Fracture toughness of bleached enamel: Effect of applying three different nanobiomaterials by nanoindentation test

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Abstract

Background: Despite the absence of dispute about the efficacy of bleaching agents, a prime concern is about their compromising effect on the enamel structure. This *in vitro* study investigated whether the addition of three different biomaterials, including nano-bioactive glass (n-BG)/nano-hydroxy apetite (n-HA)/nano-amorphous calcium phosphate (n-ACP), to bleaching agents can affect the fracture toughness (FT) and vickers hardness number (VHN) of bovine enamel. **Materials and Methods:** The crowns of the newly extracted permanent bovine incisors teeth were separated from the root and sectioned along their central line; one half serving as the control specimen and the other half as the test specimen. After mounting and polishing procedure, all the control specimens (C) were subjected to nano-indentation test to obtain the baseline values of FT. Then, the control specimens were exposed to a 38% hydrogen peroxide for four times, each time for 10 min. The test specimens were divided into three groups and treated as follows, with the same protocol used for the control specimens: Group 1; ACP + hydrogen peroxide (HP) mixed gel; Group 2 BG + HP mixed gel; and Group 3 HA + HP mixed gel. FT measurements with nano-indentation were carried out subsequent to bleaching experiments. Data were analyzed using SPSS and Kruskal–Wallis test ($\alpha = 0.05$). **Results:** A significant difference in young's modulus (YM), VHN, and FT at baseline and subsequent to bleaching in control group was observed. However, no significant differences were found in YM, VHN, and FT between the test groups, compared to the respective baseline values. **Conclusion:** Under the limitations of the current study, it can be concluded that the n-HA, n-ACP, and n-BG could be potential biomaterials used to reduce the adverse effects of tooth bleaching.

Keywords: Biomaterials, enamel, fracture toughness, hardness, tooth whitening

Introduction

Among various esthetic procedures for improving tooth colors, vital tooth bleaching is a well-accepted method of treating discolored tooth due to its conservative approach and higher cost-benefit ratio compared with other methods.[1] Carbamide peroxide (CP: 35–37%) or hydrogen peroxide (HP: 30–40%) in high concentrations is most commonly used bleaching agent used in office bleaching technique.[2] These reactive agents break into water and nacent oxygen, with low molecular weight and thereby infiltrate through enamel and dentine gaps. This rupture the weak bonds between chromatogenic molecules and organic matrix and convert it into smaller and lighter molecules.[3]

Though efficacious, the bleaching agents may weaken the enamel structure. Some studies have reported no significant effects of bleaching agents on human enamel.[3,4] However, the findings indicative of bleaching as an erosive process are supported by several studies, showing morphological changes and modification of microstructural integrity of enamel in deeper regions.[5] The variation caused in the inorganic composition of dental structures, decline in the quantity of calcium and phosphorus, modification in the morphology of many crystrals in the superficial layer are noticed after treatment with peroxide based bleaching agents, in comparison with nontreated enamel.[2] Moreover, the commonly used carriers in bleaching agents (except

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glycerin or carbopol) may themselves act as demineralizing agents.[6] Therefore, it becomes imperative to undo any harm caused by these bleaching agents by the application of mineralizing agents on affected tooth surfaces.[5]

Nowadays, several mineralizing agents are used to reinforce the mineralized dental tissues including enamel and dentin. Hydroxy apetite (HA) can remineralize altered enamel surfaces, helps in healing of tooth microfractures and brightens and whitens the teeth.[7] According to a recent report, HA paste when applied can repair early carious lesions and microscopic pores.[8] HA nanocrystals were found to be growing impeccably between the enamel and paste interface.[9]

Amorphous calcium phosphate (ACP) is one of the promising remineralizing agents. On application of ACP on incipient enamel lesions, there is a quick precipitation of ACP in the tooth surface defects, hydrolyzing into apatite, and filling the defects. This process leads to a possible remineralizing of dental tissue[10] and also improve the luster and smoothness of teeth.[11] The latest research dwells in biomimetic, materials-like, CPP-ACP and their effectiveness in reduction of mineral loss, remineralization of white spot lesions and maintenance of structural integrity of teeth.[12] A recent study illustrated that using CPP-ACP on enamel surface prior to bleaching could also bring about protective effects.[13]

More recently, some bioactive glass (BG) materials have been introduced as tooth remineralizing agents. BG, initially found to be a bone regenerative material,[11] has a composition of silicon, sodium, calcium, and phosphorus oxides in defined amount; it was introduced as 45S5 Bioglass in 1969 by Larry Hench.[14] Claims have been made regarding its remineralizing potential and that it prevents demineralization.[15] It is believed to form hydroxycarbonate apatite akin to biological mineral. Further, its alkaline nature reduces demineralization by buffering the acidic bleaching products, on mixing with HP.[16]

Several *in vitro* tests have been introduced to evaluate the mechanical properties of dental hard tissues. The fracture toughness (FT) test (K_{IC} test) is employed to assess the fracture resistance of brittle materials. It is a sign of material resistance to crack initiation and unstable propagation. It is a superior measure of tooth fracture resistance that conventional tests (flexural strength test).[17] K_{IC} being an intrinsic property, it is an accurate measure of structural integrity, unaffected by different loading conditions or specimen geometries. The effect of bleaching agents on enamel and dentine fracture resistance is found to be contentious. Seghi and Denry[18] confirmed a 30% reduction in FT of tooth with 10% CP bleaching for 12 h, whereas no considerable reduction in toughness was found in another study on bleaching the enamel specimens for 70 h with 6.5% HP or 20% CP.[19]

Hence, the aim of this present study was to investigate the effect of addition of each of the three remineralizing agents – nano BG/HA/ACP to bleaching agents. The effect was assessed in terms of FT of bovine enamel. The null hypothesis stated that FT of bleached enamel was unaffected with the addition of nano-BG (n-BG) or HA or ACP to the bleaching agents.

Materials and Methods

Specimen preparation

Newly extracted permanent bovine incisors were collected. The teeth were cleaned thoroughly and stored at 4°C in 0.2% thymol solution until required. The teeth were cross-sectioned with a diamond blade (Speedy, Prodont, Holliger, Vence, France) in a low-speed hand piece, separating the crown from the root. The crown of each tooth was sectioned along the central line with a slow-speed water-cooled diamond saw; one half served as the control specimen and the other half as the test specimen. Each specimen was used as its own control to reduce the effects of natural variation of enamel on the results of the experiment. Each half crown was embedded in self-cured acrylic resin (Dentsply Ltd., Surrey, England) so that the buccal surface remained exposed to remove the outermost enamel layer. To obtain a flat and smoothly polished surface for the nano-indentation test, the labial surfaces were sequentially ground wet with 3000- and 5000-grit silicon carbide abrasive papers (Silicium Carbide, Matador, Wasserfest 991A, Softflex). During polishing procedures, the bottom of the embedded specimens was aligned parallel to the polished surface. Subsequently, the specimens were inspected under a stereomicroscope (SP10.0224, Motic Instrument Inc., CA, USA) to exclude those with cracks or defects.

Bleaching procedure

Prior to initiating the bleaching treatment, all the control specimens were subjected to nano-indentation test to obtain the baseline values of FT (C−). After the "initial" measurements were made, the control specimens were exposed to a 38% HP bleaching agent (Opalescence Xtra Boost, Ultradent Products, South Jordan, UT, USA) with a pH value of 7.0 (C+). This product is packaged as two syringes, one containing the liquid HP and the other a chemical activator. To mix the two components, the syringes were joined together and back and forth movements were made twenty times to thoroughly mix the agents according to manufacturer's instructions. After mixing, the syringes were separated and the mixture was applied as a 1-mm-thick layer on the enamel surface. The treatment procedure was repeated four times, two times on the 1st day and two times on the 5th day, each time for 10 min according to the manufacturer's instructions, so that the total application time added up to 40 min. Between treatment sessions, the residual treatment gel was removed under running distilled water.

During the treatment period, the specimens were stored in an incubator under 37°C in distilled water.

The test specimens (T) were divided into three groups ($n = 3$) and treated as follows:

Group 1 (ACP group): A mass fraction of 7.5% was used to make ACP + HP mixed gel.[2]

Group 2 (BG group): A mass fraction of 7.5% was used to make BG + HP mixed gel.[16]

Group 3 (HA group): A mass fraction of 2% was used to make HA + HP mixed gel.[1]

In each group, the prepared gel was applied on the test specimens with the same protocol used for control specimens.

All the materials used in this study are described in Table 1, according to their compositions manufacturers.

Nano-indentation test

At baseline (C−), FT measurements were carried out, and control (C+) and test measurements were carried out subsequent to bleaching experiments. Nano-indentation testing was performed by the CSM indentation tester (Nano-Hardness Tester, CSM, Switzerland) with maximum load of 100 mN by a diamond Berkovich indenter tip. According to the literature, the Poisson's ratio of 0.28 was assumed for calculating the elastic modulus. Five indentations per test were performed on each specimen at every experimental stage.

The K_{IC} was calculated by the following equation:

$$K_{IC} = 0.016\left(\frac{E}{HV}\right)^{\frac{1}{2}} \frac{P}{C^{\frac{3}{2}}}$$

where E is the elastic modulus measured in the nano-indentation test (GPa), HV is the Vickers microhardness (GPa), P is the applied indentation load (mN), and C is the indentation crack length (m).

Atomic force microscope (AFM) was used to evaluate the surface topography and indentation crack length of enamel. AFM is one kind of scanning probe microscopes which uses a microscale cantilever with a fine tip to scan the surface of the samples and the deflection of the cantilever is utilized to gather information about the surface properties.

Statistical analysis

Statistical analysis was performed using the SPSS 18.0 software (SPSS Inc., Version 18.0, Chicago, IL, USA). For intertreatment analyses comparing the differences in mean index scores observed between the positive control or test and baseline group, a nonparametric test (Npar test) was used as the statistical measure. Data assessed intratreatment product effects in the test group compared with baseline using the Kruskal-Wallis test. The null hypothesis for the Npar test stated that there is no difference between baseline scores and the scores collected after treatment in the positive control or test groups. The null hypothesis for intratreatment comparisons was that there is no difference in the observed effect caused by either reinforcing/remineralizing agents. All the hypotheses were tested at $\alpha = 0.05$.

Results

Young's modulus (YM), vickers hardness number (VHN), and FT value of samples at the baseline, after bleaching in positive control and test groups are presented in Figure 1a-c and Tables 2-4.

The results demonstrated that the mechanical properties of enamel decreased after bleaching with 38% HP. There was a significant difference in YM between baseline and positive control group ($P = 0.015$); and a significant difference in VHN ($P = 0.008$) and FT ($P = 0.037$) was found between baseline and positive control group. However, no significant differences were found in YM, VHN, and FT of the test, between any of the groups, compared to the respective baseline values. Comparisons of the ΔYM, ΔVHN, ΔFT (Δ: Test value subtracted from the baseline value) within each one of nono-ACP (n-ACP), nBG, and nano-HA (n-HA) groups revealed a significant difference only in ΔVHN ($P = 0.50$), whereas nBG group showed greater ΔVHN than n-ACP and n-HA groups.

Figure 2 compares the AFM images of baseline, positive control, and test specimens. As seen in the figure, there is a slight decrease in roughness of enamel surfaces of the test groups, especially for the n-HA group.

Table 1: Description of materials used in this study

Treatment agents	Composition*	Product name	Manufacturer
Opalescence Xtra Boost 38%	Hydrogen peroxide, chemically activated agent	Opalescence Xtra Boost	Ultradent Products, South Jordan, UT, USA
Bioactive glass (45S5 bioglass)	45% SiO_2, 24.5% Na_2O, 24.5% CaO, 6% P_2O_5	NovaBone	NovaBone Products LLC Alachua, Florida, USA
Hydroxyapatite	$3Ca_3(PO_4)_2 \cdot Ca(OH)_2$	Nanopowder	Sigma, Aldrich, Spain
Amorphous calcium phosphate	$Ca_2O_7P_2H_2O$	Puruma p.a	Sigma, Aldrich, Spain

*According to the information provided by the manufacturers

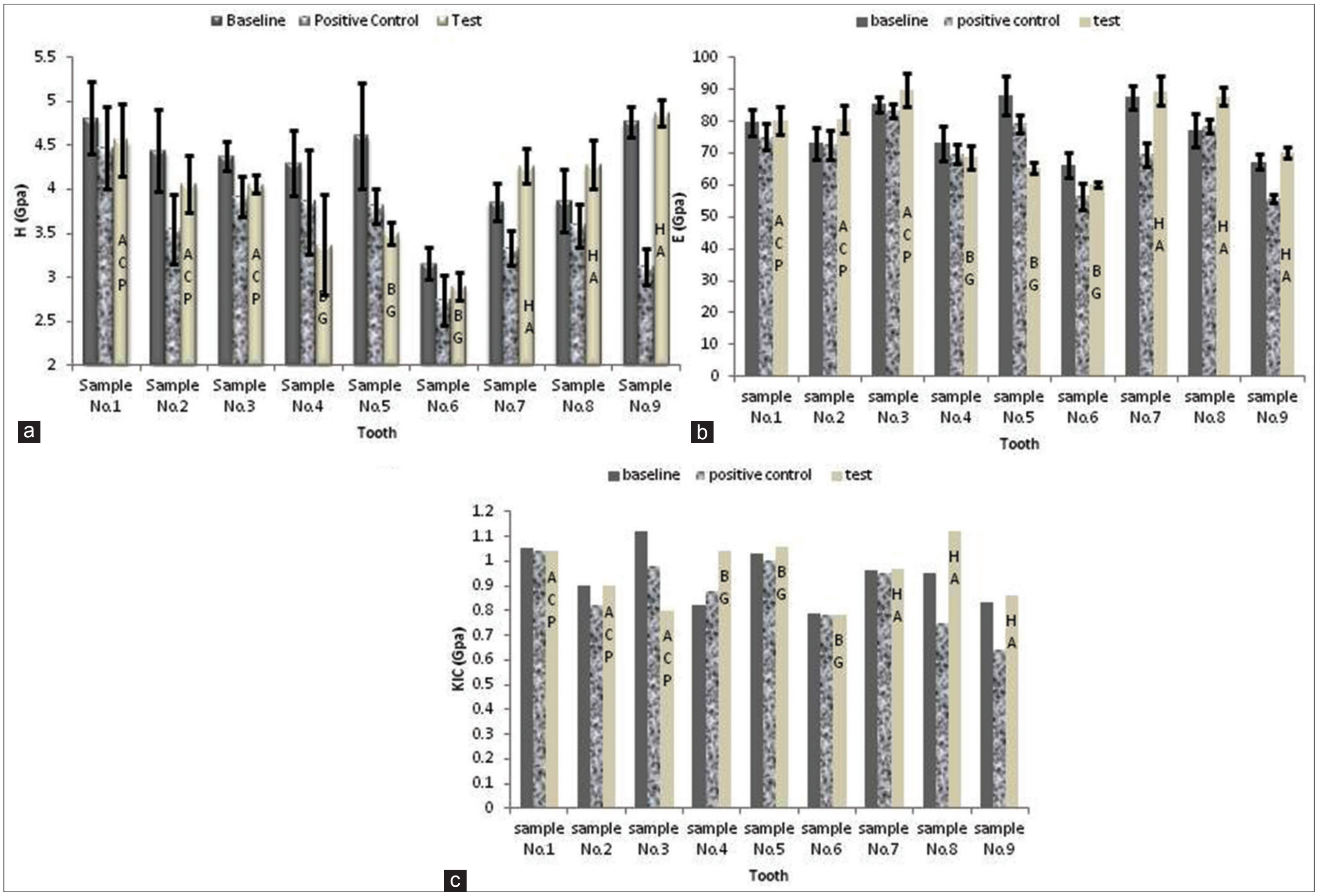

Figure 1: Comparison between baseline control and test sample values of (a) hardness (b) elastic modulus (c) fracture toughness of the enamel

Table 2: Mean±standard deviation of the hardness[a]

Sample number (GPa)	1 (ACP)	2 (ACP)	3 (ACP)	4 (BG)	5 (BG)	6 (BG)	7 (HA)	8 (HA)	9 (HA)
Baseline	4.81±0.41	4.44±0.47	4.38±0.16	4.30±0.37	4.61±0.60	3.16±0.18	3.86±0.21	3.87±0.36	4.77±0.17
Control positive	4.47±0.47	3.55±0.40	3.92±0.23	3.86±0.59	3.81±0.20	2.74±0.28	3.33±0.20	3.59±0.25	3.12±0.21
Test	4.56±0.41	4.06±0.32	4.06±0.10	3.37±0.57	3.50±0.13	2.90±0.16	4.27±0.20	4.28±0.28	4.87±0.15

[a]All values are mean±SD. SD: Standard deviation; ACP: Amorphous calcium phosphate; BG: Bioactive glass; HA: Hydroxyapetite

Table 3: Mean±standard deviation of the elastic modulus[a]

Sample number (GPa)	1 (ACP)	2 (ACP)	3 (ACP)	4 (BG)	5 (BG)	6 (BG)	7 (HA)	8 (HA)	9 (HA)
Baseline	79.68±4.06	73.09±5.09	85.36±2.35	73.12±5.36	88.11±6.20	66.44±3.93	87.5±3.60	77.11±5.30	67.36±2.50
Control positive	75.12±4.25	72.62±4.43	83.22±2.10	69.66±3.10	79.27±2.80	56.59±4.20	69.67±3.70	78.45±2.10	55.71±1.20
Test	80.31±4.43	80.81±4.34	89.82±5.40	68.70±3.60	65.40±1.59	60.05±0.90	89.56±4.70	87.84±2.90	70.33±1.70

[a]All values are mean±SD. SD: Standard deviation; ACP: Amorphous calcium phosphate; BG: Bioactive glass; HA: Hydroxyapetite

Table 4: Indentation results of the fracture toughness[a]

Sample number ($MPam^{1/2}$)	1 (ACP)	2 (ACP)	3 (ACP)	4 (BG)	5 (BG)	6 (BG)	7 (HA)	8 (HA)	9 (HA)
Baseline	1.05	0.90	1.12	0.82	1.03	0.79	0.96	0.95	0.83
Control positive	1.04	0.82	0.98	0.88	1	0.78	0.95	0.75	0.64
Test	1.04	0.90	0.80	1.04	1.06	0.78	0.97	1.12	0.86

[a]All values are mean. ACP: Amorphous calcium phosphate; BG: Bioactive glass; HA: Hydroxyapetite

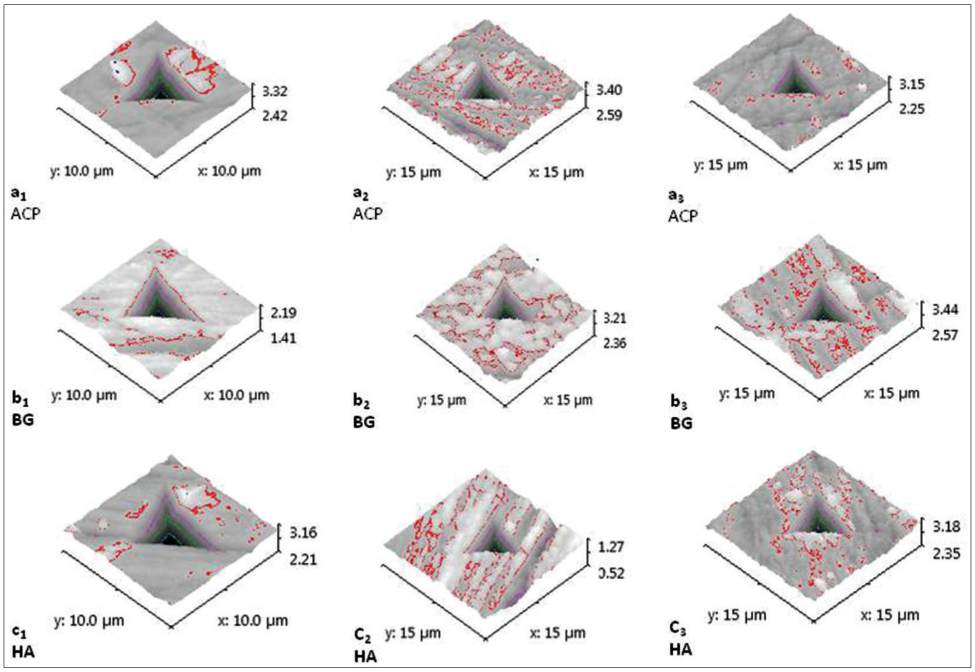

Figure 2: Atomic force microscope images of specimen before (baseline) (1), after bleaching in positive control (2) and after bleaching in test groups (3)

Discussion

Tooth whitening is a widespread dental treatment; however, there are still concerns about its different side effects. Various investigations into the probable adverse effects of bleaching agents to cause morphological changes in enamel structure yielded incongruous results. These can be attributed to the difference in the duration of bleaching process and concentration of bleaching agents.[3-5] The treatment with peroxide containing bleaching agents causes changes in the inorganic composition and therefore, demineralization is a possible mechanism for reduction in mechanical properties.[20] Application of remineralizing agents on affected tooth surfaces might reverse these alterations. Remineralization, defined as mineral gain, is caused by precipitation of minerals on the surface of enamel, where calcium and phosphate ions are deposited in the crystals defects of demineralized enamel.[21] Many remineralizing materials viz. fluorides, casein calcium phosphatides, calcium sodium phosphosilicates (bioglass), synthetic or natural HA, and few others that help in enamel remineralization are available. In the present study, nano-indentation is applied to test the effect of remineralization materials - HA, ACP and BG on mechanical properties of enamel.

The baseline values of Young's modulus, hardness, and K_{IC} were found to be variable among teeth [Figure 1]. It is supported by Hairul Nizam, who found that mechanical properties of teeth depend on age and health conditions of donors.[22] K_{IC} value in negative control was comparable with the normal value for enamel and to the control group of another study.[22] The null hypothesis of this study was rejected because in the positive control group bleaching-induced alterations in YM, VHN, and K_{IC} were significant compared with baseline, whereas these alterations in the test group were not significant. In fact, incorporating nano-remineralizing agents into bleaching agents compromised the mechanical properties of enamel surface. In addition, the AFM images confirmed these findings [Figure 2]. This result might be attributed to the deposition of nano-remineralizing agents in the defects of enamel surface. As enamel, in its structural make-up has small intercrystalline spaces, enamel cracks and few other defects, some ions and molecules can somewhat penetrate the enamel, inspite of its density.[23] Although the critical value for clinically significant reduction in enamel fracture resistance is not known, even a little decrease is considered detrimental keeping in mind the stress and fatigue a tooth bears in its entire lifetime. More significant for this study is to monitor the change in the value of this property, rather than noting the accurate value, as the change amounts to the effect of the bleaching agent on nano-mechanical tooth properties.

Many significant changes in enamel properties by the use of bleaching agents (morphological alterations, increased porosity, nonuniform changes) have been studied in the past,[3-5] which can considerably decrease the mechanical properties. It can also cause drying and desiccation of dental structures, thereby increasing the brittleness of coronal tooth structure.[24] The present study also support these findings as seen in the reduction of young's modulus, hardness, and K_{IC}, after bleaching with 38% HP due to its undermining effect on nano-mechanical properties of teeth although the exact mechanism of HP affecting the dentin and enamel has yet to be fully clarified. The acidity of HP is responsible for the morphological changes and demineralization accompanied with organic matter damage is the reason behind the loss of microhardness of teeth.[16]

The refurbishing of enamel porosities have been accomplished with the use of nano-sized hydroxyapatite crystals paste in some studies.[8,9] HA, being alkaline, increases the total pH of mixture when combined with HP, thereby decreasing the demineralization caused by acidic HP. The bleaching process is also aided due to the formation of free radical of HP.[1] HA particles also forms an smooth protective layer on enamel, thus diminishing the direct effect of HP on enamel structure. Even under higher magnification, the enamel surface in case of HA + HP showed little variations.[1] The reduction in the particle size of HA into nanometric range enhances its remineralizing effect. Undeniably, the interaction of nanoparticles with dentin and enamel is more effectual due to the increased surface-to-volume ratio.[25]

American dental association foundation developed ACP for remineralization and treatment of early enamel caries lesion.[26] This biologically active ACP, release plenty of calcium and phosphate ion, to maintain a supersaturated state, in this manner causing remineralization. Srinivasan *et al*. found enamel microhardness to increase by 64.25%, on treatment with CPP-ACP.[27] A similar study compared the remineralization caused by the toothpastes containing NovaMin (BG) and CPP-ACP and noticed an increase in calcium and phosphorus in surface layer of enamel, which was found to be amorphous in nature.[28] ACP when added to 7.5% and 9.5% HP gels, was unable to prevent mineral loss and also in a study conducted by da Costa and Mazur.[29] a combination of 10% CP and ACP was unable to avoid the reduction in enamel microhardness, whereas the value decreased with the addition of fluoride or HA during bleaching which act by preventing the loss of mineral content from enamel during bleaching treatment.[1] This was observed in contradiction to this study where ACP mixed with bleaching gel could prevent significant alterations in mechanical properties during the bleaching period.

BG is a highly bioactive, biocompatible inorganic material, which on reacting with aqueous environments releases calcium, sodium and phosphate ions.[15] At the liquid – glass interface, the swift exchange of sodium ions with H + and HCO_3 - ions, facilitates the discharge of calcium and phosphate ions, thus forming a supersaturated ion pool around the enamel surface. Gradually, they crystallizes and convert this layer into carbonate-enriched HA (HCA).[30] This ionic dissociation of BG is found to be much more rapid in rate and magnitude in HP, in comparison with distilled water.[16] These acidic conditions also facilitate the deeper penetration of mineral ions into enamel structure, as supported by a prior study.[30] Therefore, an increase in protection is observed with the use of BG and HP. However, the long reaction time is a drawback in its application as an effective remineralization agent.

The calcium and phosphate ions, lost during bleaching, may be regained with the use of remineralizing agents in the form of an initial new ACP layer deposited on enamel surface. It gradually crystallizes on reacting with hydroxyl ions, carbonate, and fluoride in oral cavity.[27-30]

It is important to consider that our study evaluated the effect of using the mentioned remineralizing agent on YM, VHN, and FT among mechanical properties. Therefore, further investigations are necessary to substantiate and compare the probable beneficial effect of incorporating these remineralizing agents into tooth bleaching products on other mechanical and esthetic properties.

Conclusion

Under the current *in vitro* experimental conditions, it can be concluded that the HA, ACP, and BG could be potential biomaterials used for tooth bleaching. Although literature supports that the ions lost during bleaching can be refurbished from salivary electrolytes, yet these can exacerbate into erosive-abrasive lesions. Therefore, a gentle, noninvasive bleaching procedure with the integration of remineralizing agents, may prevent the irremediable alterations of enamel surface.

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Conflicts of interest

There are no conflicts of interest.

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