In Vitro Enamel Remineralization Efficacy of Calcium Silicate-Sodium Phosphate-Fluoride Salts versus NovaMin Bioactive Glass, Following Tooth Whitening

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Abstract

Objectives This study aimed to evaluate the effect of in-office bleaching on the enamel surface and the efficacy of calcium silicate-sodium phosphate-fluoride salt (CS) and NovaMin bioactive glass (NM) dentifrice in remineralizing bleached enamel.

Materials and Methods Forty extracted premolars were sectioned mesio-distally, and the facial and lingual enamel were flattened and polished. The samples were equally divided into nonbleached and bleached with 38% hydrogen peroxide (HP). Each group was further divided according to the remineralization protocol (n = 10); no remineralization treatment (nontreated), CS, or NM, applied for 3 minutes twice/day for 7 days, or CS combined with NR-5 boosting serum (CS+NR-5) applied for 3 minutes once/day for 3 days. The average Knoop hardness number (KHN) and surface roughness (utilizing atomic force microscopy) were measured. Surface topography/elemental analysis was analyzed by using scanning electron microscopy/energy dispersive X-ray analysis. All the tests were performed at baseline, after bleaching, and following each remineralization protocol. Data were statistically analyzed by two-way analysis of variance and Bonferroni post hoc multiple comparison tests (α = 0.05).

Results HP significantly reduced KHN and increased roughness (p < 0.05). All remineralization materials increased the hardness and reduced the surface roughness after bleaching except NM, which demonstrated significantly increased roughness (p < 0.05). Ca/P ratio decreased after bleaching (p < 0.05), and following treatment, CS and CS+NR-5 exhibited higher remineralization capacity in comparison to NM (p < 0.05).

Conclusion Although none of the material tested was able to reverse the negative effect of high-concentration in-office HP on enamel completely, the remineralization efficacy of CS and CS+NR-5 was superior to that of NM.

Keywords ► bleaching  ► enamel  ► remineralization  ► scanning electron microscopy  ► energy dispersive X-ray analysis  ► calcium  ► silicate-sodium phosphate-fluoride  ► NovaMin

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Introduction

Among the aesthetic management modalities of vital and nonvital discolored teeth, dental bleaching has proven to be a highly conservative and simple treatment option. At present, several bleaching agents are commercially available and are mainly based on different concentrations of hydrogen peroxide (HP) or carbamide peroxide (CP). These products are available for use at home or in the dental office, possibly aided by various types of light sources to intensify the oxidation reaction.1

The process of bleaching teeth involves free radicals generation such as oxygen and perhydroxyl because of the decomposition of HP. The presence of these radicals generates an oxidation-reduction reaction.2 Due to their exceedingly electrophilic nature, free radicals diffuse through the matrices of enamel and dentin to bombard the pigment molecules and ultimately gain stability, and this is possible due to the permeability of dental tissue. Following this reaction, the organic macromolecules—of which the pigments are formed—are broken down into less complex, smaller, and lighter molecules.3 Due to the unspecific nature of this reaction, several studies have investigated its undesirable effects on tooth structure such as mineral loss and alterations to surface topography which can, in turn, alter the biomechanical properties of enamel.4,5

Morphological changes of enamel surface were reported, mainly due to the erosive nature of the bleaching process.6 Bleaching affects the organic components of enamel and produces changes in the mineral phase, which ultimately creates visible morphological changes on the enamel surface.7,8 A study by Ushigome et al described that HP selectively erodes rod sheath areas while CP causes mineral dissolution and decalcification, which results in etch-like erosion of surface and subsurface enamel.9 The erosive effects are in view of the acidity of the bleaching agents, where some bleaching products have been reported to have a pH as low as 2.4.10

The application of remineralizing agents was found to restore the morphological defects caused by bleaching.11 Currently, among the available remineralization promoting agents, fluoride-based formulations remain the most commonly used anti erosive materials. They enhance the precipitation of calcium phosphates and in turn promote the formation of fluoro-hydroxyapatite crystals in dental tissues.12

A dentifrice containing calcium silicate, sodium phosphate, and fluoride salts (CSSPF) has been introduced. This technology was first proposed to supplement the natural process of mineralization by human saliva, which was possible by providing auxiliary calcium and phosphate that nucleates hydroxyapatite (HAP) formation. This mechanism seems to promote the remineralization and repair of softened enamel and aids in protecting enamel from acid challenges.13 The CSSPF-based toothpaste was introduced with a boosting serum, which was claimed to enhance the power of enamel remineralization.

Another class of commercially available agents that have been indicated in the treatment of dental hypersensitivity and enamel remineralization is bioactive glass, specifically, NovaMin. Primarily, NovaMin is a ceramic material that consists of amorphous sodium-calcium-phosphosilicate as a powder of fine particle size that is highly reactive in water. NovaMin works in two mechanisms. One mechanism is that the fine powder itself is capable of occluding dentinal tubules,14 and the second is by its reaction when exposed to the tooth’s aqueous environment. The production of calcium and phosphate ions from the glass is brought about by the rapid exchange of particles between the sodium ions in NovaMin and hydrogen cations from the aqueous environment of the tooth.15 The release of sodium at primary exposure of the material to water leads to a transitory and localized increase in pH. This effect encourages the precipitation of the supplementary calcium and phosphate ions delivered by the NovaMin ultimately forming a layer of calcium phosphate. This layer then crystalizes into carbonate-enriched hydroxyapatite (HCA).16 The newly formed HCA combines with the residual NovaMin particles, remineralizing the enamel surface and hinders further demineralization.14

The remineralization of bleached enamel has been scarcely tackled in the literature. Therefore, this study was conducted to investigate the remineralizing capacity of two anti erosive/ remineralizing agents (namely CSSPF and NovaMin bioactive glass) on the bleached enamel, by assessing their effect on the enamel surface hardness and roughness. The null hypothesis being that the tested remineralizing agents have no effect on the surface hardness and surface roughness of the bleached enamel.

Materials and Methods

Samples Preparation

The compositions of the tested materials are listed in Table 1. Forty sound human premolars extracted for orthodontic purposes were used in this study after ethical approval by the University of Sharjah research ethics committee and after patient consent. The teeth were examined under a stereomicroscope for the presence of cracks, decay, or any other defect, and teeth with defects were excluded from the study. Any soft tissues remnants were removed by using an ultrasonic scaler. The roots were sectioned at the cemento-enamel junction using a high-speed dental handpiece and a diamond bur and then the pulp tissues were removed. The crown of each tooth was sectioned mesio-distally into two halves using a diamond saw (Isomet 1000; Buehler, Lake Bluff, Illinois, United States) to obtain 80 specimens. The specimens were embedded in self-cured acrylic resin (Fastray, Harry J. Bosworth Co.; Skokie, Illinois, United States) with the enamel surface facing upward. The enamel surfaces were polished using a grinding/polishing machine (EcoMet 30; Buehler, Lake Bluff, Illinois, United States) with 400, 600, and 1200 grit water-cooled sandpaper discs to attain flat enamel surfaces and then polished using Synthetic Polishing Cloth (Super-Snap Buff Disk, Shofu Dental Corp.; Kyoto, Japan) soaked with 1 µm diamond suspension (Buehler). After polishing, the specimens were cleaned in an ultrasonic bath with deionized water for 5 min.
The enamel surface microhardness (baseline KHN) was determined by using a microhardness tester (HMV-2; Shimadzu Corporation, Tokyo, Japan). Five indentations per sample were performed at distances of 100 µm from each other using a microindentor (Knoop diamond, 100 g/15 sec. An average of five readings for each specimen was recorded as its KHN value. According to KHN values at the baseline, data from 10 specimens (n = 10) were averaged so that the average baseline KHN in the eight experimental groups was nearly equal (Table 2).

### Remineralization of Enamel

Four groups (nonbleached groups) were randomly selected and did not receive any bleaching treatment. In contrast, the other four groups (bleached groups) were treated with a 38% HP bleaching gel (Opalescence R Boost, Ultradent Products, Inc.; South Jordan, Utah, United States). Bleaching of the enamel samples was done following the manufacturer’s instructions, and each sample was subjected to three consecutive 20-min applications. Following each bleaching gel application, the sample surface was washed with a strong stream of air and water for 20 sec. After the third session, the bleached surfaces were resubmitted to KHN microhardness test to obtain the degree of softening.

### Table 1

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Composition</th>
<th>pH value</th>
<th>Lot</th>
</tr>
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<tbody>
<tr>
<td>Opalescence Boost</td>
<td>Ultradent Products, South Jordan, Utah, United States</td>
<td>38% hydrogen peroxide, 3% potassium nitrate, and 1.1% fluoride ions (10,000 ppm)</td>
<td>7.52³⁰</td>
<td>B069</td>
</tr>
<tr>
<td>Sensodyne Repair &amp; Protect</td>
<td>GSK, Middlesex, United Kingdom</td>
<td>Glycerin, PEG-8, silica, calcium sodium phosphosilicate (NovaMin), sodium lauryl sulfate, aroma, titanium dioxide, carborner, potassium acetatesulfame, limonene, contains sodium monofluorophosphate 1.08% w/w (1,450 ppm fluoride)</td>
<td>8.63²¹</td>
<td>B7034603</td>
</tr>
<tr>
<td>Regenerate Enamel Science</td>
<td>Unilever; London, United Kingdom</td>
<td>Glycerin, calcium silicate, PEG-8, hydrated silica, trisodium phosphate, sodium phosphate, aqua, PE-60, sodium lauryl sulfate, sodium monofluorophosphate, aroma flavour, synthetic fluorlogophite, sodium saccharin, polyacrylic acid, tin oxide, limonene</td>
<td>9.02²¹</td>
<td>4175CCC</td>
</tr>
<tr>
<td>Regenerate Enamel Science -Advanced Enamel Serum</td>
<td>Unilever; London, United Kingdom</td>
<td>NR-5 serum: glycerin, calcium silicate, peg-8, trisodium phosphate, sodium phosphate, aqua, PE-60, sodium lauryl sulfate, sodium monofluorophosphate, aroma/flavour, hydrated silica, synthetic fluorlogophite, sodium saccharin, polyacrylic acid, tin oxide, CI 77891, Limonene Activator gel: Aqua, glycerin, cellulose, gum, sodium fluoride, benzyl alcohol, ethylexyglycerin, phenoxyethanol, CI 42090</td>
<td>Not available</td>
<td>42038CA</td>
</tr>
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</table>

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline (KHN)</th>
<th>Bleached (KHN)</th>
<th>Re-min (KHN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonbleached</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>342.7 ± 34.2</td>
<td>321.4 ± 33.4</td>
<td></td>
</tr>
<tr>
<td>NM</td>
<td>341.4 ± 53.6</td>
<td>332.5 ± 22.5</td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>346.6 ± 35.4</td>
<td>338.5 ± 23.9</td>
<td></td>
</tr>
<tr>
<td>CS+NR-5</td>
<td>347.3 ± 42.1</td>
<td>328.8 ± 43.2</td>
<td></td>
</tr>
<tr>
<td>Bleached</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>346.1 ± 29.9</td>
<td>281.1 ± 47.3</td>
<td>272.1 ± 33.7</td>
</tr>
<tr>
<td>NM</td>
<td>348.5 ± 21.0</td>
<td>274.9 ± 25.4</td>
<td>293.6 ± 36.2</td>
</tr>
<tr>
<td>CS</td>
<td>343.0 ± 42.8</td>
<td>272.2 ± 37.6</td>
<td>324.2 ± 42.6</td>
</tr>
<tr>
<td>CS+NR-5</td>
<td>349.1 ± 46.3</td>
<td>276.5 ± 43.5</td>
<td>335.0 ± 31.5</td>
</tr>
</tbody>
</table>

Abbreviations: CS, regenerate enamel science; NR-5, regenerate enamel boosting serum; KHN, Knoop hardness number; NM, sensodyne repair and protect; NT, no treatment.

Note: Within a column, different superscript capital letters indicate a significant difference (p < 0.05). Within a row, different superscript small letters indicate a significant difference (p < 0.05).
control. Both groups were kept in artificial saliva for 24 h and did not receive any remineralization treatment. Groups 2 and 6 were subjected to remineralization treatment with NovaMin-based toothpaste (NM), and groups 3, 4, 7, and 8 were subjected to CSSPF-based toothpaste (CS). Group 4 and 8 received additional treatment with NR-5 boosting serum (CS+NR-5).

The remineralization procedure in groups 2, 3, 4, 6, 7, and 8, consisted of 3-min application of a solution of toothpaste/water in 1:2 ratio two times a day for 7 days. The toothpaste solution was applied by using a soft brush and minimal pressure. In Groups 4 and 8, after treatment with the toothpaste, the remineralizing NR-5 gel was prepared by mixing equal amounts of NR-5 serum with the activator gel (1:1 ratio) on a glass slab with a plastic mixing stick. This mixture was applied to the specimens’ surfaces and kept undisturbed for 3 minutes once daily for 3 consecutive days.

After each application of toothpaste or serum gel, the specimens were rinsed in distilled water for 5 sec and kept in the freshly prepared artificial saliva in an incubator at 37°C. The artificial saliva was prepared following Holland’s protocol,17 (composition: 0.4 g NaCl, 0.4 g KCl, 0.795 g CaCl2·2H2O, 0.78 g NaH2PO4·2H2O, 0.005 g Na2S·9H2O, 1 g urea, and 1,000 ml distilled water), and the pH of the saliva was adjusted at 7 ± 0.2.

Knoop Surface Hardness Test
As mentioned earlier, the Knoop surface microhardness (KHN) of the enamel surfaces before and after bleaching was recorded. Subsequently, the test was repeated following each remineralization treatment. The number of indentions per sample and the testing procedures were standardized throughout the study. At each testing point, data of each experimental group from 10 specimens were averaged.

Surface Roughness Test
Forty additional enamel samples were prepared and polished, as mentioned above. The baseline surface roughness of each specimen was evaluated under an atomic force microscope (AFM; Flex-Axiom, Nanosurf AG, Liestal, Switzerland). AFM was used in a noncontact mode by using an AFM cantilever with magneto-resistive sensors integrated into its tip (7 µm thickness, 225 µm length, and 38 µm width) under a constant force of 48 N/m.

On each polished surface, four standardized rectangular spots (25 × 25 µm each) were scanned. The changes in the vertical position provide the height of the images, registered as bright and dark regions. The tip-specimens distance was maintained stable through constant oscillation amplitude (set-point amplitude). AFM micrographs were analyzed by using a scanning probe microscopy data analysis software (C3000 control software; version 3.7.2.8, Nanosurf AG, Liestal, Switzerland). The average surface roughness (Sa), valley depth (lowest value - Sv), and peak height (highest value - Sp) of the unbleached enamel surfaces were recorded as the baseline readings and were expressed as numeric values in nanometers.

Then the samples were divided into eight equal groups (n = 5) according to the experimental design mentioned above. The surface roughness measurements were repeated after each allocated treatment for each experimental group.

Micromorphological and Energy Dispersive X-Ray Analysis
Three representative samples from each experimental group were dehydrated in a desiccator containing dehydrated silica gel at room temperature for 24 h. The micromorphological analysis was performed after sputter coating with 100 Å Gold-Palladium (EMS 7620 Mini Sputter Coater; Electron Microscopy Sciences, Hatfield, Pennsylvania, United States) to create a conductive specimen surface and to reduce electron charging, which may reduce the quality of the image. Observations were performed under different magnifications up to ×5000 by using a scanning electron microscope (SEM) (VEGA3 XM–TESCAN; Kohoutovice, Czech Republic) operating at 20 kV acceleration voltage and 10 to 25 mm working distance.

SEM-coupled energy-dispersive X-ray spectroscopy (AZtecLive, Oxford Instruments; Abingdon, United Kingdom) operating at a take-off angle of 35 degrees with map mode was conducted at the same operating voltage. The average main elements % weight content of the experimental groups (calcium and phosphorus), and the calcium to phosphorus ratio (Ca/P) per each group was calculated.

Statistical Analysis
Results were analyzed with statistical software (SPSS software; version 15.0, SPSS Inc., Chicago, Illinois, United States) by using a two-way analysis of variance and Bonferroni post hoc multiple comparison tests (α = 0.05) to determine the effect of the variables (bleaching and the type of the remineralizing agent) on the surface hardness and surface roughness of enamel, and to determine the effect of bleaching and type of the remineralizing agent on the surface hardness and surface roughness of enamel.

Results
The results of the Knoop microhardness test of the experimental groups are listed in ►Table 2. The post hoc test revealed a statistically significant difference (p < 0.05) between the surface hardness of the enamel specimens before and after bleaching, with a significant reduction in the enamel surface hardness by 18 to 21%. NM showed inferior ability to restore the reduced surface hardness of enamel, compared with CS and CS+NR-5 groups, which showed no significant difference (p > 0.05) between the KHN of enamel at baseline and after remineralization with these two groups.

The results of the AFM surface roughness test of the experimental groups are listed in ►Table 3 and illustrated in ►Fig. 1. A statistically significant difference (p < 0.05) between the surface roughness of the enamel specimens before and after bleaching was evident, with more than threefold increase in the enamel surface roughness. Treatment of enamel with NM significantly increased the surface roughness of both bleached and nonbleached enamel (p < 0.05). Meanwhile, the

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Table 3  Mean area surface roughness in nm (± standard deviation) of bleached and nonbleached enamel surfaces after no treatment, treatment with sensodyne repair & protect, regenerate enamel science, or regenerate enamel-advanced enamel serum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline (nm)</th>
<th>Bleached (nm)</th>
<th>Re-min (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonbleached</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>42.6 ± 8.6</td>
<td>42.4 ± 5.5a</td>
<td></td>
</tr>
<tr>
<td>NM</td>
<td>46.7 ± 7.4a</td>
<td>84.0 ± 7.6b, b</td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>44.7 ± 5.9</td>
<td>51.6 ± 7.4a</td>
<td></td>
</tr>
<tr>
<td>CS+NR-5</td>
<td>42.9 ± 6.8</td>
<td>54.7 ± 8.4a</td>
<td></td>
</tr>
<tr>
<td>Bleached</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>44.3 ± 5.1a</td>
<td>136 ± 9.6a</td>
<td>144.7 ± 9.4c,b</td>
</tr>
<tr>
<td>NM</td>
<td>40.8 ± 8.3a</td>
<td>130 ± 7.6a</td>
<td>173.6 ± 11.3b,c</td>
</tr>
<tr>
<td>CS</td>
<td>44.2 ± 9.2a</td>
<td>140 ± 8.2b</td>
<td>89.7 ± 8.2b,c</td>
</tr>
<tr>
<td>CS+NR-5</td>
<td>46.3 ± 8.4a</td>
<td>142 ± 9.3b</td>
<td>82.4 ± 9.1b,c</td>
</tr>
</tbody>
</table>

Abbreviations: CS, regenerate enamel science; NR-5, regenerate enamel boosting serum; NM, sensodyne repair and protect; NT, no treatment. Note: Within a column, different superscript capital letters indicate a significant difference (\(p < 0.05\)). Within a row, different superscript small letters indicate a significant difference (\(p < 0.05\)).

Fig. 1 Scanning electron microscope and atomic force microscope microphotographs of bleached and nonbleached enamel surface after no treatment, or treatment with sensodyne repair and protect (NM), regenerate enamel science (CS) or CS followed by regenerate enamel boosting serum (CS+NR-5).
CS and CS+NR-5 groups showed a significant decrease in surface roughness of the bleached enamel ($p < 0.05$).

The average element content (wt%) of calcium, and phosphorus, and Ca/P ratio of the experimental groups are listed in Table 4. Within the nonbleached groups, there was no significant difference in the main element wt% and the Ca/P ratio between NT and the treated groups. A significant reduction in the Ca/P ratio was recorded after bleaching ($p < 0.05$). In comparison to the PC group, an increase in the Ca/P ratios was measured for the bleached groups after treatment with NM, CS, and CS+NR-5.

Sample SEM microphotographs of the investigated groups are presented in Fig. 1. The surface morphological analysis showed a typical honeycomb appearance in all the bleached groups, the pattern is similar to that of the acid-etched enamel. Compared with the NT bleached group, this pattern was less evident after treatment with the tested remineralizing agents. None of these treatments were able to completely reverse the effect of bleaching, compared with the nonbleached treated groups.

**Discussion**

Teeth whitening, although comparatively prevalent, is still considered controversial regarding the morphological changes brought upon enamel, accompanying the procedure. The significance of this concern has manifested as the recommendation of using a remineralizing agent after bleaching. The effectiveness of the various remineralizing agents remains in question. In this study, the effectiveness of CSSPF and NovaMin-based dentifrice to remineralize bleached human enamel were tested, using the Knoop microhardness test and analyzing mean surface roughness values with an AFM. This study has observed that the tested remineralizing products have positively affected the microhardness and surface roughness of the bleached enamel; thus, the null hypothesis tested should be rejected.

Conflicting results were reported in the literature regarding the effect of bleaching on enamel. Cadenaro et al, in an in vivo study, found no significant effect of 38% HP in-office bleaching on the roughness of enamel. In contrast, Hosoya et al reported morphological changes to enamel following bleaching with a high concentration of HP, and concluded a strong association between bleaching and the increased enamel roughness and Streptococcus mutans adhesion. While Basting et al reported a significant reduction by 10 to 23% KHN of bleached enamel, Rodrigues et al reported a 47% reduction in KHN of bleached enamel. These inconsistencies may be due to differences in study design, namely, the magnitude and time of loading used in the hardness testing or the different concentration and time of application of the bleaching agent.

In the current study, a significant reduction in surface hardness and an increase in surface roughness of enamel was found after bleaching, and these results were confirmed by the SEM morphological analysis and the AFM micrographs (Fig. 1). Our findings are in agreement with a study by Lewinstein et al, who reported a reduction of 13% KHN for home bleaching and up to 25% for in-office bleaching.

These changes in surface roughness and hardness after enamel bleaching can be attributed to either the low pH or high concentration of the peroxides used with the in-office bleaching. The bleaching material used in this study has a pH value of 7.52, which is considered neutral or slightly alkaline. Therefore, the increased porosity of enamel can be attributed to the effect of the nascent oxygen of the high concentration HP rather than the acidic erosion of the hydroxyapatite.

The EDX elemental analysis (Table 4) revealed a significant reduction in the Ca/P ratio of the bleached enamel in comparison to the baseline readings, which in agreement with the findings of several previous studies.

The application of CSSPF showed a significant increase in microhardness compared with values obtained after bleaching. This finding is consistent with other investigations; an in situ study by Joiner et al placed acid-challenged tooth enamel inserts onto patients’ partial dentures. The inserts were then treated with four different agents. The study found that the combination of calcium, phosphate, and fluoride ions from

**Table 4** Energy dispersive X-ray analysis elemental analysis of Ca and P (wt%) and Ca/P ratio of bleached and nonbleached enamel surfaces after no treatment, treatment with sensodyne repair & protect, regenerate enamel science, or regenerate enamel-advanced enamel serum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Element composition (wt %)</th>
<th>Ca/P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonbleached</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>Ca 37.80 ± 1.3a</td>
<td>P 17.20 ± 0.7a</td>
</tr>
<tr>
<td>NM</td>
<td>Ca 36.20 ± 1.1a</td>
<td>P 17.60 ± 0.9a</td>
</tr>
<tr>
<td>CS</td>
<td>Ca 36.10 ± 1.1a</td>
<td>P 17.10 ± 1.3a</td>
</tr>
<tr>
<td>CS+NR-5</td>
<td>Ca 35.30 ± 0.5a</td>
<td>P 16.40 ± 0.8a</td>
</tr>
<tr>
<td>Bleached</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>Ca 20.00 ± 1.2a</td>
<td>P 11.70 ± 1.0a</td>
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<tr>
<td>NM</td>
<td>Ca 25.00 ± 1.0a</td>
<td>P 11.50 ± 1.1b</td>
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<tr>
<td>CS</td>
<td>Ca 25.80 ± 0.8a</td>
<td>P 11.90 ± 0.9b</td>
</tr>
<tr>
<td>CS+NR-5</td>
<td>Ca 24.50 ± 1.1a</td>
<td>P 11.00 ± 0.8b</td>
</tr>
</tbody>
</table>

Abbreviations: CS, regenerate enamel science; CS+NR-5, regenerate enamel boosting serum; NM, sensodyne repair & protect; NT, no treatment.

Note: Within a column, different superscript letters indicate a significant difference ($p < 0.05$).
the sodium fluoride and mono-fluoro-phosphate resulted in an increase in microhardness, which was consistent along with all evaluation intervals. Moreover, another experiment conducted by Hornby et al studied the effectiveness of CSSPF versus other agents under conditions of pH cycling within the normal range of demineralization and remineralization, and they concluded that CSSPF proved significant effectiveness versus different formulations included in their study. The superior effects of CSSPF can be attributed to its mode of action, which includes multiple mechanisms. Calcium silicate has the ability to release calcium ions in an acidic environment, which deprives the area of protons while simultaneously enriching the local calcium concentration. This mechanism increases the degree of saturation and theoretically inhibits demineralization. Moreover, in the presence of phosphate salts, calcium silicate has been shown to deposit directly onto the enamel surface and maintain its affinity even after rinsing. This affinity, aided by the presence of phosphate ions, results from the nucleation and eventual precipitation of HAP on the surface of the calcium silicate particles. This nucleation takes place after only one exposure and at pH values as low as 4. Moreover, the newly formed HAP may act as the first defense against future acid attacks, keeping the underlying enamel protected. These explanations were supported by the findings of the EDX elemental analysis, where an increase in the calcium and phosphate wt% was recorded after treatment with CS. Finally, the efficacy of fluoride alone, in remineralization, is directly dependent on the availability of calcium and phosphate at the site of action. This dependency is absent in calcium silicate and phosphate technologies.

It is worth noting that none of the tested materials was able to reverse the effect of bleaching on enamel roughness completely. This may be attributed to the negative effect of the abrasive particles that are added to them to increase their mechanical ability to remove plaque.

**Conclusion**

Physical and chemical structural changes brought upon enamel must be a matter of serious concern to any practitioner utilizing bleaching procedures for improved aesthetics. Also, knowledge of the different remineralizing agents and their efficacy is useful in enhancing treatment outcome and long-term patient satisfaction. The application of CSSPF for 3 min, two times a day and for 7 days was able to significantly re-harden acid-eroded enamel and greatly decrease its surface roughness.

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**Conflict of Interest**

None declared.

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