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Effect of hydrogen peroxide and its combination with nano-hydroxyapatite or nano-bioactive glass on the enamel demineralization and tooth color: An *in vitro* study

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ABSTRACT

Background: Patient demand for esthetic dental treatments is increasing, and among different techniques, tooth bleaching is a popular procedure for smile improvement. There is a controversy over the demineralizing effect of hydrogen peroxide (HP) containing bleaching agents on tooth enamel. The aim of this study was to evaluate the effect of HP and its combinations with hydroxyapatite (HA) and bioactive glass (BG) on enamel demineralization and tooth color changes.
Materials and Methods: Three groups of 20 teeth were used. Bleaching regimens included HP alone, HP + HA, and HP + BG. Bleaching was repeated at six periods of 15 min. Energy dispersive spectrometry was performed to evaluate calcium, phosphorus, sodium, magnesium, and fluoride content of superficial enamel before and after bleaching. Tooth color was evaluated by spectrophotometer before and after bleaching and ΔE values were calculated. Data were statistically analyzed using SPSS version 17.
Results: Ca and P content was increased significantly in group HP + BG ($P < 0.05$). There was no significant difference in ΔE values between the three groups ($P > 0.05$). (p value = 0.34).
Conclusion: Addition of BG to HP can increase superficial enamel mineral content after bleaching and has no effect on tooth color changes in comparison to HP alone.

Key Words: Bioglass, hydrogen peroxide, hydroxyapatites, tooth bleaching

INTRODUCTION

Discoloration of teeth due to extrinsic or intrinsic origins might compromise the beauty of patient's appearance, increasing the demand for esthetic dental procedures. Among different therapies, tooth bleaching is an easy, cost-effective, and conservative method to enhance tooth appearance.[1,2]

The bleaching agents usually contain hydrogen peroxide (HP) or carbamide peroxide (CP) that cause the oxidation of colored organic and inorganic compounds in tooth structure by the decomposition of peroxide into free radicals.[3,4] However, the reaction is unspecific, and the organic and inorganic materials of the enamel and dentin may be affected,

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resulting in undesirable topographic effects or mineral loss.[5]

Although studies are controversial on the effect of bleaching on tooth surface's morphological changes, there is a general agreement that peroxide can change tooth mineral content.[6-10]

In a few studies, fluoride has been incorporated into bleaching agents to decrease demineralization and expedite the rehardening of enamel.[11,12] Santos *et al*. reported the addition of calcium or fluoride to the bleaching gel reduced the erosion effect of HP.[12] Furthermore, some researchers have added nano-HA or nano-bioactive glass (BG) to the bleaching materials to reduce demineralization.[5,13,14]

Santos *et al*. found no anti-demineralizing effect by adding nano-HA to HP.[12] However, Khoroushi *et al*.[1] and Jiang *et al*.[15] showed that the addition of nano-HA to HP could increase enamel mineral content.

The aim of this study was to describe the effect of HP and its combination with nano-HA or nano-BG on the levels of inorganic elements in enamel and tooth color changes. The null hypothesis was that combining HP with nano-HA or nano-BG had no effects on enamel mineral content or tooth color changes.

MATERIALS AND METHODS

This study was developed in accordance with the Code of Ethics in Research (IR.SSU.REC.1396.165). This is an experimental and *in vitro* study.

Sixty intact and noncarious human premolar teeth extracted for orthodontic reasons were stored in 0.5% chloramine-T solution at 4°C and used within 2 months of extraction.[16]

Teeth were examined by a stereomicroscope (ZTX-20-W, Huaguang, China) at ×20 magnification to exclude any teeth with cracks or structural defects.[17] Teeth were allocated to the three following groups of twenty teeth using random numbers table:

- Group 1 (HP): Bleaching with 40% HP (Boost PF, Ultradent, USA)
- Group 2 (HP + HA): Bleaching with custom gel containing 40% HP (Boost PF) + hydroxyapatite (HA) 2%wt (Sigma-Aldrich, Spain)
- Group 3 (HP + BG): Bleaching with custom gel containing 40% HP (Boost PF) + BG 7.5%wt (NovaBone, Florida, USA).

Final pH of the bleaching agent in each group was measured with pH meter before the application of the material.

After embedding the root in acrylic resin, the buccal surfaces of the tooth crowns were abraded with 600-grit and 1000-grit silicon carbide abrasive papers and subjected to energy dispersive spectrometry (EDS) (Pro-X, Switzerland) analysis to measure Ca, P, Na, Mg, and F contents.

Bleaching procedure was performed following the manufacturer's instructions.

The bleaching agent was applied on the buccal surface of each tooth with a brush for 15 min. Then, the bleaching agent was removed from the surface with a brush, and the samples were rinsed with tap water for 1 min to remove the bleaching gel and were stored in 37°C distilled water until the next bleaching treatment. This was repeated three times in one session for a total of 45 min (3 min × 15 min). The treatment was repeated 1 week later (total six treatments on each tooth). EDS analysis was repeated after the completion of treatment.

Tooth shade was evaluated before and after the bleaching procedure using VITA Easyshade (VITA Zahnfabrik, Germany). L*, a*, and b* parameters were acquired for a reference point 5 mm gingival of the cusp tip three times on each tooth and the mean of the three assessments was used for Δ E calculation.[18]

The data were statistically analyzed using ANOVA, Tukey *post hoc*, and paired *t*-test in SPSS software ($P < 0.05$).(version 17.0, Chicago, IL, USA).

RESULTS

The pH of the bleaching agents was 5.0 for group HP, 7.0 for group HP + HA, and 9.5 for group HP + BG. Mean and standard deviation of studied elements are presented in Table 1. Ca and P content differed significantly between groups after bleaching (ANOVA, $P = 0.013$ and $P = 0.016$, respectively), but other studied elements did not show a significant difference. Tukey *post hoc* showed that both Ca and P contents were significantly higher after bleaching in group HP + BG than group HP + HA ($P = 0.014$ and $P = 0.032$, respectively), but groups HP and HP + HA and groups HP and HP + BG did not have a significant difference.

Mean Δ E (±standard deviation) was 7.8 ± 2.4 in group HP, 8.1 ± 2.5 in group HP + HA, and 6.8 ± 3.5

Table 1: Mean±standard deviation (μg/g) of the studied elements in experiment groups

	Ca		P		Ca/P		Na		Mg		F	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
HP	41.5±7.7	44.1±5.5	25.1±2.7	26.8±2.4	1.7±0.2	1.7±0.2	2.3±1.2	2.2±1.0	2.7±1.4	3.0±0.9	3.9±0.7	4.1±1.4
HP + HA	38.6±8.8	42.9±6.9	22.1±5.1*	25.4±3.8*	1.7±0.2	1.7±0.2	2.0±0.8	2.1±1.0	2.8±1.1	2.7±1.0	3.8±0.8	3.4±0.9
HP + BG	40.5±8.9*	48.5±5.6*	23.1±3.8*	28.0±1.7*	1.7±0.2	1.7±0.2	1.8±1.3	1.6±0.9	2.5±1.3	2.4±1.0	4.3±0.9*	3.3±1.0*

*Significant difference between before and after bleaching values (μg/g). Paired-samples *t*-test, $P<0.05$. HP: Hydrogen peroxide; HA: Hydroxyapatite; BG: Bioactive glass

in group HP + BG. There was no significant difference between the three study groups.

DISCUSSION

The aim of this study was to evaluate the effect of HP with or without nano-HA or nano-BG on enamel demineralization and tooth color change. The null hypothesis was that addition of nano-HA and nano-BG to HP did not affect enamel demineralization and tooth color. According to the results, the null hypothesis was rejected.

The use of HP alone did not change the concentration of studied elements. Loss of enamel mineral content following the application of a bleaching agent is controversial. Efeoglu *et al*. found that office bleaching can reduce the mineral content of enamel extending to a depth of 250 μm.[19] Furthermore, Bizhang *et al*. concluded that the use of bleaching agents induced mineral loss in bovine tooth enamel.[20] Our study did not confirm these results. A possible explanation may be the inclusion of fluoride compounds (as sodium fluoride) in the bleaching agent, which prevents mineral loss.[21-24]

Studies about the effect of bleaching on elemental changes are limited. However, some researchers have claimed enamel hardness change is related with its mineral content.[19] Sulieman *et al*. reported that *in vitro* use of 35% HP on enamel for 30 min had no effects on enamel or dentin hardness.[25] Faraoni-Romano *et al*. showed surface microhardness of bovine enamel increased after application of various concentrations of HP and CP.[21] In contrast, Lewinstein *et al*.[26] and Al-Salehi *et al*.[9] concluded that enamel microhardness decreases after the use of HP.

Loss of enamel superficial elements has also been investigated in the form of enamel surface roughness changes.[20,21] Faraoni-Romano *et al*. reported that the application of different bleaching agents on tooth enamel reduced its surface roughness.[21] McGukin observed a slight increase in enamel surface roughness in his study, but Hunsaker *et al*. and Gürgan *et al*. did not seen no surface roughness change.[20] It seems that morphological alterations of enamel are mainly due to the acidity of HP, while decreased microhardness is due to the effect of both demineralization and destruction of organic part by HP.[11]

The different results of studies could be due to different study conditions, including tooth substrates (e.g., human or bovine), bleaching materials, pH, preparation times,[9] procedures (e.g., application of phosphoric acid before bleaching[27] or different specimen storage conditions during experiment),[26,28] and discrepancy in performance and measurement methods.[1]

The addition of nano-HA and nano-BG to the bleaching agent increased enamel mineral content in the current study, which is in accordance with Khoroushi *et al*.[1] and Jiang *et al*.[15]

Furthermore, Sasaki *et al*. found the addition of HA to HP increased surface enamel microhardness than samples treated by HP alone.[29] Deng *et al*. also found similar results using a bleaching agent consisting of HP and BG.[11] Furthermore, it has been shown that application of BG after bleaching caused remineralization by increasing Ca and *P* values.[14]

Nanoparticle remineralizing materials were used in this study. It has been reported that the use of nano-sized HA increases the remineralization effect because the surface-to-volume ratio increases and the nanoparticles interact with enamel and dentin more effectively.[30] Furthermore, nano-sized BG has been shown to increase dentin remineralization[31-34] and overcome its long reaction time as an efficient remineralizing material.[11]

The superficial aprismatic enamel is more resistant to demineralization because it is usually hypermineralized and rich in fluorapatite,[35] so we removed this layer and polished it to gain uniform

mirror-like surface and minimize the difference among samples.[11]

In the present study, we kept the teeth in distilled water at intervals between bleaching periods. We did not use artificial saliva because it might have a remineralizing effect and interact with HA and BG due to its ionic content.[11,19]

All bleaching regimens will be considered effective since a minimum Δ E value of 3.3 is known to be visually comprehensible.[11] Although it is thought that BG deposits may interfere with the penetration of peroxide and whitening efficacy, this speculation was rejected by the current study, which is in accordance with Deng *et al*.,[11] Jiang *et al*.,[15] and Sasaki *et al*.[29] also found that the addition of HA did not interfere with the whitening results.

Future studies are required to evaluate whether BG or HA application will not decrease bleaching efficacy *in vivo*.

CONCLUSION

Within the limitations of the current study, it can be concluded that fluoride-containing HP bleaching material may not induce loss of superficial enamel mineral content. The addition of nano-HA or nano-BG to the bleaching agent may improve the mineral content (Ca and P) of superficial enamel and will not affect bleaching efficacy.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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