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Effect of Two Different Remineralizing Agents on the Surface Roughness of Bleached Enamel.

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ABSTRACT

To evaluate the effects of nano hydroxyapatite (NHAP) and amorphous calcium phosphate (ACP) remineralizing agents on the surface roughness of bleached bovine enamel. A total of 25 bovine anterior teeth were prepared for this study. They were randomly divided into two main groups the control group (n=5) and the experimental group (n=20) which received the photo-activated and chemically-activated bleaching agents. Then the experimental group was further divided into two subgroups receiving NHAP and ACP remineralizing agents. Surface roughness measurements were performed for all groups using image analysis software. One Way-ANOVA showed that the nano hydroxyapatite group specimens increased the enamel surface roughness after bleaching with photo-activated and chemically-activated bleaching agents in comparison with amorphous calcium phosphate. There was no statistically significant difference between both remineralizing agents. The application of remineralizing agents following bleaching procedures did not significantly alter the enamel surface roughness.

Keywords: Nano Hydroxyapatite, amorphous calcium phosphate, surface roughness, enamel bleaching.

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INTRODUCTION

Teeth discoloration had been considered a main esthetic problem facing dentists in the era of restorative dentistry. The demand of teeth whitening had increased rapidly and bleaching systems had a wide interest by the dental practitioners to achieve esthetic results. Bleaching agents are available in a huge number in all markets. They differ according to their concentrations, compositions and their techniques of applications [1].

Teeth discoloration can be categorized into extrinsic and intrinsic discolorations. Extrinsic discoloration occurs as a result of indirect stains as chlorhexidine mouthwash or direct stains from coffee, cigars or tea [2]. While, intrinsic discoloration might be due to changes in the thickness of enamel and dentin and/or changes in their chemical composition.

Although bleaching is considered to be a non-destroying and safe method for teeth discoloration, several studies reported its side effects following its application as increased surface roughness [3,4]. Studies stated that the use of remineralizing agents after bleaching procedures could repair early carious lesions considering its structural and chemical similarity with the tooth minerals [2].

Application of amorphous calcium phosphate (ACP) remineralizing agent on the tooth surface following bleaching procedure could stabilize the calcium and phosphate ions level, through maintaining a state of super saturation [5]. The morphological structure and chemical properties of nano hydroxyapatite might play an important role in remineralizing the enamel surface [6]. According to its various properties as non-toxicity, biocompitability and osteoconductivity, it can be used in many applications, among them it can be used as a remineralizing agent [7].

Thus, the purpose of that study was to evaluate the effects of nano hydroxyapatite and amorphous calcium phosphate remineralizing agents on the surface roughness of bleached enamel.

MATERIALS AND METHODS

Two different bleaching agents and two different remineralizing agents were used in this study. Their description, composition, manufacture and patch number were listed in Table 1.

Table 1: Materials Description, composition, manufactures and batch numbers of the two bleaching agents and the amorphous calcium phosphate remineralizing agent.

Material	Description	Composition	Manufacturer	Batch Number
White Smile photo-activated bleaching agent.	In-Office Photo - activated bleaching agent. The colour of the mixed gel is green.	32% hydrogen peroxide concentration.	Discuss Dental, Germany	14029
Power bleaching of White Smile bleaching agent.	In-Office chemically-activated bleaching agent. The colour of the mixed gel is yellow.	40% hydrogen peroxide concentration.	Discuss Dental, Germany	13138
Amorphous Calcium Phosphate.	Relief gel. (Remineralizing agent).	Sodium Fluoride, Potassium nitrate, Water.	Discuss Dental, LLC Cul Vercity, CA	13294026

Study design

A total of 25 bovine anterior teeth were selected and prepared to be used in this study for the surface roughness test. Specimens were divided into two main groups. First group (n=5) was used as a control; where the teeth were not subjected to any treatment (Baseline). While the second group (n=20) represented the experimental group. The experimental group was then divided into two subgroups of 10 specimens each representing two different bleaching agents used in the study (White Smile Light activated bleaching agent and White Smile chemically-activated bleaching agent). Each subgroup was further divided into two divisions of

five specimens each; representing the two different remineralizing agents applied in the study (amorphous calcium phosphate and an experimentally prepared nano hydroxyapatite remineralizing agents).

Preparation of Nano hydroxyapatite powder

Pure grade chemicals were used without further purification. Calcium hydroxyapatite (CaHAP) was prepared from Ca (OH) . 8 H_2O and H_3PO_4 [8]. 0.25 M of Ca $(OH)_2$. $8H_2O$ and 0.03 M of H_3PO_4 were prepared as stock materials. H_3PO_4 solution was added to the calcium hydroxide with continuous stirring at 100 °C under nitrogen gas atmosphere. Phosphoric acid was added with low rate (4 cm^3/min) to avoid the local depression in the pH of the solution. The resultant white suspension was aged at 100 °C for 16 hours, and the formed precipitates were filtered off. The precipitates were washed with de-ionized water and methanol, and then dried at the room temperature for 16 hours.

Characterization of the prepared Nano-hydroxyapatite

The obtained particles were characterized by the following techniques:

Fourier transformer infrared (FTIR) spectroscopy analysis

Infrared absorption spectra (IR) were performed by the Potassium Bromide (KBR) disc technique using a Fourier transformer infrared spectrometer (Nexus 670 FTIR, USA) in the range between 400 to 4000 cm^{-1}.

X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) was carried out by Brukur D8 advance diffractometer (Germany) device, using CuKα radiation with wavelength of 1.54 Aº, in the 2Θ range 5-60º with a step – size of 0.02º and a step time of 0.5 second.

Transmission electron microscope (TEM) analysis

The morphology of nano hydroxyapatite powder, particles size and degree of crystallinity were evaluated utilizing a transmission electron microscope (TEM) at accelerating voltage of 200 KV with magnetic imaging resolution: 22 Aº.

Teeth selection and preparation

Twenty-five bovine anterior teeth were selected for this study. The teeth were extracted and washed under running water using a brush to be cleaned from any debris, and then the teeth were further cleaned ultrasonically from any deposits or extrinsic stains. The roots of all teeth were cut using a low-speed double-side cutting disc, and the pulp tissues were removed and the pulp chambers were sealed with a piece of cotton to avoid in or out passage of the fluids. Teeth crowns were fixed over double adhesive tapes. These tapes were fixed over the surface of a glass slab after being labeled palatally using nail polish for identification.

Whitening Vehicle application

White Smile photo-activated bleaching agent

The material was dispensed from the barrel syringe by attaching the mixing tip to the top of the dual syringe of the white smile light whitening gel. The gel was applied directly to the labial surfaces of each specimen in 1-2 mm thick layer. The whitening lamp (The whitening lamp LED technology with highly focused light spectrum, Discuss Dental, Germany) was placed as close as possible to the teeth and the session time was adjusted for 15 minutes for photo-activation. After 15 minutes, the bleaching gel was removed from the surfaces of the specimens using dry cotton rolls without rinsing with water to be ready for the application of another fresh layer. This procedure was repeated three times and each time the whitening lamp was adjusted for 15 minutes resulting in a total of 45 minutes treatment of the application of white smile light activated bleaching agent. Then the bleaching gel was removed from the labial surface of each specimen using dry

cotton rolls and the specimens were finally stored in tap water in tightly sealed containers for 24 hours before the surface roughness test was carried out.

White Smile chemically-activated bleaching agent

The material was dispensed from the barrel syringe by attaching the mixing tip to the top of the dual syringe of the white smile light whitening gel. The gel was applied directly to the labial surfaces of each specimen in 1-2 mm thick layer. The gel was left undisturbed on the teeth surfaces without photo activation for 15 minutes. After 15 minutes, the bleaching gel was removed from the surfaces of the specimens using dry cotton rolls without water rinsing to be ready for the application of another fresh layer. This procedure was repeated three times and each time the gel was left undistributed on the teeth labial surface for 15 minutes without photo activation, resulting in a total of 45 minutes treatment of the application of white smile chemical activated bleaching agent. The bleaching gel was then removed from each specimen surface using dry cotton rolls and finally stored in tap water in tightly sealed containers for 24 hours before the surface roughness test was carried out.

Application of the remineralizing agents

Amorphous calcium phosphate (ACP relief gel)

ACP relief gel remineralizing agent was applied on the labial surfaces of the bleached specimens. The applied layer was 1-2 mm thick. The gel was left undisturbed on the surfaces for 10 to 15 minutes according to the manufacturer's instructions. Then, the gel was rinsed with water, dried and then stored in a tightly sealed container containing tap water for 24 hours before the surface roughness test was carried out.

Experimental nano hydroxyapatite powder

The prepared nano hydroxyapatite powder was mixed with distilled water to obtain a suspension of a flowable consistency to be able to be applied to the labial surfaces of the bleached specimens. 0.5 mg of the powder were dispensed and measured using digital balance ADAM equipment (AE ADAM^{R}) then, each 0.5 mg of the powder were mixed with 5 ml of distilled water resulting in a suspension, which was applied to the labial surfaces of the all bleached specimens using a brush. The applied mix was left undisturbed on the specimens' surfaces for 10 to 15 minutes. Then the material was rinsed with water from the surfaces, dried with cotton rolls and then stored in a tap water tightly sealed containers for 24 hours before the surface roughness test was carried out.

Surface roughness measurement

All prepared specimens were subjected to surface roughness measurements using a digital camera (C 5060, Olympus, Japan) which was mounted on a stereo microscope (BX 60, Olympus, Japan) connected to computer software. Specimens were placed over clay and the lens was adjusted at the middle 1/3 with magnification of 9X for capturing images. Then images were transferred to the computer and analyzed using software system (Image J, 1.4 1a, NIH, USA). Calculations of the arithmetic means of elevations and depressions (Ra) were done for analysis according to the light reflection.

Statistical analysis

Data was presented as mean and standard deviation (SD) values. One Way-ANOVA was used to study the effect of application of different remineralizing agents on mean enamel surface roughness (Ra). Duncan's post-hoc test was used for pair-wise comparison between the means when ANOVA test is significant. Independent t-test was used to study the difference between different bleaching agents tested as well as the remineralizing agents used.

Statistical analysis was performed with the program IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 22 for Windows [9].

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RESULTS

NHAP Fourier transformer infrared (FTIR) spectroscopy analysis

IR spectra of the prepared specimens were represented in Figure 1. Abroad band at ~ 3425.3 - 3440 cm^{-1} had appeared. Also a strong band at 1000 – 1100 cm^{-1} beside a week one at ~ 930 cm^{-1}. Two other sharp bands at ~ 580 and 558 cm^{-1} were specified. In addition, a sharp band had appeared at ~ 1420 cm^{-1}

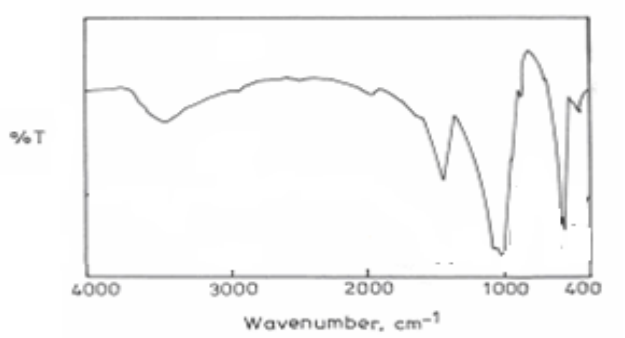

Figure 1: IR spectrum band of the prepared nano hydroxyapatite.

NHAP X-ray diffraction (XRD)

The results of the X-ray diffraction were presented in Figure 2. It showed the specific peaks of hydroxyapatite (JCPDS 86-0740.)

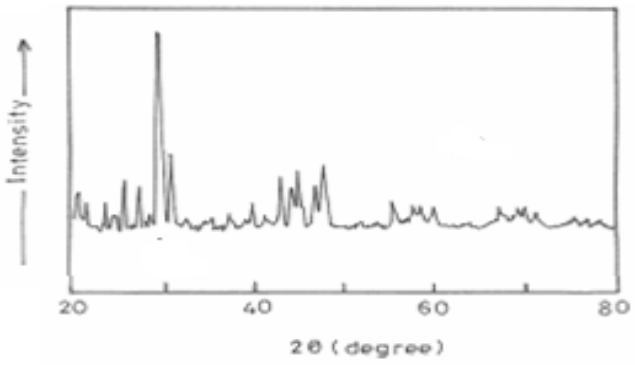

Figure 2: X-ray pattern of the prepared nano Hydroxyapatite

NHAP transmission electron microscope (TEM)

Figure 3 displayed TEM images of the prepared specimens. The average article size estimated from the TEM image was from 30 to 100 nm. On other hand, it was observed that irregular short rod shape particles had appeared.

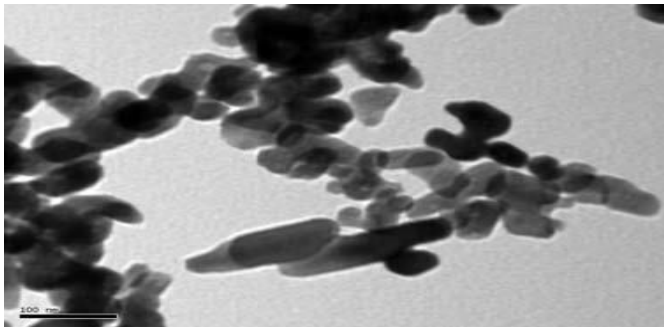

Figure 3: TEM image of the prepared nano hydroxyapatite

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Results of surface roughness

Means and standard deviations (SD) of surface roughness values for the two different bleaching agents and the control groups were presented in Table 2. White smile photo-activated bleaching agent group showed the highest mean surface roughness value (123.85±1.93). While, the lowest mean surface roughness value was recorded for the control group (121.75±6.25) a*t* *p*= 0.494. There was no statistically significant difference between the two groups. White smile power activated bleaching agent showed the highest mean roughness value (122.63±8.74). While, the lowest mean surface roughness value was recorded for the control group (121.75±6.25) at *p*= 0.860. There was no statistically significant difference between the two groups at *p*= 0.05.

Table 2: The effect of different bleaching agents on the mean enamel surface roughness

	Control		After Bleaching		*p*-value
	Mean	SD	Mean	SD	
Photo-activated bleaching agent	121.75	6.25	123.85	1.93	0.494 NS
Chemically-activated bleaching agent	121.75	6.25	122.63	8.74	0.860 NS
NS= Not statistically significant					

Means and standard deviations (SD) of the two different remineralizing agents within each of the two bleaching agents were presented in Table 3. For White Smile photo-activated bleaching agent; the amorphous calcium phosphate (ACP) remineralizing agent group showed the lowest mean surface roughness value (125.52±2.67) while, the highest mean roughness value was recorded for nano hydroxyapatite (NHAP) remineralizing agent group (127.67±2.54). There was no significant difference between both groups at *p*= 0.228. While for White Smile chemically-activated bleaching agent; the ACP group showed the lowest mean surface roughness value (123.49±5.03) while, NHAP group recorded the highest mean roughness value (125.06±2.22). There was no significant difference between both groups at *p*= 0.326.

Table 3: The effect of the two different remineralizing agents within each of the two different bleaching agents on the mean enamel surface roughness.

	ACP		NHAP		*p*-value
	Mean	SD	Mean	SD	
Photo-activated bleaching agent	125.52	2.67	127.67	2.54	0.228 NS
Chemically-activated bleaching agent	123.49	5.03	126.06	2.22	0.326 NS
NS=Not statistically significant					

DISCUSSION

The present study provided data about changes in the roughness of the enamel surface resulting from hydrogen peroxide application to the labial surface of bovine teeth followed by an exposure to remineralizing agents. Bovine teeth were selected to be used in this study because they have large flat surfaces and the structural changes and mineral distribution in bovine teeth are similar to those of human teeth [10] so that; bovine teeth could be accepted as an efficient substitute for the hard dental structures of human teeth [11].

In spite of the worldwide use spread of bleaching agents and remineralizing agents, there are still debates and controversial studies about their impact on the enamel surfaces following their applications [12-14]. This might be attributed to a variety of methodologies used, different materials concentrations and different techniques of applications [15].

In the current study two different bleaching agents with two different concentrations and two different remineralizing agents were used. ACP works through a unique method that uses ACP compounds in a carbonated solution to crystallize and form hydroxyapatite. These crystals then fill in the microscopic surface defects making the teeth looking smoother and stronger [16]. NHAP has a bioactive and biocompatibility factors which enable it to act as a template once it is applied to the enamel surface and filling the micropores

by attracting a large amount of the phosphorus and calcium ions from the remineralizing solution [17]. Surface roughness measurements were assessed for all specimens using image analysis software. Measurements were automatically calculated in pixels in terms of Ra which is the arithmetic mean value of the movement of the profile above and below the center line of the surface.

Band at ~ 3425.3-3440 cm^{-1} attributed to the vibration of the OH^- group in the lattice [8]. Band at 1000-1100 cm^{-1} and at ~ 930 cm^{-1} might be due to the stretching vibrations of PO_4^{-3} group. Sharp bands at ~ 580 and 558 cm^{-1} were specified due to the deformation vibrations of the phosphate ions $(PO_4)^{-3}$ [16]. A sharp band had appeared at ~ 1420 cm^{-1} corresponding to CO_3^{-2} ions incorporated within the OH^- sites on the surface of hydroxyapatite. Cheng et al. [18] suggested that the presence of the CO^{-3} group in the lattice of hydroxyapatite might be due to the action of the atmospheric CO_2. In general, the IR spectrum indicated that the NHAP was formed in agreement with the previous work [19].

The results indicated that the formed NHAP have a nanoparticle size in the range between 30 and 100 nm and it was an amorphous material except for the main peaks of hydroxyapatite; so the product can be used in many applications. Moreover; it can be mixed with other materials to improve these properties.

Both white smile photo-activated and chemically-activated bleaching agent groups showed a slight increase in the enamel surface roughness values; (123.85±1.93) and (122.63±8.74) respectively; in comparison with the control group (121.75±6.25) (Table 2). Furthermore; there was no significant difference between the two bleaching agent groups which indicated that photo-activated and chemically-activated bleaching agents almost had the same effect on the enamel surface and both bleaching agents had slightly altered the enamel surface roughness. This might be due to the close chemical composition of both agents. These results were in agreement with Mendonca et al., Navimipour et al., Azrak et al., and Basting et al. [15,20-22]. They concluded that; using different bleaching systems or using bleaching agents with high concentrations of hydrogen peroxide might cause superficial alterations on the enamel surface and affecting its roughness. Moreover; in a study conducted by Abouassi et al. [14] they claimed that; bleaching treatments were minimally invasive procedures and non-destructive to the enamel surface as they might cause a slight increase in the surface roughness of the enamel.

Such increase in the enamel roughness might be due to the oxidation process of the enamel surface and the loss of the organic matrix which constitutes less than one percent of the enamel composition [23] due to the exposed high concentration of hydrogen peroxide bleaching agent that might have caused dissolution of the superficial layer of the enamel surface and the low pH values (high acidity) of the bleaching agent because of the higher concentration of the hydrogen peroxide [24].

The results of the current study showed a slightly lower surface roughness in enamel specimens when they were treated with amorphous calcium phosphate (ACP) remineralizing agent (125.52±2.67) after bleaching with photo-activated and chemically-activated bleaching agents compared to enamel specimens treated with nano hydroxyapatite remineralizing agent (NHAP) (127.67±2.54) following bleaching with photo-activated and chemically-activated bleaching systems (Table 3). On the other hand; there was no statistically significant difference between both remineralizing agent groups indicating that using these two remineralizing agents following bleaching procedures did not produce a significant roughness on the enamel surface.

These results were in disagreement with Yamaguchi et al. [10] and Geiger et al. [25]. They stated that; ACP had the highest rate of formation and remineralization under oral physiologic environment. Once ACP is localized at the surface, it could buffer the free phosphate and calcium ions activities maintaining a super-saturation state by decreasing demineralization and promoting remineralization.

Rahiotis and Vougiouklakis [26] mentioned that ACP could maintain the calcium and phosphate ion levels on the tooth surface by high concentration gradients of phosphate and calcium ions enhancing the remineralization process.

Moreover; it has been revealed by studies that ACP is an excellent delivery vehicle available in a slow release of an amorphous form to localize calcium and phosphate ions at the enamel surface and has a synergetic effect in enamel remineralization [27,28].

Both ACP and the experimental NHAP remineralizing agents were applied to the bleached enamel surface for the same duration, which was 10-15 minutes according to ACP manufacturer instructions. Since NHAP slightly increased the surface roughness of bleached enamel specimens compared to ACP, this might be attributed to the differences in the application techniques and forms of both agents to the enamel surface. ACP relief gel was applied in a consistent form to the enamel surface leading to more release of the calcium and phosphate ions, however such ions might have diffused more easily inside the enamel pores filling the microscopic gaps and cracks rendering the teeth smoother and less sensitive [29].

On the other hand, the experimental NHAP was prepared in a form of suspension (0.5 mg powder + 10 ml distilled water), so that; once it was brushed over the enamel surface, more calcium and phosphate ions might have been released and precipitated on the bleached enamel surface, thus; creating a slight remineralization effect and leaving a slightly rougher enamel surface.

Huang et al. [17] were in agreement with the results of this study. They concluded that; the use of a suspension of 10 % NHAP appeared to be an optimal concentration for remineralization of enamel surface. As the concentration of the NHAP increased, the rate and amount of NHAP precipitation is increased at the surface along with the precipitation of calcium and phosphate ions and enhancing the remineralization effects [30].

Nevertheless; Selivany and Hano [31] were in disagreement with the results of the current study. They reported that using NHAP remineralizing toothpaste following bleaching procedure reduced the roughness of enamel surface. This might be due to the physical properties of nano hydroxyapatite that had a greater surface area than micro-sized hydroxyapatite and their ability of being deposited into the defects of the enamel surface reducing its roughness and making it smoother. As the particle size of NHAP is small, it can easily enter the enamel surface and fill the position of crystal enamel. The difference in the results between their study and the current study might be due to the difference in the application techniques, forms, consistencies and application time used for the remineralizing agent.

However, studies conducted by Jiang et al [32] and Khoroushi et al. [33] claimed that; ACP and NHAP are alkaline salts and might reduce the surface roughness of the enamel surface if they were mixed with hydrogen peroxide bleaching agent. The more alkalinity is the bleaching agent, the shorter will be the times need for the exposure and the better will be the improved surface roughness. Since the main mineral compound of the tooth structure is hydroxyapatite; then its rational was to be used as a remineralizing agent. The hydroxyapatite crystals had adhered to the tooth surface homogenously forming a protective layer to the underlying enamel; thus decreasing the direct contact of the hydrogen peroxide with the enamel surface.

CONCLUSIONS

Within the limitation of this study the following could be concluded:

- Application of remineralizing agents following bleaching procedures did not significantly alter the enamel surface.
- Although remineralization has been considered a major area of investigation, it is still difficult to determine exactly the efficacy and different remineralization techniques.

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