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Effect of Toothpaste Application Prior to Dental Bleaching on Whitening Effectiveness and Enamel Properties

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Clinical Relevance

Dental bleaching promotes physical alteration in enamel, although these alterations might decrease when toothpaste is applied prior to the whitening procedures.

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SUMMARY

Objective: The purpose of this study was to investigate the effects on the enamel properties and effectiveness of bleaching using 35% hydrogen peroxide (HP) when applying toothpastes with different active agents prior to dental bleaching.

Methods: Seventy enamel blocks (4 × 4 × 2 mm) were submitted to *in vitro* treatment protocols in a tooth-brushing machine (n=10): with distilled water and exposure to placebo gel (negative control [NC]) or HP bleaching (positive control [PC]); and brushing with differing toothpastes prior to HP bleaching, including potassium nitrate toothpaste (PN) containing NaF, conventional sodium monofluorophosphate toothpaste (FT), arginine-based toothpastes (PA and SAN), or a toothpaste containing bioactive glass (NM). Color changes were determined using the CIE L*a*b* system (ΔE, ΔL, Δa, and Δb), and a roughness (Ra) analysis was performed before and after treatments. Surface microhardness (SMH) and cross-sectional microhardness (CSMH) were

analyzed after treatment. Data were analyzed with repeated measures ANOVA for Ra, one-way ANOVA (SMH, ΔE, ΔL, Δa, and Δb), split-plot ANOVA (CSMH), and Tukey post hoc test ($\alpha<0.05$). The relationship between the physical surface properties and color properties was evaluated using a multivariate Canonical correlation analysis.

Results: Color changes were statistically similar in the bleached groups. After treatments, SMH and CSMH decreased in PC. SMH increased significantly in the toothpaste groups vs the negative and positive control (NM > PA = SAN > all other groups) or decreased HP effects (CSMH). Ra increased in all bleached groups, with the exception of NM, which did not differ from the NC. The variation in the color variables (ΔL, Δa, and Δb) explained 21% of the variation in the physical surface variables (Ra and SMH).

Conclusion: The application of toothpaste prior to dental bleaching did not interfere with the effectiveness of treatment. The bioactive glass based toothpaste protected the enamel against the deleterious effects of dental bleaching.

INTRODUCTION

Because the demand for esthetic dentistry has recently increased, treatment protocols for altering tooth color have been developed, with tooth bleaching becoming an attractive treatment.[1] The whitening mechanism is unclear, although it most likely involves the diffusion of hydrogen peroxide (HP) through the enamel, where it reacts with the chromogens responsible for dental discoloration.[2] HP, as an oxidizing agent, breaks down the pigment molecules and makes them small enough to be removed from the dental structure through diffusion, which indirectly promotes the reduction of light absorption. This reduction in light absorption produces a significant reduction in the yellowness of dentin and an increase in whiteness for the tooth.[3]

Tooth bleaching is currently considered a safe treatment.[4] However, the effects of bleaching agents on dental tissues are not completely understood. *In vitro* studies show that changes in the morphology and properties of dental tissues can happen, such as 1) an increase in permeability and surface roughness[5-7] and 2) a decrease in surface and subsurface microhardness.[8,9] Despite favorable results and effectiveness of bleaching agents, some studies have reported side effects, with the intensity and frequency of these side effects associated with different experimental designs.[10]

Tooth sensitivity from dental bleaching is considered the most common adverse effect.[11,12] This effect starts at the beginning of the procedure and ends after discontinuing the use of the product. To eliminate the adverse effects in the morphology and properties of dental tissues, the use of fluoride remineralization systems during or after treatment has been suggested.[13-15] Additionally, the use of bleaching gel that includes different systems has been discussed; Haywood and others[16] demonstrated that the use of potassium nitrate toothpaste reduced bleaching sensitivity.

Conversely, dentifrices containing arginine or calcium sodium phosphosilicate (bioactive glass) have been studied for preventing and treating dentinal hypersensitivity,[17,18] as these compounds could promote beneficial effects and enamel rehardening,[19,20] resulting in a potential benefit for bleaching therapy.[21,22] However, there are no studies regarding the application of remineralizing compounds associated with dentifrices containing desensitizing agents prior to dental bleaching in the prevention of adverse effects caused by dental bleaching, mainly in relation to enamel properties and the effectiveness of bleaching.

The present study investigated the effects on enamel for toothpastes with different active or desensitizing agents used prior to dental bleaching with 35% HP by evaluating the effectiveness of bleaching, using color analysis, microhardness, and surface roughness on the properties and morphology of enamel. The null hypotheses tested were that the toothpaste application prior to dental bleaching would not affect the whiteness effectiveness and would not protect enamel against any deleterious effects of HP.

METHODS AND MATERIALS

Sample Preparation

Young bovine teeth were stored in a 0.01% thymol solution at 4°C for 30 days until use. Enamel/dentin blocks of 4 × 4 × 2 mm, with 1 mm of enamel and 1 mm of dentin, were obtained from the middle third of the buccal surface using a low speed water-cooled diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA). The specimens were then subsequently serially ground with 600-, 1000-, and 2000-grit SiC papers (Buehler Ltd) and polished with cloths and diamond spray (1, 0.5, and 0.25 μm, Buehler Ltd).

Table 1: *Products, Manufacturers, and Components of Toothpastes According to the Manufacturer's Information.*

Toothpaste	Manufacturer	Active agent	Fluoride	Other components
Sensodyne™ Fresh Impact (PN)	GlaxoSmithKline Brasil Ltda, Rio de Janeiro, Brazil	5% potassium nitrate	Sodium fluoride (NaF) 1426 ppm	Water, hydrated silica, sorbitol, glycerin, cocamidopropyl betaine, xanthan gum, titanium dioxide, sodium sacharin, sucralose, mentha piperita, D-limonene
Colgate™ Fluoridated Toothpaste (FT)	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
Colgate™ Sensitive Pro relief Pro-Argin™ Technology (PA)	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
Colgate™ Maximum Cavity Protection PLUS Sugar Acid Neutralizer™ (SAN)	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
Sensodyne™ Repair & Protect Novamin™ Technology (NM)	SmithKline Beecham Consumer Heathcare, 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

Between the polishing steps and at the end of this procedure, all samples were placed in an ultrasonic machine for 10 minutes (Marconi, Piracicaba, São Paulo, Brazil) to remove residual particles and smear layers to obtain a standardized enamel surface. The surfaces of the specimens, with the exception of the enamel surface, were protected with acid-resistant varnish (Risqué Colorless, Taboão da Serra, Brazil). Prior to (24 hours) and during the experiment, all prepared specimens were stored in artificial saliva in a 37°C incubator, with artificial saliva renewed every day during the study. The artificial saliva contained 1.5 mM Ca, 0.9 mM P, 150 mM KCL, 0.05 μg F/mL, and 0.1 M Tris buffer, set to a pH of 7.0.[23]

Sample Allocation

Seventy enamel-dentin blocks were allocated into seven groups (n=10). The initial L* value of each sample was used to stratify and allocate specimens into all groups, because the L* value is a significant parameter when making comparisons under the study design,[24] which aimed to reduce the initial variability among the groups. The evaluation method of the L* coordinate is described below.

Toothpaste Treatment

The specimens were submitted to simulated brushing using toothbrush heads (Oral-B Indicator 40 Soft, Gillette do Brasil Ltd, Manaus, Brazil) coupled to an automatic tooth-brushing machine (Equilabor, Piracicaba, Brazil) with a static axial load of 200 *g* and a speed of 5 movements/second, at 37°C.[25,26] One month of tooth brushing was simulated using 825 cycles.

Enamel blocks were brushed with toothpaste slurry (1:3) or distilled water. Information on the toothpastes used, including manufacturers and components, are detailed in Table 1. Additionally, the pH of the toothpastes was determined in triplicate using a pH meter (Procyon, São Paulo, Brazil), and the fluoride concentration (total fluoride, ionizable fluoride, and total soluble fluoride) in

Table 2: *Fluoride Concentration and pH of Toothpastes*

Toothpaste	Fluoride	Manufacturer's information (ppm)	Total fluoride (ppm)	Total soluble fluoride (ppm)	Ionic fluoride (ppm)	pH
PN	NaF	1426	1426.8	1440.1	1416.4	7.26
FT	MFP	1450	1486.8	1081.7[a]	247.8[a]	9.44
PA	MFP	1450	1524.5	1154.5[a]	338.1[a]	9.07
SAN	MFP	1450	1390.7	1127.8[a]	161.0[a]	9.36
NM	MFP	1426	1412.3	1411.3	64.6[a]	9.64

Abbreviations: FT, fluoride as MFP; NM, bioactive glass; PA, 8% arginine; PN, potassium nitrate (with NaF).
[a] *Statistical difference between the fluoride present in toothpastes in relation to the manufacturer's declaration using a one sample* t*-test (*$p>0.05$*).*

the toothpastes was determined as previously described,[27] using a specific ORION 96-06 electrode and an EA 940 ion analyzer (Orion, Boston, MA, USA). These results are presented in Table 2. All samples were randomly divided into seven groups according to the treatments (n=10): brushing with distilled water and placebo gel (negative control [NC]); brushing with distilled water and bleaching with 35% HP (positive control [PC]); brushing with potassium nitrate toothpaste containing NaF and bleaching with 35% HP (PN); brushing with conventional sodium monofluorophosphate (MFP) fluoridated toothpaste and bleaching with 35% HP (FT); brushing with arginine-based toothpaste (8% arginine, Pro-Argin Technology) and bleaching with 35% HP (PA); brushing with arginine-based toothpaste (1.5% arginine, Sugar Acid Neutralizer™) and bleaching with 35% HP (SAN); and brushing with toothpaste containing bioactive glass (Novamin) and bleaching with 35% HP (NM).

After each brushing treatment, the specimens were washed with distilled water for 10 seconds and stored in artificial saliva for 24 hours, before each dental bleaching procedure.

Bleaching Procedure

The bleaching treatment was performed using 35% HP (Whiteness HP, FGM, Joinville, Brazil), according to the manufacturer's instructions. The bleaching agent was applied to the enamel surface three times for 15 minutes each. The negative control group was exposed to a treatment with a placebo gel (Proderma, Piracicaba, Brazil), composed of distilled water, neutralized carbopol, glycerin, and triethanolamine, which was buffered to pH 6.0 (which was similar to the initial pH of the commercial bleaching gel used in this study). The specimens were then washed with distilled water and analyzed. The initial and final pH of the bleaching agent was measured in triplicate: initial pH = 5.64; after 15 minutes, pH = 4.87.

Color Measurements

Color reading of each specimen was performed at an ambient light condition (GTI MiniMatcher MM 1, GTI Graphic Technology, New York, NY, USA) in standardized daylight. The color was measured using a spectrophotometer (CM 700d, Minolta, Osaka, Japan). The spectral distribution was measured based on the CIE L*a*b* system, using On Color software (Konica Minolta). L* represents the luminosity (white-black) axis, a* represents the green-red axis, and b* represents the blue-yellow axis. Before measurement, the spectrophotometer was calibrated using white and black reflectance standards, according to the manufacturer's protocol. The differences in the L*, a*, and b* values between initial (baseline) and final (after 24 hours of bleaching) were expressed (ΔL, Δa, and Δb), and any color change was calculated using the following equation: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Surface Roughness

For enamel roughness analysis (Ra), each sample was rinsed in distilled water prior to profilometer measurements. The Ra was analyzed using a profilometer at two times: before (baseline) and 24 hours after bleaching (Surf-Corder 1700, Kosaka, Tokyo, Japan). Three different equidistant directions were measured on the surface of each specimen, with a cutoff of 0.25 mm, a reading length of 1.25 mm, and a velocity of 0.1 mm/s.

Microhardness Analysis

The enamel surface microhardness (SMH) was analyzed after the treatments using a Knoop indenter with a load of 50 *g* and time of 5 seconds in a microhardness tester (HMV-2000, Shimadzu, Tokyo, Japan). Five indentations were made in each sample, 100 μm apart, and the average was calculated to determine the Knoop hardness number (KHN). For the cross-sectional microhardness

Table 3: *Mean (SD) for ΔL, Δa, Δb, and ΔE Based on Treatment Group (n=10)*[a]

Toothpaste	ΔL	Δa	Δb	ΔE
Negative control	0.14 (1.0)[b]	−0.14 (0.3)[ab]	0.72 (0.9)[b]	1.45 (0.5)[b]
Positive control	2.53 (1.0)[a]	−0.45 (0.4)[b]	−5.31 (0.8)[a]	5.96 (1.0)[a]
PN	2.37 (1.0)[a]	0.16 (0.5)[a]	−4.90 (0.8)[a]	5.54 (0.9)[a]
FT	1.85 (0.9)[a]	0.14 (0.4)[ab]	−4.90 (0.6)[a]	5.31 (0.7)[a]
PA	2.48 (1.4)[a]	−0.09 (0.3)[ab]	−5.48 (1.3)[a]	6.18 (1.2)[a]
SAN	2.41 (1.4)[a]	−0.06 (0.6)[ab]	−5.25 (1.3)[a]	5.92 (1.5)[a]
NM	2.42 (1.0)[a]	−0.10 (0.5)[ab]	−4.61 (1.3)[a]	5.27 (1.5)[a]

[a] *Identical lowercase letters indicate no significant difference (p>0.05) among different groups in the same column. Abbreviations: FT, fluoride as MFP; Negative control, unbleached; NM, bioactive glass; PA, 8% arginine; PN, potassium nitrate (with NaF); Positive control, bleaching with 35% hydrogen peroxide (HP); SAN, 1.5% arginine.*

(CSMH) tests, the specimens were longitudinally sectioned through the center, and one-half was embedded in acrylic resin, exposed, and gradually polished as previously described. Three columns of five indentations were made in the central area of the slab using the microhardness tester and a load of 50 *g* for 5 seconds, with measurements occurring 10, 25, 50, 75, and 100 μm from the enamel surface. The mean values at all three measuring points at each distance were then determined.

The indentations can change the topography of the enamel surface and modify the light reflectance pattern, with negative impact on color and roughness analysis. Therefore, the SMH was evaluated only at the final time point, and the experimental groups were statistically compared with control groups.

Statistical Analysis

After exploratory analysis using the SAS software (Release 9.1, 2003, SAS Institute Inc, Cary, NC, USA), the data were subjected to one-way analysis of variance (ANOVA) (SMH, ΔE, ΔL, Δb, and Δa), ANOVA using models for repeated measures (Ra), and split-plot ANOVA (CSMH) followed by the Tukey test, at a 5% level of significance. The analysis of fluoride concentration in toothpaste was performed using a one-sample *t*-test.

A power calculation of the color variables with the following parameters was performed: $\alpha = 0.05$ and a power setting of 0.8. The power calculation computed a sample size of a minimum of nine considering the difference values proposed by Alghazali and others[28] being 1.9 ΔE* units for assessment of perceptibility and 4.2 ΔE* units for clinical acceptability of color differences.

The relationship between the physical surface properties (Ra, SMH) and color properties (ΔE, ΔL, Δb, and Δa) was performed using a multivariate Canonical correlation analysis. The correlations were tested separately by the approximate F test and associated with Wilks' lambda, Pillai's trace, Hotelling-Lawley trace, and the Roy 's greatest root ($\alpha<0.05$).

RESULTS

When considering Table 2, the FT, PA, SAN, and NM groups presented a slightly alkaline pH. Overall, all groups showed that the total fluoride present in toothpastes was statistically similar to what the manufacturer declared ($p>0.05$). In relation to total soluble fluoride, only PN and NM showed no difference between the values that the manufacturer declared ($p>0.05$). Furthermore, the PN group demonstrated higher values for ionic fluoride concentration, which was similar to the manufacturer's information ($p=0.85$).

Based on color analysis (Table 3), the positive control statistically differed from the negative control for the ΔL, Δb, and ΔE values ($p<0.001$), with increasing L* values and decreasing b* values; the Δa values did not differ between the negative and positive controls. The toothpaste groups demonstrated a statistical difference for ΔL, Δb, and ΔE values compared with the negative control (unbleached). However, the Tukey test did not demonstrate a statistical difference between the toothpaste groups and the positive control, indicating that these toothpastes did not act directly on the ΔL, Δb, and ΔE values of the specimens during dental bleaching. The PN demonstrated Δa values that differed statistically from the positive control ($p<0.05$), although the ΔL, Δb, and ΔE values were similar to toothpaste groups and positive control.

From the means of roughness values (Table 4), the results of the positive control showed increased Ra values compared with the initial values and the

Table 4: *Mean (SD) for Initial and Final Roughness Values (Ra) Based on Treatment Group (n=10)*[a]

Toothpaste	Initial Ra	Final Ra
Negative control	0.11 (0.02)^{Aa}	0.10 (0.02)^{Ac}
Positive control	0.11 (0.01)^{Ba}	0.14 (0.01)^{Ab}
PN	0.11 (0.02)^{Ba}	0.15 (0.01)^{Ab}
FT	0.11 (0.01)^{Ba}	0.14 (0.03)^{Ab}
PA	0.11 (0.02)^{Ba}	0.13 (0.01)^{Ab}
SAN	0.11 (0.01)^{Ba}	0.23 (0.02)^{Aa}
NM	0.11 (0.02)^{Aa}	0.11 (0.02)^{Ac}

[a] *Means followed by different letters (uppercase in rows and lowercase in columns) are different by PROC-MIXED ANOVA and Tukey test ($p<0.001$). Abbreviations: FT, fluoride as MFP; Negative control, unbleached; NM, bioactive glass; PA, 8% arginine; PN, potassium nitrate (with NaF); Positive control, bleaching with 35% hydrogen peroxide (HP); SAN, 1.5% arginine.*

negative control ($p<0.001$), although no statistically significant difference was found between the PN, FT, and PA groups ($p>0.05$). Nevertheless, the negative control did not increase or decrease the surface roughness, with no statistically significant differences between times ($p>0.05$). In the toothpaste groups, the NM treatment did not affect the enamel roughness, with no statistical difference compared with the negative control or between times ($p>0.05$). In the groups comparison, NM showed lower Ra values that statistically differed from the other groups ($p<0.001$). Furthermore, the SAN group increased in surface roughness, which was statistically different from the initial values, the other toothpastes, and the control groups ($p<0.001$). All groups (with the exception of NM) showed statistically significant differences in relation to the negative control ($p<0.05$) and their respective initial Ra values ($p<0.05$).

In the positive control group, bleaching caused a statistically significant loss of SMH (Table 5, SMH results) compared with the negative control. In contrast, although bleached, the toothpaste groups increased in SMH, which was also statistically different from the negative control (unbleached) and positive control ($p<0.001$). FT promoted a slight increase in KHN values compared with the negative control while statistically differing from the SAN and PA groups (intermediary values). However, these groups did not differ from the PN group. The highest microhardness values were found in the NM group, which showed statistically different values compared with the positive and negative controls and the other experimental groups ($p<0.001$).

The results of CSMH (Table 5) showed that bleaching (negative control) reduced the microhardness of the positive control, up to a depth of 100 μm, whereas this depth was not statistically different between all groups ($p>0.05$). There were no statistically significant differences between the toothpaste groups and the negative control at all depths ($p>0.05$). All toothpaste groups presented improved CSMH values compared with the positive control at 10 μm, whereas only the FT, SAN, and NM groups were statistically different from the positive control at depths of 25 and 50 μm ($p<0.05$). PA and PN did not statistically differ from the negative and positive controls at depths of 25 and 50 μm ($p>0.05$). Finally, no experimental group differed from the positive or negative controls at depths of 75 and 100 μm. No differences were found among the toothpaste groups at all depths ($p>0.05$).

The results showed a high correlation between $\Delta E \times \Delta L$ ($r=0.74$, $p<0.0001$) and $\Delta E \times \Delta b$ ($r=-0.94$, $p<0.0001$). The Canonical correlation was based on the determination of orthogonal canonical variables, as these variables must be linearly independent. The

Table 5: Mean (SD) for Enamel Surface Microhardness (SMH) and Cross-sectional Microhardness (CSMH) Values Based on Treatment Group (n=10)[a]

Toothpaste	SMH	CSMH				
		10 μm	25 μm	50 μm	75 μm	100 μm
Negative control	350.5 (14.5)^{d}	395.6 (10.6)^{Aa}	395.3 (13.2)^{Aa}	402.8 (13.2)^{Aa}	396.3 (12.5)^{Aa}	395.7 (12.2)^{Aa}
Positive control	304.0 (20.7)^{e}	339.1 (17.6)^{Cb}	359.3 (18.4)^{Bb}	361.4 (21.0)^{Bb}	369.2 (18.5)^{Bb}	378.8 (16.2)^{Aa}
PN	395.5 (19.7)^{bc}	386.6 (15.0)^{Aa}	382.2 (21.1)^{Aab}	395.1 (15.9)^{Aa}	385.4 (9.7)^{Aab}	382.1 (12.1)^{Aa}
FT	376.7 (9.0)^{c}	387.6 (14.9)^{Aa}	390.1 (17.7)^{Aa}	385.0 (15.4)^{Aa}	382.0 (16.5)^{Aab}	387.3 (13.0)^{Aa}
PA	406.3 (17.6)^{b}	379.6 (15.3)^{Aa}	382.8 (15.4)^{Aab}	382.7 (20.8)^{Aab}	393.1 (19.2)^{Aab}	387.0 (16.9)^{Aa}
SAN	409.5 (16.3)^{b}	386.6 (15.5)^{Aa}	387.1 (9.7)^{Aa}	387.9 (11.8)^{Aa}	387.1 (13.2)^{Aab}	386.9 (11.6)^{Aa}
NM	436.3 (17.6)^{a}	394.0 (11.9)^{Aa}	393.7 (12.5)^{Aa}	391.8 (10.0)^{Aa}	387.3 (7.5)^{Aab}	388.0 (8.2)^{Aa}

[a] *Means followed by different letters (SMH, lowercase in column; CSMH, uppercase in rows and lowercase in columns) are statically different ($p<0.05$). Abbreviations: FT, fluoride as MFP; Negative control, unbleached; NM, bioactive glass; PA, 8% arginine; PN, potassium nitrate (with NaF); Positive control, bleaching with 35% hydrogen peroxide (HP); SAN, 1.5% arginine.*

correlation showed the necessity of eliminating the ΔE variable from the ultimate analysis. The data showed a significant canonical correlation (canonical axis) relating the physical surface variables and color variables (F=3.59; p=0.0025; p value of Wilk's lambda=0.0025). The variation in the color variables (ΔL, Δa, and Δb) explained 21% of the variation in the physical variables (Ra, SMH). The r values of color variables that influenced the physical surface properties were ΔL (r=0.2297), Δa (r=0.1509), and Δb (r=−0.4179).

DISCUSSION

The null hypotheses tested in this study were partially accepted because the bioactive glass-based toothpaste protected the enamel against the deleterious effects of HP, and no toothpaste affected the effectiveness of bleaching. In the present study, enamel blocks were obtained from bovine teeth. These specimens were used because their physical and chemical properties are very similar to those of human teeth, and young bovine teeth are considered a practical model for evaluating bleaching procedures.[29]

The effect of remineralization agents has been confirmed in previous investigations.[13-15,21-22] Fluoridation regimens with toothpaste or gel have been shown to be effective in increasing the enamel microhardness and preventing microhardness loss during bleaching.[14,15] Other studies have shown a similar beneficial effect for products with Pro-Argin[21] or bioactive glass (Novamin)[22] when used in conjunction with dental bleaching. Nevertheless, there are no studies that have evaluated the effects of different toothpastes prior to bleaching.

This study proposed the use of an active control with NaF (PN), because it has been proven with *in vitro* results,[30] which is important because fluoride is currently used as a remineralizing agent.[31] Additionally, the MFP toothpaste acts as a non-active control of arginine-based toothpastes (PA and SAN) and toothpastes containing bioactive glass (NM), because the sodium monofluorophosphate requires enzymatic hydrolysis to release free fluoride[32]; therefore, minimal effects have been described with *in vitro* studies. This current study model allows for the investigation of nonfluoride remineralization systems (PA, SAN, and NM) and a comparison with an efficient fluoridating agent (PN). Additionally, the fluoride concentration analysis (Table 2) showed that the actual fluoride present in the studied toothpastes was very similar to what was declared by the manufacturers. However, the MFP toothpaste has a small amount of free fluoride that acts on the enamel surface explaining the small increase in SMH found in the current study when comparing the FT group with the control groups.

From the results of the SMH and CSMH evaluations, bleaching with 35% HP led to a slight but significant decrease in superficial and cross-sectional enamel microhardness (with an exception at a depth of 100 μm) without prior application of toothpaste. This decrease in microhardness is possibly due to the acidity of the bleaching gel. The lower pH of the bleaching agent may cause demineralization of the enamel.[33] This finding is in accordance with a number of previous studies[8,9] that demonstrated a decrease in microhardness during dental bleaching. In the current study, the toothpaste treatment of bleached surfaces prevented a reduction in microhardness, with an observed increase in SMH, indicating an obvious protective effect of NM, arginine-based toothpastes (PA and SAN) or potassium nitrate toothpaste containing NaF, in this order.

When comparing the therapeutic effects of the arginine treatments (SAN and PA) prior to dental bleaching with the negative, positive, and toothpaste controls, there was an increase in SMH values. Arginine is an aminoacid,[34] with a slightly alkaline pH (Table 2). Based on the mechanism of action proposed by Kleinberg,[35] the combination of arginine and calcium carbonate may provide an alkaline environment that encourages endogenous calcium and phosphate ions to precipitate on dental tissues. Therefore, inorganic electrolytes contained in saliva (calcium, phosphate, fluoride) may be important participants in the remineralization/demineralization process associated with dental bleaching. However, there is a previous study that discussed the acid solubility of the arginine-carbonate deposits.[36] When calcium carbonate dissolves slowly, the released calcium is bioavailable to remineralize the tooth and the carbonate dissolution may give a slight rise in the local pH. This process might happen during the bleaching procedures, as the initial pH of the bleaching gel used in the current study was 5.64, which decreased after 15 minutes of application. This decrease to a lower pH is potentially related with enamel demineralization.[33] Thus, carbonate dissolution may have mediated less variation of pH in the bleaching gel, decreasing the effects of loss of CSMH and SMH in the SAN and PA groups. Although the arginine-carbonate technology has been reported to be useful

as a therapeutic agent when applied after dental bleaching,[21] more studies are required to investigate the role of the arginine-carbonate complex on the enamel properties associated with this treatment, especially *in situ* and *in vivo* studies.

A previous study suggests that the NM particles react with the enamel surface to increase the concentration of Ca and P ions, resulting in the repair of the demineralized surface.[22] It is important to note that NM is regarded as a highly biocompatible material and that it has the potential to prevent demineralization and increase remineralization.[19,37,38] In this current study, it is likely that the bioavailable NM on the enamel surface after toothbrushing reacted with saliva to release calcium (Ca^{2+}), phosphate (PO_4^{3-}), and sodium (Na^+) ions.[38] This process could allow for the deposition of free calcium and phosphate, together with nonreacted particles of bioactive glass, which would form a protective layer on the enamel surface. Burwell and others[19] reported that this precipitate could result in the formation of carbonate enriched hydroxyapatite, a mineral similar to that found in natural teeth, which might be potentiated with the association of fluoride. This mechanism would explain the higher SMH values or surface roughness results of this treatment group.

According to the results of this present study, the sole use of bleaching agent (positive control) increased the enamel surface roughness, as previously described.[5,7] One concern remains with regard to the roughness change due to abrasive toothpastes. However, the brushing protocol applied in this current study (1 month, 825 cycles) may be considered minimal compared with the protocol used for simulating 1 year of brushing (10,000 cycles).[26] This current study determined that brushing created minimal or no significant abrasive effects, with the only abrasive effects noted in the SAN group. Although the stated composition of the abrasive in toothpastes is similar between the FT, SAN, or PA and NP and NM groups, the degree of abrasiveness is associated with the size and characteristics of the particles, which can be different in different toothpastes, even if they use the same component.[39] The NM group did not suffer effects from the HP and tooth brushing, which was not different from the unbleached group, indicating that there was less mineral loss, possibly due to the protective effect previously described. Regardless of the dental bleaching effects, it may be considered that the level of abrasion or increase in surface roughness are clinically less relevant than other wear processes that could occur in the oral environment.

The alteration of physical properties is likely due to the demineralization effects that are caused by the diffusion of HP and the acidic pH of the bleaching gel.[33,40] Furthermore, changes in the topography can promote a surface alteration (increase in roughness) associated with a modification of light reflectance,[41] because the observed color of the tooth is a combination of optical phenomena, including the light reflected from the enamel surface, transmitted light, and the degree of light absorption of the dental substrates.[42]

The application of toothpastes prior to dental bleaching presented an effect on the enamel properties in the present study; however, the toothpastes did not affect the effectiveness of the bleaching treatments, because all bleaching groups demonstrated increasing L* values and decreasing b* values while also presenting ΔE values that were statically similar to the positive control (only bleached). These results support a safe indication for toothpastes, especially for those that contain potassium nitrate, which was proposed by Haywood and others[16] for controlling sensitivity during dental bleaching.

In the present study, the variable correlation was statistically confirmed using multivariate Canonical correlation analysis, which demonstrated a correlation of 21% between the color and physical properties. These findings indicated that specimens with low Δb and high ΔL and Δa were associated with a rougher surface and an increase in microhardness. Therefore, the present results showed that dental bleaching (increased L* values and decreased b* values) promoted an increase in roughness. In addition, the direct correlation of 21% was not high due to the roughness variable, whereas the negative control (unbleached) and bioactive glass-based toothpaste group (NM) did not present with alterations in the surface roughness. Conversely, the correlation of high microhardness could be associated with the study model design and with the presence of toothpaste use. Thus, dental bleaching, when not associated with toothpaste treatment, promoted mineral loss and a decrease of microhardness (positive control). The correlation analysis is an important method for evaluating hypotheses, principally those that are associated with different physical properties of enamel. Within this context, the bleaching procedure promoted simultaneous changes in reflectance color, roughness, and enamel mineral loss. The multivariate correlation analysis is

a breakthrough for the study of properties and their relationship in *in vitro* models. The evaluation of physical properties and the correlation between the results of this study are very important for improving the bleaching procedure to develop a safer and more effective approach.

It can be concluded that bleaching with 35% HP resulted in morphologic changes and a significant loss of microhardness of enamel. Therefore, toothpaste treatment could decrease these changes to the enamel properties. Among these compounds, arginine-based toothpastes and potassium nitrate toothpaste (with NaF) increased superficial microhardness and did not affect dental bleaching. The toothpaste containing bioactive glass did not provide an increase in surface roughness. Furthermore, NM promotes an enhancement of the microhardness values compared with unbleached enamel, demonstrating a potential benefit for bleaching therapy.

CONCLUSION

The application of toothpaste prior to dental bleaching did not interfere with the effectiveness of treatment. The bioactive glass-based toothpaste protected the enamel against the deleterious effects of the whitening procedure.

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Conflict of Interest

The authors 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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