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EFFECT OF ADDITION OF CALCIUM PHOSPHATE NANOPARTICLES TO BLEACHING AGENTS ON SURFACE HARDNESS AND COLOR CHANGE OF ENAMEL. AN IN VITRO STUDY

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ABSTRACT

Purpose: The objective of this study was to evaluate the effect of incorporation of nanocalcium phosphate into the bleaching agent on microhardness and color change of enamel.

Materials and Methods:30 maxillary anterior teeth were divided into three groups: group (1): no bleaching agent, group (2): bleached with 40% carbmide peroxide, and group (3): bleached with 10 wt% of prepared nanocalcium phosphate modified carbamide peroxide. All samples were tested for color change using spectrophotometer, and hardness was evaluated using Vickers hardness test before and after bleaching.

Results: No significant change in color was observed for both groups although group (2) showed yellowish color change. Hardness was increased for group (3) that was confirmed by SEM and EDEX results.

Conclusion: Bleaching using nanocalcium phosphate particles had the highest hardness results and at the same time improved the remineralization effect on the tooth surface.

KEYWORDS: calcium phosphate nanoparticles, carbamide peroxide, hardness, color change, bleaching agents

INTRODUCTION

The increasing demand to have white teeth and thus a more pleasant smile has become the primary need of patients today and having discolored teeth is considered unacceptable to patients [(1)]. Treatment protocols of discoloration varies from the most conservative procedure as bleaching to extensive partial coverage veneer. In general, overcoming

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or treatment of the discolored teeth depends on different factors as the etiology, common habits, medical history, and dietary supplements of the patient. Tooth discolorations may vary in etiology, localization, appearance, severity, and adherence to tooth structure, and they are classified as intrinsic or extrinsic discoloration. Tooth whitening products as a solution to discolored teeth are commonly divided into three categories known as dentist supervised home bleaching products, in office bleaching products and over the counter whitening products. Hydrogen peroxide (HP) or its precursor carbamide peroxide (CP) is the most common active ingredients in tooth whitening products with different concentrations. Lower concentrations of CP or HP are used for home bleaching whereas higher peroxide concentrations are used for in office procedures [(2)].

Effects of bleaching on teeth were studied, but the findings are controversial. Studies reported that the exposure of enamel to bleaching agents can alter their surfaces and affect the microhardness, however, tooth bleaching doesn't create macroscopically visible defects, some studies reported some undesirable microscopic alterations which may include[(3-5)]: changes in the chemical composition of teeth, changes in the mineral content of dental structures such as calcium and phosphate, changes in enamel fluoride content, topographic changes, increase in enamel porosity and exposure of enamel prisms [(6)], rough surfaces are prone to extrinsic staining, biofilm formation, bacterial adhesion and periodontal disease [(7)].

Suggestions to incorporate elements that can reverse the adverse effect of bleaching agents are being suggested; several attempts were introduced to modify the bleaching agent with minerals that may counteract the side effects of bleaching, fluoride, as a remineralizing agent, was introduced and highly used clinically, but its efficacy is still controversial as the fluoride incorporation within the bleaching gel may raise a concern about the adverse interaction between the fluoride and carbamide peroxide; since CP may hinder the remineralizing effect of fluoride [(8, 9)].

Modifications of bleaching agents using bioactive material has been investigated lately. Some of the studies reported minimal effect of addition of these bioactive materials on the whitening effect of the bleaching agents, as well as their inhibition of restraining capability [(10)]. The scarcity of studies in this topic needs more research to assure that there was no adverse effect of such combination. Thus, the objective of this study was to evaluate the effect of nano-hydroxy apatite (n-HAP) addition to bleaching agents, and its effect on alteration of surface hardness and the staining after dental bleaching.

Research problem

The inability to benefit from bleaching agents as a remineralizing agent in addition to its main goal (teeth whitening), together with the drawbacks of frequent bleaching as it affects the surface properties of enamel and may lead to further sensitivity leaving a rough surface is also prone to discoloration, stating the problem has led to the need to improve the quality of tooth surface upon the use of whitening agents to solve the problems associated with it.

MATERIAL AND METHODS

Preparation and characterization of nano calcium phosphate[(11)]

Preparation:

For preparation of pure calcium phosphate nanofillers, calcium nitrate tetrahydrate and diammonium hydrogen phosphate were used as the starting materials at room temperature. Calcium nitrate was dissolved in distilled water then diammonium hydrogen phosphate solution was added dropwise. To obtain a precipitate, an ammonia solution was added to the mixture dropwise with stirring till reaching pH 10-11. After 24 hours, the precipitate was filtered then washed several times with distilled water and finally dried in an oven at 120-200 ºC [(12)].

Characterization

Synthesis of calcium phosphate nanofillers: calcium phosphate nanofillers were prepared by using sol gel method using titanium tetraisopropoxide as a start material mixed with HCL, ethanol and water. The exact surface area of the powder was examined using X-ray diffraction (XRD) analysis, Fourier transform infrared (FTIR) spectroscopy analysis, Scanning electron microscopic (SEM) analysis, energy dispersive X-ray spectroscopy (EDX) analysis, Transmission electron microscopy (TEM) analysis and Thermal gravimetric analysis (TGA).

Bleaching gel preparation

The bleaching gel (Ultradent Opalescence Boost Whitening Gel 40% SKU: UD4751 40 % concentration) was modified by the addition of 10% nano calcium phosphate (nCaP) that was dispersed using zeta sizer and mixed with nCaP over a magnetic stirrer (Fig. 1).

Sample preparation

Thirty maxillaries fully erupted anterior were collected from institute of diabetes, extracted for periodontally compromised reasons. Sample size was estimated based on the study done by Roza Haghgoo R et al (2016) Teeth were inspected carefully under light to exclude cracks, fractures, dental fluorosis, or enamel defects as attrition, abrasion, or erosion. The teeth were ultrasonically cleaned from all debris, stains and calculus using ultrasonic scalers, they were further disinfected and stored in deionized water for one week until being used [13].

Samples grouping

Specimens were divided randomly into three groups (10 each group)

Group 1: was not subjected to any bleaching procedures (-ve control).

Group 2: bleaching using opalescence (+ve control)

Group 3: bleaching using opalescence combined with 10 wt% calcium phosphate nano particles (Ncp).

Group 2 and 3 were bleached with the bleaching agent according to manufacturer's instructions (Fig. 2). About 1 mm thick layer of the gel was applied on the labial surface at room temperature, after 30 mins it was removed using wet cotton pellets, washed under running water then dried with cotton pellets and reimmersed in artificial saliva, the storage media was renewed daily.

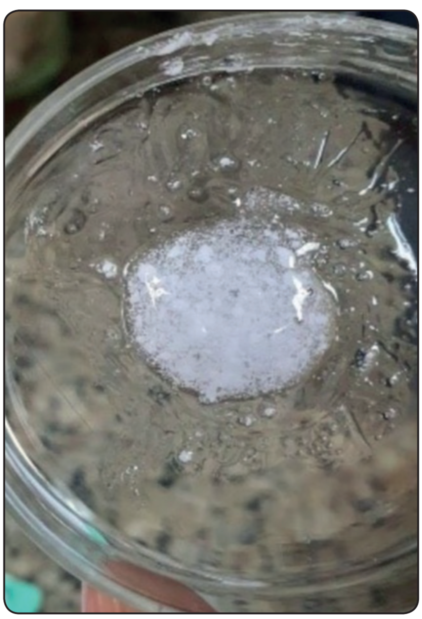

Fig. (1): Dispersion of nanocalcium phosphate in bleaching gel.

Fig. (2): Application of bleaching agent

Testing of samples

Color analysis: All the prepared specimens' colors were all subjected to spectrophotometric analysis, and shade was determined for all teeth at the base line before any treatment to determine their color parameters then were stored in artificial saliva that was renewed daily.

Color stability evaluation: The spect-rophotometer and the International Commission on Illumination (CIE) concept were used to carry out this test by measuring the light absorption of each specimen before and after treatment. The apparatus used in measurements was Agilent Cary 5000 spectrophotometers provided from Agilent Technologies (USA).

According to the L*, a* and b* values, the color change (ΔE) was calculated as:

$$\Delta E= [(\Delta L^*)^2 +(\Delta a^*)^2 +(\Delta b^*)^2]$$

Where ΔL, Δa and Δb are the differences in L, a and b values before and after treatment. The samples were fixed to the spectrophotometer holder; the machine was turned on then the samples were tested. Data were collected, tabulated, and statistically analyzed using one-way ANOVA test.

Hardness test: For each tooth, the root was sectioned1 mm below the cemento-enamel junction using a water-cooled diamond saw (Beltec Mini, Araraquara, SP, Brazil). Polishing prior to bleaching was done with polishing brush and polishing paste (manufactured by DHARMA research, INC, USA) and washed thoroughly. Specimens were then stored in artificial saliva that were renewed daily.

Crowns of all the specimens, with the labial surface faced upward, were individually embedded in chemically cured acrylic resin (Acrostone) (Acrostone Manufacturing and Import Co., Cairo, Egypt.). Specimens from each group were examined by Vicker hardness testing machine at load 100 gm, and dual time 15 seconds, the specimen's hardness was tested before and after bleaching [14].

The results were statistically analyzed using the software program (SPSS 18; SPSS, Chicao, IL, USA). Values were then presented as mean, standard deviation (SD) and confidence intervals Data were explored for normality using Kolmogorov-Smirnov test. For parametric data, one way analysis of variance (ANOVA) test and Tukey's post hoc test were used for comparison between groups. The level of significance was set at $P \leq 0.05$.

Scanning electron microscope and EDEX

Samples were coated with a layer of gold prior to SEM assessment in a Quick Coater vacuum evaporator (Type SC-701; Sanyu Electron Co., Tokyo, Japan) to prevent build-up of electrostatic charge. Evaluation of surface was done using Field Emission-Scanning Electron Microscope (FE-SEM) (QUANTA FEG250) accelerated voltage at 20KeV, Holland.

RESULTS

Color change results

Data showed normal distribution when tested using Shapiro Wilk test. Independent t-test used to compare between tested groups for all color parameters. Significant level was set at 0.05. The mean ΔE, ΔL, Δa, and Δb values were also assessed for the groups [Table 1]. The mean Δa, ΔE, and ΔL was not statistically significant between the initial and final readouts. The initial and final mean of Δb values were significantly different indicating a more bluish color of group 3.

Hardness test results

The highest mean value was recorded in group 3, followed by control group (group 1) (352±9.85), with the least value recorded in group 2 (247.57±18.20). ANOVA test revealed that the difference between groups was statistically significant (p=0.000). Tukey's post hoc test revealed a significant difference between each 2 groups (Table 2, Fig. 3)

TABLE (1) Descriptive statistics and comparison of color

		Group 2 (+ve control)	Group 3	p-value
Δ L	Mean	2	2.2	0.788 NS
	SD	0.7	1	
Δ a	Mean	-0.4	-0.5	0.515 NS
	SD	0.1	0.1	
Δ b	Mean	-2.6	-3.3	0.004*
	SD	0.2	0.3	
Δ E	Mean	3.40	4	0.123 NS
	SD	0.60	0.6	

**=significant, NS=non-significant*

TABLE (2) Descriptive statistics and comparison of hardness

	Mean	Std. Dev.	Std. Error	95% Confidence Interval for Mean		Min.	Max.	F	P
				Lower Bound	Upper Bound				
Group 1 (-ve Control)	352.00[b]	9.85	3.72	342.89	361.11	342	368	169.59	.000*
Group 2 (+ve control)	247.57[c]	18.20	6.88	230.74	264.40	213	271		
Group 3	371.14[a]	10.93	4.13	361.03	381.25	352	386		

*Significance level p≤0.05, *significant, groups with different superscript letters are significantly different*

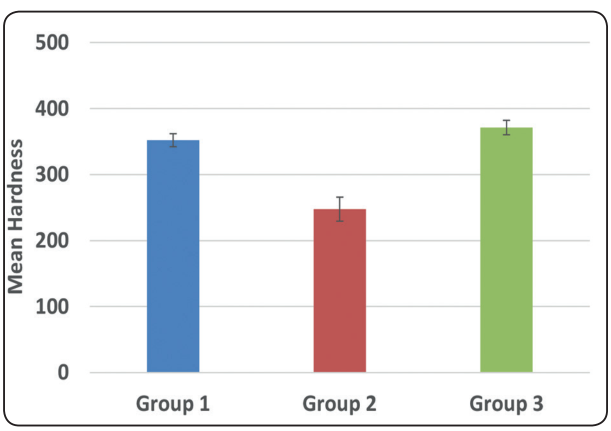

Fig. (3): Bar chart illustrating mean value of hardness recorded in different groups

SEM results:

Group 1 showed normal enamel pattern (Fig. 4). While group 2 revealed significant erosion, pattern close to type II etching pattern with loss of prism core and maintaining of the periphery (interprismatic dissolution) (Fig. 5). On the other hand, the demineralization in group 3 was less distinct with minor loss of prism core and periphery that revealed presence of free rare deposits of cap as confirmed by the EDEX results, showing signs of remineralization (Fig. 6). EDEX revealed large amounts of calcium and phosphate deposition on enamel surface (Fig. 7).

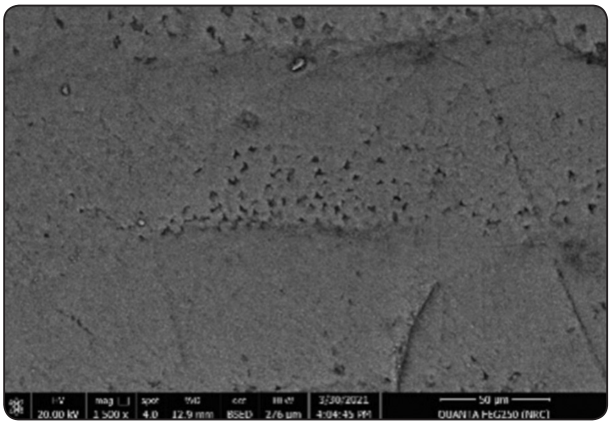

Fig. (4): Intact enamel under SEM

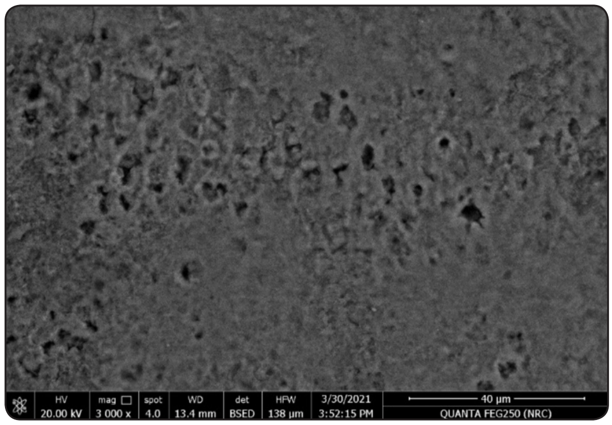

Fig. (5): Enamel surface after bleaching

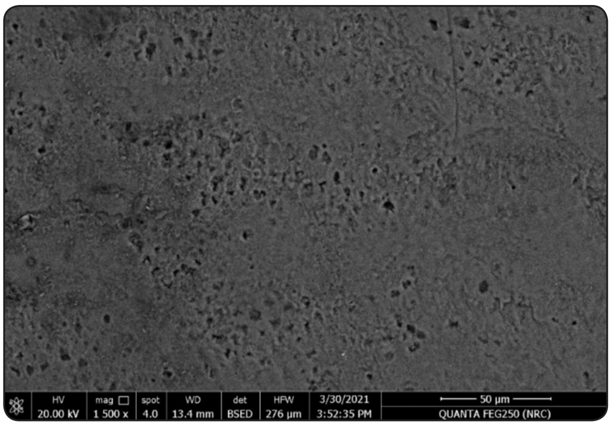

Fig. (6): Deposition of calcium and phosphate on enamel surface

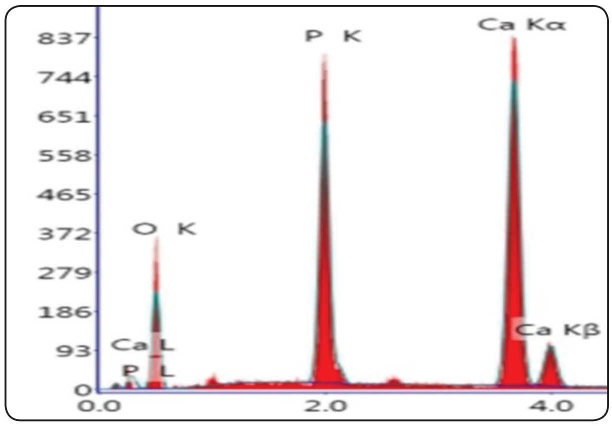

Fig. (7): EDEX results indicating ion release

DISCUSSION

In this study human anterior teeth were selected. The CIELAB system was used to measure of the color difference in bleached teeth. This system has three axes: the L* axis represents lightness and extends from 0 (black) to 100 (white); and a* and b* represent the redness-greenness and yellowness-blueness axis, respectively. The color change is mostly affected by the parameters of L* and b*, where L* values increase and b* values tend to decrease [15].

Analysis of ΔE, Δa and ΔL showed no significant difference between GP2 and GP3 and the results showed that both bleaching protocols were effective, meanwhile the addition of nano cap to the bleaching agent had no effect on the whitening efficiency but tended to increase the bluish tint of teeth. The changes might be contributed to the removal of pigments through the activity of the bleaching agents.

Oral environment has ideal conditions for remineralization of enamel. After enamel demineralization enhanced by bleaching agents facilitates ion exchange, consequently absorption of minerals may occur to substitute the lost minerals during bleaching[16]. In the present study, the microhardness of all the samples were evaluated at baseline and after bleaching procedures. Based on the results, the use of the nano-calcium phosphate particles during bleaching procedures with 40% HP resulted in higher mean enamel microhardness values, compared to the other groups. This could be explained by the fact that the microporosities formed on the surface of enamel due to bleaching might enhance remineralization in a process similar to caries arrest [(17)].

In agreement with our study, Orilisi G et al. (2021) concluded that the application of the commercial bleaching agents, containing nano-hydroxyapatite does not alter the morphological and chemical composition of the enamel surface and maintains its crystallinity and microstructure [(18)]. In addition, Llena, C. et al. (2019) found that the application of calcium phosphate for five minutes on the enamel surface after bleaching is effective in restoring the lost calcium after the bleaching process, while their effect in dentin could not be demonstrated [(19)].

CONCLUSION

Within the limitations of the present study, it can be concluded that, modifying the bleaching agent with nano-calcium phosphate particles enhanced the surface hardness of enamel without compromising the effect of bleaching agent.

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Null hypothesis

There is no significant difference between the use of bleaching agent alone or combined with remineralizing agents (calcium phosphate nano particles) on enamel surface microhardness and staining.

RECOMMENDATIONS

1. Further research on the remineralization potential of nano hydroxy apatite added to the bleaching agents.
2. Assessment of color stability of bleaching agents containing calcium phosphate particles over long period of time.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

Final Code: RE-PD-21-14

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