EGYPTIAN
DENTAL JOURNAL

Vol. 64, 265:275, January, 2018
I.S.S.N 0070-9484

Oral Medicine, X-Ray, Oral Biology and Oral Pathology

www.eda-egypt.org • Codex : 149/1801

# THE EFFECT OF DIFFERENT BIOMIMETIC MATERIALS AS A REMINERALIZATION PROTOCOL ON MICROHARDNESS AND ULTRAMORPHOLOGY OF BLEACHED ENAMEL

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## *ABSTRACT*

**Aim:** To compare the effect of Nano-HA and Nano-BG as remineralizing protocol on the microhardness and structural morphology of bleached enamel.

**Materials and methods:** Two different biomimetic materials were used; Nano-bioactive glass powder and Nanohyroxyapatite powder**.** Five extracted sound human central incisors were selected. The labial of each tooth was divided longitudinal into two equal halves. The mesial half was assigned for BG group and the distal half was assigned for HA group. A total number of 20 Specimens were randomly divided into 4 groups (5specimens each). Group1 represent sound unbleached enamel, group2 represent bleaching enamel surface, and group3 represents remineralization by BG and group4 represents remineralization by HA. The specimens in group2 were bleached using 40% Hydrogen Peroxide following the manufacturers' instructions. For Nano-BG group: a mixture of bioactive glass 45S5 powder with particle size (25-120 um) with poly-acrylic acid powder was done (PAA –BAG). Then, one milliliter of the artificial saliva was added then was applied on the bleached enamel surface followed by rinsing for one minute.For Nano-HA group: HA mixed with distilled water then was applied to bleached enamel surface followed by rinsing. All specimens were subjected to de and remineralization cycles. Surface Micro-hardness of the specimens (before bleaching after bleaching and after remineralization protocol) was determined and the mean microhardness values of the specimens were calculated, tabulated and statistically analyzed using one way analysis of variance (ANOVA) and Tukey post hoc tests were used to study the significance. The surface morphology of two representative sample of each tested group were examined using scanning electron microscope attached with energy dispersive X-ray analyzer.

**Results:** Microhardness results revealed that the greatest mean value was recorded in unbleached enamel, followed by remineralized enamel with Nano- BG, then remineralized enamel with Nano- HA, with the least value in bleached enamel. ANOVA test revealed that the difference was extremely statistically significant ($p<0.0001$). Tukey's post hoc test revealed no significant difference between unbleached enamel and remineralized enamel with Nano- BG. Moreover, there was no significant remineralized enamel with Nano- HA and remineralized enamel with Nano- BG. This was associated with some morphological changes in the enamel surfaces between different tested groups.

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**Conclusions:** The use of bleaching agents greatly affects the structural integrity of enamel surface and resulted in great mineral loss. It is strongly recommended to perform remineralization protocol after any bleaching procedure. The biomimetic materials are very promising due to their ability to perfectly reconstruct partially damaged bleached enamel surface. Bioactive glass material was able to perform almost complete recovery of the structural integrity of bleached enamel. Also, Nanohydroxyapatite successfully restore the damaged bleached enamel surface but to less extend compared to bioactive glass.

**KEY WORDS:** biomimetics, nano bioactive glass, nano hydroxyapatite, bleaching.

## INTRODUCTION

Tooth staining is a common aesthetic problem. Bleaching treatment is considered as conservative approach of esthetic dental treatments. Despite the tooth whitening esthetic benefits, some side-effects have been reported, including: Tooth sensitivity, and structural changes such as decrease in microhardness (MH) and increased roughness (1). In office bleaching commonly involves the application of 30% hydrogen peroxide (HP). It is supposed that peroxide diffuses through the enamel, and may react with organic colored materials found within the tooth structures leading to a reduction in color. Although there is little question about their efficacy, the enhancement in tooth color may be at the expense of the enamel structure may be weakened by the bleaching agent. Numerous studies (2-6) have evaluated the effects of peroxide-containing products on the physical and chemical properties of tooth enamel. However, the research in this area has been controversial (1). Some studies (2-5) reported that there was no evident change in enamel microhardness and morphology after bleaching treatment. But others have found calcium loss, alterations in surface morphology, changes in chemical composition and decrease in microhardness of enamel.(7,9)

It should be pointed out that the remineralization and protective benefits of saliva may overcome the detrimental bleaching effects in vivo.(1) However, it is still necessary to reduce the risk of any minor damage caused by the bleaching agents. Enamel consists of a highly organized hierarchical microstructure made up of carbonated hydroxyapatite nanocrystals, 50–70 nm in width, 20–25 nm in thickness, with a length to width aspect ratio over 1000. These features provide dental enamel its high strength and extreme hardness. Moreover, mature enamel is acellular, and has a mineral content of 95 mass % and does not remodel. All of these factors contribute to the difficulty of repairing dental enamel tissue. (8,10)

In order to minimize such demineralization after contact with bleaching agents, some ingredients such as fluoride(11-13) , calcium(14) or amorphous calcium phosphate(11) are added into the bleaching gel by manufacturers. Some biomimetic materials such as nano hydroxyapatite and nano structured bioactive glass have been advocated as new techniques for enamel reconstruction that have been a challenging topic of research in material sciences and dentistry.(15) Beneficial effects of these biomimetic materials may help in protection of the precious enamel from demineralization during the bleaching procedure.

Synthetic nano-hydroxyapatite (Nano-HA) is an attractive biomaterial owing to its chemical and structural similarity with natural tooth mineral. It has been suggested that the Nano-HA would lead to a considerably superior remineralization. (16-20) Nano- Bioglass®45S5 (Nano-BG) is a bioactive implant material that stimulates bone repair. In an aqueous environment, this material undergoes a series of reactions, resulting in the formation of a surface layer made of hydroxy-apatite and/or hydroxycarbonate apatite.(21-33) However, the remineralization potential of bioactive glass has so far not been evaluated.

Therefore, the aim of this study was to compare the effect of Nano-HA and Nano-BG as remineralizing protocol on the microhardness and structural morphology of bleached enamel.

## MATERIALS AND METHODS

### Biomimetic materials as Remineralizing agents:

Two different biomimetic materials were used in this study for a proposed remineralization protocol after bleaching procedure:

1) **Nano-bioactive glass powder** (Calcium sodium phosphor-silicate 45S5) (Nano Streams – 6th October city-Egypt) with particle size (25-120 um) having a composition of 45 wt% Silica, 25 wt% Calcium Oxide, 25 wt% Sodium Oxide and 5 wt% Phosphorus Pentaoxide.
2) **Nanohyroxyapatite powder** Rod-like hydroxyapatite particles (diameter < 100 nm, aspect ratio 2-3) (Nano Streams – 6th October city-Egypt)

### Specimens preparation:

Five extracted sound human central incisors having coronal labial dimensions of 8mm in width×11mm in length that was checked by a digital caliper (Mitutoyo, Tokyo, Japan) were selected for this study. The selected teeth were extracted either from diabetic patients or patients with periodontal disease. The teeth were washed thoroughly under tap water and polished with fluoride free polishing paste to remove any plaque or soft remnants. The teeth were examined under stereomicroscope to ensure that they were free from cracks, fractures or any defects and were then stored in physiologic saline solution at room temperature till the beginning of the experiment. The labial of each tooth was divided longitudinal into two equal halves Each tooth was mounted in an automated diamond saw (Isomet 4000, Buehler Ltd., Lake Bluff, IL, USA), using 0.6 mm diamond disc at 2500 rpm with feeding rate 16.5 mm /min under copious water coolant to be cut longitudinal into two equal halves. The mesial half was assigned for BG group and the distal half was assigned for HA group.

A total number of 20 Specimens were randomly divided into 4 groups (5specimens each) according to enamel surface treatment performed. **Group1** represent sound unbleached enamel, **group2** represent bleaching enamel surface, and **group3** represents remineralization by BG and **group4** represents remineralization by HA .

The coronal segment were then separated from their root with 0.5 mm thickness diamond disk (Buehler, USA) low speed hand piece with sufficient coolant, to obtain an enamel/dentin block (E/D). Each E/D block were then mounted on chemically activated acryl resin block.

### Bleaching procedures:

In the current study ***White Smile Power Bleaching (White Smile, Germany)*** was used. It is a peroxide-based bleaching gel containing 40% Hydrogen Peroxide as the active ingredient but without any remineralizing components having pH 8.

The specimens in **group2** were bleached following the manufacturers' instructions. 1-2 mm thickness of the bleaching gel was applied on the enamel specimen was applied three times, each for 15 minutes then were rinsed with distilled water for one minute.

### Remineralization protocol

**For Nano-BG group**: a mixture of bioactive glass 45S5 powder with particle size (25-120 um) with poly-acrylic acid powder **(1250 Mw, El Gomhrya for Drugs Trade)** was done as follow :for each 60 grams of nano bioactive glass powder, 40 grams of poly-acrylic acid powder was added. Mixing of the two powders was done by placing both powders in a plastic jar then putting a magnet in the jar in order to stabilize the jar over the magnetic Jenway Hot Plate and Stirrer model number 1000 at 300 rpm for 5 minutes. A digital weight scale **(A&D**

**limited GF-3000, Japan)** was used to weight twenty milligram (0.02 g) of the used poly-acrylic acid-bioactive glass (PAA –BAG). Then, one milliliter of the artificial saliva was added to the used mix using a dropper and then mixed using a disposable micro-brush. This mix was applied on the bleached enamel surface of specimens of group of Bio-active glass and applied manually by rubbing with disposable micro-brush for one minute with gentle pressure, followed by copious rinsing with distilled water for one minute.

**For Nano-HA group:** HA mixed with distilled water in a ratio of 2 g powder to 1ml liquid. A similar quantity of nano-HA paste was applied to bleached enamel surface manually by rubbing with disposable micro-brush for one minute with gentle pressure, followed by copious rinsing with distilled water for one minute

All specimens were subjected to de and remineralization cycles as follow. Specimens were first subjected to remineralization in artificial saliva for 3 hours then they would be demineralized in sodium acetate for 15 minutes and then storage in artificial saliva for the rest of the day. This procedure was repeated three times daily for five consecutive days**.** The artificial saliva and the acetate buffer were prepared at the laboratory of Biochemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt. The artificial saliva was prepared having the following composition methyl-p-hydroxybenzoate: 2.00g/l, sodium Carboxymethyl cellulose: 10.0g/l, MgCl2.6H2O: 0.059g/l, CaCl2.2H2O: 0.166g/l, K2HPO4: 0.804g/l, KH2PO4: 0.326g/l, KCl: 0.625g/l and NaF: 0.05 ppm with pH=7.2. ***Torres, C.P et al, 2010.*** The sodium acetate buffer was prepared having the following composition Ca (CaCl2): 1.5mM, PO4 (KH2PO4): 0.9 mM and acetate: 0.050 M with pH=5.

### Microhardness test procedure

Surface Micro-hardness of the specimens (before bleaching after bleaching and after remineralization protocol) was determined using Digital Display Vickers Micro-hardness Tester (Model HVS-50, Laizhou Huayin Testing Instrument Co., Ltd. China) with a Vickers diamond indenter and a 20X objective lens. A load of 100g was applied to the surface of the specimens for 20 seconds. Three indentations, which were equally placed over a circle and not closer than 0.5 mm to the adjacent indentations, were made on the surface of each specimen. The diagonals length of the indentations were measured by built in scaled microscope and Vickers values were converted into micro-hardness values.

Micro-hardness calculation;

Micro-hardness was obtained using the following equation:

$$HV=1.854\ P/d2$$

where, HV is Vickers hardness in Kgf/mm$^2$ , P is the load in Kgf and d is the length of the diagonals in mm.

### statistical analysis:

The mean microhardness values of the specimens were calculated, tabulated and statistically analyzed using one way analysis of variance (ANOVA) and Tukey post hoc tests were used to study the significance. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with SPSS 19.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows.

### Ultramorphological analysis of enamel surface

The surface morphology of two extra representative sample of each tested group were examined using scanning electron microscope attached with energy dispersive X-ray analyzer (SEM-EDXA Unit, Main Defense Chemical Laboratory; Cairo, Egypt). For SEM examination, the collected specimens were examined at 30kV using the secondary electron LFD detector under the magnification (X 4000) with a (spot size 4.7-5.3nm).

## RESULTS

### Microhardness results

The greatest mean value was recorded in unbleached enamel, followed by remineralized enamel with Nano- BG, then remineralized enamel with Nano- HA, with the least value in bleached enamel. ANOVA test revealed that the difference was extremely statistically significant ($p<0.0001$). Tukey's post hoc test revealed no significant difference between unbleached enamel and remineralized enamel with Nano- BG. Moreover, there was no significant remineralized enamel with Nano- HA and remineralized enamel with Nano- BG (Table 1, Fig. 1)

### Percent change in microhardness

The mean percent change (decrease) in microhardness after bleaching in relation to control was (-17.63±7.33). After remineralization, the percent change of microhardness in relation to control was (-5.77±1.7) in Nano- HA group and (-0.044±-0.018) in Nano- BG group.

Unpaired t test revealed a significant difference in the percent change obtained by the 2 remineralizing agents ($p<0.0001$), (Table 2, Fig.2)

### Ultramorphological analysis of enamel surface after different treatment protocol

Scanning electron microscopic examination of sound unbleached enamel revealed smooth enamel surface and some micro porosities are apparent (fig.3A).

Scanning electron microscopic examination of bleached enamel showed loss of uniform structure of enamel with uniform mineral depletion. Enamel rods and interprismatic structures appeared that may be due to demineralization giving etching-like appearance. Besides increasing in the enamel microporosities and pitting erosion giving honey combed appearance (fig.3B).

TABLE (1) Microhardness of enamel (ANOVA test)

| Groups | Mean | Std. Dev | Std. Error | 95% Confidence Interval for Mean | | Min | Max | F | P |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | | | |
| Unbleached enamel (control) | 283.59$^{a}$ | 23.38 | 7.39 | 266.86 | 300.31 | 267.45 | 327.50 | 30.381 | .000* |
| Bleached enamel | 232.27$^{c}$ | 8.50 | 2.69 | 226.19 | 238.35 | 223.54 | 247.32 | | |
| Remineralized enamel with Nano- HA | 266.14$^{b}$ | 10.11 | 3.20 | 258.91 | 273.37 | 254.70 | 277.10 | | |
| Remineralized enamel with Nano- BG | 282.21$^{a,b}$ | 5.41 | 1.71 | 278.34 | 286.08 | 276.58 | 290.65 | | |

TABLE (2) Comparison of percent change of microhardness (in relation to control) after treatment with Nano- HA and Nano- BG (unpaired t test)

| | Mean | Std. Dev | Std. Error Mean | Mean Diff. | Std. Error of diff. | 95% C.I. Diff. | | t | P |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper | | |
| Remineralized enamel with Nano- HA | -5.77 | 1.727 | 1.906 | -5.72 | 2.69 | -11.38 | -.064 | -2.125 | .000* |
| Remineralized enamel with Nano- BG | -.044 | **0**.018 | 1.903 | | | | | | |

*Significance level $p<0.05$, *significant, ns= non-significant* *95 % C.I. diff= 95% Confidence Interval of the Difference*

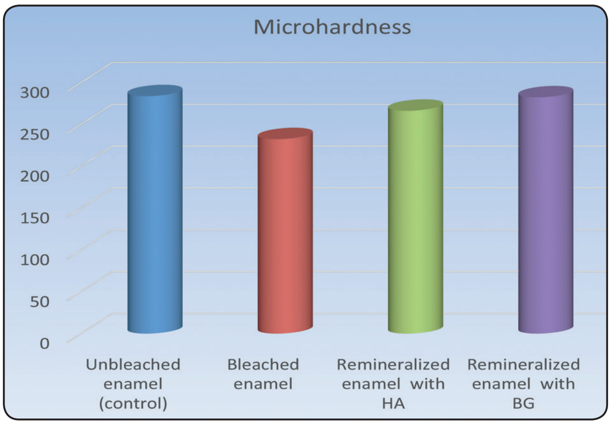

Fig. (1) Column chart showing mean microhardness of enamel

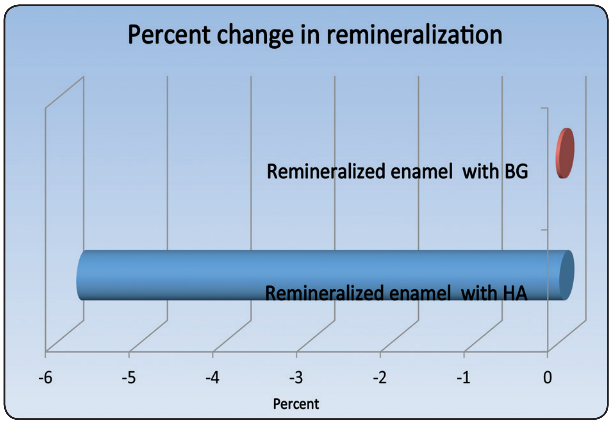

Fig. (2) Bar chart showing percent change of microhardness (in relation to control) after treatment with HA and BG

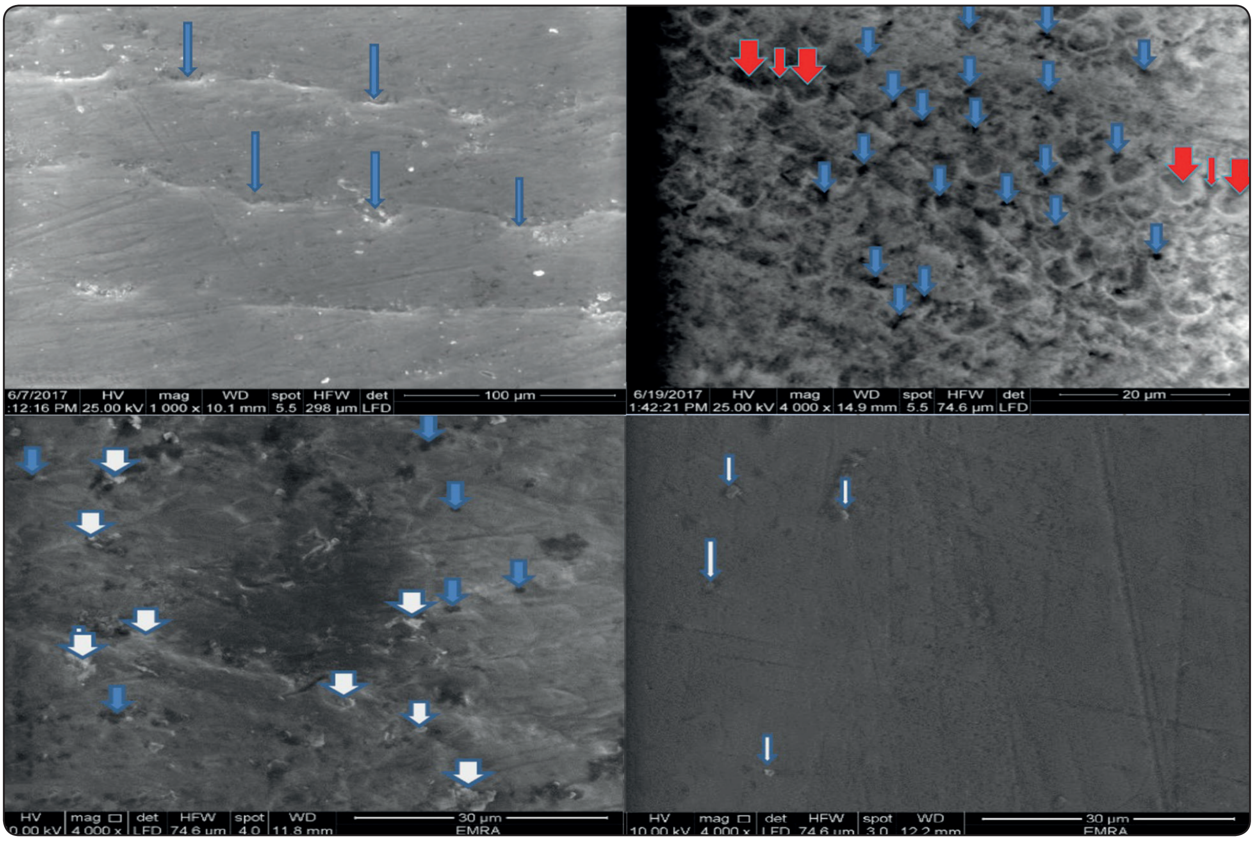

Fig. 3A: Scanning electron micrograph of sound unbleached enamel of control group revealed a smooth enamel surface and some micro porosities are apparent (blue arrows). Fig. 3B: scanning electron micrograph of bleached enamel revealed increasing enamel microporosities and pitting erosion (blue arrows), enamel prisms and interprismatic structures appeared giving honey combed appearance(red arrows). Fig. 3C: scanning electron micrograph of bleached enamel remineralized by Nano- HA revealed few microporosities (blue arrows), mineral layer blocking the surface porosities (white arrows). Fig. 3D: scanning electron micrograph of bleached enamel remineralized by Nano- BG revealed smooth enamel surface with mineral layer blocking some porosities (white arrows).

Scanning electron microscopic examination of bleached enamel remineralized by Nano- HA revealed appearance of few microporosities with the presence of mineral layer blocking the surface porosities and interprismatic structure (fig.3C ).

Scanning electron micrograph of bleached enamel remineralized by Nano- BG showed that the enamel surface appeared to be near to the normal where smooth enamel was shown with mineral layer blocking some porosities and intact enamel prims (fig.D).

## DISCUSSION

Dental bleaching agents greatly affect the mineral content of dental enamel. This effect varied according to the concentration of peroxide as well as application time.(4,5) It is known that the oxidation-reduction reaction of the bleaching agent might cause dissolution of the organic and inorganic dental matrix until only carbon dioxide and water remain. Additionally, it has been shown that bleaching with 35-38% hydrogen peroxides may change enamel morphology and alter mineral content(3,4,8-10). Thus, efforts have been made to achieve a protocol capable of remineralizing bleached enamel, recovering microhardness loss and surface alterations(11-15). This study was designed to assess and compare the effect of two remineralizing protocol using innovative biomimetic materials (Nano-BG and Nano-HA) on the structural integrity of bleached enamel surface, manifested by microharness and morphological changes in enamel surface.

Biomaterials are natural or synthetic materials used in biological systems to reconstruct or replace tissues, organs or bodily functions thus improving the quality of life of patients. These materials are often used in dentistry due to their ability to interact with dental tissues, inducing the remineralization process(29,31).

Hydroxyapatite is a biomaterial found as a natural mineral constituent found in bone, representing 30 to 70% of the mass of bones and teeth. Laboratory studies have demonstrated the ability of nano-HA crystals in repairing microscopic enamel defects(19).

Bioactive glass has numerous novel features essentially its ability to act as a biomimetic mineralizer matching the body's own mineralizing traits while also affecting cell signals in a way that benefits the restoration of tissue structure and function. Bioactive glasses (BAGs) as opposed to most technical glasses are characterized by the reactivity in water and in aqueous liquids. This compound in an aqueous environment release bioavailable calcium, sodium and phosphate ions contributing to the remineralization process.(31)

In the current study, all specimens were subjected to de- and remineralization cycles for five consecutive days using artificial saliva and sodium acetate in a trial to simulate the natural oral conditions. Artificial saliva was also chosen, as the bioactive glasses need calcium and phosphorus rich medium to enhance the precipitation and transformation of hydroxy apatite crystals promoting the bioactive glass action(30).

In the present study, the results of microhardness test and SEM analysis, as expected, revealed that HP alone could result in significant microhardness loss and morphological change of enamel. These findings are consistent with studies (3,8-10) that suggested that morphological change of enamel is due to the demineralization caused by acidic HP, while microhardness loss is due to the combined effects of demineralization and destruction of organic matter by HP. hydrogen peroxide induced enamel mineral loss after bleaching and this process can lead to demineralization of the enamel surface and compromise its mechanical properties.(1,9,10)

Our SEM micrographs showed that many surface defects were produced on the enamel surface when bleaching gel was applied three times, this is in agreement with other study (2) who reported significant alterations of the prismatic structure of enamel when the bleaching protocol was applied three or four times.

In the current study, the ultrastructural results of the bleached enamel surface showed loss of uniform structure of enamel with uniform mineral depletion. Erosive lesions were seen in prismatic enamel as characteristics pattern of demineralization with dissolution of prismatic and interprismatic substances giving honeycomb appearance. These results may be due to the intrinsic decalcifying effect of hydrogen peroxide. As the bleaching agents release oxygen radicals that affect inter and intra prismatic structure of the enamel, providing moderate de-protienation so that any minerals associated with protiens are removed as well. This may explain the loss of calcium and phosphate from enamel when subjected to bleaching regimen.

In the current study, Poly acrylic-bioactive glass powder was the intervention material, it is a mixture of poly acrylic acid powder with bioactive glass powder (BAG 45S5) consisted of silicon 21%, calcium 18%, phosphorus 3%, sodium 18%, oxygen 40 % elements. It had been used in this study due to its chemical reactivity in the presence of aqueous solutions such as saliva that leads to the formation of hydroxyapatite (HA) structures.

PAA-BAG has been selected due to its reduced cutting efficiency when compared to BAG abrasive powder(15). Poly acrylic acid (PAA) has been added to bio-active materials in order to mimic the functional role of non-collagenous proteins in binding the calcium and Phosphate ions to form nano-precursors, small enough to penetrate the defective dental structure more effectively.(26) Not only that, but also the bioactive glass particles by their nature have the tendency to deposit hydroxyl carbonate apatite, a mineral that is very close in the chemical structure from the hydroxyapatite (30).

The results of the present study revealed that the application of both nano-HA and Nano-BG could recover microhardness values. The results of the current study using remineralizing protocol by Nano-HA increased the microhardness of bleached enamel that might indicate that Nano-HA could provide a high mineral deposition on the bleached enamel. This finding was in accordance with other study (15) that suggested that nanohydroxyapatite crystals could have been immediately incorporated to the superficial enamel after bleaching the teeth with hydrogen peroxides, favoring mineral uptake and hardness recovery. Accordingly, it has been documented that nano-HA and nanocarbonate apatite penetrate into the intercrystalline spaces and rod sheaths. Therefore, these nanoparticles enhance the superficial enamel smoothness and block up surface defects (16,17).

Moreover, the application of Nano-BG could provide a great microhardness values that indicates more mineral deposition in samples shortly after its application. This was in accordance with studies (31,32) that reported that regardless of formulation bioactive glass materials or mode of their application technique, it was found to be more effective in enamel remineralization compared to other topical agents such as fluoride and CPP-ACP. Furthermore, bioactive glass was able to routinely form a protective layer rich in calcium and phosphate content as it was suggested that in saliva, sodium ions from the bioactive glass particles readily react with hydrogen cations (in the form of H3O+) from saliva inducing the release of calcium and phosphate (PO4 -) ions from the glass. This was associated with a localized, transient increase in pH during the initial exposure of the material to saliva due to the release of sodium. This increase in pH would help in precipitation of the extra calcium and phosphate ions provided by the bioactive glass material to form a calcium phosphate layer and hence, this layer would crystallize into hydroxyapatites (31). Also, the great amount of Ca2+ and PO3-4 released by the biomaterial formed a supersaturated ionic reservoir for the enamel apatite. Bioactive glass particles have been also reported in other in vitro studies (31) to attach to the tooth surface and continue to release ions and transform into hydroxy apatite (HA) for up to two weeks.

However, the most surprising results in the present study were that nano-BG recorded higher microhardness value than Nano-HA. This may be related to the amount of the soluble compound of calcium and phosphate in nano-BG that was greater than that of Nano-HA. This was in agreement with other study (16) that reported that the highly crystalline HAP particles are classified as nonresorbable materials and that It has been shown that the degradation of biomaterials strongly depends on their solubility. It was also supposed that The highly crystalline HAP particles are classified as non-resorbable materials and that It has been shown that the degradation of biomaterials strongly depends on their solubility and that as the calcium content in treatment solution increase, the remineralization would increase too(15,-17). Therefore, Nano-BG dissolves readily in the oral cavity and redeposits on the damaged enamel.

Our SEM micrographs demonstrated that many surface defects were produced on the enamel surface as a result of bleaching process, while the nanoparticles reconstructed the surface topography. The morphological appearance of the demineralized enamel has been previously reported and was comprised of thin and irregular enamel rods with indistinct borders and wide inter-rod zones**.** In the present study the SEM photomicrographes of bleached enamel with HP bleaching agent were un-uniformly mineral depleted and loss of uniform structure of enamel. Erosive lesions are seen in prismatic enamel as characteristics pattern of demineralization with dissolution of prism cores and interprismatic substances giving honeycomb appearance. While in bleaching with agent comprising fluoride, the morphological changes were minimal presented as surface discontinuity. These results may be due to the intrinsic decalcifying effect of hydrogen peroxide. As the bleaching agents release oxygen radicals that affect inter and intra prismatic portion of the enamel, providing moderate de-protienation. Any minerals associated with protiens are removed as well. This may explain the loss of calcium and phosphate from enamel when subjected to bleaching regimen.

The bleached enamel tends to return back to the normal after remineralizing protocols. This indicates the reverse action of biomimetic materials that reverse the mineral loss by supplementing calcium and phosphorous ions, keeping a balance between the bleached enamel and its remineralization. Nano-BG was able to reconstruct the damaged bleached enamel surface morphology and that no morphological changes were detected compared to control unbleached enamel. However, Nano-HA also recovers the damaged bleached enamel surface with minimal morphological changes denoting the insufficient release of calcium and phosphate compared to Nano-BG.

## CONCLUSIONS

Within the limitations of this study, the following conclusions could be derived:

1. The use of bleaching agents greatly affects the structural integrity of enamel surface and resulted in great mineral loss.
2. It is strongly recommended to perform remineralization protocol after any bleaching procedure.
3. The biomimetic materials are very promising due to their ability to perfectly reconstruct partially damaged bleached enamel surface.
4. Bioactive glass material was able to perform almost complete recovery of the structural integrity of bleached enamel.
5. Also, Nanohydroxyapatite successfully restore the damaged bleached enamel surface but to less extend compared to bioactive glass.

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