Effect of three nanobiomaterials on the surface roughness of bleached enamel

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Abstract

Background: The ever-increasing demand for enhanced esthetic appearance has resulted in significant developments in bleaching products. However, the enamel surface roughness (SR) might be negatively affected by bleaching agents. This *in vitro* study was undertaken to compare the effects of three nanobiomaterials on the enamel SR subsequent to bleaching. **Materials and Methods:** The crowns of six extracted intact nonerupted human third molars were sectioned. Five dental blocks measuring 2 mm × 3 mm × 4 mm were prepared from each tooth and placed in colorless translucent acrylic resin. The enamel areas from all the specimens were divided into five groups ($n = 6$): Group 1 did not undergo any bleaching procedures; Group 2 was bleached with a 40% hydrogen peroxide (HP) gel; Groups 3, 4, and 5 were bleached with a 40% HP gel modified by bioactive glass (BAG), amorphous calcium phosphate, and hydroxyapatite, respectively. The enamel SR was evaluated before and after treatment by atomic force microscopy. The data were analyzed by Kruskal–Wallis and Mann–Whitney tests. **Results:** SR increased significantly in the HP group. SR decreased significantly in the HP gel modified by BAG group as compared to other groups. **Conclusions:** Within the limitations of this study, incorporation of each one of the three test biomaterials proved effective in decreasing enamel SR subsequent to in-office bleaching technique.

Keywords: Amorphous calcium phosphate, bioactive glass, bleaching, hydroxyapatite, roughness

Introduction

There is growing demand for esthetic appearance all over the world. Of all the various features of human beings, a beautiful smile is of great significance. Tooth bleaching procedures as conservative, effective, and cost-effective techniques have become very popular choices. However, like all other treatment modalities, they are associated with some side effects. In recent years, in-office bleaching has become an easy choice after the introduction of bleaching gels containing hydrogen peroxide (HP) in concentrations up to 35−45%. As a result of these high concentrations, in-office bleaching can be completed in a short time, making it a suitable choice for patients who desire immediate whitening.[1]

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Nevertheless, a large number of studies have shown that bleaching agents can give rise to chemical, structural, and mechanical alterations in surface, as well as subsurface enamel structures.[2,3] Scanning electron microscope observations have shown microscopic changes in tooth structures consisting of an increase in porosity, depression and surface irregularities,[4] an increase in surface roughness (SR),[5] and a decrease in hardness,[6] as well as fracture resistance.[7] Bleaching exerts a direct effect on the organic (protein) content of the tooth, but this changes the mineral phase and leads to morphological changes on the tooth surface.[8]

Tooth bleaching has also been shown to increase SR.[9] SR underwent alterations during or after treatment, which was a function of HP concentration.[10]

Researchers believe that SR leads to an increase in susceptibility to bacterial adhesion and staining.[6,11,12] Subsequent to bleaching, pigments adhere to the rough surfaces, especially to those of enamel, more easily than to the original tooth surfaces, resulting in more discoloration.[13,14]

A wide array of remineralizing agents has been introduced to prevent these adverse outcomes. During remineralization, calcium and phosphate ions from an external source reach

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the tooth, resulting in an increase in deposition of ions in the crystal voids in demineralized enamel, increasing net mineral gain.[15]

Fluoride use has been considered in bleaching techniques[16] or added to bleaching gels;[17] however, its influence on bleached enamel is a matter of controversy. In addition, its low solubility results in rapid deposition of fluorapatite on enamel; therefore, it cannot penetrate into deeper layers[18] resulting in remineralization being confined to superficial layers.[19]

A study showed no significant differences in SR between samples exposed to fluoride gels or HP with or without calcium, or in the initial, and final roughness characteristics of specimens.[20]

Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is another option. The calcium and phosphate ions are released from the CPP complex and penetrate into the enamel rods, resulting in an increase in the density of hydroxyapatite (HA) crystals,[15,21] and at least preventing demineralization by subsequent acid attacks.[22]

There is insufficient data available on the effects of in-office bleaching agents, which have been modified by remineralizing agents, on the enamel SR. Therefore, this *in vitro* study was undertaken to investigate the efficacy and influence on enamel SR of in-office bleaching technique in association with the use of three nanobiomaterials as hardening/remineralizing agents: ACP, HA, and bioactive glass (BAG).

The null hypothesis tested was that incorporation of each one of ACP, HA, and BAG into HP gel would not affect enamel SR.

Materials and Methods

Preparation of the specimens

Six nonerupted human third molars, extracted for orthodontic reasons, were selected. The buccal surfaces had no stains, enamel cracks or fractures, caries, or other defects. The teeth were cleaned thoroughly and stored in 0.5% thymol until used for the purpose of the study.

Five dental blocks measuring 2 mm × 3 mm × 4 mm were prepared from each tooth using a low-speed saw under water cooling. The dental blocks were separately placed in colorless translucent acrylic resin, with enamel surfaces exposed for application of bleaching agents. The enamel samples were flattened with the use of a polishing machine with 400-, 600-, and 1200-grit abrasive paper under water cooling followed by polishing with diamond pastes, as well sequentially decreasing granulations (6 μm, 3 μm, 1 μm, and 1/2 μm), using felt discs under mineral oil cooling and gentle manual pressure. The enamel thickness was not measured, but all the specimens underwent visual examinations for the existence of a very thin enamel layer or dentin exposure. Samples not suitable for the evaluation of roughness were excluded from the study.

Bleaching procedure

The 40% (Opalescence® Xtra® Boost, Ultardent Products Inc., USA) in-office bleaching protocols were performed for 2 weeks, with one session each week. Each session consisted of three 15-min periods with a 5-min interval between them. Wet cotton pellets were placed on the specimen surfaces to prevent dehydration between treatment procedures. Five groups were evaluated with the following protocols:

- Group 1 ($n = 6$): No treatment (control)
- Group 2 ($n = 6$): Bleaching gel
- Group 3 ($n = 6$): Bleaching gel + BAG Nova Bone Products LLC, Alachua, Florida, USA)
- Group 4 ($n = 6$): Bleaching gel + ACP (Sigma, Aldrich, Spain)
- Group 5 ($n = 6$): Bleaching gel + HA (Sigma, Aldrich, Spain).

Surface roughness test

Atomic force microscopy (AFM) testing machine (Brukernano scale 1.1) was used for roughness test before and after the bleaching procedures, using 1 μm × 1 μm images. AFM software, Image Plus 2.9 by Gwyddion were used to calculate Ra for each specimen.

Statistical analysis

The differences in enamel SR data before and after bleaching procedures in each group were statistically analyzed using Kruskal–Wallis and Mann–Whitney tests. Statistical significance was set at $P < 0.05$.

Results

Average SR values before and after treatments in each group are presented in Table 1 and Figure 1.

Table 1: Mean values (standard deviations) of baseline and final surface roughness measurements for each group and the surface roughness change

Groups numbers and definitions	SR (B)	SR (F)	SRC
(1) Control	23.51 (10.49)	23.51 (10.49)	0.00 (0.00)[a]
(2) Bleached	22.43 (7.86)	31.47 (14.04)	9.04 (6.94)[b]
(3) Bleached + BAG	23.64 (7.26)	9.05 (1.55)	−14.59 (6.28)[c]
(4) Bleached + ACP	22.62 (8.72)	19.98 (10.14)	−2.64 (9.51)[a]
(5) Bleached + HA	24.55 (8.77)	21.68 (6.29)	−2.87 (8.62)[a]

SR (B) is the average of the baseline surface roughness measurements, SR (F) the average of final surface roughness values and SRC surface roughness change. Different superscripts (a, b and c) indicate mean values that are significantly different. ACP: Amorphous calcium phosphate; HA: Hydroxyapatite; BAG: Bioactive glass

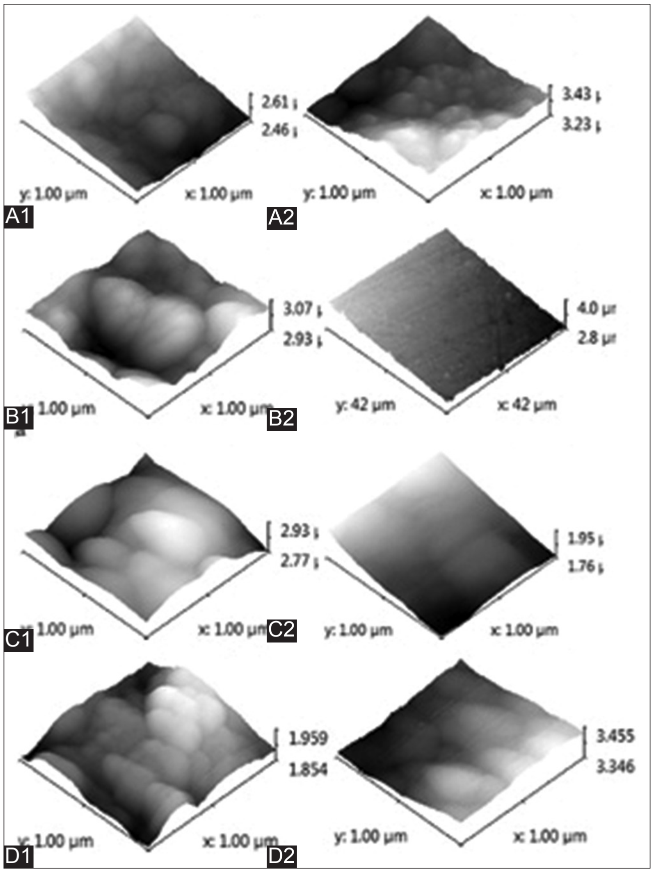

Figure 1: Atomic force microscopy images: Left column (A1-D1): Intact enamel. Right column: A2 (bleached enamel with a 40% hydrogen peroxide), B2 (bleached enamel with a 40% hydrogen peroxide gel modified by bioactive glass), C2 (bleached enamel with a 40% hydrogen peroxide gel modified by amorphous calcium phosphate), and D2 (bleached enamel with a 40% hydrogen peroxide gel modified by hydroxyapatite)

There were significant differences in SR alterations between the five study groups ($P = 0.003$). There were significant differences between the control group and HP ($P = 0.004$) and BAG + HP ($P = 0.002$) groups. SR increased significantly in the HP group when compared with other groups. There were no significant differences between the control group and HA + HP ($P = 0.99$) and ACP + HP ($P = 0.394$) groups. SR decreased significantly in the BAG + HP group as compared to other groups.

Discussion

The first hypothesis evaluated in this investigation was validated since combinations of HP with ACP, HA, and BAG did not affect enamel SR.

A large number of previous studies have shown that bleaching agents exert negative effects on enamel hardness, roughness, and surface morphology.[6,9,23] In this study, AFM was used to determine enamel SR before and after treatment with HP and HP modified by incorporation of ACP, HA, and BAG.[24]

AFM was applied to investigate tooth surfaces to compare particle distribution patterns in the superficial layer of tooth surfaces.[25] It was found that AFM yields high-contrast and high-resolution images and is an important tool to provide new structural information: Tapping mode AFM (TM-AFM) images can demonstrate net differences between exposed and unexposed enamel areas.[26]

In a number of studies, 20%, 25%, and 35% H_2O_2 bleaching systems resulted in significant reductions in enamel SR as compared to the control group.[27,28] However, two clinical studies with the use of noncontact profilometric measurements of surface replicas did not reveal any significant differences in enamel SR between the test and control groups subsequent to bleaching with 35% carbamide peroxide (CP) or 38% H_2O_2.[29,30]

Another study showed that both the in-office bleaching technique with 38% HP for 45 min and the at-home technique using 10% CP for 7 days resulted in no decrease in micro hardness and SR of the enamel.[28] Bleaching with 10% HP and 10% CP did not result in changes in the enamel SR, but bleaching treatment combined with the use of abrasive dentifrices significantly increased roughness values.[31] Other investigations showed that tooth bleaching can increase SR.[9,32] The discrepancies in the results might be attributed to differences in study conditions, including tooth substrates (human or bovine), bleaching agents, pH values, treatment times, procedures, and different methods used for measurements.

There are still concerns regarding the negative effect of HP on enamel.[19] In some recent studies remineralizing agents have been incorporated to prevent or at least minimize potential damages to enamel by bleaching agents.[16,33,34]

Ideally, a remineralization system is expected to furnish calcium, phosphate, and fluoride ions that can affect subsurfaces rather than deposition only in the superficial layer.[21]

Based on the results of the present study, use of the remineralizing agents ACP, BAG, and HA, during bleaching procedures with 40% HP resulted in a decrease in mean enamel SR values, when compared to the positive control group (Group 2), in which no remineralizing agent was used. This result was significant in Group 3, in which BAG was added to the bleaching gel, resulting in a decrease in SR in comparison with the control group.

It has been reported that there is an increase in the remineralization effect when nano-sized HA is used. In fact, the interaction of nanoparticles with dentin and enamel is more effective, which is attributed to an increase in surface-to-volume ratio;[35] therefore, we used nano-sized remineralizing agents to promote the penetration of the agents as reinforcing agents into porosities resulting from the probable mineral loss due to bleaching.

In relation to the bleaching agent pH, it must be pointed out that the more alkaline the agent, the shorter the exposure time needed, resulting in better bleaching efficacy.[36] However, the alkalinity of the material reduces the expiry date, explaining why these materials are marketed with an acidic pH.[32] ACP, BAG, and HA, as alkaline salts, might buffer the acidity of HP and reduce demineralization when mixed with HP.[19]

Since the main mineral complex of the tooth is HA, it is rational to use it as a remineralizing agent.[37] The HA particles adhere homogeneously to the enamel surface, forming a protective layer for the underlying enamel, decreasing the direct contact of HP with enamel surface. The solution around the enamel surface might soon become supersaturated with enamel apatite.[38] In a recent clinical trial use of 6% HP with 2% nano-HA resulted in the lower sensitivity as compared to the bleaching product without nano-HA.[39]

Another study revealed that ACP renders the tooth less sensitive to heat, cold, air pressure, and tactile stimulation when it is applied topically by dental practitioners or by patients themselves.[40] This might be attributed to the fact that ACP has the capacity to obliterate the dentinal tubules by rapidly depositing calcium phosphate ions on the surface and within the dentinal tubules.[41,42]

ACP might be a promising biomimetic adjunct for bleaching procedures to prevent/restore the enamel damage induced by bleaching agents.[19] In the present study, BAG + HP decreased enamel surface roughness, with significant differences from the control group.

BAGs consist of oxides of calcium, sodium, phosphorus, and silica.[8] It has been reported that in aqueous media glasses have the capacity to form a layer of HA on their surface[43] that occludes the dentinal tubules, inhibits dentin demineralization, and increases dentin remineralization.[44]

In the aqueous environment of the tooth, sodium ions are rapidly exchanged with hydrogen cations (in the form of H_3O^+), resulting in the release of calcium and phosphate ions from the glass.[45] In addition, the release of sodium results in a transient increase in pH, which promotes precipitation of extra calcium and phosphate ions from BAGs. Then, this layer crystallizes into carbonate-enriched HA, preventing further demineralization.[46]

It should be noted that the present study was carried out *in vitro*, and further studies are necessary to substantiate the hypothesis that remineralizing agents can decrease SR when combined with high concentrations of bleaching agents.

Conclusion

Within the limitations of this *in vitro* study, it can be concluded that bleaching procedures have a detrimental effect on enamel SR, which can be minimized by the subsequent use of BAG, ACP, and HA powders as remineralizing agents.

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Conflicts of interest

There are no conflicts of interest.

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