agent RelyX™ U200 Automix (3M ESPE, São Paulo, Brazil). The photoactivation of the cement was carried out for 20 seconds using the light-emitting diode radio photoactivator (SDI Limited, Bays water, Victoria, Australia).

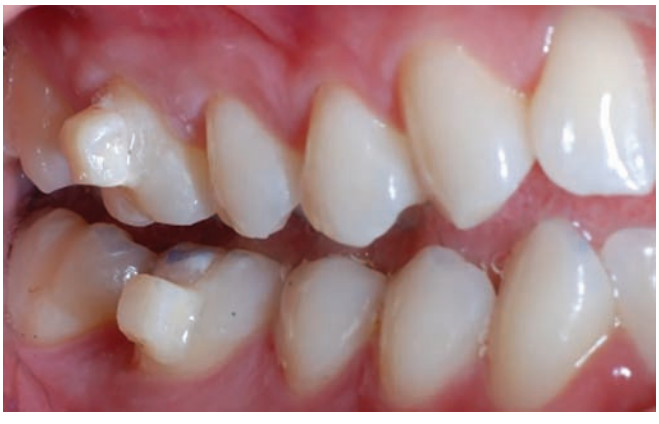


Fig. 1: Specimens fixed to the buccal surface of the upper and lower first molars

Division of Groups

The specimens were randomly divided into three groups, according to the treatment condition (n = 20): CG—negative control group: No bleaching treatment; HP35%—positive control group: The specimens were cleared with Whiteness HP35% (FGM, Joenville, SC, Brazil); and nano-HAP: The specimens were whitened with Whiteness HP35% + paste nano-HA.

The bleaching agent application protocol followed the manufacturer's specifications. There were three bleaching sessions with a 7-day interval. Three 15-minute applications were made in each session with a 5-minute interval between each application. Twenty-four hours after the last bleaching session, fragments were removed with the help of orthodontic pliers (Quinelato, Rio Claro, São Paulo, Brazil) and submitted to final laboratory analysis.

The nano-HAP paste was applied to the dental surface for 10 minutes prior to the application of the bleaching agent. The slurry was prepared from 1 gm of nano-HAP powder with 1 mL of distilled water.

Ion Chromatography Test

The chemical quantification analysis was performed after the bleaching treatment due to the destruction of the samples. Five specimens were used from each group, where the dentin layer was removed with diamond drill bit 4138 (KG Sorensen, São Paulo, Brazil) coupled to the high-speed turbine under refrigeration (KaVo, Joinville, Santa Catarina, Brazil), leaving only one layer of enamel left. The specimens from each group were ground together to obtain a homogeneous mass, then autoclaved to decontaminate the sample and then subjected to the acid digestion process by microwave radiation (MarsXpress—CEM, São Paulo, Brazil). The samples were digested in polytetrafluoroethylene (Teflon®) vials previously decontaminated in HNO_3 nitric acid (10% v/v) and then washed with distilled/deionized water. The samples were digested with 0.1 gm of ground sample, with the addition of 3 mL of nitric acid (HNO_3), 1 mL of hydrochloric acid (HCl), and 1 mL of hydrogen peroxide (H_2O_2), and after release of the gases produced with the chemical reaction of the acids, samples were placed on the reel itself and taken to the microwave oven that was previously programmed to heat according to inorganic samples (800 W for 10 minutes + 400 W for 5 minutes and 10 minutes of sample cooling). The samples were then transferred to polyethylene vials, with a final volume of 50 mL. The sample was diluted to ×303 from ×50, drawing 0.165 mL and measuring up to 50 mL with distilled/deionized water. The samples were analyzed in the ion chromatograph (ICS 2000

DUAL—DIONEX, Sunnyvale, California, USA) for quantification of the chemical elements calcium, fluorine, and phosphorus.

Knoop Microhardness

KHN readings were performed before and after the bleaching treatment using a microdrometer (FM-700, Future Tech Corp., Tokyo, Japan) with a loading of 25 gf for 5 seconds. Five penetrations were made on each surface with 100 µm distance between each indentation. Samples with microhardness between 272 and 440 KHN were selected.

After the five indentations were measured, the initial mean and final mean were calculated. Subsequently, the percentage of microhardness loss (KHN%) was found using the following formula:

$$KHN\% = \frac{KHN(I) - KHN(F)}{KHN(I)} \times 100$$

where KHN (I) is the initial mean KHN of the group and KHN (F) is the final mean KHN of the group.

Tristimulus Colorimetry

The sample color was measured using a TC using the CIE L*a*b* system. The specimens were placed on an opaque black surface so that the reflected light did not interfere with the colorimeter values. To evaluate the color change between the initial and final results, ΔE was calculated using the following formula:

$$\Delta E = \{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\}^{1/2}$$

where $\Delta L = L - L_0$, $\Delta a = a - a_0$, $\Delta b = b - b_0$. ΔL, Δa and Δb represent the variation of the coordinates in black-white, red-green and blue-yellow respectively.

Statistical Analysis

The statistical software BioEstat 5.0 was used, with a significance level of 5%. For significance analysis between the initial and final mean of KHN and TC among the groups, one-way ANOVA was used and Tukey test.

RESULTS

Iron Chromatography Analysis

Table 2 represents the quantitative analysis of IC. Was showed that the nano-HAP group presented the highest percentage of F, followed by CG and HP35% groups, respectively. The nano-HAP and HP35% groups presented reduction of Ca and phosphorus ions when compared with CG.

Table 3 shows the mean and standard deviation of KHN of the groups analyzed. One-way ANOVA and Tukey's test showed that there was no statistically significant difference between the initial and final KHN means between the control and nano-HAP groups ($p > 0.05$); however, the HP35% group presented a statistically significant reduction of the final KHN mean when compared with the initial KHN mean ($p < 0.05$).

Table 2: Percentage of ions fluoride (F), calcium (Ca) and phosphorus (P)

	Quantification of ions		
Group	*F*	*Ca*	*P*
CG (negative control)	0.23%	85.13%	60.46%
HP35% (positive control)	0.13%	42.76%	20.10%
nano-HAP	0.29%	72.75%	48.05%

Table 3: Mean (standard deviation) of initial and final KHN of the groups under study

Group	*Initial*	*Final*
CG (negative control)	375.61 ± 2.95 Aa	372.28 ± 2.73 Aa
HP35% (positive control)	375.15 ± 2.82 Aa	318.22 ± 4.98 Bb
nano-HAP	374.27 ± 7.88 Aa	369.49 ± 2.23 Aa

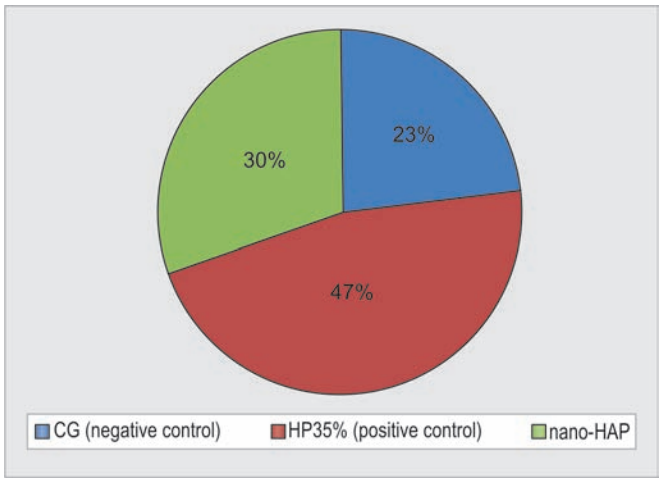
Different lowercase letters indicate differences in the initial and final KHN values, while uppercase letter indicate differences among the groups ($p < 0.05$)

Graph 1 shows the KHN% of the groups. The HP35% group presented higher KHN%, followed by nano-HAP and negative control.

Tristimulus Colorimetry

The mean and standard deviations in ΔE are presented in Table 4. One-way ANOVA and Tukey's test ($p < 0.05$) showed a statistically significant difference between the mean ΔE of the control group and the other groups analyzed, but the mean of ΔE and HP35% did not differ statistically from nano-HAP ($p > 0.05$).

The mean values of the coordinates L*, a*, and b* are shown in Graphs 2 to 4. The nano-HAP group showed a



Graph 1: Knoop microhardness percentage for the groups

Table 4: Means and standard deviations of E in the groups under study

Groups	*n*	*Mean and standard deviation*
CG (negative control)	5	0.56 ± 0.12 a
HP35% (positive control)	5	12.82 ± 1.09 b
nano-HAP	5	12.64 ± 0.50 b

Different letters indicate significant differences in E (p <0.05)

statistically significant change only in the L* coordinate compared with the other groups.

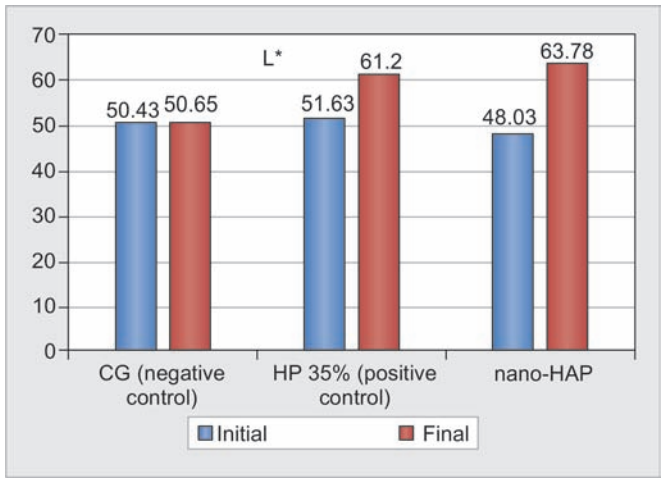
DISCUSSION

The H_0 tested in this study for HF and KHN was discarded since the nano-HAP paste promoted a lower loss of minerals and KHN. For CT, the H_0 was accepted because the nano-HAP paste did not interfere in the bleaching efficacy.

Ion chromatography is considered a versatile, sensitive, and selective technique for separation and determination of a series of ions present in low concentrations. Due to these characteristics, ionic chromatography has been used in different areas of scientific research, such as in the health, food, pharmaceutical, and environmental industries.[30]

The nano-HAP particles have physical properties that can make it similar to the size of the enamel crystal. Studies have shown[31] that nano materials can adhere easily to the surface and have bioactive and biocompatible properties. The effect of remineralization is increased when the reduction of the HAP particle is reduced to nanometric range.[31,32]

Synthetic HAP has properties of biocompatibility[33] and osseointegration, which makes it a substitute for human bone in implants and prostheses.[34,35] The HAP formula is $Ca_{10}(PO_4)_6(OH)_2$, Ca/P ratio equal to 1.67 and has the most stable and least soluble calcium phosphate of all.[18] The structure of HAP allows the replacement of cations and isomorphic ions with great ease.[36] Its hydroxyl group (OH) can be replaced by Fa, so the hexagonal structure becomes more stable and less soluble than stoichiometric HAP.[18]

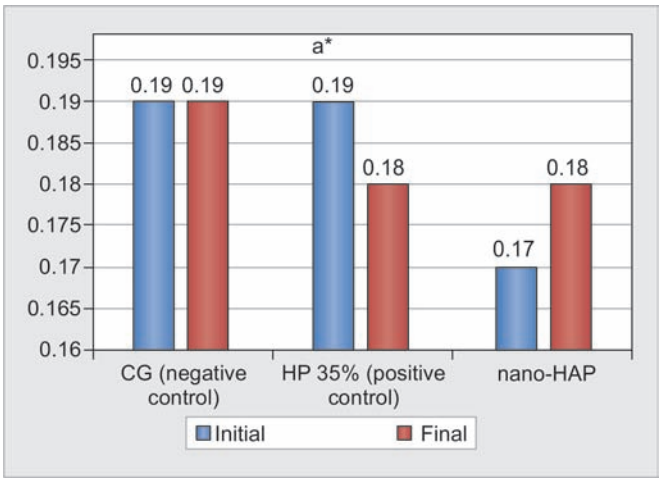


Graph 2: Color coordinate L*

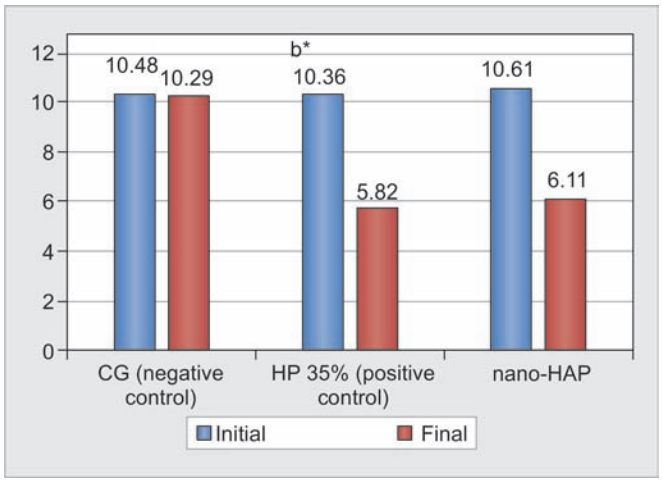
The nano-HAP paste used in this study is a synthetic biomaterial capable of being deposited on dental structures that have undergone mineral loss.[34] Studies have shown that bleaching agents can cause structural changes in the enamel, such as the formation of erosions and depressions.[16,37] Because it contains manometric particles, nano-HAP is able to penetrate these erosion areas to avoid further demineralization and promote remineralization with the deposition of:[38,39]

$$Ca_{10}(PO_4)_6(OH)_2$$

The chemical and biological characteristics of the nano-HAP described above may justify the increase of the F ions percentage when compared with the other groups analyzed, and the lower loss of Ca and P ions in relation to the group just cleared.



Graph 3: Color coordinate a*



Graph 4: Color coordinate b*

The HP can cause changes in the superficial layer, as well as the inter- and intraprismatic portions of the enamel, promoting a moderate deproteinization.[39] The previous application of the nano-HAP paste can promote the formation of a protective layer, reducing the possibility of enamel demineralization caused by HP.[40]

The nano-HAP group presented higher KHN mean in comparison to the HP35% group; however, both groups did not differ significantly from the CG, showing that this protective layer may have preserved the enamel. The nano-HAP group had the lowest percentage of microhardness loss (KHN%) compared with the HP35% group, showing that the nano-HAP paste favored the lowest loss of KHN.

The CIE system L*a*b*, developed by the International Commission on Illumination (CIE) in 1976[41] was used, where the coordinate L* represents the degree of luminosity and its values vary between 0 (black) and 100 (white). The coordinate a* represents the green/red color change, and the b* coordinate represents the blue/yellow color change. It has been reported that the limits of ΔE values that become visible to the human eye vary widely in the literature and depend on each observer.[39] However, with uncontrolled clinical intraoral factors, values lower than 3.7 are considered clinically acceptable.[42-44]

The mean ΔE obtained from the bleached groups was higher than this value, revealing the efficacy of the bleaching treatment. The HP35% and nano-HAP groups did not show significant differences in ΔE values when compared with CG, revealing that the nano-HAP paste did not influence the bleaching efficacy. The coordinate a* was considered the axis with the least capacity to influence bleaching, corroborating previous studies.[45-47]

The results showed a decrease in the values of the coordinate b*, indicating the reduction of the yellow color and revealing bleaching effectiveness. The decrease of the b* coordinate values can be explained by the removal of the chromogenic pigments from the tooth during bleaching.[21] Although enamel is considered as an achromatic tissue, the present result suggests that whitening can decrease opalescence and make them bluish. This assertion is based on increasing the amount of blue light in reflectance mode after bleaching.[48] The values of the L* coordinate demonstrate bleaching effectiveness, where the groups clarified with HP showed the increase of the values. The increase in the values of the coordinate L* represents that the tooth tends to white.[46,47,49,50] Application of the nano-HAP paste did not interfere with bleaching effectiveness.

Several studies suggest that saliva may influence the loss of mineral components caused by tooth whitening because salivary flow stimulation increases the presence of components, such as carbonic acid, hydrogen carbonate, hydrogen phosphate, and calcium fluoride, which are associated with increased buffering capacity and maintenance of the balance between demineralization and remineralization.[51,52] *In situ* studies about bleaching are important to demonstrate the interaction between bleaching agents, saliva, soft tissues, and tooth structure.[53-55]

CONCLUSION

With the limitations of the present study, it was concluded that previous application of the nano-HAP paste was able to preserve the KHN, to promote less loss of minerals and did not interfere in bleaching effectiveness.

REFERENCES

1. Arévalo PM, VC. Larrucea Recidiva del color por té, café y vino. *In vitro*. Rev Clin Periodoncia Implantol Rehabil Oral 2012 Aug;5(2):57-65.
2. Ma X, Li R, Sa Y, Liang S, Sun L, Jiang T, Wang Y. Separate contribution of enamel and dentin to overall tooth colour change in tooth bleaching. J Dent 2011 Nov;39(11):739-745.
3. Attin T, Manolakis A, Buchalla W, Hannig C. Influence of tea on intrinsic colour of previously bleached enamel. J Oral Rehabil 2003 May;30(5):488-494.
4. Berger SB, Coelho AS, Oliveira VA, Cavalli V, Giannini M. Enamel susceptibility to red wine staining after 35% hydrogen peroxide bleaching. J Appl Oral Sci 2008 May-Jun;16(3):201-204.
5. Kwon SR, Wertz PW. Review of the mechanism of tooth whitening. J Esthet Restor Dent 2015 Sep-Oct; 27(5):240-257.
6. Kamath U, Sheth H, Mullur D, Soubhagya M. The effect of Remin Pro® on bleached enamel hardness: an *in-vitro* study. Indian J Dent Res 2013 Nov-Dec;24(6):690-693.
7. Gjorgievska E, Nicholson JW. Prevention of enamel demineralization after tooth bleaching by bioactive glass incorporated into toothpaste. Aust Dent J 2011 Jun; 56(2):193-200.
8. Zeczkowski M, Tenuta LMA, Ambrosano GMB, Aguiar FHB, Lima DANL. Effect of different storage conditions on the physical properties of bleached enamel: an *in vitro* vs. *in situ* study. J Dent 2015 Sep; 43(9):1154-1161.
9. Sun G. The role of lasers in cosmetic dentistry. Dent Clin North Am 2000 Oct;44(4):831-850.
10. Mendonça LC, Naves LZ, Garcia LF, Correr-Sobrinho L, Soares CJ, Quagliatto PS. Permeability, roughness and topography of enamel after bleaching: tracking channels of penetration with silver nitrate. Braz J Oral Sci 2011 Jan; 10(1):1-6.
11. Karadas M, Seven N. The effect of different drinks on tooth color after home bleaching. Eur J Dent 2014 Apr;8(2):249-253.
12. Ferreira Sda S, Araújo JL, Morhy ON, Tapety CM, Youssef MN, Sobral MA. The effect of fluoride therapies on the morphology of bleached human dental enamel. Microsc Res Tech 2011 Jun;74(6):512-516.
13. Cavalli V, Arrais CA, Giannini M, Ambrosano GM. High-concentrated carbamide peroxide bleaching agents effects on enamel surface. J Oral Rehabil 2004 Feb;31(2):155-159.
14. Bistey T, Nagy IP, Simó A, Hegedus C. *In vitro* FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel. J Dent 2007 Apr;35(4):325-330.

15. Cavalli V, Rodrigues LK, Paes-Leme AF, Brancalion ML, Arruda MA, Berger SB, Giannini M. Effects of bleaching agents containing fluoride and calcium on human enamel. Quintessence Int 2010 Sep;41(8):e157-e165.
16. Attin T, Schmidlin PR, Wegehaupt F, Wiegand A. Influence of study design on the impact of bleaching agents on dental enamel microhardness: a review. Dent Mater 2009 Feb;25(2):143-157.
17. Tam LE, Bahrami P, Oguienko O, Limeback H. Effect of 10% and 15% carbamide peroxide on fracture toughness of human dentin *in situ*. Oper Dent 2013 Mar-Apr;38(2):142-150.
18. Costa ACFM, Lima MG, Lima LHMA, Cordeiro VV, Viana KMS, Souza CV, Lira HL. Hidroxiapatita: Obtenção, caracterização e aplicações. Rev Eletr Mat Proc 2009;4(3):29-38.
19. Sasaki RT, Catelan A, Bertoldo Edos S, Venâncio PC, Groppo FC, Ambrosano GM, Marchi GM, Lima DA, Aguiar FH. Effect of 7.5% hydrogen peroxide containing remineralizing agents on hardness, color change, roughness and micromorphology of human enamel. Am J Dent 2015 Oct;28(5):261-267.
20. Lia Mondelli RF, Garrido Gabriel TR, Piola Rizzante FA, Magalhães AC, Soares Bombonatti JF, Ishikiriama SK. Do different bleaching protocols affect the enamel microhardness? Eur J Dent 2015 Jan-Mar;9(1):25-30.
21. Justino LM, Tames DR, Demarco FF. *In situ* and *in vitro* effects of bleaching with carbamide peroxide on human enamel. Oper Dent 2004 Mar-Apr;29(2):219-225.
22. Arcari GM, Baratieri LN, Maia HP, De Freitas SF. Influence of the duration of treatment using a 10% carbamide peroxide bleaching gel on dentin surface microhardness: an *in situ* study. Quintessence Int 2005 Jan;36(1):15-24.
23. de Arruda AM, dos Santos PH, Sundfeld RH, Berger SB, Briso AL. Effect of hydrogen peroxide at 35% on the morphology of enamel and interference in the de-remineralization process: an *in situ* study. Oper Dent 2012 Sep-Oct;37(5):518-525.
24. Zero DT. *In situ* caries models. Adv Dent Res 1995 Nov; 9(3):214-230.
25. Navazesh M, Kumar SK; University of Southern California School of Dentistry. Measuring salivary flow: challenges and opportunities. J Am Dent Assoc 2008 May; 139 (Suppl): 35S-40S.
26. Lima AA, Figueiredo MA, Krapf SM, Souza FR. Velocidade do fluxo salivar e pH salivar após radioterapia da região de cabeça e pescoço. Rev Bras Cancerol 2004 Sep;50(4):287-293.
27. Moritsuka M, Kitasako Y, Burrow MF, Ikeda M, Tagami J, Nomura S. Quantitative assessment for stimulated saliva flow rate and buffering capacity in relation to different ages. J Dent 2006 Oct;34(9):716-720.
28. Boanini E, Torricelli P, Gazzano M, Giardino R, Bigi A. Nanocomposites of hydroxyapatite with aspartic acid and glutamic acid and their interaction with osteoblast-like cells. Biomaterials 2006 Sep; 27(25):4428-4433.
29. 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 Nov; 36(11):907-914.
30. Michalski R. Environmental applications of ion chromatography in Eastern and Central Europe. J Chromatogr Sci 2010 Aug; 48(7):559-565.
31. Kim YS, Kwon HK, Kim BI. Effect of nano carbonate apatite to prevent re-stain after dental bleaching *in vitro*. J Dent 2011 Sep;39(9):636-642.
32. Generosi A, Rau JV, Albertini RV, Paci B. Crystallization process of carbonate substituted hydroxyapatite nanoparticles in toothpastes upon physiological conditions: an *in situ* time-resolved X-ray diffraction study. J Mater Sci Mater Med 2010 Feb;21(2):445-450.
33. Haghgoo R, Rezvani MB, Zeinabadi MS. Comparison of nano-hydroxyapatite and sodium fluoride mouthrinse for remineralization of incipient carious lesions. J Dent (Tehran) 2014 Jul; 11(4):406-410.
34. Sun L, Berndt CC, Gross KA, Kucuk A. Material fundamentals and clinical performance of plasma-sprayed hydroxyapatite coatings: a review. J Biomed Mater Res 2001 Oct;58(5):570-592.
35. Eanes ED. The influence of fluoride on the seeded growth of apatite from stable supersaturated solutions at pH 7.4. J Dent Res 1980 Feb;59(2):144-150.
36. Ma QY, Logan TJ, Traina SJ. Lead immobilization from aqueous solutions and contaminated soils using phosphate rocks. Environ Sci Technol 1995 Apr;29(4):1118-1126.
37. Torres CR, Koga AF, Borges AB. The effects of anti-oxidant agents as neutralizers of bleaching agents on enamel bong strength. Braz J Oral Sci 2006;5(16):971-976.
38. Huang SB, Gao SS, Yu HY. Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion *in vitro*. Biomed Mater 2009 Jun;4(3):034104.
39. de Moraes IQ, Silva LN, Porto IC, de Lima Neto CF, Dos Santos NB, Fragoso LS. Effect of in-office bleaching with 35% hydrogen peroxide with and without addition of calcium on the enamel surface. Microsc Res Tech 2015 Aug;78(11):975-981.
40. Türkün M, Celik EU, Aladag A, Gökay N. One-year clinical evaluation of the efficacy of a new daytime at-home bleaching technique. J Esthet Restor Dent 2010 Apr; 22(2):139-146.
41. Billmeyer FW, Saltzman M. Principles of color technology. New York: John Wiley & Sons; 1981.
42. Douglas RD, Steinhauer TJ, Wee AG. Intraoral determination of the tolerance of dentists for perceptibility and acceptability of shade mismatch. J Prosthet Dent 2007 Apr;97(4):200-208.
43. Gomes MN, Francci C, Medeiros IS, De Godoy Froes Salgado NR, Riehl H, Marasca JM, Muench A. Effect of light irradiation on tooth whitening: Enamel microhardness and color change. J Esthet Restor Dent 2009 Dec; 21(6):387-394.
44. Sulieman M, MacDonald E, Rees JS, Newcombe RG, Addy M. Tooth bleaching by different concentrations of carbamide peroxide and hydrogen peroxide whitening strips: an *in vitro* study. J Esthet Restor Dent 2006 Feb; 18(2):93-100.
45. de Vasconcelos AA, Cunha AG, Borges BC, Machado CT, dos Santos AJ. Tooth whitening with hydrogen/carbamide peroxides in association with a CPP-ACP paste at different proportions. Aust Dent J 2012 Jun;57(2):213-219.
46. Goodson JM, Tavares M, Sweeney M, Stultz J, Newman M, Smith V, Regan EO, Kent R. Tooth whitening: tooth color changes following treatment by peroxide and light. J Clin Dent 2005;16(3):78-82.
47. Eimar H, Siciliano R, Abdallah MN, Nader SA, Amin WM, Martinez PP, Celemin A, Cerruti M, Tamimi F. Hydrogen peroxide whitens teeth by oxidizing the organic structure. J Dent 2012 Dec;40(Suppl 2):e25-e33.
48. Park HJ, Kwon TY, Nam SH, Kim HJ, Kim KH, Kim YJ. Changes in bovine enamel after treatment with a 30% hydrogen peroxide bleaching agent. Dent Mater J 2004 Dec;23(4):517-521.
49. Karadas M, Duymus ZY. *In vitro* evaluation of the efficacy of different over-the-counter products on tooth whitening. Braz Dent J 2015 Jul-Aug;26(4):373-377.
50. Schmeling M, Maia HP, Baratieri LN. Opalescence of bleached teeth. J Dent 2012 Jul;40(Suppl 1):e35-e39.

51. Iizuka J, Mukai Y, Taniguchi M, Mikuni-Takagaki Y, Ten Cate JM, Teranaka T. Chemical alteration by tooth bleaching of human salivary proteins that infiltrated subsurface enamel lesions – experimental study with bovine lesion model systems. Dent Mater J 2014;33(5):663-668.
52. Sa Y, Sun L, Wang Z, Ma X, Liang S, Xing W, Jiang T, Wang Y. Effects of two in-office bleaching agents with different pH on the structure of human enamel: An *in situ* and *in vitro* study. Oper Dent 2013 Jan-Feb;38(1):100-110.
53. Xu B, Li Q, Wang Y. Effects of pH values of hydrogen peroxide bleaching agents on enamel surface properties. Oper Dent 2011 Aug; 36(5):554-562.
54. Li Y, Greenwall L. Safety issues of tooth whitening using peroxide-based materials. Br Dent J 2013 Jul; 215(1):29-34.
55. Meireles SS, Fontes ST, Coimbra LA, Della Bona Á Demarco FF. Effectiveness of different carbamide peroxide concentrations used for tooth bleaching: an *in vitro* study. J Appl Oral Sci 2012 Mar-Apr;20(2):186-191.