©Operative Dentistry, 2019, **44-1**, 76-87

Effect of Remineralizing Gels on Microhardness, Color and Wear Susceptibility of Bleached Enamel

E Crastechini • AB Borges • CRG Torres

Clinical Relevance

Tooth bleaching reduces enamel microhardness and increases tooth-wear susceptibility. The use of remineralizing gels during bleaching procedures recovers enamel microhardness and reduces abrasive wear, increasing bleaching safety without compromising efficacy.

SUMMARY

Objectives: To evaluate the effect of a remineralizing gel combining fluoride and calcium silicate/phosphate or a sodium fluoride gel on bleached enamel microhardness, color, and wear susceptibility.

Methods and Materials: Two hundred forty bovine enamel-dentin samples were prepared. Baseline analysis of Knoop microhardness, color coordinates (L*a*b*), and surface profile were performed. According to the baseline microhardness values, specimens were stratified into six groups (n=40): NC (negative control)—no treatment; BL (positive control)—bleaching with 40% hydrogen peroxide gel (Opalescence Boost, Ultradent); BL/Rs—bleaching + application of calcium silicate/phosphate gel (Regenerate Serum, Unilever - Rs); Rs/BL—Rs + bleaching; Rs/BL/Rs—Rs + bleaching + Rs; and BL/F—bleaching + 2% sodium fluoride gel. After the treatment described for each group, color change (ΔE) and microhardness were evaluated again. To evaluate abrasion susceptibility, samples were randomly divided into two subgroups, according to the toothpaste used (Cp—Close Up or Rt—Regenerate), and underwent 100,000 brushing strokes. The profile of each sample was evaluated and the mean wear calculated. The data were analyzed by ANOVA and Tukey tests.

Results: All bleached groups showed a significant reduction of microhardness in relation to the negative control. The groups treated with remineralizing gels showed a significantly higher microhardness and less wear than the positive control, although nonsignificant differences were observed among them. Nonsig-

Érica Crastechini, DDS, MSc, PhD, São Paulo State University—UNESP, Institute of Science and Technology, Department of Restorative Dentistry, Avenida Engenheiro Francisco José Longo, 777, Jardim São Dimas, São José dos Campos, Brazil

Alessandra Bühler Borges, DDS, MSc, PhD, associate professor, São Paulo State University—UNESP, Institute of Science and Technology, Department of Restorative Dentistry

*Carlos Rocha Gomes Torres, DDS, PhD, associate professor, São Paulo State University—UNESP, Institute of Science and Technology, Department of Restorative Dentistry, São José dos Campos, Brazil

*Corresponding Author: Avenida Engenheiro Francisco José Longo, 777, Jardim São Dimas, São José dos Campos, SP 12245-000, Brazil; e-mail: carlosrgt@ict.unesp.br

DOI: 10.2341/17-150-L

nificant differences in ΔE were found among bleached groups. The groups brushed with Regenerate toothpaste showed significantly less wear than those brushed with Close Up toothpaste.

Conclusions: The remineralizing gels did not interfere with bleaching efficacy. However, all the treatments minimized the surface hardness reduction caused by the bleaching procedure and enamel loss after abrasion. Regenerate toothpaste resulted in less enamel abrasion.

INTRODUCTION

Some studies have demonstrated that tooth bleaching agents can produce changes in the enamel surface properties,[1-6] while others showed no alterations.[7,8] The changes were attributed to the low pH of bleaching gels,[2,3,9] but other studies reported that even neutral pH bleaching gels can cause morphological alterations in the enamel surface.[1,10] These changes were characterized by increased surface porosity, degradation of the organic matrix, and loss of calcium and phosphate, causing reduction in surface microhardness.[1,4-6] This reduction may increase enamel wear produced by tooth brushing. The intensity of this wear depends on the pH of the bleaching gels, the abrasive potential of the toothpaste, and the timing of tooth brushing after bleaching.[11-13] All these factors lead to increased surface roughness and staining susceptibility.[14]

With the intention of reversing the demineralizing effects of bleaching agents on dental enamel, several studies have investigated the effects of treatment using remineralizing gels after bleaching to recover enamel surface microhardness. Many substances were tested, such as sodium fluoride,[15,16] nanohydroxyapatite, hydroxylated apatite, and casein phosphopeptide-amorphous calcium phosphate.[17] Fluoride is widely used in postbleaching treatment, since fluoride-containing agents promote the deposition of calcium fluoride on the enamel surface.[18] This increases the incorporation of fluoride ions into the demineralized area in the form of fluorapatite or fluoridated hydroxyapatite.[19,20]

Other remineralizing substances have been added to bleaching gels in an attempt to protect the enamel surface. Borges and others[13] added 0.5% calcium gluconate to the bleaching gel and concluded that this supplementation reduced surface wear by abrasion. In addition, da Costa and Mazur[21] tested different carbamide peroxide bleaching gels containing 0.11% sodium fluoride or amorphous calcium phosphate (ACP), as well the effect of subsequent application of 1.23% acidulated phosphate fluoride gel for 5 minutes after tooth bleaching. They concluded that fluoride or ACP in the bleaching agent did not prevent reduction in microhardness after bleaching. However, the final application of a fluoride gel led to significant microhardness recovery.

Recently, an enamel remineralizing system has been described, indicated for treatment of dental erosion. It consists of a specific association of calcium silicate and sodium phosphate salts with fluoride ions. The same active ingredients are available as a gel (boosting serum) and as a remineralizing toothpaste.[22-24] Previous investigators, using this system and comparing it with the use of regular fluoride toothpaste after erosive cycles, have concluded that gel application followed by the use of remineralizing toothpaste produced a significantly higher enamel remineralization than regular toothpaste alone.[22-24] It was also shown that this system formed hydroxyapatite on the surface of the demineralized enamel.[25] Considering the remineralizing potential of this product, it can be speculated that the application of this remineralizing gel immediately before or after dental bleaching may be favorable for protecting or remineralizing.

Considering that bleaching may promote demineralization and reduction of enamel microhardness, thus increasing its abrasion susceptibility,[1,4-6] and that some postbleaching treatments may remineralize the enamel surface, the incorporation of this step in the bleaching protocol seems highly advisable.[15,16] In this sense, the use of fluoride or other remineralizing agents that induce deposition of hydroxyapatite on the demineralized surface is quite promising.[15,16]

The aim of this study was to evaluate the protective and remineralizing potential of the new calcium silicate/phosphate system and the 2% sodium fluoride gel on bleached enamel microhardness and wear with different toothpastes. The interference of remineralizing agents on the bleaching efficacy was also investigated. The null hypothesis tested was that treatment with remineralizing gels would not result in significant differences in relation to enamel microhardness reduction, color change (ΔE), or wear after bleaching and that the kind of toothpaste would not influence abrasive wear.

METHODS AND MATERIALS

Specimen Preparation

Freshly extracted bovine incisors were obtained from animals with an average age of 3 years. The teeth were cleaned and stored in a 0.1% thymol solution, pH 7.0, throughout the period of preparation of the enamel-dentin specimens.[26]

Two hundred forty cylindrical specimens (enamel-dentin) were obtained from the labial surface of the teeth. They were cut with a diamond trephine mill having a 3-mm internal diameter. All specimens were embedded in acrylic resin using a silicone mold 6 mm in diameter and 3.1 mm deep. At the bottom of the mold, there was a cavity on the second level 3 mm in diameter and 0.1 mm deep. In the lateral area of the mold, there was a curved projection for producing a lateral groove in the specimen, allowing for correct positioning of the sample in the profilometer. The specimens were placed inside this internal cavity with the enamel surface facing the bottom of the mold. The mold was then filled with self-curing acrylic resin (Classico, Campo Limpo Paulista, SP, Brazil) and taken to a pressure chamber (City, Guarulhos, SP, Brazil), filled with water, and subjected to a pressure of 30 psi for 10 minutes until total polymerization. The resin in which the samples were embedded worked as a reference for wear measurement, since it was not subjected to the abrasive challenge.

The sample side corresponding to the enamel surface was polished with silicon carbide sandpapers P1200, P2400, and P4000 (Extec Corp, Enfield, CT, USA) under cooling water for 30, 60, and 120 seconds, respectively.[27] After each sanding and final polishing, the specimens were cleaned in an ultrasonic bath of deionized water (Odontobrás, Ribeirão Preto, SP, Brazil) for 10 minutes to remove waste and abrasive grains.

Two parallel scratches were then made on the resin at the sides of the embedded tooth. These scratches served as guides to orient superimposition of the baseline and posttreatment profiles. To avoid a wrong color evaluation at the baseline due to enamel/dentin dehydration during specimen preparation, the teeth were immersed in artificial saliva for 7 days, using the formulation proposed by Klimek and others.[28]

Baseline Color, Microhardness, and Surface Evaluation

Baseline color evaluation was performed using a colorimetric reflectance spectrophotometer (CM 2600d, Konica Minolta, Osaka, Japan), collecting the color data according to the CIE L*a*b* color space. The device was connected to a microcomputer and the SpectraMagic NX software (Konica Minolta) was used to control the device and perform the readings. The apparatus was adjusted to the standard illuminant D65, small area view mode, with the specular component included. The observer angle was set to 2°. The device was adjusted to perform three consecutive readings to obtain the mean values of L*, a*, and b* per sample.

Baseline microhardness (Knoop hardness number [KHN]) of the enamel surfaces for all specimens was measured with a microhardness tester (FM-700, Future-Tech, Tokyo, Japan) using a Knoop indenter under a 50-g load and 10-second dwell time.[27] Three indentations were performed on each specimen with a 100-μm distance between them, and the mean value was calculated. The indentations were made in an area away from the reading path of the profilometric evaluation.

The baseline profiles of the enamel surfaces were measured. In order to ensure the exact repositioning of the samples during the surface analyses both before and after the experimental procedure, the contact profilometer (MarSurf GD 25, Mahr, Göttingen, Germany) was equipped with a custom-made jig. The diamond stylus of the profilometer moved 4.2 mm from the resin surface reference on one side, passing over the first scratch, and then toward the enamel and over the resin and second scratch on the other side. Three profile measurements were performed for each specimen at intervals of 0.25 mm, the second one being exactly at the center of the enamel surface.

Distribution of Experimental Groups

According to the baseline microhardness values, specimens were stratified into six groups (n=40) to avoid initial differences among the groups. The groups were NC (negative control) no treatment performed; BL (positive control) application of 40% hydrogen peroxide (H_2O_2) bleaching gel (Opalescence boost, Ultradent, South Jordan, UT, USA); BL/Rs bleaching gel, followed by the application of calcium silicate/phosphate fluoride gel Regenerate Boosting Serum (Unilever, Le Meux, France); Rs/BL application of the calcium silicate/phosphate fluoride gel followed by bleaching; Rs/BL/Rs application of calcium silicate/phosphate fluoride gel followed by bleaching and a new application of calcium silicate/phosphate fluoride gel; and BL/F bleaching followed by application of 2% sodium fluoride gel. Additional information about the products tested are shown in Table 1.

Table 1: *Composition, Manufacturer, and Batch Number of the Products Used*

Product	Composition	Manufacturer	Batch No.
Opalescence Boost 40%	40% hydrogen peroxide, 3% potassium nitrate, 1.1% sodium fluoride (4950 ppm fluoride ions), thickener, pH regulators	Ultradent, South Jordan, UT, USA	BBWBT
Regenerate Boosting Serum	NR-5 Serum: glycerin, calcium silicate, PEG-8, trisodium phosphate, sodium phosphate, water, PEG-60, sodium lauryl sulfate, aroma/flavor, hydrated silica, synthetic flurophlogopite, sodium saccharin, polyacrylic acid, CI 77891, limonene, sodium monofluorophosphate (1450 ppm fluoride ions) Activator gel: Water, glycerin, cellulose gum, benzyl alcohol, ethylhexylglycerol, phenoxyethanol, CI 42090, sodium fluoride (1450 ppm fluoride ions)	Unilever, Le Meux, France	41929CYB
Flugel Neutral 2%	2% sodium fluoride (9000 ppm fluoride ions), sodium saccharin, hydroxyethyl cellulose, propylene glycol, glycerin, deionized water	DFL, Rio de Janeiro, RJ, Brazil	L16101442
Regenerate Enamel Science Advanced toothpaste	Glycerin, calcium silicate, PEG-8, silica hydrate, trisodium phosphate, sodium phosphate, water, PEG-60, sodium lauryl sulfate, aroma, synthetic fluorphlogopite, sodium saccharin, polyacrylic acid, tin oxide, limonene, CI 77891, sodium monofluorophosphate (1450 ppm fluoride ions)	Unilever, Le Meux, France	L: 50348CB
Close Up Bioactive Protection Toothpaste	Calcium carbonate, water, sorbitol, hydrated silica, sodium lauryl sulfate, aroma, cellulose gum, potassium citrate, benzyl alcohol, sodium silicate, sodium saccharin, CI12490, CL74160, lanolin, sodium monofluorophosphate (1450 ppm fluoride ions)	Unilever, Ipojuca PE, Brazil	118210322019

Surface Treatments

Opalescence boost 40% bleaching gel was applied according to the manufacturer's instructions. The product comprises two syringes (red and clear), which were attached to mix the two components and create the gel. Next, all the content was transferred to one of the syringes and applied on the enamel surface for 20 minutes. About 6.20 mg bleaching gel was applied per specimen. After this period, the gel was aspirated and a new application was performed for another 20 minutes. In total, three consecutive applications of bleaching gel were performed, resulting in 60 minutes of gel contact time with the enamel surface. After the final application, the specimens were washed with an air-water spray.

The Regenerate Boosting serum, a calcium silicate/phosphate and fluoride-based remineralizing gel, is presented in two separate tubes, one containing the serum gel and the other the activator gel. Before using, equal amounts of the gels were weighed on a precision scale and mixed for application. The mixture was applied over the enamel surface for 3 minutes, then removed with an air-water spray. The gel was applied immediately before or after the bleaching agent, depending on the experimental group. In the BL/F group, a 2% neutral sodium fluoride gel (Flugel, DFL, Rio de Janeiro, Brazil) was applied for 3 minutes after the bleaching agent and then washed off. In the negative control group, the samples received no treatment.

Immediately after the treatments, the final microhardness was measured to verify the immediate effects of the treatments on the enamel surface. The percentage of surface microhardness change (SMC) was calculated using the formula $\%SMC = ([SM_{baseline}-SM_{final}]/SM_{baseline})*100$. The specimens were then immersed in artificial saliva for 7 days for rehydration, and a final color reading was performed. The posttreatment readings were performed in the same way and with the same parameters used at baseline.

Based on the data of the color coordinates L*, a*, and b*, obtained before bleaching and after the procedures for each sample, the ΔE was calculated using the formula $\Delta E = ([\Delta L^*]^2 + [\Delta a^*]^2 + [\Delta b^*]^2)^{1/2}$. This was conducted according to the instructions of Commission Internationale de l'Eclairage.[29] ΔE values greater than 3.3 indicate a perceptibility threshold for more than 50% of the observers.[30]

Abrasive Wear

After the final microhardness and color readings, the specimens were submitted to abrasive wear. For this, each group was randomly divided into two

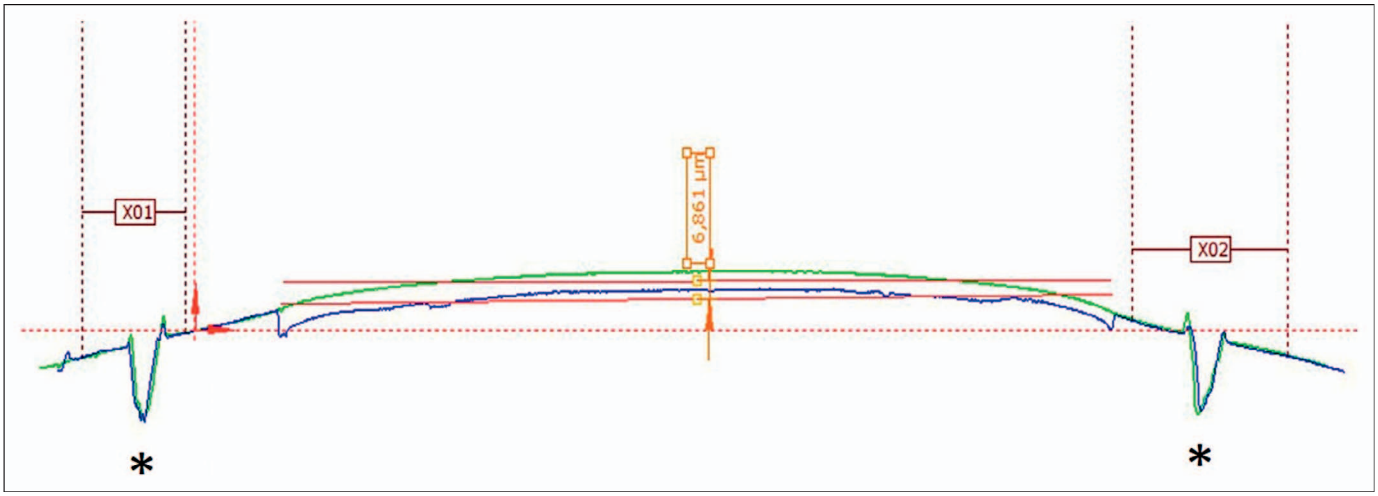

Figure 1. *Superimposition of the baseline (blue) and final (green) profiles for quantification of wear using the scratches marked with asterisks as guides. Regression lines for each profile are shown in red. The surface loss is determined by the distance between the regression lines.*

subgroups (n=20). In the first subgroup (Rt), the remineralizing toothpaste Regenerate (Unilever, Le Meux, France) was used, while in the second subgroup (Cp) a regular fluoride-containing toothpaste, Close Up Bioactive protection (Unilever, Ipojuca, PE, Brazil), was used. The specimens were placed on a holder with a metal perforated mask protecting the acrylic resin and the scratches of the abrasion, exposing only the enamel area. They were placed into a brushing machine (MEV-2T, Odeme, Luzerna, Santa Catarina, Brazil) and underwent 100,000 brushing strokes[12] receiving 50,000 strokes for two consecutive days, with a load of 200 g, according to ISO/TS 14569-1.[31] During brushing, the samples were immersed in a toothpaste slurry, prepared with toothpaste diluted in artificial saliva (1:2 w/w).[12] The specimens were stored in a closed container at 100% relative humidity overnight.

In order to obtain a homogeneous wear, the toothbrushes were fixed in an automatic brushing machine so that their long axes were at an angle of 12° to the brushing direction. This avoided the formation of nonbrushed tracks induced by the bristles on the enamel surface.[32] In addition, the brushing direction was perpendicular to the reading direction of the profilometer.

After tooth brushing, the final profile reading of each sample was performed. The final profiles were superimposed on the corresponding baseline ones, using dedicated software for contour evaluation (MarSurf XCR 20, 4.50-07 SP3, Mahr, Göttingen, Germany). The parallel scratches were used as a reference for profile superimposition. For each pair of superimposed profiles in the area corresponding to the wear, regression lines were calculated in both the baseline and final profiles. The distance between these lines was measured in micrometers by the same software (Figure 1). Three values were obtained for each specimen, and the mean was calculated.

Statistical Analysis

The baseline and after-treatment microhardness data, as well the percentage of surface microhardness change, were compared with one-way ANOVA, followed by the Tukey test. To analyze the baseline color of the samples and the ΔE after bleaching, one-way ANOVA was performed followed by the Tukey test. For the wear analysis, data were submitted to two-way ANOVA (treatment x type of toothpaste), followed by the Tukey test. The software Statistica for Windows (Statsoft, Tulsa, OK, USA) was used for all the analyses with a significance level of 5%.

RESULTS

There were no statistically significant differences among the groups in relation to the baseline microhardness ($p=0.1119$) or color coordinates ($p>0.05$ for L*, a*, and b*). After the treatments, an ANOVA test showed statistically significant differences for absolute microhardness values ($p=0.0001$), percentage of surface microhardness change ($p=0.0001$), and ΔE ($p=0.0001$) among the groups. All groups that were bleached showed a significant reduction of final microhardness in relation to the negative control (Table 2). However, all groups that received the treatment with remineralizing gels showed a significantly smaller percentage of microhardness reduc-

Table 2: *Means (Standard Deviation) of Baseline and Final Microhardness, Percentage of Surface Microhardness Change (SMC), Color Difference, and Wear*

Groups*	Baseline Microhardness (KHN)	Final Microhardness (KHN)	% SMC	Color Difference (ΔE)	Wear (μm)**
BL	325.68 (13.14) a	246.28 (30.46) a	23.16 (9.15) a	3.75 (1.04) b	3.36 (0.84) c
Rs/BL/Rs	318.25 (12.18) a	263.55 (18.66) b	17.12 (5.87) b	3.72 (1.28) b	2.19 (0.70) a
BL/Rs	323.83 (11.47) a	264.06 (28.54) b	18.43 (8.55) b	3.87 (0.91) b	2.23 (0.91) a
Rs/BL	320.54 (13.26) a	265.22 (23.58) b	17.19 (7.32) b	3.88 (0.94) b	2.13 (0.85) a
BL/F	323.51 (16.34) a	269.10 (25.66) b	16.73 (7.82) b	4.03 (1.22) b	2.12 (0.71) a
NC	325.68 (13.14) a	323.28 (10.96) c	0.70 (1.66) c	1.31 (0.58) a	2.85 (0.98) b

** Groups followed by the same letters in columns do not present significant differences according to the Tukey test.*
*** Results of Tukey test for the factor treatment in relation to wear.*

tion than the positive control, which received only the bleaching gel. Regarding color difference, the bleached groups showed a significantly higher ΔE than did the negative control group. The ΔE was produced by the reduction of values on the b* coordinate and an increase on the L* coordinate, which means a reduction of chroma and an increase of lightness. No significant differences in ΔE were found among bleached groups, regardless of whether remineralizing gels were used (Table 2).

Results of the two-way ANOVA test concerning abrasive wear showed significant differences among the treatments ($p=0.0001$) and between toothpastes ($p=0.0001$). No interactions were observed between the two factors ($p=0.4860$). Results of the Tukey test for the factor treatment showed that the groups treated with remineralizing gels had significantly less wear than did the group only bleached. However, nonsignificant differences were observed among them. Only the positive control, just bleached, showed greater wear than did the negative control group (Table 2). In relation to the factor type of toothpaste, the groups brushed with Regenerate showed significantly less wear (2.04±0.80 μm) than those that brushed with the regular toothpaste (2.92 ±0.88 μm). The means (μm) of surface loss for each group and toothpaste are shown in Figure 2.

DISCUSSION

In the present study, dental bleaching caused a significant reduction in enamel microhardness of about 23.16%. Similar percentages were found in other studies, ranging from 11.12% to 25%, increasing with application time.[10,33] The literature is inconsistent as to which percentage of enamel microhardness reduction is considered clinically relevant. Acid etching of enamel surfaces for adhesive purposes, a common clinical procedure in daily dental practice, effects a microhardness reduction of 73.78%.[34] In a previous in vitro study, the correlation between microhardness of eroded enamel surfaces with toothbrush wear was investigated. The specimens were immersed in carbonated beverage (pH=2.91) for 1, 5, or 15 minutes before brushing (8000 strokes).[35] The percentage of microhardness reduction and wear after treatments were, respectively, 11.52% (0.63μm), 14.18% (1.19 μm), and 30.69% (2.22 μm). Regression analysis showed a significant negative linear correlation between hardness and abrasive wear. Therefore, even a microhardness reduction of only 11% can significantly influence enamel wear.

Another study showed a positive correlation between peroxide concentration and microhardness reduction.[36] We tested the most concentrated product on the market, containing 40% H_2O_2, which is a nonspecific oxidizing agent that acts not only on the decomposition of chromophore molecules but also on tooth structure.[2] H_2O_2 can penetrate enamel structure mainly via proteins (amelogenin and enamelin), causing fragmentation of the polypeptide chain due to cleavage of the peptide bonds.[37] As the organic

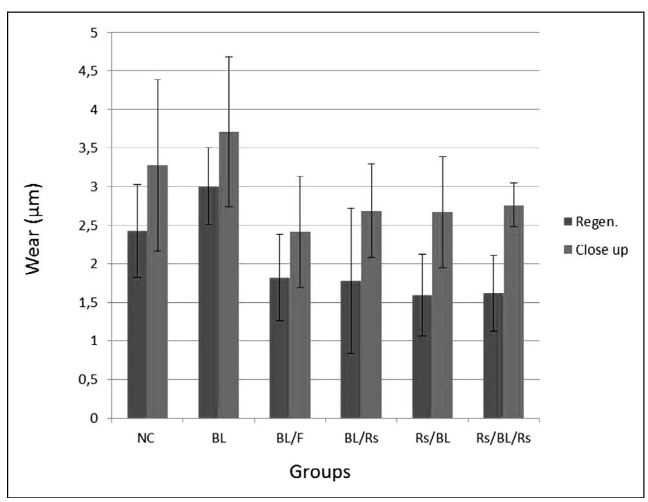

Figure 2. *Means of wear for all groups with the two different toothpastes.*

content also affects enamel integrity, its changes by oxidization may also indirectly alter the mineral content.[2]

It has been reported that bleaching gels with an acidic pH cause more demineralization and reduction of enamel surface microhardness than those with a higher pH.[2,3,10] However, some bleaching agents with a pH close to neutral also cause some changes in the enamel by decreasing its surface microhardness.[1,10] In the present study, Opalescent Boost bleaching gel has a pH of 7.4, yet it reduced enamel microhardness. It is speculated that, besides the nonspecific oxidative effect, the low concentration of calcium and phosphate in the whitening gel formulation makes it undersaturated compared with hydroxyapatite, promoting its demineralization regardless of the pH.[10]

When a solid is immersed in a solution, the interface between the two phases becomes a site for continuous exchange of the components from solid (in the case of enamel, Ca^{2+}, PO_4^{3-}, and OH^- ions).[38] The ions are released from the solid surface and dissolve in the solution (dissolution) and, in the same way, other ions leave the solution to attach to the solid surface (crystal growth). When the rate at which ions enter the solution equals that at which they attach to the crystal surface, the solid and solution are in a state of equilibrium and there is no net change in the solid. At equilibrium, the solution is said to be saturated with respect to the solid.[38] If the solution is undersaturated, the solid loses ions to the solution until a balance is reached, and if the solution is supersaturated, the solution loses ions to the solid until, again, equilibrium is reached.[38] Remarkably, this balance varies according to the pH and background ion concentration.[38] Lussi and Hellwig[39] studied the effects of various beverages on promoting enamel softening during the erosive process and observed that it depends not only on the pH of the substances, but also on the concentration of Ca^{2+} ions in their composition.

Studies have found that remineralization by saliva can recover the microhardness reduced by the bleaching procedure, repairing the lost calcium and phosphate ions.[40] However, this process can take up to 15 days before the surface microhardness returns to baselines values.[41] During this period in which the enamel is not completely repaired, teeth would be more susceptible to loss of structure due to abrasive wear, such as during toothbrushing.[12,13,42] The abrasive wear can be intensified, for example, if highly abrasive toothpastes and strong brushing forces are used. A study suggested delaying tooth brushing at least 1 hour after bleaching in order to reduce enamel wear.[13] However, more studies are needed to confirm the effectiveness of brushing delay.

The application of fluoridated agents in a gel or solution on the enamel surface after bleaching is being recommended for remineralization and increasing microhardness.[15,21,33,43-45] This procedure can promote formation of calcium fluoride deposits on the enamel surface.[18] When the local pH decreases, F^- ions are released from calcium fluoride deposits; this release is accompanied by Ca^{2+} ion release from the tooth surface due to demineralization. The presence of these ions changes the fluoride content into hydroxyapatite molecules by replacing OH^- ions for F^- ions, forming fluorapatite or fluoridated hydroxyapatite.[46] Fluorapatite is formed when all hydroxyl ions of the hydroxyapatite molecule are replaced by fluoride, while fluoridated hydroxyapatite is formed when this replacement by fluoride is partial.[47] Fluorapatite is less soluble and more acid resistant than fluoridated hydroxyapatite.[47] These ionic changes lead to a greater tendency for the ions to join the structure, repair the enamel surface, and produce an increase in microhardness.[19,20] Lewinstein and others[33] observed that exposure, even to a low concentration of fluoride, can restore surface microhardness after bleaching. However, China and others[15] concluded that only a neutral fluoride increases the microhardness of enamel bleached with 35% H_2O_2, while the application of acidulated fluoride causes an even greater reduction. In the present study, the effect of a neutral 2% fluoride gel[33] (9000 ppm fluoride ions) on the surface of the bleached enamel was tested. It is a highly concentrated fluoride compound commonly applied in-office for caries prevention. This postbleaching application increased the surface microhardness compared with the positive control group, but the surface did not return to its baseline surface microhardness values.

In an attempt to protect the enamel during bleaching, 1.1% of sodium fluoride (4950 ppm of fluoride ions) was incorporated in the bleaching gel analyzed in this study. However, it did not prevent enamel surface demineralization, indicating that something was interfering with its protective effect, possibly an interaction with H_2O_2 or other ingredients of the formulation.

The calcium silicate/phosphate fluoride gel was tested as a protective agent when applied before bleaching, as a remineralizing agent when applied after, and with both actions when applied before and after the bleaching procedure. This double application was tested in an attempt to intensify its effect on the enamel microhardness. In all cases, its application resulted in statistically higher final values of microhardness compared with the group that was only bleached. Based on this, in relation to microhardness, the null hypothesis was rejected. The use of this gel before bleaching significantly reduced the demineralizing effect of the H_2O_2 gel, while its use afterward promoted enamel remineralization. However, the use prior and after did not show any significant differences in relation to when it was used only once.

The remineralization process using this system starts when calcium silicate is deposited onto the enamel surface, causing an exchange of calcium ions with the hydrogen ions in the oral fluid, leading to the formation of silanol groups (Si-OH) in the surface layer of deposited calcium silicate. This causes an increase in the local pH, generating a negatively charged surface with the formation of silicon oxide (Si-O) groups. The calcium ions in the oral fluid are attracted to the surface of the calcium silicate layer. Consequently, the concentration of calcium and phosphate ions in the oral fluid is high enough to nucleate hydroxyapatite on the calcium silicate surface.[20,25,48] The formation of hydroxyapatite over the enamel using this system has been demonstrated in both *in vitro* and *in situ* studies.[25,49]

The mode of action of calcium silicate/phosphate fluoride formulations on enamel remineralization by the deposition of hydroxyapatite has been investigated. This effect was observed after the use of toothpaste alone[24,25] and after the use of toothpaste followed by the boosting gel.[22,23] Both the gel and the toothpaste have fluoride in their composition, which may allow an additional mode of action for repair and protection by promoting the formation of calcium fluoride deposits on the enamel surface.[18] A previous study found that the minimum concentration of fluoride necessary for the formation of calcium fluoride deposits on the enamel surface is 0.1%, and the first globules will be precipitated in the first 20 seconds.[49] These two mechanisms acting together may increase remineralization of the enamel surface. It must be noted that applying calcium silicate/phosphate fluoride gel over the bleached enamel, even though containing only 1450 ppm of fluoride ions, did not show statistically different mineralization effects in relation to the neutral 2% fluoride gel containing 9000 ppm of fluoride ions (Table 1). This might be related to the synergistic action of the hydroxyapatite formation with the fluoride mineralization mechanism of the boosting serum gel.

It could be supposed that the mineralizing potential of boost serum gel, applied before bleaching, could interfere with tooth enamel permeability, or even prevent the penetration of H_2O_2 into the tooth structure and reduce its bleaching effect. Additionally, when applied after bleaching, some ingredient in both gels' formulation could produce some kind of tooth staining, impairing the whitening results. In the present study, the previous application of calcium silicate/phosphate fluoride gel did not interfere with the ΔE promoted by bleaching. Studies evaluating the use of desensitizing agents containing 2% sodium fluoride before bleaching with 35% H_2O_2 also showed no interference with the ΔE.[50] However, no previous studies have analyzed the interference of the calcium silicate/phosphate fluoride gel on the bleaching effect. It has been observed that the application of both remineralizing gels postbleaching did not interfere with the ΔE of the tooth structure. Sodium fluoride gel is colorless, while calcium/phosphate silicate gel is slightly milky white. All bleached groups, despite the remineralizing treatments, presented statistically similar ΔE overall. The bleaching effect was related to a reduction in the b* coordinate and an increase in the L* coordinate. The a* value was not significantly influenced by the bleaching procedure. Thus, in relation to ΔE, the null hypothesis was accepted.

Regarding abrasive wear, the group that received only bleaching treatment showed a significantly higher wear than the nonbleached one (Table 2). A negative correlation between hardness and wear was observed, since the reduction of surface microhardness caused by bleaching increases the enamel loss by toothbrush abrasion. Other studies showing the relation between enamel bleaching and wear have obtained similar results.[11-13] In addition, the bleached groups in which the remineralizing gels were applied showed less wear than did the group that was only bleached, indicating that this procedure could offer some clinical benefits to the patients subjected to this kind of treatment. Thus, in relation to wear, the null hypothesis was rejected.

Another important aspect in relation to abrasive wear is that the groups that were bleached and received the remineralizing gels showed less wear than did the negative control group (Table 2). This might be due to the fact that after bleaching treatments and before the color reading, the specimens were immersed in artificial saliva for 7 days. The artificial saliva may have interacted with any calcium fluoride deposited on the enamel, forming fluorapatite or fluoridated hydroxyapatite, or continued to promote hydroxyapatite formation/deposition when the calcium silicate/phosphate fluoride gel was used.[40,41] Both these processes may have further increased remineralization and enamel surface microhardness, leading to less enamel wear than the bleached as well as the nonbleached groups.

Significant differences were observed in the wear caused by the toothpastes tested. The groups that were brushed with Regenerate showed less wear than those brushed with Close Up. The use of a less abrasive toothpaste after bleaching might be clinically relevant, avoiding additional tooth wear on those patients receiving this kind of treatment, although this should be confirmed in controlled clinical trials. Thus, in relation to the kind of toothpaste, the null hypothesis was rejected. Two suppositions could be made about this result. The first is that the remineralizing effect of Regenerate could protect the surface from abrasion by hydroxyapatite formation,[25,48] increasing enamel microhardness.[33] The second is probably related to lower abrasivity of the abrasive particles in this toothpaste.

One factor that impacts toothpaste abrasivity is the relative hardness of the abrasive particles. Regenerate contains hydrated silica as an abrasive, while the Close Up used in this study has a combination of hydrated silica and calcium carbonate. Moh's hardness is used to measure material hardness on a scale of 0 to 10, wherein 10 represents the hardness of a diamond. In this scale, the hydrated silica has a hardness of 2.5 and the calcium carbonate, 3.[51] However, previous studies have tested various toothpastes having calcium carbonate and hydrated silica alone or in combination, but no statistically significant differences between the toothpastes in terms of enamel wear were found.[52-54] In contrast, the relation between toothpaste abrasiveness and wear was evaluated by Borges and others,[13] who subjected bleached enamel and dentin to brushing abrasion with slurries having different degrees of abrasivity; they observed that the higher the abrasiveness, the greater the tooth wear. However, it should be noted that the relationship between toothpaste abrasivity and dental wear is complex; it depends on the concentration and size of the abrasive particles.[53] In addition, the presence of the acquired pellicle has been shown to reduce enamel wear from toothpaste abrasion.[54]

It should be emphasized that this is an *in vitro* study, which has limitations in reproducing conditions found in the oral cavity, eg, the presence of saliva. The acquired pellicle and its action, together with bleaching and remineralization treatment, may modify the results of the current study such that complementary investigations are necessary to confirm their impact. The importance of clinical studies to evaluate the performance of calcium silicate/phosphate fluoride formulations or fluoride gels for longer periods of use after the bleaching treatment is worthy of further study.

CONCLUSIONS

Considering the limitations of this *in vitro* study, we concluded that

1. Bleaching reduced enamel microhardness and increased abrasion suitability.
2. Although the remineralizing gels tested did not prevent microhardness reduction and wear of bleached enamel, they did bring about their decrease without interfering with the bleaching efficacy.
3. Regenerate toothpaste resulted in less enamel abrasive wear than did Close Up.
4. There were no significant differences among the remineralizing gels in their effect on microhardness, color, or wear.

Acknowledgements

The authors have no financial interests in the companies whose materials are included in this article. The authors thank CAPES (coordination for the improvement of higher education personnel), for the first author's scholarship.

Conflict of Interest

The authors of the manuscript entitled "Effect of Remineralizing Gels on Microhardness, Color, and Wear Susceptibility of Bleached Enamel" declare no conflict of interest in relation to this article.

(Accepted 5 March 2018)

REFERENCES

1. de Moraes IQ, Silva LN, Porto IC, de Lima Neto CF, Dos Santos NB, & Fragoso LS (2015) Effect of in-office bleaching with 35% hydrogen peroxide with and without addition of calcium on the enamel surface *Microscopy Research and Technique* **78(11)** 975-981 http://dx.doi.org/10.1002/jemt.22561

2. Jiang T, Ma X, Wang Y, Tong H, Shen X, Hu Y, & Hu J (2008) Investigation of the effects of 30% hydrogen peroxide on human tooth enamel by Raman scattering

and laser-induced fluorescence *Journal of Biomedical Optics* **13(1)** 014019 http://dx.doi.org/10.1117/1.2870114

3. Sa Y, Sun L, Wang Z, Ma X, Liang S, Xing W, Jiang T, & Wang Y (2013) Effects of two in-office bleaching agents with different pH on the structure of human enamel: An in situ and in vitro study *Operative Dentistry* **38(1)** 100-110 http://dx.doi.org/10.2341/11-173-L

4. Al-Salehi SK, Wood DJ, & Hatton PV (2007) The effect of 24h non-stop hydrogen peroxide concentration on bovine enamel and dentine mineral content and microhardness *Journal of Dentistry* **35(11)** 845-850, http://dx.doi.org/10.1016/j.jdent.2007.08.001

5. Souza RO, Lombardo GH, Pereira SM, Zamboni SC, Valera MC, Araujo MA, & Ozcan M (2010) Analysis of tooth enamel after excessive bleaching: A study using scanning electron microscopy and energy dispersive x-ray spectroscopy *International Journal of Prosthodontics* **23(1)** 29-32.

6. Cakir FY, Korkmaz Y, Firat E, Oztas SS, & Gurgan S (2011) Chemical analysis of enamel and dentin following the application of three different at-home bleaching systems *Operative Dentistry* **36(5)** 529-536 http://dx.doi.org/10.2341/11-050-L

7. Borges AB, Zanatta RF, Barros AC, Silva LC, Pucci CR, & Torres CR (2015) Effect of hydrogen peroxide concentration on enamel color and microhardness *Operative Dentistry* **40(1)** 96-101 https://doi.org/10.2341/13-371-L

8. Parreiras SO, Vianna P, Kossatz S, Loguercio AD, & Reis A (2014) Effects of light activated in-office bleaching on permeability, microhardness, and mineral content of enamel *Operative Dentistry* **38(5)** 225-230, https://doi.org/10.2341/13-031-L

9. Sun L, Liang S, Sa Y, Wang Z, Ma X, Jiang T, & Wang Y (2011) Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide *Journal of Dentistry* **39(10)** 686-692, http://dx.doi.org/10.1016/j.jdent.2011.07.011

10. Magalhães JG, Marimoto AR, Torres CR, Pagani C, Teixeira SC, & Barcellos DC (2012) Microhardness change of enamel due to bleaching with in-office bleaching gels of different acidity *Acta Odontologica Scandinavica* **70(2)** 122-126 http://dx.doi.org/10.3109/00016357.2011.600704

11. Wiegand A, Otto YA, & Attin T (2004) In vitro evaluation of toothbrushing abrasion of differently bleached bovine enamel *American Journal of Dentistry* **17(6)** 412-416.

12. Trentino AC, Soares AF, Duarte MA, Ishikiriama SK, & Mondelli RF (2015) Evaluation of pH levels and surface roughness after bleaching and abrasion tests of eight commercial products *Photomedicine and Laser Surgery* **33(7)** 372-377, http://dx.doi.org/10.1089/pho.2014.3869

13. Borges AB, Santos LF, Augusto MG, Bonfiette D, Hara AT, & Torres CR (2016) Toothbrushing abrasion susceptibility of enamel and dentin bleached with calcium-supplemented hydrogen peroxide gel *Journal of Dentistry* **49** 54-59 http://dx.doi.org/10.1016/j.jdent.2016.03.009

14. Cavalli V, Arrais CA, Giannini M, & Ambrosano GM (2004) High-concentrated carbamide peroxide bleaching agents effects on enamel surface *Journal of Oral Rehabilitation* **31(2)** 155-159.

15. China AL, Souza NM, Gomes Ydo S, Alexandrino LD, & Silva CM (2014) Effect of fluoride gels on microhardness and surface roughness of bleached enamel *Open Dentistry Journal* **8** 188-193 http://dx.doi.org/10.2174/1874210601408010188

16. Moosavi H, & Darvishzadeh F (2016) The influence of post bleaching treatments in stain absorption and microhardness *Open Dentistry Journal* **10** 69-78 http://dx.doi.org/10.2174/1874210616021000069

17. Heshmat H, Ganjkar MH, Miri Y, & Fard MJ (2016) The effect of two remineralizing agents and natural saliva on bleached enamel hardness *Dental Research Journal (Isfahan)* **13(1)** 52-57 http://dx.doi.org/10.4103/1735-3327.174713

18. Featherstone JD, Cutress TW, Rodgers BE, & Dennison PJ (1982) Remineralization of artificial caries-like lesions in vivo by a self-administered mouthrinse or paste *Caries Research* **16(3)** 235-242.

19. Moreno EC, Kresak M, & Zahradnik RT (1974) Fluoridated hydroxyapatite solubility and caries formation *Nature* **247(5435)** 64-65.

20. Li X, Wang J, Joiner A, & Chang J (2014) The remineralisation of enamel: A review of the literature *Journal of Dentistry* **42(Supplement 1)** 12-20 http://dx.doi.org/10.1016/S0300-5712(14)50003-6

21. da Costa JB, & Mazur RF (2007) Effects of new formulas of bleaching gel and fluoride application on enamel microhardness: An in vitro study *Operative Dentistry* **32(6)** 589-594 http://dx.doi.org/10.2341/06-166

22. Joiner A, Schäfer F, Naeeni MM, Gupta AK, & Zero DT (2014) Remineralisation effect of a dual-phase calcium silicate/phosphate gel combined with calcium silicate/phosphate toothpaste on acid-challenged enamel in situ *Journal of Dentistry* **42(Suppliment 1)** 53-59 http://dx.doi.org/10.1016/S0300-5712(14)50008-5

23. Jones SB, Davies M, Chapman N, Willson R, Hornby K, Joiner A, & West NX (2014) Introduction of an interproximal mineralisation model to measure remineralisation caused by novel formulations containing calcium silicate, sodium phosphate salts and fluoride *Journal of Dentistry* **42(Suppliment 1)** 46-52 http://dx.doi.org/10.1016/S0300-5712(14)50007-3

24. Hornby K, Ricketts SR, Philpotts CJ, Joiner A, Schemehorn B, & Willson R (2014) Enhanced enamel benefits from a novel toothpaste and dual phase gel containing calcium silicate and sodium phosphate salts *Journal of Dentistry* **42(Supplement 1)** 39-45 http://dx.doi.org/10.1016/S0300-5712(14)50006-1

25. Sun Y, Li X, Deng Y, Sun JN, Tao D, Chen H, Hu Q, Liu R, Liu W, Feng X, Wang J, Carvell M, & Joiner A (2014) Mode of action studies on the formation of enamel minerals from a novel toothpaste containing calcium

silicate and sodium phosphate salts *Journal of Dentistry* **42(Supplement 1)** 30-38 http://dx.doi.org/10.1016/S0300-5712(14)50005-X

26. Attin T, Wegehaupt F, Gries D, & Wiegand A (2007) The potential of deciduous and permanent bovine enamel as substitute for deciduous and permanent human enamel: Erosion-abrasion experiments *Journal of Dentistry* **35(10)**773-777 http://dx.doi.org/10.1016/j.jdent.2007.07.007
27. Ávila DM, Zanatta RF, Scaramucci T, Aoki IV, Torres CR, & Borges AB (2017) Influence of bioadhesive polymers on the protective effect of fluoride against erosion *Journal of Dentistry* **56** 45-52 http://dx.doi.org/10.1016/j.jdent.2016.10.015
28. Klimek J, & Ahrens G (1982) Fluoride uptake and retention in the dental enamel of adults after the application of NaF tablets and NaF gel in vivo *Caries Research* **16** 156-161.
29. Commission internationale de l'Eclairage (2004) Colorimetry Publication CIE, No. 15.3, Central Bureau of the CIE, Vienna, Austria.
30. Ruyter IE, Nilner K, & Moller B (1987) Color stability of dental composite resin materials for crown and bridge veneers *Dental Materials* **3(5)** 246-251, http://dx.doi.org/10.1016/S0109-5641(87)80081-7
31. International Organization for Standardization. ISO/TS 14569-1:2007. Dental materials – Guidance on testing of wear – Part 1: Wear by toothbrushing. Geneva: International Organization for Standardization, 2007.
32. Wiegand A, Kuhn M, Sener B, Roos M, & Attin T (2009) Abrasion of eroded dentin caused by toothpaste slurries of different abrasivity and toothbrushes of different filament diameter *Journal of Dentistry* **37(6)** 480-484 http://dx.doi.org/10.1016/j.jdent.2009.03.005
33. Lewinstein I, Fuhrer N, Churaru N, & Cardash H (2004) Effect of different peroxide bleaching regimens and subsequent fluoridation on the hardness of human enamel and dentin *Journal of Prosthetic Dentistry* **92(4)** 337-342, http://dx.doi.org/10.1016/j.prosdent.2004.07.019
34. Collys K, Slop D, Cleymaet R, Coomans D, & Michotte Y (1992) Load dependency and reliability of microhardness measurements on acid-etched enamel surfaces *Dental Materials* **8(5)** 332-335 https://doi.org/10.1016/0109-5641(92)90110-X
35. Attin T, Koidl U, Buchalla W, Schaller HG, Kielbassa AM, & Hellwig E (1997) Correlation of microhardness and wear in differently eroded bovine dental enamel *Archives of Oral Biology* **42(3)** 243-250, https://doi.org/10.1016/0003-9969(06)00073-2
36. Lia Mondelli RF, Garrido Gabriel TR, Piola Rizzante FA, Magalhães AC, Soares Bombonatti JF, & Ishikiriama SK (2015) Do different bleaching protocols affect the enamel microhardness? *European Journal of Dentistry* **9(1)** 25-30 http://dx.doi.org/10.4103/1305-7456.149634
37. Hegedüs C, Bistey T, Flóra-Nagy E, Keszthelyi G, & Jenei A (1999) An atomic force microscopy study on the effect of bleaching agents on enamel surface *Journal of Dentistry* **27(7)** 509-515.
38. Shellis RP, Featherstone JD, & Lussi A (2014) Understanding the chemistry of dental erosion *Monographs in Oral Science* **25** 163-179, http://dx.doi.org/10.1159/000359943
39. Lussi A, & Hellwig E (2014) Risk assessment and causal preventive measures *Monographs in Oral Science* **25** 220-229 http://dx.doi.org/10.1159/000360612
40. Basting RT, Rodrigues AL Jr, & Serra MC (2003) The effects of seven carbamide peroxide bleaching agents on enamel microhardness over time *Journal of the American Dental Association* **134(10)**1335-1342 http://dx.doi.org/10.14219/jada.archive.2003.0047
41. Araujo NC, MU, da Costa Soares Nery MM, Sales WS, & Gerbi ME (2013) Effect of pH values of two bleaching gels on enamel microhardness *General Dentistry* **61(4)** 55-58.
42. Mondelli RF, Azevedo JF, Francisconi PA, Ishikiriama SK, & Mondelli J (2009) Wear and surface roughness of bovine enamel submitted to bleaching *European Journal of Esthetic Dentistry* **4(4)** 396-403.
43. Attin T, Kielbassa AM, Schwanenberg M, & Hellwig E (1997) Effect of fluoride treatment on remineralization of bleached enamel *Journal of Oral Rehabilitation* **24(4)** 282-286.
44. Borges AB, Yui KC, D'Avila TC, Takahashi CL, Torres CR, & Borges AL (2010) Influence of remineralizing gels on bleached enamel microhardness in different time intervals *Operative Dentistry* **35(2)**180-186 http://dx.doi.org/10.2341/09-117-L
45. Salomão D, Santos D, Nogueira R, Palma-Dibb R, & Geraldo-Martins V (2014) Acid demineralization susceptibility of dental enamel submitted to different bleaching techniques and fluoridation regimens *Operative Dentistry* **39(4)**178-185 http://dx.doi.org/10.2341/13-140
46. Vogel GL, Carey CM, & Ekstrand J (1992) Distribution of fluoride in saliva and plaque fluid after a 0.048 mol/L NaF rinse *Journal of Dental Research* **71(9)**1553-1557 http://dx.doi.org/10.1177/00220345920710090201
47. Meyer-Lueckel H, Paris S, & Ekstrand KR (2016) *Caries Management—Science and Clinical Practice* Thieme, New York.
48. Parker AS, Patel AN, Al Botros R, Snowden ME, McKelvey K, Unwin PR, Ashcroft AT, Carvell M, Joiner A, & Peruffo M (2014) Measurement of the efficacy of calcium silicate for the protection and repair of dental enamel *Journal of Dentistry* **42(suppl 1)** 21-29, http://dx.doi.org/10.1016/S0300-5712(14)50004-8
49. Petzold M (2001) The influence of different fluoride compounds and treatment conditions on dental enamel: A descriptive in vitro study of the CaF(2) precipitation and microstructure *Caries Research* **35(suppl 1)** 45-51 http://dx.doi.org/49110

50. do Carmo Públio J, D'Arce MB, Ambrosano GM, Aguiar FH, Lovadino JR, Paulillo LA, & Lima DA (2015) Efficacy of tooth bleaching with the prior application of a desensitizing agent *Journal of Investigative and Clinical Dentistry* **6(2)**133-140 http://dx.doi.org/10.1111/jicd.12074

51. Newbrun E (1997) The use of sodium bicarbonate in oral hygiene products and practice *Compendium of Continuing Education in Dentistry* **18(21)** 2-7.

52. Turssi CP, Faraoni JJ, Rodrigues Jr AL, & Serra MC (2004) An in situ investigation into the abrasion of eroded dental hard tissues by a whitening dentifrice *Caries Research* **38(5)** 473-477 http://dx.doi.org/10.1159/000079629

53. Pickles MJ, Joiner A, Weader E, Cooper YL, & Cox TF (2005) Abrasion of human enamel and dentine caused by toothpastes of differing abrasivity determined using an in situ wear model *International Dental Journal* **55(3 Supplement 1)**188-193.

54. Ferreira MC, Ramos-Jorge ML, Delbem AC, & Vieirac R de S (2013) Effect of toothpastes with different abrasives on eroded human enamel: An in situ/ex vivo study *Open Dentistry Journal* **7** 132-139, http://dx.doi.org/10.2174/1874210601307010132