Original Article

Effect of the calcium silicate and sodium phosphate remineralizing products on bleached enamel

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Abstract

Context and Aims: This study evaluated the effect of calcium silicate and sodium phosphate (CSSP) dentifrice and serum on the surface of enamel bleached with hydrogen peroxide (H_2O_2).

Materials and Methods: A total of 160 bovine enamel slabs were bleached with 35% H_2O_2 and treated with sodium fluoride (NaF) dentifrice-GI, CSSP dentifrice-GII; CSSP dentifrice + CSSP serum-GIII, or NaF dentifrice + NaF gel-GIV. The dentifrices were applied using a brushing machine three times daily for 7 days. After brushing, sodium phosphate gel and CSSP serum were applied. The microhardness (KNH, $n = 14$), surface roughness (Ra, $n = 14$), energy dispersive spectroscopy ($n = 6$), and scanning electron microscopy ($n = 6$) were assessed at t_0 (before bleaching), t_1 (after bleaching), and t_2 (after postbleaching treatments).

Statistical Analysis Used: The data were subjected to a two-way analysis of variance and Bonferroni's test.

Results: The KNH decreased at t_1 ($P < 0.001$) but recovered at t_2 for all treatments, although only GII showed restored baseline values ($P = 0.0109$). The surface roughness increased at t_1 ($P < 0.001$) and reduced at t_2 ($P < 0.001$) for all groups, with no significant differences among groups. Enamel composition and morphology did not differ after the treatments, except for silicon accumulation in GIII.

Conclusions: Postbleaching treatment with CSSP dentifrice and serum yielded superior remineralizing effects on bleached enamel.

Keywords: Calcium silicate; dental bleaching; enamel; sodium phosphate

INTRODUCTION

Dental bleaching is a procedure mainly used for esthetic purposes.[1-3] In office settings, the most common techniques involve applying concentrated hydrogen peroxide (H_2O_2) gels to enamel due to their practicality and effectiveness, even for limited periods or on discolored teeth.[4]

However, the enamel surface is not immune to these applications, and numerous adverse effects have been reported, including erosion, decreased microhardness (KNH), increased dental sensitivity, surface roughness, porosity, tooth wear, and staining susceptibility.[5-8] Diminished KNH may lead to clinical defects, including compromised shear bond strength of restorations, which can compromise their longevity.[6] In addition, enamel demineralization due to bleaching can cause greater biofilm retention, increase the risk of caries, and cause discomfort.[9,10] Although saliva is important for remineralization, its actions may not be sufficiently rapid.[11]

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To prevent and reverse these damaging impacts, studies have investigated the potential benefits of the use of fluoridated gels and dentifrices.[12,13] The main goal of using substances such as sodium fluoride (NaF), hydroxylated apatite, nano-hydroxyapatite, calcium gluconate, or casein phosphopeptide amorphous calcium phosphate is to form deposits of ions that can remineralize enamel and produce fluorapatite and fluoridated hydroxyapatite particles that can be incorporated into its composition.[12] However, the application of 2% NaF has not been shown to fully recover enamel KNH after bleaching procedures. The use of common fluoridated gels before, during, or after bleaching has also not shown better effects.[14,15]

Aiming to provide an alternative solution, different mechanisms of enamel remineralization have been created, including a dentifrice and serum containing calcium silicate and sodium phosphate (CSSP). *In vitro* and *in situ* studies have tested the effects of these substances on eroded enamel, with promising results. Evaluation of the remineralizing effects of the CSSP dentifrice and serum in erosive challenges showed their capability to red-harden enamel and form hydroxyapatite crystals.[16-18] In this context, the use of these substances may have beneficial effects on bleached enamel. However, few studies have evaluated the effects of CSSP dentifrice and serum on the serum properties of bleached enamel.[19,20] Assessment of the remineralizing potential of the CSSP serum and dentifrice for enamel KNH, staining, and abrasion susceptibility showed recovery of enamel KNH and reduced susceptibility to wear and color alteration.[19] Furthermore, a single clinical study showed that the use of CSSP dentifrice reduced dentine hypersensitivity and was more effective than sodium monofluorophosphate dentifrice.[20]

However, more studies are needed to better understand the effects of CSSP products on enamel properties. The objective of this study was to evaluate the effect of CSSP dentifrice and serum on the surface of enamel bleached with H_2O_2. Four null hypotheses were designed: (1) bleaching and postbleaching treatments would not alter enamel KNH; (2) surface roughness would remain unaltered after bleaching and postbleaching procedures; (3) enamel mineral composition values during the testing periods would not change; and (4) bleaching would not cause alterations in the enamel morphology and postbleaching treatments would not cause further changes.

SUBJECTS AND METHODS

Sample size

A pilot study with $n = 3$ was conducted to estimate the number of dental specimens required to observe differences among at least one experimental group. The literature was also checked to estimate the effect size.[19] Power analysis was performed using G*Power software (Heinrich Heine University, Düsseldorf, Bundesland, Germany) (alpha = 0.05; power = 0.85), which indicated a requirement for a minimum of 13 specimens.

Specimen preparation

Bovine teeth were cleaned and stored in 0.1% thymol solution at 4°C during preparation. The teeth were cut using a diamond disk and a cutting machine (IsoMet 1000, Buehler, Lake Bluff, Illinois, USA) to produce 160 square fragments (2 per tooth) (5 mm length × 5 mm width × 3 mm thickness) from their labial surfaces.

Fragments were polished with Silicon (Si) Carbide Sandpaper (Norton, São Paulo, Brazil) to reduce granulation (#600, #800, #1200) for 60 s under cooling water (APL-4; Arotec, Cotia, São Paulo, Brazil). They were then further polished with alumina pastes (Arotec, São Paulo, Brazil) at various granulation levels (6, 3, 1, and 0.25 µm) using polishing felt (ATM, Germany) and cleaned with deionized water in an ultrasonic bath (Ultrasonic Cleaner, Ribeirão Preto, SP, Brazil). Tooth fragments were embedded in PVC molds and filled with self-curing acrylic resin (JET-Clássico, Campo Limpo Paulista, SP, Brazil), with the enamel surface outward.

The measurements were taken for all groups at three different periods: T_0 (baseline-without bleaching), t_1 (after bleaching), and t_2 (posttreatments).

Bleaching procedure

Specimens were stored in artificial saliva (DaTerra, Ribeirão Preto, São Paulo, Brazil) containing 2 g Methyl-p-hydroxybenzoate, 10 g sodium carboxymethylcellulose, 0.625 g KCl, 0.059 g MgCl2.6H2O, 0.166 g CaCl2.2H2O, 0.804 g K2HPO4, and 0.326 g KH2PO4 per liter. pH was adjusted to 6.75 using KOH, and specimens were kept at 37°C until bleaching. Enamel surfaces were bleached with 35% H_2O_2 gel (Whiteness HP 35%, FGM, Joinville, Santa Catarina, Brazil). The gel was mixed following the manufacturer's 3:1 ratio, applied in three 15-min sessions with stirring to prevent bubbles, and rinsed during 5-min intervals.

Experimental groups

The specimens were divided into four groups ($n = 20$), according to the postbleaching applications: GI: NaF (1450 ppm F) dentifrice (Colgate Total®12, Colgate-Palmolive, Osasco, São Paulo, Brazil); GII: CSSP dentifrice (Regenerate Enamel Science, Unilever, Le Meux, France); GIII: CSSP dentifrice and CSSP serum; and GIV: NaF (1450 ppm F) dentifrice and 2% NaF gel (Fluogel Neutral 2%, DFL. Rio de Janeiro, Rio de Janeiro, Brazil).

Surface treatments

Different treatments were applied to each group. Dentifrices were applied using soft toothbrush heads (Slim Soft, Colgate-Palmolive, Osasco, São Paulo, Brazil) with a 200 g load, placed in a brushing simulation machine (Biopdi, São Carlos, São Paulo, Brazil). Specimens underwent 7 days of brushing, three times daily, at two movements per second for 120 s each, totaling 4 min and 40 s, equivalent to 1 month of brushing.[21] Specimens were immersed in a slurry of toothpaste and distilled water (1:2 weight ratio), changed, and rinsed with distilled water every minute. During intervals, specimens were kept in artificial saliva at 37°C.

Remineralizing gel and serum were applied on the third and final day of brushing, totaling three 3-min applications. Two percent NaF gel was applied to the enamel using an extra-fine microbrush (KG Sorensen, Cotia, São Paulo, Brazil). The CSSP serum (Regenerate Boosting Serum, Unilever, Le Meux, France) consists of two tubes, an activator gel and a serum gel, equal parts mixed and applied using the provided instrument. Specimens were cleaned with a microbrush dampened with distilled water posttreatment.

Microhardness test

Before the KNH test, all specimens were inspected using an optical microscope to detect any possible fractures and then subjected to an ultrasonic bath with deionized water for 2 min (Ultrasonic Cleaner, T-1449-D, OdontoBras, Ribeirão Preto, SP, Brazil). The KNH was measured using a tester (HMV-2000; Shimadzu KNH tester, Kyoto, Japan) and a Knoop indenter on the enamel surface, at a static load of 50 g and a 10-s dwell time. Three tests were performed on each specimen, with the indentations made perpendicular and within 100 μm of each other. The median value was calculated and used for the statistical analysis.

Surface roughness test

The analysis was conducted using a confocal laser microscope (LEXT OLS 4000, Olympus, Tokyo, Japan). The specimens were placed parallel to the microscope table and images were taken at ×20. After the images were obtained, the surface roughness was evaluated, and the data were given in μm using the software provided by the microscope manufacturer.

Energy dispersive spectroscopy and scanning electron microscopy

For these tests, specimens were prepared as described but not embedded in PVC molds. Instead, they were placed in PLEXIGLAS® acrylic plates (Darmstadt, Germany) and underwent the same surface treatments. After treatments, five specimens per group were selected and immersed in 2.5% glutaraldehyde solution with 0.1M sodium cacodylate trihydrate (pH 7.4) for 12 h at 4°C. Subsequently, they were washed in an ultrasonic bath (Ultrasonic Cleaner, T-1449-D, OdontoBras, Ribeirão Preto, SP, Brazil) for 10 min and dehydrated using increasing ethanol concentrations: 25%, 50%, and 75% for 20 min each, followed by 95% and 100% for 60 min each.

The scanning electron microscope (EVO 50; Carl Zeiss, Cambridge, England) used an accelerating voltage of 20 kV, 10 nA beam current, and a 10 mm working distance. EDS analysis involved two random points per sample to determine mean values of oxygen (O), phosphorus (P), and calcium (wt%). Specimens were fixed on metal stubs with adhesive tape and gold-coated for 120 s before scanning electron microscopy (SEM) analysis. Representative images depicting treatment effects on enamel were captured at ×10,000.

Data analysis

Statistical analysis utilized IBM SPSS Statistics, version 25.0 (IBM Corp., New York, USA). Data distributions were assessed with Shapiro–Wilk tests, and sphericity was checked with the Mauchly test at $\alpha = 5\%$. The two-way ANOVA (posttreatment vs. periods) and Bonferroni posttests ($\alpha = 5\%$) analyzed KNH, surface roughness, and O, P, and Ca percentages. SEM images were examined by two calibrated examiners ($\kappa > 0.9$).

RESULTS

Microhardness

The data analysis showed no significant differences in the baseline KNH values among the tested groups. However, after bleaching, all groups showed significant reductions ($P < 0.0001$). The values increased after surface treatment ($P < 0.0001$). CSSP dentifrice + serum was the only surface treatment that showed a return of the KNH to baseline values. The interaction between the factors was significant ($P = 0.0109$). The mean values and standard deviations of the KNH data are presented in Table 1.

Surface roughness

No differences in baseline surface roughness values were observed. The roughness values increased after bleaching ($P < 0.0001$) in all groups. Every surface treatment tested in this study showed a statistically significant decrease in values between t_2 and t_1 values ($P < 0.0001$). In addition, a significant interaction was observed between the factors ($P = 0.0344$). The mean values and standard deviations of the surface roughness for all groups are shown in Table 2.

Energy dispersive spectroscopy

The relative percentage weight values for all the elements tested, as well as the Ca/P ratio, are shown in Table 3. Neither the bleaching nor the surface treatments affected the O%, Ca%, P%, and Ca/P ratios among GI, GII, and GIV. Although the software was unable to identify quantifiable amounts of other elements, the graphics indicated an accumulation

Table 1: Microhardness (Knoop hardness number) mean values (standard deviations) of experimental groups after the different periods

Groups	Without bleaching (t_0)	After bleaching (t_1)	Posttreaments (t_2)
NaF dentifrice	372.0±25.36^{A,a}	195.2±15.99^{C,a}	273.7±53.07^{B,b}
CSSP dentifrice	366.1±22.02^{A,a}	209.9±39.88^{C,a}	258.0±43.64^{B,b}
CSSP dentifrice + serum	342.3±39.05^{A,a}	223.4±42.55^{B,a}	331.7±57.03^{A,a}
NaF dentifrice + NaF gel	355.8±23.37^{A,a}	201.1±36.02^{C,a}	280.1±33.14^{B,b}

Values followed by different upper-case letters in rows presented significant differences ($P<0.05$)· Values followed by different lower-case letters in columns presented significant differences ($P<0.05$). NaF: Sodium fluoride· CSSP: Calcium silicate sodium phosphate· KHN: Knoop hardness number

Table 2: Surface roughness (μm) mean values (standard deviations) of experimental groups after the different periods

Groups	Without bleaching (t_0)	After bleaching (t_1)	Posttreaments (t_2)
NaF dentifrice	0.362±0.178^{A,a}	0.539±0.070^{B,a}	0.345±0.141^{A,a}
CSSP dentifrice	0.218±0.063^{A,a}	0.566±0.070^{B,a}	0.183±0.107^{A,a}
CSSP dentifrice + serum	0.233±0.059^{A,a}	0.432±0.142^{B,a}	0.327±0.087^{A,a}
NaF dentifrice + NaF gel	0.157±0.039^{A,a}	0.576±0.098^{B,a}	0.303±0.076^{A,a}

Values followed by different upper-case letters in rows presented significant differences ($P<0.05$), Values followed by different lower-case letters in columns presented significant differences ($P<0.05$). NaF: Sodium fluoride, CSSP: Calcium silicate sodium phosphate

Table 3: Percentage values atomic percentage (wt%) (standard deviations) of calcium· phosphorous· and oxygen in enamel composition after the different periods

Chemical elements	Without bleaching	Only bleached	NaF dentifrice	CSSP dentifrice	CSSP dentifrice + serum	NaF dentifrice +2% NaF
Oxygen	46.99±2.01	42.66±2.54	49.24±1.89	50.20±2.21	50.38±1.24	51.23±2.45
Phosphorous	20.10±0.47	21.03±0.22	19.20±0.74	19.43±0.78	21.57±1.47	18.75±1.51
Ca	32.89±1.53	36.31±2.32	31.55±1.15	30.35±1.43	30.02±2.79	30.02±3.72
Ca/phosphorous	1.70±0.03	1.78±0.008	1.71±0.03	1.64±0.006	1.60±0.03	1.67±0.05

Ca: Calcium· NaF: Sodium fluoride· CSSP: Calcium silicate sodium phosphate

of Si in the specimens in GIII (CSSP dentifrice + CSSP Serum) [Figure 1].

Scanning electron microscopy

SEM images at ×10,000 are shown in Figure 2. The evaluation of images achieved a kappa coefficient with perfect agreement (intra-examiner kappa A = 1, B = 1; inter-examiner kappa A × B = 0.96). No significant morphology difference was found on the specimens' surfaces. All slabs showed a regular and homogeneous surface, with small and shallow irregularities and a smear layer covering the enamel surface. A protective layer consisting of the apparent accumulation of calcium fluoride deposits was observed in GI and GIV and silanol (Si-OH) in GII and GIII due to the presence of calcium silicate. Dentifrice particles were also observed.

DISCUSSION

Dental bleaching can alter enamel's mineral and mechanical properties, affecting KNH, a key test for such evaluations.[4,8] Various factors, including exposure to acidic environments, can disrupt these properties, leading to demineralization.[22] Enamel demineralization from bleaching primarily stems from ionic differences between the enamel and the bleaching solution.[23] This interaction results in the exchange of substances like calcium, phosphate, and hydroxyl ions.[1,10] The undersaturation of the bleaching solution compared to enamel prompts ion release to attain equilibrium, depending on calcium availability and pH levels.[19] Such reactions can significantly impair enamel's mechanical performance. The use of remineralizing agents aims to rectify the chemical imbalance induced by bleaching and offer beneficial effects.[24,25]

Acidic challenges can decrease enamel KNH by 25%,[26,27] with lower pH gels exacerbating KNH loss and damage.[11,28] The reduction in KNH led to increased susceptibility to dental wear and abrasion after tooth brushing simulations.[19] Fluoridated substances help create a saturated condition for enamel, reducing mineral component loss.[29] Saliva's mineralizing capabilities, although effective, may take time to restore enamel to ideal conditions, posing risks for further demineralization, especially in patients with certain habits or using abrasive toothpaste.[11,15]

In our study, alterations in KNH values were observed, likely due to the nonspecific mode of action of bleaching agents, leaving behind reactive oxygen species that oxidize proteins and affect the enamel's inorganic counterpart.[10] Thus, CSSP remineralizing dentifrices and gels showed positive results in restoring KNH,[12] although not reaching control group levels.[19] A comparison of the efficacy of CSSP dentifrice/serum and a bioactive glass dentifrice in enamel bleached with a fluoridated H_2O_2 gel (10.000 ppm) found that the CSSP combination provided better surface remineralization.[30] This result may have been due to boosting the remineralizing properties of the bleaching gel.[31] All surface treatments in our study increased KNH

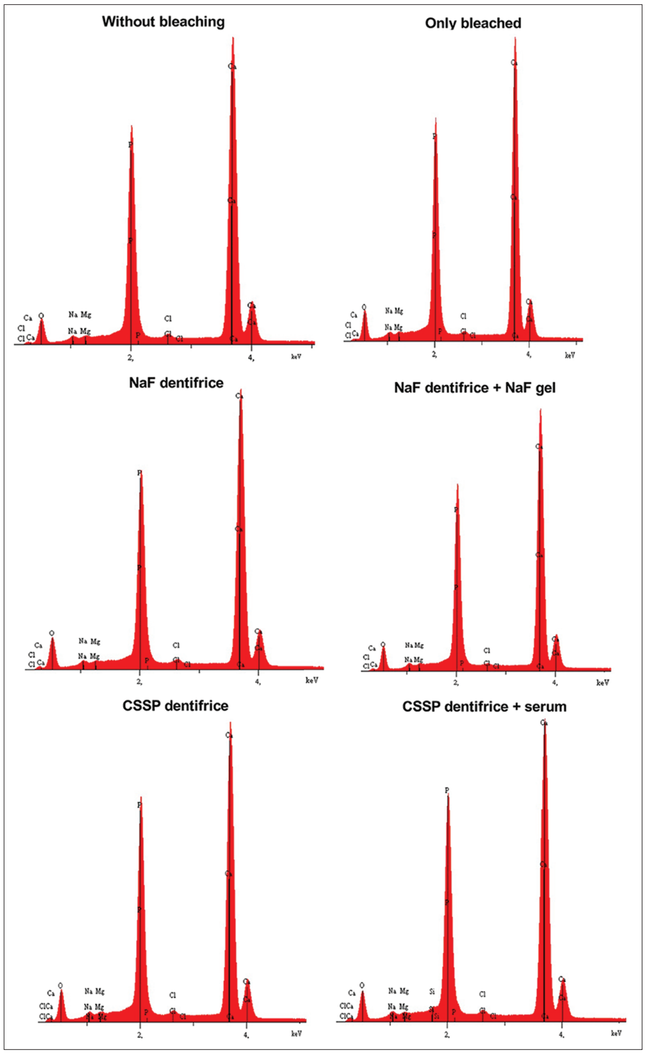

Figure 1: Energy dispersive spectroscopy analysis conducted in the present study: Without bleaching; after bleaching; sodium fluoride (NaF) dentifrice; NaF dentifrice + NaF gel; CSSP dentifrice; CSSP dentifrice + serum. CSSP: Calcium silicate and sodium phosphate, NaF: Sodium fluoride

values, with the CSSP dentifrice + serum combination being the sole treatment to restore KNH to baseline values, rejecting the null hypothesis. This may be attributed to CSSP's mode of action, forming silanol deposits on enamel, attracting calcium ions for remineralization, and hydroxyapatite crystal formation.[12,18,19,32] The salts originating from the combination of CSSP also have a great affinity to the enamel surface, reinforcing its ability to provide remineralization.[9,30] EDS and SEM analyses confirmed these findings.[32,33]

Furthermore, a reduction in abrasive wear was previously observed after simulated tooth brushing,[19] with no difference between CSSP dentifrice/gel and fluoridated products. These findings are consistent with those in the current study, in which CSSP dentifrice + serum showed efficacy. This was a result of the significant interaction

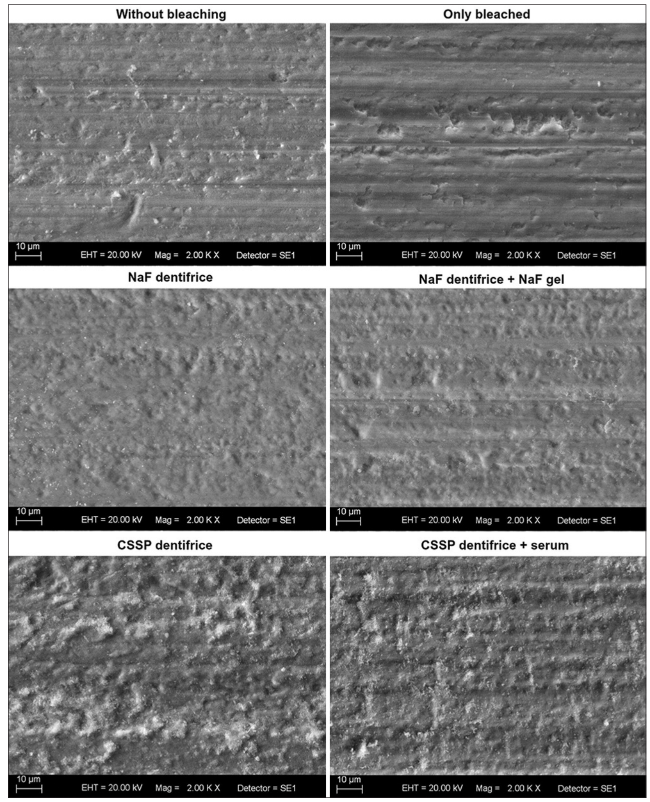

Figure 2: Scanning electron microscopy images of the enamel surfaces: Without bleaching; only bleached; sodium fluoride (NaF) dentifrice; NaF dentifrice + NaF gel; CSSP dentifrice; CSSP dentifrice + serum. CSSP: Calcium silicate and sodium phosphate, NaF: Sodium fluoride

in the KNH test. CSSP products were also effective in reducing mineral loss and dentin permeability compared to fluoridated treatments under citric acid exposure, suggesting their protective role.[34] However, CSSP did not offer protection against abrasive-erosive wear.[35]

Increased surface roughness favors bacterial colonization, increasing biofilm formation,[36,37] and tooth staining susceptibility.[38] Dental bleaching is linked to increased porosity and enamel crystal distribution changes.[38] Our study's postbleaching treatments effectively reduced roughness, aligning with CSSP products' remineralization potential. These findings are supported by studies that reported comparable results using CSSP dentifrice and serum on bleached enamel, as well as other methods of enamel remineralization.[30,36] The null hypothesis was rejected, likely due to surface homogenization postbrushing simulation. The large particle size and fluoride in CSSP products may have masked their effectiveness.[31,34,39] The presence of fluoride at concentrations associated with remineralization may have masked the otherwise more effective use of the CSSP products. Although another study utilizing a nonfluoridated dentifrice showed worse protective effectiveness compared to CSSP in eroded enamel,[40] the same outcome cannot be extrapolated to bleached teeth. Future studies should consider nonfluoridated dentifrices and control groups for a clearer assessment of CSSP's potential.

The Ca/P ratio assesses mineralized tissue composition,[41] but the impact of enamel bleaching on it varies in the literature.[42-44] Few studies have evaluated the effect of the application of remineralizing treatments on mineral content. The application of dentifrices containing bioactive glass and arginine-carbonate after bleaching prevented mineral loss.[41] The use of CSSP dentifrice and serum, as well as bioactive glass, also increased the Ca/P ratio after bleaching.[30] Another study found that the use of H_2O_2 gels, with or without calcium, did not interfere with or cause a loss of calcium or phosphorous in enamel.[43] The current study also found no significant changes postbleaching and treatment, supporting the null hypothesis, likely due to short H_2O_2 exposure and concentrated fluoride in all groups.

In our study, despite the potential for bleaching agents to cause irregularities on enamel surfaces,[45] we observed a homogeneous surface with minor irregularities. Interestingly, SEM images differed from previous studies,[18,31,32] showing a protective layer in CSSP groups, possibly indicating the effectiveness of CSSP dentifrice and serum gel.[32] This layer likely facilitated hydroxyapatite nucleation and ion balance restoration, as well as reduced dentin permeability, as seen in another study.[35] Clinical use of CSSP dentifrice for dentine hypersensitivity yielded better results than fluoride,[20] suggesting the advantages of calcium silicate-containing products.

Definitive correlation between mechanical and morphological tests on enamel and clinical outcomes remains elusive, despite observed bleaching effects potentially leading to impractical results. Given the limitations of this *in vitro* study and current literature, utilizing CSSP remineralizing agents could benefit bleached teeth by mitigating KNH loss better than NaF products. CSSP also demonstrated effectiveness against erosion and dentine hypersensitivity. Nonetheless, further clinical investigations are imperative to evaluate factors like saliva's influence accurately.

CONCLUSIONS

Considering the limitations of our study, we have drawn the following conclusions:

- Bleaching reduced KNH and increased roughness values while remineralizing had the opposite effect. The application of CSSP dentifrice + serum treatment restored KNH to baseline levels
- Neither bleaching nor remineralizing treatments affected enamel mineral content according to EDS analysis. However, SEM revealed that CSSP dentifrices and gels might form a protective enamel layer.

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Conflicts of interest

There are no conflicts of interest.

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