ORIGINAL ARTICLE

Effect of Nanohydroxyapatite Incorporation in Hydrogen Peroxide Bleaching Agent on Color, Microhardness, and Microscopical Features of Dental Enamel

Asraa S. Abtan* , **Nibal M. Hoobi**

Pediatric and Preventive Dentistry Department, College of Dentistry, University of Baghdad, Baghdad, Iraq

*Corresponding Author: Asraa S. Abtan
Email: asraasabah90@gmail.com

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Abstract

Purpose: The present study aimed to evaluate the effectiveness of incorporating nanohydroxyapatite into hydrogen peroxide bleaching material on color, microhardness, and morphological features of dental enamel.

Materials and Methods: 33 sound maxillary first premolar were used for the study. Enamel blocks (7mm× 5mm×3mm) were prepared from the middle third of the buccal halves of each tooth. Each dental block was embedded in self-curing acrylic resin with an exterior enamel surface exposed for various applications. The dental blocks were randomly divided into three groups (n=11) according to the bleaching technique. The groups were designed as follows: control; Hydrogen Peroxide (HP) and hydrogen peroxide with nanohydroxyapatite (HP-nHAp) groups. Color measurements and microhardness tests were conducted before and after treatment using the Vita Easy Shade spectrophotometer and Vickers hardness test, respectively. One sample representing each group was selected for morphological analysis using scanning electron microscope.

Results: The results showed that both HP and HP-nHAp groups induced color changing. Enamel microhardness loss of the HP group was significantly higher than that of the HP-nHAp and control groups. The enamel morphological changes were only observed in the HP group.

Conclusion: nHAp could significantly reduce the enamel microhardness loss caused by HP while preserving enamel surface morphological features without affecting bleaching efficacy.

Keywords: Bleaching; Enamel; Hydrogen Peroxide; Microhardness; Morphological Changes; Nanohydroxyapatite.

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1. Introduction

Dental aesthetics, such as tooth color, is highly significant for most people and any discoloration or staining can have a detrimental effect on their quality of life. Tooth discolorations may vary in etiology, localization, appearance, severity, and adherence to tooth structure, and they are classified as intrinsic or extrinsic discolorations [1]. Tooth discoloration is commonly treated with bleaching, which is considered the safest, most conservative, and effective procedure available for extrinsic discoloration [2]. Tooth bleaching products are commonly classified as: home bleaching, in-office bleaching, and over-the-counter whitening products. HP 30-40% associated with chemical or physical catalysts is the most common bleaching agent utilized in in-office treatment [3]. Dental bleaching mechanism is dynamic and complex, and involves penetration and diffusion of HP into enamel and dentin. This leads to the production of free radicals that oxidize chromophores, which are large organic compounds absorbing certain wavelengths of visible light. Free radicals attack chromophores, breaking their double bonds and making them colorless, resulting in a lighter appearance of the tooth [4].

Despite its proven efficacy, widespread apprehension persists regarding the adverse effects of HP on enamel. Several investigations found that the HP bleaching agents could induce chemical, structural and mechanical alterations to enamel such as increased roughness, decreased microhardness, alteration in mineral content, as well as damaging the pulpal tissue [3; 5- 8]. which could potentially be influenced by factors such as composition, concentration, pH values, and different application techniques of bleaching agents. Based on this finding, further research is required to develop a treatment that exhibits enhanced efficacy, durability, and efficiency to achieve the optimal whitening result without any detrimental side effects.

Various remineralizing agents, such as fluoride, calcium, and amorphous calcium phosphate can be applied to minimize the negative impact of bleaching treatment on enamel [2]. nHAp is considered one of the most important materials to overcome dental enamel demineralization. Hydroxyapatite with the chemical formula Ca_{10} (PO_4) OH_2 is a calcium phosphate compound with a calcium phosphorus ratio of 1:67 [9]. nHAp refers to the hydroxyapatite particles ranging from 8-39 nm [10] and the crystalline structure of natural hydroxyapatite in teeth closely resembled that of synthetic nHAp. The nHAp has a high affinity to demineralized surfaces because of its capacity to adhere to pores formed by acid attack. After adherence, nHAp proliferates and arranges into microclusters to create a consistent apatite layer that could fully cover both interprismatic and prismatic enamel [11]. Thus, the present study aimed to assess the effect of nHAP incorporating in 40% HP gel on color, microhardness, and morphological changes of dental enamel.

2. Materials and Methods

This comparative in vitro study was performed in the Department of Pedodontics and Preventive Dentistry, University of Baghdad/Collage of Dentistry, Baghdad, Iraq, and it was approved by the scientific and the ethical committee at the College of Dentistry / University of Baghdad (Approval Number: 686322 in 10/11/2022).

2.1. Preparation of Specimens

Thirty-three human maxillary premolar teeth were extracted for orthodontic purposes. These teeth were then thoroughly washed and cleaned using tap water, and then polished with a non-fluoridated pumice slurry. The polishing process was carried out for each tooth sample using a rubber cup attached to a slow-speed handpiece. The teeth were then immersed in a 0.1% thymol solution, which serves as an antimicrobial agent to prevent bacterial growth until they were ready to be used. The teeth were examined under a magnifying lens (10X), and any tooth with visible cracks, caries, or structural enamel defects was discarded. Enamel samples were prepared by horizontally cutting the teeth in the cervical area to separate crowns from roots. The crown of each tooth was then longitudinally sectioned in a mesio-distal direction to separate buccal and lingual parts. Enamel blocks of uniform dimensions (7×5mm and 3mm) thickness were prepared from the middle third of the buccal surface of each tooth using a double-sided diamond disk (Dental Lab Diamond disc, Airgoesin, Brazil) and a micro-motor handpiece with water cooling. These blocks were calibrated using an electronic digital caliper for accurate measurements. Each enamel block was individually inserted into self-curing acrylic resin with the buccal enamel surfaces

exposed (Figure 1). The exposed outer enamel surfaces were grounded and polished by using silicon carbide abrasive grit 1200 for 10 s, 2500 for 10 s, and 4000 for 4 minutes positioned in water cooling rotary polishing machine (HERGON, ITALY) to obtain a flat and smooth surface for the microhardness test [12]. Microscopical samples were obtained without grinding to preserve the microstructure [13]. All samples were then stored individually in plastic containers containing de-ionized water ready for baseline measurements.

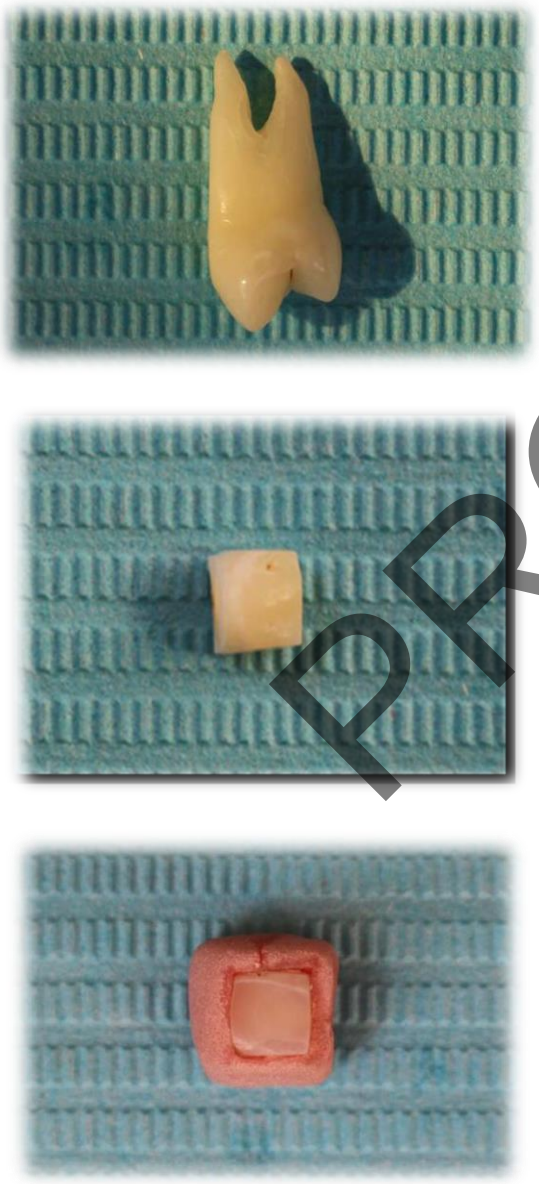

Figure 1. Enamel sample preparation. A: Sound extracted maxillary first premolar. B: Enamel block. C: Enamel block embedded in self-curing acrylic resin

2.2. Sample Grouping

According to the bleaching technique, enamel samples were divided into three groups as follows:

1. Control group: the samples did not undergo any treatments and only stored in de-ionized water during the experimental period.

2. HP group: the samples were treated with 40% HP bleaching gel.

3. HP-nHAp group: the samples were treated with 40% HP bleaching gel modified by the incorporation of nHAp powder.

All the enamel samples were stored in de-ionized water throughout the experiment as storage media except during the bleaching procedure [14].

2.3. Bleaching Procedure

2.3.1. Hydrogen Peroxide Bleaching Technique

Hydrogen peroxide (40% Opalescence Boost, USA) bleaching gel was used in accordance with the manufacturer's instructions. The product was activated by syringe-to-syringe mixing. A 1 mm thick layer of HP gel was applied to enamel samples and left for 20 minutes. The gel was then rinsed with deionized water for 10 seconds and dried with gauze. This process was repeated two more times during the same session for each sample resulting in a total of 60 minutes of application. The bleaching procedure was repeated after one week to obtain a total of two bleaching sessions.

2.3.2. Hydrogen Peroxide Modified by Nanohydroxyapatite Bleaching Technique

For the preparation of hydrogen peroxide-nanohydroxyapatite mixture (HP-nHAp), 2g of nHAp powder (Ald rich, Germin) was mixed with 1 ml of deionized. After that, they were mixed with 1 ml of 40% HP [15]. The HP-nHAp mixture was applied to the enamel surface for 20 minutes and then washed with deionized water for 10 seconds. This procedure was repeated two more times to have a total of 60 minutes of application. The bleaching procedure was repeated after one week to obtain a total of two bleaching sessions.

2.4. Color Measurement

Color measurements were conducted before and after the bleaching procedure using the Vita Easyshade Advance 4.0 spectrophotometer (Vita Zahnfabrik, Bad Sackingen, Germany), following the CIE $L^*a^*b^*$ color space system. The $L^*a^*b^*$ system represents the lightness (L^*) which is the white-black axis, the red-green axis (a^*), and the blue-yellow axis (b^*) of the color space [16]. During the measurement process, each sample was placed on a white background to prevent any potential absorption effect on color parameters [17]. The measurements were done by placing the tip of the device at a right angle contact with the samples. For each specimen, measurements were repeated 3 times and the average of the readings was calculated. The overall color change of each sample in every group was determined using the following equation [16] (Equation 1):

$$\Delta E = [(\Delta L)2 + (\Delta a)2 + (\Delta b)2]\ ½ \quad (1)$$

Where:

- ΔE: Color Change.
- ΔL: (L* after treatment- L* baseline).
- Δa: (a* after treatment- a* baseline).
- Δb: (b* after treatment- b* baseline).

2.5. Microhardness Measurement

The microhardness measurement was carried out by a digital Vickers microhardness tester (TH715, SN: 0003, Beijing Time High Technology Ltd, China) at the baseline and after the treatment. The measurements were taken by applying Vickers diamond indenter with a 100 g load vertically to the enamel surface for 15 seconds. Three indentations were taken for each specimen, and then the mean for these three records was obtained [18]. The percentage of microhardness loss was calculated as follows (Equation 2):

$$PMHL = [(SMHbaseline\ SMHfinal) /SMHbaseline] \times 100\% \quad (2)$$

2.6. Scanning Electron Microscope Analysis

Scanning Electron Microscopy (SEM) was carried out using (Axia-ChemiSEM, Thermo Fisher Scientific, USA). Three enamel samples were selected for SEM analysis, one for the control group, one of the HP bleached enamel sample, and one for the bleached enamel with HP-nHAp specimen. The specimens were dried with gauze, fixed to a metal stump, and then sputter-coated with gold using a vacuum system coating machine. SEM microphotographs of the enamel surface of each specimen at 5000X magnification were obtained.

2.7. Statistical Analysis

The statistical analysis was carried out utilizing (SSPS version-22). Shapiro-Wilk test was used to evaluate the normality of data. The data was further analyzed using One-Way ANOVA and Tukey's post-hoc multiple comparison tests. Independent t-test was used to assess the enamel microhardness difference between baseline and after treatment. The level of significance for all statistical tests was $p<0.05$.

3. Results

3.1. Color Measurement

The ΔE mean values of the three groups are shown in Figure 2. The highest ΔE was shown in the HP group, followed by the HP-nHAp group and then the least ΔE was shown in the control group.

3.2. Microhardness Measurement

The Vickers Hardness Numbers (VHN) before and after treatment as well as PMHL for the three groups are shown in Table 1. The result revealed that the highest reduction was recorded in the HP group followed by HP-nHAp and then the control group. According to One-Way ANOVA and Tukey multiple comparison tests, a significant difference in PMHL was found among the three groups ($p <0.05$). In the HP group, PMHL was statistically and significantly higher than that of the HP-nHAp and control groups ($p <0.001$), while there was no significant difference between the HP-nHAp and control groups ($p >0.05$).

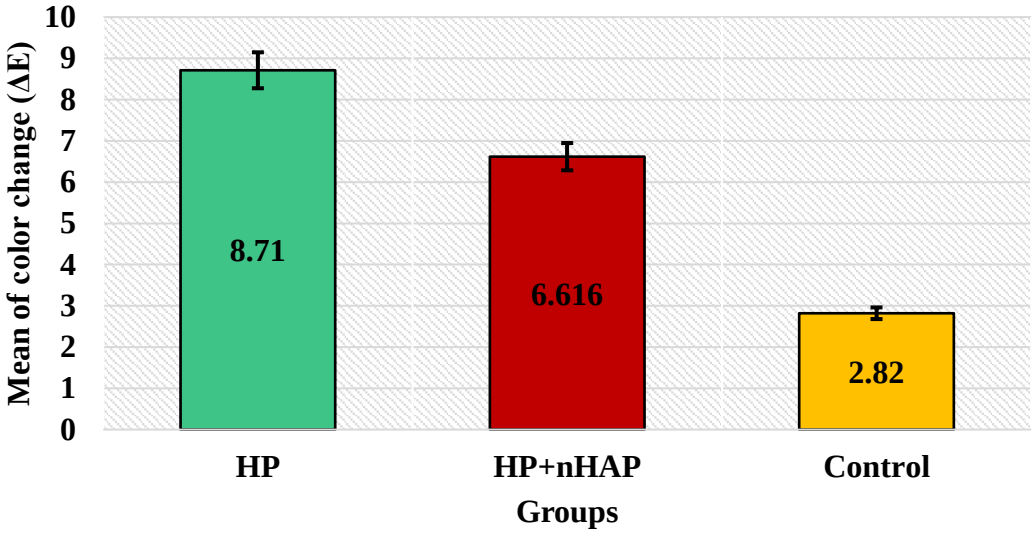

Figure 2. Mean of color change (ΔE) for all groups

Table 1. Microhardness(VHN) before and after treatment and percentage of microhardness loss (mean ±SD) and statistical difference among various groups

Groups	VHN(B) Mean±SD	VHN(F) Mean±SD	PMHL(%) Mean±SD
Control	372.74 ± 36.25	370.82 ± 36.51	0.529 ± 0.372
HP	376.93 ± 37.67	320.67 ± 37.76 ^	17.867 ± 5.915[a]
HP-nHAp	371.34 ± 43.03	357.02 ± 42.44	4.064 ± 1.827

(^) a statistically significant difference between sound and bleached enamel surface [Independent T-Test $p < 0.05$]. [(a)] A statistically significant difference between the HP-treated group with control and HP-nHAp groups [Tukey post-hoc test, $p < 0.05$]. HP: Hydrogen peroxide. HP-nHAp: Hydrogen peroxide modified by nanohydroxyapatite. VHN: Vikers hardness number. PMHL: Percentage of enamel microhardness loss

3.3. Scanning Electron Microscope Analysis

Representative SEM micrographs of the enamel surface of the three groups are shown in Figure 3. The obvious alterations in enamel morphology were observed in the enamel surface of the HP group when compared to the other two groups. It revealed a distinct structure of enamel presented as enamel irregularities and roughness. No special alteration was found on the enamel surface in the HP-nHAp treated group. A smooth, homogenous surface with well-defined fish scale appearance of enamel rods can be seen.

4. Discussion

Tooth whitening treatment is gaining popularity, and multiple bleaching agents are available in the market with different techniques [19]. The bleaching material is based on various peroxide compounds, which may have some detrimental effects [2]. Therefore, nHAp material was used, as it is considered one of the most biocompatible and bioactive materials making it widely used in medicine and dentistry due to its structural and chemical similarity to the natural bone and tooth minerals, and also not having irritation effects. The nHAp has a high affinity to demineralized surfaces due to its ability to penetrate the enamel pores and act as a template in remineralization, enhancing crystal growth and integrity [20]. Enamel samples were stored in deionized water between bleaching sessions and did not store in an artificially created saliva solution because artificial saliva contains calcium (Ca) and phosphate (P) allowing enamel remineralization that reverses the effect produced by the bleaching procedure and thereby acting as confounding factor which masks the actual effects of

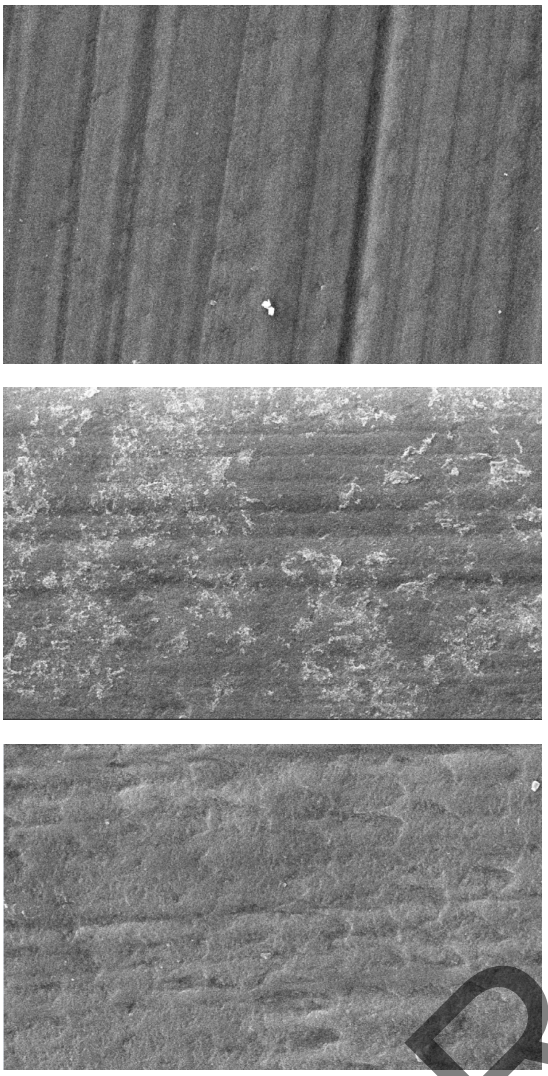

Figure 3. Microscopical features of the outer enamel surface using scanning electron microscope in the control group (A), HP group (B), HP-nHAp group (C) at 5000X

bleaching on minerals containing enamel and its microhardness [14, 21, 22].

Various methods can be used to assess the effectiveness of teeth whitening procedures. In the present study, the VITA easy shade spectrophotometer was used for color measurement, the instrumental color analysis offers a potential advantage over other methods [23]. This device provides accurate, repeatable, and rapid measurement of spectral color space. This technique measures the chromatic space between two points by calculating the ΔE and objectively evaluating the color changes at different time periods during whitening treatment. In this regard, values of ΔE E ≥ 3.3 are known to be visually perceptible [24, 25]. The result of the current study revealed that each of the HP (8.710± 3.443) and HP-nHAp treated (6.616±2.074) groups produced satisfactory results in color change. This finding was in agreement with the results of many studies [19, 24, 26, 27]. These studies reported that the addition of nHAp to the HP did not interfere with the whitening results and presence of nHAp did not interfere with the substrate characteristics and the HP reaction.

Microhardness is considered an appropriate test to evaluate minor changes, such as mineral loss in the enamel surface. In this study, Vicker's hardness test was chosen because it is simple, quick, and only requires a small portion of the sample buccal surface to test. In addition, evidence shows that it has a high sensitivity for monitoring of enamel mineral loss [28]. In the current study, the results showed that there was a statistically significant loss in the microhardness of samples that were subjected to HP bleaching compared to the other two groups. Moreover, the HP PMHL (17.867±5.915) was higher than that recommended as acceptable by ISO 28399, which states "the acceptable reduction of microhardness promoted by bleaching agent should be not higher than 10%" [29]. The decrease of enamel microhardness can be explained by the combined effects of demineralization and degradation of organic components particularly when exposed to high concentrations of HP [30]. The reactive and non-selective oxygen-free radicles of HP gels act not only on the chromophores but also interact with the organic component, oxidizing the proteins in its composition (amelogenins and enamelins) [31, 32]. This causes alterations in the protein matrix situated within the enamel crystallites, disrupting the crystal structure and hence increasing its vulnerability to mechanical stress, resulting in decreased microhardness and fracture toughness. Moreover, the absence of saliva leads to improper mineral recovery, resulting in a structural decrease in microhardness [30]. This finding is in agreement with research conducted by Khoroushi *et al.* [15], Ata [33] and Vieira *et al.* [3], in which a significant loss of enamel microhardness was reported. In contrast to the study done by Cvikl *et al.* [34] who concluded that using a relatively high concentration of HP bleaching gels with a shorter application time might be less harmful to the enamel surface. Moreover, these findings were supported by SEM analysis characterized by the loss of the uniform appearance of the enamel surface associated with the increase in roughness and surface irregularities. These

findings were in agreement with Altınışık *et al.* [7] who observed "serious surface changes, such as the complete removal of the aprismatic layer and an increased depth of enamel irregularities".

On the other hand, nHAp played an essential role in preserving the enamel structure during the bleaching treatment. nHAp is an alkaline salt that reduces the acidity of HP solution by increasing its pH from approximately 3.2 to 5.4 [9]. In addition, nHAp particles may adhere evenly to the enamel surface, creating a protective coating for underlying enamel that reduces direct contact between HP and the enamel [19]. In addition to the fact that nHAp can help to remineralize tooth surface by delivering Ca and P to the area of demineralized enamel [35]. The Ca and P supplementation of bleaching products aims to form a supersaturated gel with Ca and P ions, preventing their dissolution from hydroxyapatite [4]. All these effects of nHAp might significantly minimize enamel demineralization induced by HP and thereby reduce microhardness loss. This came in agreement with the results of many studies [15, 19, 26]. Results of SEM examination agreed with those of microhardness values in group HP-nHAp which showed the enamel surface with no morphological changes and the enamel surface preserving a sound enamel structure. In fact, it has been reported that the nHAp plays a crucial role during bleaching treatment for maintaining enamel structure [9]. nHAp particles multiply and aggregate to create microclusters, leading to a uniform apatite coating that can completely cover prismatic and inter-prismatic enamel. Thus, nHAp restores the altered enamel morphology by maintaining the crystallinity of the enamel [36]. This finding is consistent with Monterubbianesi *et al.* [19] who affirmed: "that the presence of nHAp prevents alterations of the enamel structure". The absence of using saliva as a storage medium could be a limitation of this study, since saliva plays an important role in protecting the substrate from excessive mineral loss, allowing enamel remineralization.

5. Conclusion

From this in vitro study, it can be concluded that the incorporation of nHAp in an HP bleaching agent could significantly minimize the microhardness loss of enamel caused by 40% HP alone and keep the enamel surface morphology unchanged without affecting bleaching efficacy. nHAp is a promising revolutionary material to minimize the undesirable effects associated with tooth bleaching and does not interfere with the whitening performance of the bleaching agent. In addition to its effectiveness, nHAp is considered a biologically safe material making it desirable to be used in various fields of dentistry.

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