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MICRO HARDNESS OF BLEACHED HUMAN ENAMEL FOLLOWING APPLICATION OF CONVENTIONAL VERSUS NANO ACTIVE BIOGLASS: AN INVITRO STUDY

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ABSTRACT

Objectives: The aim of this study was to compare the effect of conventional vesrsus nanosized bioactive glass on the microhardness values of bleached enamel.

Methods: Forty-five fresh extracted human incisors were divided into three main groups (15 each) according to the bleaching technique (B); unbleached (B_0) as a Control, light activated bleaching (B_1) and chemical activated bleaching (B_2). Each group was further divided equally into three subgroups (5 each) according to application of remineralizing agent (R); either conventional bioactive glass (R_1), nano bioactive glass (R_2) and without application of any remineralizing agent as a control (R_0). Teeth bleaching was done as per the manufacturer's instructions, while, remineralizing agents were applied according to wang et al 2011. Microhardness assessment was done after bleaching as well as after remineralization using Digital Display Vickers Microhardness Tester.

Results: The unbleached group showed the highest mean microhardness followed by chemically activated bleaching group, then the light activated bleaching group. Regarding remineralization; nano bioactive glass groups showed higher microhardness results than conventional bioactive glass groups.

Conclusions: Bleaching has a deleterious effect on enamel microhardness. Bioactive glass can counter act the adverse effect of bleaching on enamel. Nanobioactive glass is a promising material for remineralization

INTRODUCTION

Vital tooth beaching is a common aesthetic procedure used to mask tooth discoloration. Although, loss of minerals is usually accompanying the process, various remineralizing agents could be used with different efficiency to restore the resultant mineral loss.

Vital bleaching can be performed using at-home or in-office bleaching. The main advantages of in-office whitening procedure over at-home bleaching

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system include dentist control, reduced total treatment time, protection of soft tissue, avoidance of material ingestion, and greater potential for immediate results that may enhance patient satisfaction and motivations.[1]

Bleaching systems are based primarily on hydrogen peroxide or one of its precursors, notably carbamide peroxide. The mechanism of action of bleaching agents involves oxidation of organic components in which the structure to be bleached donates electrons to the bleaching agent, opening in the pigmented carbon rings and converting them to chains that are lighter in color.[2] This reaction is possible because of low molecular weight of peroxide solutions, allowing their diffusion through enamel and dentin.[3]

These materials are often used in combination with an activating agent such as heat or light. There are several types of light activation sources as lasers, light emitting diode (LEDs), plasma arc lamps (PAL) and halogen lamps.[4] Mercury halide lamp was also introduced.[5] The theoretical advantage of a light source is its ability to heat the hydrogen peroxide, thereby increasing the rate of decomposition of oxygen to form oxygen free radicals and enhancing the release of stain containing molecules.[6]

The morphological enamel surface alteration following bleaching is controversial. Many effects have been analyzed in vital teeth after hydrogen peroxide bleaching treatment.[7] An increase in roughness, a decrease in enamel microhardness values,[8] and loss of calcium has been noted using atomic force microscopy observation.[9]

Re-mineralization is like replacing the missing rungs in a rickety ladder to make it stable and strong again. Different minerals or ionic technology used for re-mineralization such as fluoride, tricalcium Phosphate (TCP), Dicalcium Phosphate Dehydrate (DCPD), Casein Phosphopeptide (CPP), Amorphous Calcium Phosphate (ACP), CPP-ACP nano complexes and Bioactive glasses .[10]

Bioactive glasses (BAGs) are characterized by the reactivity in water and in aqueous solutions. Consequently, they are widely used in medical field as for bone grafts, scaffolds and coating material for dental implants. The bioactivity of BAGs is derived from their reactions with tissue fluids resulting in formation of hydroxy-carbonate Apatite (HCA) layer on the glass.[11]

Nowadays, nano technology is considered one of the most efficient methods used in teeth re-mineralization using micro-meter-sized nano crystallites that are found to be with more effective than old traditional approaches.[12]

However, the use of remineralizing agent after bleaching may act as a repair agent reversing the demineralization effect of bleaching agent and improve the enamel microhardness values.[9] Therefore, the objective of this study was to evaluate the effect of different bleaching techniques and subsequent remineralization with conventional and nano bioactive glass on microhardness of human enamel surfaces.

MATERIALS AND METHODS

Two different bleaching approaches were used in this study, namely;

1) Light activated Chair-side Whitening Gel (Zoom2):

Zoom2 chairside whitening gel (4.6 gm) (Discus Dental, Inc., Culver City, CA, USA) consists of two adjacent tubes, one contains 25% hydrogen peroxide bleaching gel (25% HP) and the other contains peroxide activator (ferrous gluconate) and the two tubes were combined in dispenser's mixing tip.

2) Chemical activated Chair-side Whitening Gel (Dash):

Dash chair side whitening gel (2.9 gm) (Discus Dental, Inc., Culver City, CA, USA) is chemically

activated, so it requires no light for whitening. It consists of 30% hydrogen peroxide whitening gel. This gel requires no syringe-to-syringe mixing or refrigeration, and delivers the whitening gel with superior ease of use and stability.

For remineralization of human enamel after bleaching, two remineralizing agents were applied:

1. Conventional size bioactive glass: was fabricated according to Naghib et al, 2012.[13]

Synthesis of 45S5 bioactive-glass via melting technique in order to produce bioglass with conventional size is done through the following. SiO2, P2O5, CaCO3 and Na2CO3 powders were blended homogenously. The obtained blend was decarburized at 900 °C for 2 h, and then melted in an alumina plant at 1350 °C for 2.5 h and then quenched in water at room temperature. Afterwards, the produced 45S5 bioglass was milled using planetary milling (SVD15IG5-1, LG Company) for 12 h with 1500 rpm velocity in order to gain the bioactive-glass powder. The fabricated powder was screened to achieve a maximum particle size of 38 μm.

2.Nano bioactive glass as remieralizing materials: Fabricated by sol gel method according to **Durgalakshmi and S. Balakumar, 2013.[14]**

The initial procedure involves mixing of Tetraethylorthosilicate and HNO3, followed by ethanol to hydrolysis and stirred for 30 minutes to obtain the gel, the following reagents were added in the order of orthophosphoric acid, calcium nitrate and sodium hydroxide. Further, the solution was stirred for 4 hours to get a homogenous gel. The sols were aged at a temperature of 70oC for 24 hours, and sintered at 600˚C for 2 hours.

Teeth selection:

A Total of 45 fresh human incisors extracted for periodontal reason were selected and used in this study. Teeth were free of caries, restorations, surface defects or enamel cracks. Immediately after extraction, teeth were thoroughly washed and scraped to remove remnants of periodontal ligament, ultrasonically scaled to remove plaque and calculus and then the teeth were polished with rubber cup and fluoride free polishing paste under water coolant at low speed.

Teeth storage:

The teeth were stored in saline at 5ºC in a refrigerator immediately after extraction to avoid teeth dehydration and the time between extraction and bleaching procedure was no longer than three months.

Specimens preparation:

Using a low speed diamond disc under water irrigation a transverse section was made to separate the crown from the root, 2-3 mm apical to CEJ. Each crown was embedded in self-cured acrylic resin (Acrostone, Cairo, Egypt) square block of (1.5 cm X 1.5 cm X 2cm). The blocks were prepared using split mold assembly that allow for pouring of five blocks at the same time. Each tooth crown was embedded in acrylic resin while it was in soft dough stage and the crown was pressed in the acrylic so that the labial surface faced upwards till it was flushed with the top surface of the block and the lingual surface faced the acrylic resin. The embedded samples were allowed to polymerize at room temperature.[15] After acrylic setting the block was removed from the mold and checked carefully.

Grouping of specimens:

Samples were divided into three main groups (15 each) according to the bleaching technique (B) used either unbleached (B_0) as a Control, light activated bleaching (B_1) and chemical activated bleaching (B_2). Each group was further divided equally into three subgroups (5 each) according to application of remineralizing agent (R) either conventional

bioactive glass (R_1), nano bioactive glass (R_2) and without application of any remineralizing agent as a control (R_0).

Teeth bleaching:

Regarding the control group, no bleaching treatment was done. Samples were stored in saline, renewed daily, at room temperature during the entire experiment.[15] According to manufacturer's instructions the two Whitening Gels used (either light or chemical activated) in this study were stored in refrigerator at 5ºC and prior to their use they were removed from the refrigerator and allowed to come to room temperature. For the light activated bleaching, Zoom2 Chairside Whitening gel was used (Discus Dental, Inc., Culver City, CA, USA). The gel was light activated by Zoom2 Chairside Whitening Lamp that Consists of mercury halide lamp filtered to emit ultraviolet light in the 350-400 nm range. After removal of the syringe cap of Zoom2 Whitening Gel, the mixing tip was attached and secured with ¼ turn clockwise. The whitening gel was extruded into a dapping dish and mixed for 5 seconds with the provided brush. The whitening gel was applied on the labial surface of the teeth (2-3 mm thick) using the provided brush. After the gel application, the light tip was fixed at right angle as close as possible to the teeth. The distance between the light guide and the teeth was about 3cm and the light guide was adjusted to cover all the samples. The light device was turned on and the timer was activated for 15 minutes. All samples were covered with light from all directions. Countdown was displayed once the light was turned on. The lamp beeped once when three minutes were remaining and again three times on the final three seconds of the cycle. When time was zero a long beep sounded and the light turned off automatically. After the first session was completed, the whitening gel was removed with dry gauze. Then the teeth were covered again with whitening gel and the procedure was repeated for three sessions (total bleaching time was 45 minutes). After the bleaching procedure finished, the samples were washed with water and kept in saline at room temperature. Concerning the chemical activated bleaching; Dash chair side whitening gel (Discus Dental, Inc., Culver City, CA, USA) was used. The whitening accelerator was applied to the labial surface of teeth before each cycle. The syringe cap of Dash Whitening Gel syringe was removed and attach flocked tip and secure with a clockwise turn in place. The gel was applied to the facial side of teeth (1-2 mm thick). The gel was left on teeth for 15 minutes. After completion of the 15 minutes, the gel was removed by dry gauze and the cycle was repeated for three times (total bleaching time was 45 minutes). The teeth were rinsed with water and kept in saline at room temperature.

Application of the remineralizing agent:

All specimens (either bleached or not bleached) were subjected to remineralizing agent application for three minutes by rubbing the labial surface of teeth with 20 mg of bioactive glass (BAG) either conventional or nano using a wet cotton pellet for three minute followed by copious rinsing for one minute with distilled water.[16] All treated specimens were subsequently stored in Artificial Saliva AS (pH 7.4), at 37 C for one day before microhardness assessment.

Microhardness assessment:

Surface microhardness of the specimens was determined using Digital Display Vickers Microhardness Tester (Model HVS-50, Laizhou Huayin Testing Instruments Co., Ltd. China) with a Vickers diamond indentor and a 20X objective lens. A load of 200 gm was applied to the labial surface of the specimens for 15 seconds. Three indentations were equally placed over an area of one mm diameter at the middle third of the labial surfaces of the specimens. The diamond-shaped indentations were carefully observed in the microscope. Image analysis soft ware allowed the accurate digital measurements of their diagonals. Vickers values were converted into microhardness values MHV following this equation:

MHV=1.854 P/d^2

Where;

MHV is Vickers microhardness values in Kgf/mm^2

P is the load in Kgf

d is the length of the diagonals in mm

Statistical analysis:

Data presented as mean and standard deviation (SD) values. Data explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Microhardness (VHN) showed normal distribution, One Way-ANOVA used to study the effect of different bleaching material and remineralizing agent on mean microhardness (VHN). The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 22 for Windows.

RESULTS

Mean and SD of enamel microhardness of control and experimental groups of both bleached and remineralized specimens are presented in tables (1 and 2) and figures (1 and 2), respectively.

1. Effect of different bleaching techniques on enamel microhardness (VHN)

Mean and standard deviation (SD) of enamel microhardness (VHN) after application of different Bleaching techniques were presented in table (1) and figure (2).

Regarding the non remineralizing agent group, the unbleached (control) group (249.09±9.52 VHN) showed the highest statistically significant mean microhardness value (VHN) followed by chemically activated bleaching group (220.17±1.3 VHN) followed by light activated bleaching group (210.03±1.49 VHN) which had the lowest statistically significant value at p≤0.001.

Regarding the conventional bioactive glass group, the unbleached group (247.01±4.96 VHN) showed the highest statistically significant mean microhardness value (VHN) followed by chemically activated group (230.92±1.29 VHN) which had a statistically insignificant diffrerence compared to the light actaivated group (228.73±2.83 VHN) at p≤0.001.

Regarding the nano bioactive glass group, the unbleached group (254.58±4.74 VHN) showed the highest statistically significant mean microhardness value (VHN) followed by light activated group (246.01±1.23 VHN) which had a statistically insignificant difference compared to the chemically activated group (245.26±3.76 VHN) at p≤0.001.

2. Effect of different remineralizing agent on mean enamel microhardness (VHN)

Mean and standard deviation (SD) of enamel microhardness (VHN) after application of different

TABLE (1) Mean and standard deviation (SD) of enamel microhardness (VHN) after application of different bleaching techniques.

		Bleaching technique						p-value
		Unbleached (Control)		Light activated		Chemical activated		
		Mean	SD	Mean	SD	Mean	SD	
Microhardness	Without application of remineralizing agent	249.09[a]	9.52	210.03[c]	1.49	220.17[b]	1.30	≤0.001***
	Conventional bioactive glass	247.01[a]	4.96	228.73[b]	2.83	230.92[b]	1.29	≤0.001***
	Nano bioactive glass	254.58[a]	4.74	246.01[b]	1.23	245.26[b]	3.76	≤0.001***

Means with the same letter within each row are not significantly different at p=0.05. ***= Highly significant***

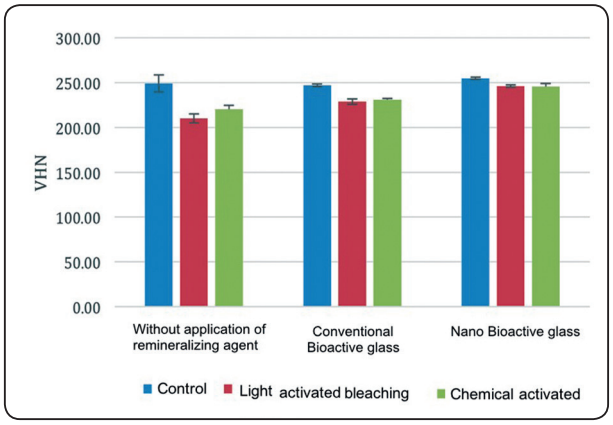

Fig. (1) Histogram showing the mean enamel microhardness (VHN) after different Bleaching techniques.

Bleaching techniques were presented in table (2) and figure (3).

Regarding the unbleached control group, there was no statistically significant difference among all remineralizing agents, p=0.311.

Regarding the light activated group, the unremineralized group (210.03±1.49 VHN) showed the lowest statistically significant mean microhardness value (VHN) followed by conventional bioactive glass group (228.73±2.83 VHN) followed by nano bioactive glass group (246.01±1.23 VHN) which had the highest statistically significant value at p≤0.001.

TABLE (2) Mean and standard deviation (SD) of enamel microhardness (VHN) after application of different remineralizing agents.

Bleaching technique \ Remineralizing agent		Without application		Conventional bioactive glass		Nano bioactive glass		**p-value**
		Mean	SD	Mean	SD	Mean	SD	
Microhardness	Unbleached (Control)	249.09	9.52	247.01	4.96	254.58	4.74	0.311 NS
	Light activated	210.03^{c}	1.49	228.73^{b}	2.83	246.01^{a}	1.23	≤0.001***
	Chemical activated	220.17^{c}	1.30	230.92^{b}	1.29	245.26^{a}	3.76	≤0.001***

Means with the same letter within each row are not significantly different at p=0.05. ***= *highly significant*

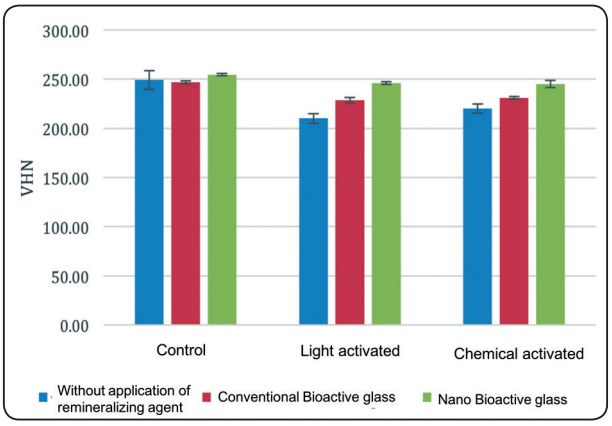

Fig. (2) Histogram showing the mean enamel microhardness (VHN) after different remineralizing agent application

Regarding the chemically activated group, the unmineralized group (220.17±1.3 VHN) showed the lowest statistically significant mean microhardness value (VHN) followed by conventional bioactive glass group (230.92±1.29 VHN) followed by nano bioactive glass group (245.26±3.76 VHN) which had the highest statistically significant value at p≤0.001.

DISCUSSION

The contemporary bleaching agents are typically either hydrogen peroxide or carbamide peroxide that are widely used with different bleaching techniques either in office or at home. With in-office bleaching techniques, the agent can be activated using different energy generators, such as light, laser, or heat to reduce the length of the procedure and accelerate the oxidizing oxygen reaction.[17]

Dental tissues could be affected by the bleaching gel as shown by many authors. enamel microhardness,[18,19] superficial roughness, [20,21] and chemical composition,[22] are the frequently affected parameters in the literatures.

Microhardness can provide indirect evidence of mineral loss or gain in the dental hard tissues.[23] Furthermore, differences in organic and inorganic content can also be verified by microhardness tests.[9]

Results of the current study revealed that both bleaching techniques decreased the microhardness of enamel significantly, and the light activated bleaching had more effect than the chemically activated one.

The chemical effect of the bleaching agents on the enamel surface such as mineral loss and changes in the calcium-phosphorus concentrations, may affect different properties as microhardness of enamel.[24] Some studies assumed that the organic matrix of enamel subsurface was altered by the oxidizing effect of peroxide radicals. These changes in the organic part might affect the mechanical properties of enamel, such as fracture toughness and microhardness.[25]

Moreover, Hegedüs et al. in 1999,[26] suggested that the peroxide-containing bleaching agents affect the organic phase of enamel surface and may extend to the subsurface of enamel. This penetration might be attributed to low molecular weight of hydrogen peroxide. Accordingly, the more oxidative effects occur in the subsurface enamel where more organic material is present changing the properties of the outer enamel and the subsurface.

Additionally, *Lewinstein et al in 2004,*[23] *reported that both chemical and power bleaching caused a significant reduction in enamel and dentin microhardness. Moreover, Alsalehi et al in 2007,*[27] *concluded that there was significant decrease in microhardness after immersion of enamel specimens in different concentration of HP solutions. Many studies concluded that* the effect on the microhardness properties is probably dependent on the concentration and light activation, combined with a high concentrated HP that could exacerbate this alteration. [21, 22]

On the other hand, many studies proved that bleaching agents (hydrogen and carbamide peroxides) showed no significant effect on the microhardness of enamel surface.[6, 22,28and 29]

Sulieman et al in 2004,[22] reported that there was no adverse effect on enamel or dentin specimens in regard to the surface hardness after power bleaching with 35%hydrogen peroxide, that might be a result of using different bleaching material than those used in the present study. Araujo *et al*. in 2010,[19] in an *in situ* study, concluded that changes in microhardness are not significant and can be recovered 14 days after bleaching, due to the absorption and precipitation of the calcium and phosphate present in the saliva.

More over, Parreiras *et al*, in 2014,[30] also reported nonsignificant enamel changes comparing light activated and nonlight activated bleaching protocols right after the bleaching process, and also reported that, after one week storage in artificial saliva, all the specimens´ microhardness were similar to their initial values.

These contradictory studies regarding the microhardness alterations can be explained by the fact that surveys have different methodologies, such as using different bleaching agents (with different concentrations, application times and methods of application), different forms of hardness evaluation (Knoop, Vickers, weight and length indentation), pH level and storage method of the specimens,[8] Thus, it becomes difficult to find concrete data to compare the results.

A recovery toward pretreatment microhardness value might be expected. Important factors such as salivary flow, buffring capacity of saliva oral hygiene and the use of topical remineralizing agents may increase the remineralization of bleached enamel.[31]

Bioactive glasses have unique remineralizing properties and are generally introduced into various dentifrices as very fine particles to provide calcium and phosphorus to the tooth surface. Bioactive glasses (BAGs) as opposed to most technical glasses are characterized by the reactivity in water and in aqueous liquids. The bioactivity of BAGs is derived from their reactions with tissue fluids resulting in the formation of hydroxy-carbonate apatite layer on the glass.

In addition, Nano-technology is one of the most important fields of researches nowadays. As the Nano particles help in the concentration and amplification of the remineralizing agents at the surface of the tooth substrates, this study was conducted to show the remineralizing effect of the conventional versus Nano-sized bioactive glass.

Artificial saliva was used as a storage medium in this study to simulate the washing effect resembling the natural oral conditions. It was also chosen, as the bioactive glasses need calcium and phosphorus rich medium to enhance the precipitation of hydroxy apatite crystals promoting the bioactive glass action.[32] *Bakry et al, 2011,*[33] recommended to use a storage medium rich in calcium and phosphorus, instead of the water, as it would help in the transformation of the precipitate formed into hydroxyapatite crystals.[34,35] 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.[36]

The results of the current study revealed that both types of bioactive glass have increased the enamel microhardness which is increased more with nano-sized BAG than the conventional one.

Bioactive glasses are silicate-based materials and can form a strong chemical bond with the dental tissues. These biomaterials are highly biocompatible and can form a hydroxyapatite layer when implanted in the body or soaked in the simulated body fluid.

Conventional bioactive glasses are exposed to contaminants during the conventional glass processing which exerts negative effects on bioactivity. *Gross and Strun, 1980,*[37] reported that impurity cations in bioactive glasses have considerable effects on tissue bonding. Due to several disadvantages, conventional glass processing method including melting of glass components was replaced by sol-gel method with a large number of benefits such as low processing temperature, higher purity and homogeneity and therefore better control of bioactivity. The sol-gel derived Nano bioactive glass has a porous structure which increases its surface area by two orders of magnitude compared to a melt-derived conventional bioactive glass. Therefore, the rate of hydroxyapatite formation for the sol-gel based Nano bioactive glass is more rapid. *LaTorre et al, 2009,*[38] demonstrated that bioactive glasses with surface areas greater than 50 m^2/g could bond to the calcified hard tissues within 24 hours of in vitro experiment. Moreover, variation in remineraliazation competence might be due to different remineralization mechanism of the two agents. In addition, differences in processing method, particle size and application technique might also affect the remineralization efficacy of each agent. Both conventional and Nano bioactive glass form HAP layer on the demineralized dental tissue. This process involves different stages; calcium ions dissolve from the bioactive glass into the body fluid while silica-rich interlayer forms on the glass surfaces. HAP nucleation is enhanced by supersaturation of the surrounding fluid due to the dissolution of the calcium ions. Additionally, favorable sites for the nucleation are provided by the dissolved silicate ion obtained from silica-rich interlayer dissolution.

The process of nucleation and growth of the HAP layer continues by the reactions of the calcium, phosphate, and hydroxide ions. Carbonate or fluoride anions present in the storage medium might be incorporated in the remineralization process, as well.[39-41] These results are in agreement with those obtained by *Vollenweider et al, 2007, Huang et al 2009, Huang et al, 2011 and Goh et al ,2013).*[42-45]

CONCLUSIONS

Under the limitations of this study, it can be concluded that:

1. Bleaching has a deleterious effect on enamel microhardness
2. Bioactive glass can counteract the adverse effect of bleaching on enamel
3. Nanobioactive glass is a promising material for remineralization hamber temperature in vitro. J

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