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Effect of Toothpaste Use Against Mineral Loss Promoted by Dental Bleaching

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Clinical Relevance

Dental bleaching promotes slight mineral loss associated with the surface changes of enamel, which might be prevented by toothpaste use prior to treatment.

SUMMARY

Aim: To investigate the effect of different toothpaste formulations used prior to dental bleaching with 35% hydrogen peroxide (HP) on the mineral content and surface morphology of enamel. Methods: Seventy bovine enamel blocks (4×4×2 mm) were submitted to *in vitro* treatment protocols using a toothbrushing machine prior to dental bleaching or a placebo procedure (n=10) as proposed in the following groups: unbleached control (PLA), bleached control (HP), and brushing with differing toothpastes prior to HP bleaching, including: potassium nitrate toothpaste containing sodium fluoride (PN), sodium monofluorophosphate/MFP toothpaste (FT), arginine-carbonate (8% arginine) (PA) or arginine-carbonate (1.5% arginine) toothpaste (SAN), and toothpaste containing bioactive glass (NM). Phosphorus concentration in gel ([P]) was evaluated (μg of P/mg of gel), and the elemental levels (wt%) of Ca, P, and Na as well as the proportion between Ca and P and spectra graphics were determined using an energy-dispersive X-ray spectrometer (EDS). The surface morphology was assessed using scanning electron microscopy (SEM). The data were subjected to analysis of variance and the Tukey test (α=0.05).

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Results: HP demonstrated the greatest [P] values in gel, being statistically different from PLA. The [P] of NM was statistically similar to PLA. HP showed a significant decrease in the Ca% and Ca/P values when compared to PLA in EDS analysis. PA showed Ca/P values statistically different from HP. In accordance with SEM analysis, the PA, SAN, and NM groups presented a smooth and uniform enamel surface, while HP and FT demonstrated some alterations in morphology. Conclusion: The toothpastes containing bioactive glass or arginine carbonate used prior to dental bleaching were effective in protecting enamel against mineral loss promoted by the whitening procedure.

INTRODUCTION

Tooth whitening has become a popular procedure for patients who seek to improve the color appearance of their teeth. Currently, dental bleaching procedures utilize hydrogen peroxide (HP) as an oxidizing agent.[1] Although the bleaching mechanism is not totally understood, it has been suggested that, when in contact with dental tissues, there is an oxidation reaction, and HP releases free radicals, hydroxyl anions, and reactive oxygen molecules,[2] all of which break up the organic pigmented molecules,[3,4] resulting in smaller and less heavily pigmented molecules that absorb less light in dentin.[1,4] This change in pigmentation causes teeth to appear lighter. With careful diagnosis and appropriate attention to the bleaching procedure, dental bleaching is considered a relatively conservative and safe approach for the treatment of discolored teeth.[5]

Dental bleaching may cause structural changes to the tooth surfaces, including changes to surface morphology and physical-chemical properties. Some studies have demonstrated enamel surface alterations following exposure to HP-based gels, suggesting that the procedure results in mineral loss that may be related to changes in surface microhardness, roughness, and the mineral content of enamel.[6-10] Calcium and phosphate ions are the main constituents of the hydroxyapatite crystal found in dental enamel, and their concentration is largely affected by the dissolution process, as occurs during a demineralization/erosive event.[11] Nevertheless, few studies have chemically assessed the concentration of these ions after tooth bleaching.[9,10] It is unknown how the bleaching procedure can change the concentration of phosphorus in the hydroxyapatite crystals of enamel. Additionally, tooth dissolution may result in defects and porosities on the enamel surface visualized under microscopic evaluation.[7,12]

Saliva and other active agents play an essential role in maintaining or creating an active environment to reduce demineralization or to promote remineralization in tooth surfaces exposed to bleaching treatment.[13,14] Currently, a commercially available bioactive glass-based toothpaste (calcium sodium phosphosilicate , NovaMin, SmithKline Beecham Consumer Healthcare, Berkshire, United Kingdom) has been promoting enamel remineralization[15] and is indicated for dentin hypersensitivity treatment.[16] Bioactive glass was originally developed as a bone regeneration material that demonstrated beneficial effects when incorporated into the bleaching gel or when used before or after dental bleaching.[17,18] Additionally, an arginine-carbonate–based toothpaste has been commercially available for caries control[19] and hypersensitivity treatment.[20] However, few studies exist in the literature that discuss the effects of arginine-carbonate toothpaste on preventing erosive surface loss[21] and its effect prior to, during, or after bleaching.[18,22] Therefore, the effect of toothpastes on the enamel surface when used prior to dental bleaching has been rarely assessed.

The aim of this study was to evaluate the potential effect of different toothpaste formulations used prior to dental bleaching on the surface morphology and mineral content of enamel. The null hypotheses tested were 1) that the dental bleaching would not affect the calcium or phosphorus content of the enamel, 2) that the toothpaste would not affect the mineral content of the enamel when used prior to treatment, and 3) that there would be no difference between the different toothpastes tested in protecting enamel against calcium or phosphorus loss promoted by dental bleaching.

METHODS AND MATERIALS

Sample Preparation

Freshly extracted and intact young bovine incisors were selected and stored in a 0.1% thymol solution for one month. Seventy enamel-dentin blocks, with dimensions of 16 mm^2 and 2 mm in height (1 mm of enamel and 1 mm of dentin), were prepared from the buccal surfaces of the teeth. The sectioning of teeth was performed using a high-concentration diamond disc (Buehler, Lake Bluff, IL, USA) coupled with a precision cutting machine (Isomet 1000, Buehler). Sectioned blocks were serially planed and polished using a water-cooled mechanical grinder and 600-, 1000-, and 2000-grit SiC papers (Buehler), and at the end, they were polished using cloths and diamond spray (1, 0.5, and 0.25 μm, Buehler). The specimens were immersed in deionized water and placed in an ultrasonic bath for 10 minutes between and after the polishing procedures to obtain a standardized enamel surface. All block surfaces, excluding the enamel surface, were protected using an acid-resistant varnish (Risqué, Taboão da Serra, Brazil). Twenty-four hours before and during the study, all prepared samples were stored in artificial saliva, which was renewed every day, in a 37°C incubator. The artificial saliva contained a known composition of 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 μg F/mL, and 0.1 M Tris buffer with a pH of 7.0.[23]

Toothpaste Treatment

Samples were submitted to linear brushing movements using toothbrush heads (Oral-B Indicator 40 Soft, Gillette do Brazil Ltda, Manaus, Brazil) attached to an automatic toothbrushing machine (Equilabor, Piracicaba, Brazil), using a static axial load of 200*g* and a speed of 5 movements/s at 37°C.[24] The samples were randomly assigned to the groups. In control groups (unbleached or bleached), the brushing was performed only with distilled water, while in the toothpaste groups, toothpaste/distilled water slurries (1:3) were used. The composition of each toothpaste and bleaching agent used, as well as the description of the experimental groups, is listed in Table 1. Toothbrushing was simulated using 825 cycles, which corresponded to one month, considering that approximately 10 to 15 strokes are performed in each toothbrushing event.[25] The blocks were washed with distilled water for 10 seconds and stored in artificial saliva for 24 hours prior to the dental bleaching procedures.

Bleaching Treatment and Gel Collection

A 35% HP gel (Whiteness HP, FGM, Joinville, Brazil) was applied to the exposed enamel area following the manufacturer's instructions. The composition of the gels is described in Table 1, with the initial and final pH of the bleaching agent measured in triplicate, thus presenting three readings for the bleaching gel (means: initial pH=5.64; after 15 minutes=4.87) and for the placebo gel (buffered to pH 6.0) using a pH meter (Procyon, São Paulo, Brazil). The bleaching or placebo gel was applied to the enamel surfaces three times for 15 minutes per application, without intervals between applications. Each application was performed using a precise amount of gel measured (0.010±0.002 g) using an analytical balance (AUW 220 d, Shimadzu, Kyoto, Japan). The weight values of the gel were used to calculate the phosphorus concentration per gram of gel. After exposure of the bleaching gel to the tooth, the specimens were placed in 0.5 mL of deionized water (gel rising water) in closed bottles and submerged in an ultrasonic bath for 30 seconds (Marconi, Piracicaba, São Paulo, Brazil). Immediately after, the specimens were submitted to 1 minute of vigorous stirring. This process was repeated three times for each sample, considering that the application of gel was performed following the manufacturer's instructions. The mixture of gel and distilled water used to rinse off the sample in each application was analyzed to assess the phosphorus concentration.

Phosphorus Measurement in Gel

Phosphorus concentration was evaluated relative to the weight of the bleaching or placebo gel used in each specimen. The phosphorus quantification for each sample proposed in this study was calculated by adding the three applications (μg of P/mg of gel), using the method described by Fiske and Subbarow.[26] In this method, the reaction consisted of the mixture of bleaching gel and distilled water, ultra-purified water, and molybdic acid solution (ammonium molybdate at 2.5% in sulfuric acid), which were vigorously vortexed. After 10 minutes, the reducing agent (1-amino-2-naphthol-4-sulfonic acid, sodium sulfite, and bisulfate) was added, and the mixture was vortexed again. After 20 minutes, the blue color intensity was measured using a spectrophotometer (DU 800, Beckman Coulter, Brea, CA, USA) at 660 nm and calibrated with standards from 0 to 24 μg P/mL. In a previous pilot study, the addition of a phosphorous standard (6 μg of P) in the mixture and the use of a control (6 μg of P+distilled water) for evaluation of the minimal variation of phosphorus concentration between the specimens of the same group were planned. In order to evaluate the variation of phosphorus concentration during bleaching procedures, the baseline values of phosphorus in gel not exposed to enamel were evaluated. The phosphorus concentration of gels at baseline was obtained by diluting the fresh gel in distilled water, thus repeating the process described for the collection methods of gel.

Energy-Dispersive X-Ray Spectrometry

Five samples per group were randomly selected for analysis by energy-dispersive X-ray spectrometry (EDS). The selected samples were submitted to sputtering under vacuum (Desk II, Denton Vacuum, Moorestown, NJ, USA) for the application of a fine

Table 1: *Groups, Products, and Toothpastes Used in This Study*[a]

Groups	Toothpaste	Treatment/Bleaching[b]	Manufacturer	Composition[b]
Unbleached control (PLA)	*Without toothpaste (distilled water)*	*Placebo gel (three times for 15 min each application)*	*Proderma, Piracicaba, Brazil*	*Water, neutralized carbopol, glycerin, triethanolamine, buffered in pH 6.0*
Bleached control (HP)	*Without toothpaste (distilled water)*	*35% HP (three times for 15 min each application)*	*Whiteness HP, FGM, Joinville, Brazil*	*35% hydrogen peroxide, thickener (carbopol), glycol, water*
Potassium nitrate toothpaste containing NaF (PN)	Sensodyne Fresh Impact	35% HP	GlaxoSmithKline Brasil Ltda, Rio de Janeiro, Brazil	5% potassium nitrate, dodium fluoride (NaF) 1426 ppm, water, hydrated silica, sorbitol, glycerin, cocamidopropyl betaine, xanthan gum, titanium dioxide, sodium sacharin, sucralose, mentha piperita, d-limonene
MFP fluoride toothpaste (FT)	Colgate Maximum Cavity Protection	35% HP	Colgate-Palmolive, São Bernardo do Campo, Brazil	Sodium monofluorophosphate (MFP) 1450 ppm, water, calcium carbonate, glycerin, sodium lauryl sulfate, cellulose gum, tetrasodium pyrophosphate, sodium bicarbonate, benzyl alcohol, sodium saccharin, sodium hydroxide
Arginine- carbonate–based toothpaste (8% arginine) (PA)	Colgate Sensitive Pro relief Pro-Argin	35% HP	Colgate-Palmolive, São Bernardo do Campo, Brazil	8% arginine, MFP 1450 ppm, water, calcium carbonate, sorbitol, arginine bicarbonate, sodium lauryl sulfate, cellulose gum, titanium dioxide, tetrasodium pyrophosphate, sodium bicarbonate, benzyl alcohol, sodium saccharin, xanthan gum, limonene
Arginine- carbonate–based toothpaste (1.5% arginine) (SAN)	Colgate Maximum Cavity Protection PLUS Sugar Acid Neutralizer	35% HP	Colgate-Palmolive, São Bernardo do Campo, Brazil	1.5% arginine, MFP 1450 ppm, water, calcium carbonate, glycerin, arginine bicarbonate, sodium lauryl sulfate, cellulose gum, titanium dioxide, tetrasodium pyrophosphate, sodium bicarbonate, benzyl alcohol, sodium saccharin, sodium hydroxide
Toothpaste containing bioactive glass (NM)	Sensodyne Repair & Protect Novamin	35% HP	SmithKline Beecham Consumer Healthcare, Berkshire, United Kingdom	5% calcium sodium phosphosilicate, MFP 1426 ppm, glycerin, silica, PEG-8, titanium dioxide, carbomer, cocamidopropyl betaine, sodium methyl cocoyl taurate, sodium saccharin, d-limonene

Abbreviation: HP, hydrogen peroxide.
[a] *Italic cells are related to the gel composition, and roman cells show the composition of toothpastes.*
[b] *According to the manufacturer's instructions.*

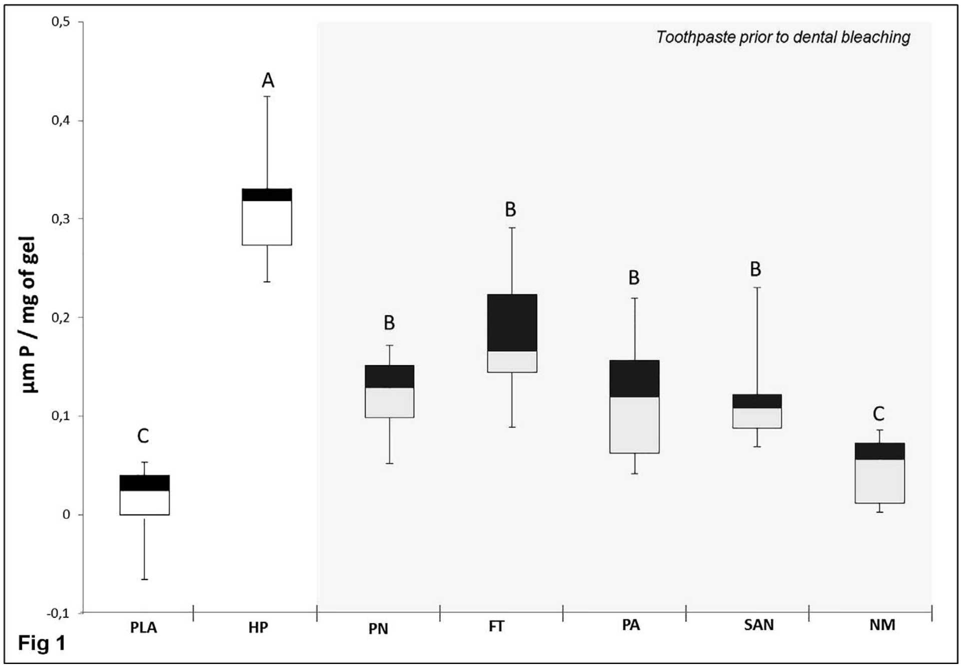

Figure 1. *Box plot of phosphorus concentration in the gel. The higher box plot means that values represent phosphorus loss of enamel with gel. PLA, placebo; HP, 35% hydrogen peroxide; PN, potassium nitrate (with NaF); FT, fluoride as MFP; PA, 8% arginine; SAN, 1.5% arginine; NM, bioactive glass.*

carbon layer. The EDS point analysis (Vantage, Acquisition Engine Co, Tokyo, Japan) was performed on the enamel surface to determine the elemental presence of Na, Mg, Al, Si, P, and Ca. The elemental levels (wt%) of Ca, Na, and P and the proportion between Ca and P were determined. For each sample, five points were randomly selected (300 μm^2 for each point), and the mean values were calculated.

Scanning Electron Microscopy

For analysis by scanning electron microscopy (SEM), another five specimens from each group were randomly selected and subjected to vacuum in a sputter coater (SCD 050, Balzers Union Aktiengesellschaft, Balzers, Liechtenstein) to deposit a thin layer of gold, equivalent to 10^{-6} mm, in order to increase the surface reflectance. Then images (4000×) of representative areas of the specimens were obtained using SEM (JSM-5600LV, JEOL, Tokyo, Japan).

Statistical Analysis

The data of phosphorus concentration in the gel and elemental levels (wt%) of Ca, Na, and P and the proportion between Ca and P were statistically analyzed using SAS software (SAS Institute Inc, Cary, NC, USA). The normal distribution of the values was verified, and a parametric analysis was performed. The data were subjected to a one-way analysis of variance and the Tukey test for multiple comparisons (α=0.05).

RESULTS

Phosphorus Concentration in Gel

Based on the phosphorus concentration [P] analysis for the gel (Figure 1), the results demonstrated that dental bleaching (HP, bleached control) resulted in greater phosphorus loss ($p<0.001$) when compared to the unbleached control (PLA) group. Furthermore, all toothpaste applications prior to dental bleaching decreased the [P] values, which differed statistically from the bleached control (HP) group; however, the toothpaste containing bioactive glass (NM) group was the only one that did not show significant difference in relation to PLA ($p>0.05$).

Elemental Levels (wt%) and EDS

The values of the relative percentage weight of calcium (Ca%) are presented in Table 2. The Ca% values of the HP group were significantly less than the PLA group ($p<0.001$). The application of NM and arginine-carbonate (1.5% arginine) toothpaste (SAN) did not significantly affect the relative Ca% compared to the PLA group ($p>0.05$) or the HP group ($p>0.05$).The Ca% values of the arginine-carbonate (8% arginine) (PA) group were statistically similar to the HP control group ($p>0.05$). The smallest values of Ca% were found in the potassium nitrate (PN)

Table 2: *Mean (Standard Deviation) of Elemental Levels (wt%) for EDS Analysis of Enamel Surface According to the Treatment Group*[a]

	Calcium	Phosphorus	Sodium	Ca/P
PLA	65.52 (0.15) A	33.68 (0.11) C		1.95 (0.01) A
HP	65.03 (0.07) B	34.10 (0.11) AB	—	1.91 (0.01) BC
PN	64.61 (0.14) C	34.31 (0.10) A	—	1.88 (0.01) B
FT	65.07 (0.19) B	34.06 (0.23) AB	—	1.91 (0.01) BC
PA	65.09 (0.26) B	33.59 (0.27) C	0.54 (0.12) A	1.94 (0.01) A
SAN	65.19 (0.22) AB	34.17 (0.21) A	—	1.91 (0.02) BC
NM	65.13 (0.25) AB	33.73 (0.15) BC	0.64 (0.03) A	1.93 (0.01) AB

[a] *Identical letters indicate no significant difference (p>0.05) among different groups (vertical). Italics indicate the control groups. — represents % wt < 0.01. Abbreviations: PLA, placebo; HP, 35% hydrogen peroxide; PN, potassium nitrate (with NaF); FT, fluoride as MFP; PA, 8% arginine; SAN, 1.5% arginine; NM, bioactive glass.*

group, which significantly differed from all other groups ($p<0.01$).

The relative percentage weight values of phosphorus (P%) are presented in Table 2. The relative P% was significantly higher in the HP group when compared to PLA group ($p<0.05$). The PA and NM groups did not significantly differ from the unbleached control; therefore, only PA differed statistically from the HP group ($p<0.05$), presenting lower values of P%. The P% values observed in the PN and SAN groups were statistically higher than the PLA, PA, and NM groups ($p<0.01$), which were statistically similar between them ($p>0.05$) and between the sodium monofluorophosphate/MFP toothpaste (FT) and HP groups ($p>0.05$).

The EDS results showed absence (wt%) of Mg, Al, or Si quantifiable by specific software. Considering the representative spectra graphics presented in Figure 2, the PA, SAN, and NM groups demonstrated the presence of sodium (Na; note the sets); therefore, the sodium (Na%) was quantified (Table 2) only in the PA and NM groups, and it was not significantly different between them ($p>0.05$). The proportion between the Ca% and P% values of the HP group was significantly less than the PLA group ($p<0.001$). PLA obtained 1.95 (Ca/P), which was statistically similar ($p>0.05$) to the PA and NM groups; in addition, the PA was the only one statistically different from the HP group ($p>0.05$).

SEM

The SEM images collected (Figures 3 and 4) present a smooth and uniform enamel surface in the groups submitted to toothpaste application prior to the whitening procedure. Superficial alterations were found in the HP (Figure 3B) and FT groups (Figure 3D), demonstrating different levels of enamel demineralization with a loss of interprismatic substance and an increase in porosity. Figures 4C,D show retained mineral particles on enamel surfaces treated with NM, presenting a surface covered with a precipitate, which was not solubilized by the whitening procedures.

DISCUSSION

Null hypotheses 1 and 2 were rejected because the dental bleaching affected the calcium and phosphorus content of enamel and the toothpastes acted in the mineral content of the bleached enamel when used prior to the whitening procedure. Null hypothesis 3 was rejected because there was a difference in the effects found in the toothpastes used for protecting enamel against slight mineral loss promoted by dental bleaching. Dental enamel is the hardest mineralized biological tissue, containing approximately 96% mineral, 3% water, and 1% organic matter by weight.[27] The principal inorganic constituent of enamel is hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, which tends to incorporate a number of elements (Na, K, Cl, F, Mg, Zn, Pb, Cu, Sn, and Al).[27,28] In this study, bovine teeth were used because the chemical and physical morphology of bovine enamel resembles that of human enamel.[29] Within this context, the mineral loss is associated with the release of calcium and phosphorus ions due to hydroxyapatite crystal dissolution, resulting in a decrease of enamel properties during dental bleaching.[6-10]

The effect of toothpaste on dental bleaching related to the mineral content of enamel is still unclear in that few studies[18,22,30] have evaluated the effect of the use of toothpastes in tooth whitening on enamel. Toothpastes are the most widespread products used in oral hygiene, and their interaction with dental treatment, as tooth bleaching, should be known. Potassium nitrate toothpaste, conventional fluoride toothpaste, arginine-based toothpaste, and

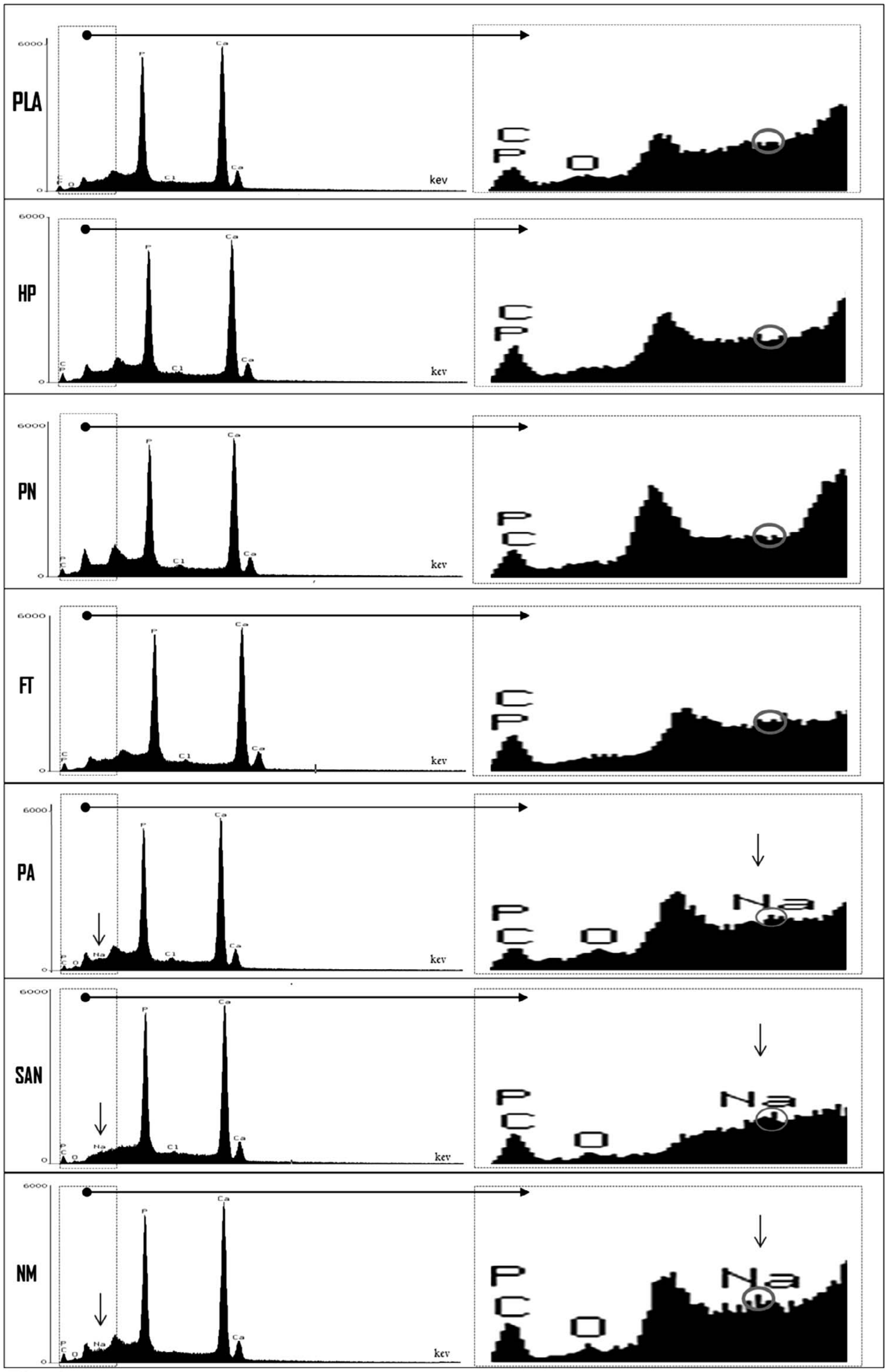

Fig 2

Figure 2. *Representative spectra graphics for EDS analysis. In image enlargement, a slight increase in the sodium peak (Na) is indicated by sets in the PA, SAN, and NM groups. PLA, placebo; HP, 35% hydrogen peroxide; PN, potassium nitrate (with NaF); FT, fluoride as MFP; PA, 8% arginine; SAN, 1.5% arginine; NM, bioactive glass.*

toothpaste containing bioactive glass assessed in the present study do not affect the whitening effectiveness of the bleaching procedure.[18] In the present study, the focus was to evaluate the potential effects of toothpaste application prior to dental bleaching by analyzing the enamel surface morphology and mineral content, especially by the new approach of quantifying the phosphorus content. The use of MFP toothpaste (FT) has been suggested as a nonactive control because MFP requires enzymatic hydrolysis

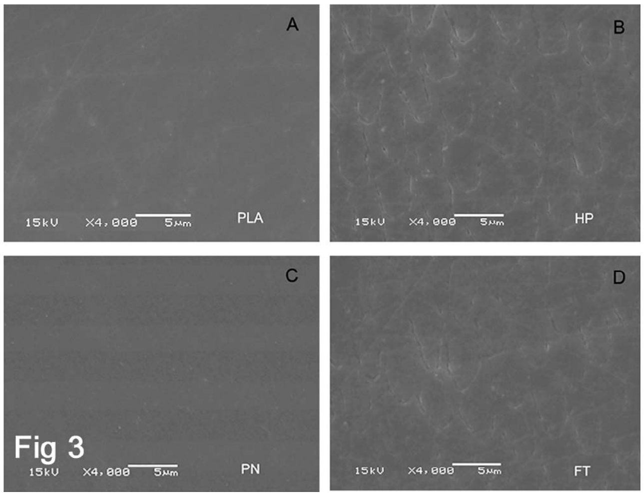

Figure 3. *Representative SEM images (4000×) of the specimens from the treatment groups. (A): Placebo (PLA). (B): Only bleached (HP). (C): Potassium nitrate toothpaste containing NaF (PN). (D): MFP fluoridated toothpaste (FT). PLA, placebo; HP, 35% hydrogen peroxide; PN, potassium nitrate (with NaF); FT, fluoride as MFP.*

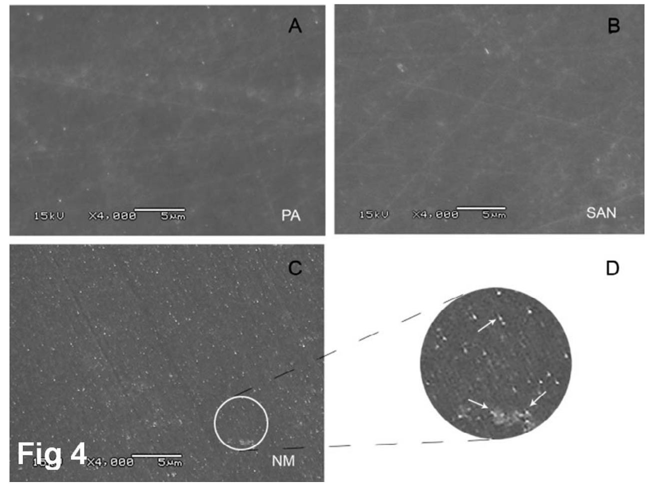

Figure 4. *Representative SEM images (4000×) of the specimens from the treatment groups. (A): 8% arginine-carbonate–based toothpaste (PA). (B): 1.5% arginine-carbonate–based toothpaste (SAN). (C): Toothpaste containing bioactive glass (NM). (D): Presence of mineral precipitates found in the NM group. PA, 8% arginine; SAN, 1.5% arginine; NM, bioactive glass.*

in human saliva and dental biofilm to release free fluoride.[31] Thus, as artificial saliva was used, the MFP had a low effect in *in vitro* models to prevent the demineralization of enamel. In another way, a NaF-based toothpaste was tested (PN group) and was considered to be an active group once this compound was ionized in aqueous solution, with fluoride ions available and active. NaF has been proven with *in vitro* results,[32] which is important because fluoride is currently used as an agent that promotes remineralization of dental hard tissues and that decreases the effects of demineralization.[32] The low effect of MFP on decreasing demineralization in *in vitro* studies was validated in SEM analysis. Figure 3 shows alterations in the enamel topography of the FT group, indicating mineral loss in the interprismatic areas and surface enamel similar to the HP group.

In dental bleaching, a mineral dissolution with loss of calcium and phosphorus occurs.[33,34] This adverse effect can be attributed to the action of free radicals resulting from the oxidation of the organic and inorganic elements on teeth[3] and also to the acidic pH of the bleaching agent applied.[35] EDS analysis showed a decrease in Ca% values for the bleached control group (HP); however, the P% values increased in the HP group. These percentage values are relative to the area analyzed in weight. The mineral gain and loss is possibly due to the crystal structure of $Ca_{10}(PO_4)_6(OH)_2$.[36] Due to the characteristics of the EDS methodology and once the amounts of all elements were presented as a mass percentage (%), a greater loss of calcium can increase the relative values of phosphorus, which exists in a small proportion in the hydroxyapatite crystal, and it also influences the analysis of other elements. Thus, an increase in calcium loss would lead to higher proportions for the P% values, but this does not mean that the bleached enamel surface incorporated phosphorus ions when compared to the placebo group. This was clearly demonstrated in the chemical analysis of gel, in which there was an increase in phosphorus loss of enamel with the gel (Figure 1).

The formation of mineral precipitates on enamel surface when using the bioactive glass toothpaste (NM) occurred due to the interaction of bioactive glass particles, Ca, P, and Na,[15] as shown by the representative images (Figure 4D) and spectra graphic (Figure 2). EDS analysis in the NM group suggested the relative percentage weight of Na (mean=0.64%) and Na presence in spectra graphics, indicating that it could be possible for the NM group to promote the formation of a hydroxycarbonate apatite layer[15] on the tooth surface. When bioactive glass is brought into contact with body fluids, there is a rapid capitation of Na, Ca, and P, thus creating precipitated minerals that are able to achieve remineralization of the damaged enamel surfaces[15,17,30,37] or reduce the effects of the demineralization process.[17,18] The results of SEM analysis (Figure 4D) show that the NM group presented uniform mineral precipitates covering the enamel surface that remained intact. The NM toothpaste did not allow a morphological change on enamel in SEM images; this finding is corroborated by the roughness results described by Vieira-Junior and others[18] that reported similarity between the unbleached enamel and the bleached enamel previously exposed to bioactive glass toothpaste. Based on SEM images, EDS analysis, and the low concentration of phosphorus in the gels, it is possible to suggest that NM decreased the mineral loss of enamel, likely due to the fact that the hydroxycarbonate apatite precipitate was not lost by dissolution with the gel application. These results indicate a beneficial effect of bioactive glass toothpaste use prior to dental bleaching therapy and are in agreement with previous studies,[17,18] suggesting that the combination of the nonreacted bioactive glass particles and the newly formed hydroxycarbonate apatite layer results in preventing further demineralization.

In relation to arginine-containing toothpastes (PA and SAN), the effects on microhardness, roughness, or the mineral content of enamel in literature are rarely assessed.[18,38] It is thought that arginine is a source of calcium since it is a positively charged amino acid[39] that binds to negatively charged dental tissues and calcium carbonate.[40] Moreover, the alkaline environment promoted by toothpaste could buffer the pH of the gel, which might encourage calcium and phosphate ions to precipitate themselves under dental tissues.[40] When considering the Ca/P ratio and the P% values by EDS analysis (Table 2), the results may indicate a lower dissolution of hydroxyapatite crystal in the PA group (8% arginine) in the case that the PA group did not differ from the unbleached control (PLA). This effect can be mediated by the arginine concentration of toothpaste since these results were not replicated in the SAN group, which is composed of 1.5% arginine. However, these findings should be carefully evaluated since EDS analysis is relative (wt%) and performed in random sections of the enamel. The role of arginine in different demineralization/remineralization processes should be evaluated in future investigations.

The observed morphological changes on the enamel surface (Figures 3 and 4) suggest that the damaging effects of dental bleaching may be decreased by prior application of different toothpastes (PN, SAN, PA, and NM). Despite the values of Ca% and P% in EDS analysis, the PN group presented a polished and unchanged enamel surface in SEM analysis, indicating no morphologic alterations resulting from dental bleaching. Added to this, it is likely that there was an incorporation of fluoride ions (from NaF) on the enamel surface (calcium fluoride/ fluorhydroxyapatite), which decreased the values of the other element %s in EDS. This proposition is corroborated, since the phosphorus concentration in gel did not show an increase of phosphorus loss for the PN group. From this statement, it is clear that EDS analysis is a semiquantitative/qualitative method that should be applied in conjunction with further methodologies for chemical quantification of enamel after bleaching, as performed in this study.

In the present study, an automatic toothbrushing machine was used in order to standardize different variables. In *in vitro* models, the effect of toothpastes on dental structure may be influenced by several factors, such as the velocity of brushing, the type of movement, and the amount of load applied and temperature. Currently, considering the frequency with and time in which people perform their toothbrushing,[41] the overall recommendation for simulating the toothbrushing under *in vitro* conditions is to perform the brushing twice a day, 10 to 15 strokes per tooth.[25,42] Thus, the purpose was to simulate approximately 30 days of brushing, using 825 cycles, to create an enamel surface treated with the different active compounds studied.

In accordance with the presented results, there were differences in the potential effects among the different toothpastes used. Specifically, the toothpastes containing bioactive glass (NM) or arginine (PA) were efficient in minimizing the negative bleaching effects related to mineral loss and changes in enamel topography. Nevertheless, natural human saliva could provide an effective environment for the remineralization of dental hard tissues demineralized by dental bleaching; therefore, the pretreatment with toothpaste helps prevent the slight enamel phosphorus loss from dental in-office bleaching procedures, which may not be clinically relevant unless the patient's own postsalivary remineralization is impaired. Further research is needed to investigate the performance of bioactive glass or arginine containing toothpastes in *in situ* and *in vivo* studies regarding the combination of toothpaste and effects of bleaching agents on dental substrates.

CONCLUSIONS

The dental in-office bleaching procedure promoted slight phosphorus and calcium loss of enamel; however, the toothpastes containing bioactive glass or arginine-carbonate used prior to treatment were effective in preventing mineral loss.

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Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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