

Effect of Application of Two Different Bleaching Agents on Human Enamel In vitro

Abdelrahman Eid¹, HanaaM. Aly², Sonia M. El- Shabrawy³, Sherif M. Hayati⁴

¹ B.D.S, MSc of Oral Biology, Faculty of Dentistry, Alexandria University, Egypt

² Professor of Oral Biology, Faculty of Dentistry, Alexandria University

³ Professor of Dental Biomaterial, Faculty of Dentistry, Alexandria University

⁴ Lecturer of Oral Biology, Faculty of Dentistry, Alexandria University

Abstract: ***Objective:** This study was done to investigate the effects of application of two different bleaching techniques and agents on enamel hardness and surface morphology. **Material and methods:** twenty six intact human first premolars extracted for orthodontic reasons were used in this split-tooth in vitro study. The specimens were randomly divided into two equal groups and sectioned buccolingually. Half of each crown was treated and the other half served as its control. For Group 1 (G1), an in-office bleaching technique was used. The specimens were treated with UV light activated H₂O₂ 25% (zoom whitening system) according to manufacturer's instructions. For Group 2 (G2), an at-home bleaching protocol was used with 20% carbamide peroxide 4 hours daily for 14 days. The effects of bleaching agents on enamel were evaluated using surface roughness analysis, microhardness test (MHT) and scanning electron microscopy (SEM). **Results:** This study revealed that bleaching resulted in significant decrease in microhardness of enamel surface. SEM investigation demonstrated that the bleaching agents affected enamel surface morphology producing erosion of outer enamel layer. These findings were pronounced in in-office bleaching more than in at-home bleaching. **Conclusion:** The high concentration of bleaching agents could affect intensively enamel hardness and alter surface morphology.*

Keywords: Bleaching, Hydrogen peroxide, Carbamide peroxide, Enamel

1. Introduction

Many types of color problems may affect the appearance of teeth, and the causes of these problems vary, as does the speed with which they may be removed. Therefore, the causes of tooth staining must be carefully assessed for better prediction of the rate and the degree to which bleaching will improve tooth color, since some stains are more responsive to the bleaching than others.^(1,2)

Extrinsic stains usually result from the accumulation of chromatogenic substances on the external tooth surface. Extrinsic color changes may occur due to poor oral hygiene, ingestion of chromatogenic food and drinks, and tobacco use. These stains are localized mainly in the pellicle and are either generated by the reaction between sugars and amino acids or acquired from the retention of exogenous chromophores in the pellicle.⁽³⁾

Intrinsic stains are usually caused by deeper internal stains or enamel defects. They are caused by aging, ingestion of chromatogenic food and drinks, tobacco usage, enamel microcracks, tetracycline medication, excessive fluoride ingestion, severe jaundice in infancy, porphyria, dental caries, restorations, and the thinning of the enamel layer. Aging is a common cause of discoloration.⁽⁴⁾

Tooth stains caused by aging, genetics, smoking, or coffee are the fastest to respond to bleaching: Yellowish aging stains respond quickly to bleaching in most cases⁽⁵⁾, whereas blue-gray tetracycline stains are the slowest to respond to bleaching⁽⁶⁾, while teeth with brown fluorescence are moderately responsive.⁽⁷⁾

The mechanism of bleaching by hydrogen peroxide is not well-understood. In-office and home bleaching gels contain hydrogen peroxide or its precursor, carbamide peroxide, as the active ingredient in concentrations ranging from 3% to 40% of hydrogen peroxide equivalent.⁽⁸⁾

There are three fundamental approaches for bleaching vital teeth: in-office or power bleaching, at-home or dentist-supervised night-guard bleaching, and bleaching with over-the-counter (OTC) products.⁽⁹⁾

First, in-office bleaching utilizes a high concentration of tooth-whitening agents (25–40% hydrogen peroxide). Here, the dentist has complete control throughout the procedure and has the ability to stop it when the desired shade/effect is achieved. In this procedure, the whitening gel is applied to the teeth after protection of the soft tissues by rubber dam or alternatives⁽¹⁰⁾, and the peroxide will further be activated (or not) by heat or light for around one hour in the dental office.⁽¹¹⁾

Second, at-home or dentist-supervised night-guard bleaching basically involves the use of a low concentration of whitening agent (10–20% carbamide peroxide, which equals 3.5–6.5% hydrogen peroxide). In general, it is recommended that the 10% carbamide peroxide be used 8 h per day, and the 15–20% carbamide peroxide 3–4 h per day. This treatment is carried out by the patients themselves, but it should be supervised by dentists during recall visits. The bleaching gel is applied to the teeth through a custom-fabricated mouth guard worn at night for at least 2 weeks. This technique has been used for many decades and is probably the most widely used.⁽¹²⁾

Finally, over-the-counter (OTC) bleaching products have increased in popularity in recent years. These products are composed of a low concentration of whitening agent (3–6%hydrogen peroxide) and are self-applied to the teeth via gum shields, strips, or paint-on product formats. They are also available as whitening dentifrices, pre-fabricated trays, whitening strips, and toothpastes.⁽¹³⁾

There is still controversy over the effects of dental bleaching on the physical properties of enamel. Many studies in the literature have investigated the effects of bleaching on enamel morphology and the surface texture morphological alteration of the enamel surface – increased porosity of the superficial enamel structure, demineralization and decreased protein concentration, organic matrix degradation, modification in the calcium:phosphate ratio, and calcium loss– thereby supporting the hypothesis that bleaching agents are chemically active components potentially able to induce substantial structural alterations in human dental enamel.^(14–18)

Some studies have reported that bleaching did not significantly affect the enamel surface.^(16,19) However, other investigations demonstrated morphological alterations in the bleached enamel surface: depressions, porosity, and increased depth of enamel grooves.^(14,15,20)

Enamel surface hardness and wear resistance after dental bleaching have also been investigated in the literature. Some studies^(21,22) showed no effects, while others^(23,24) showed significant decreases in hardness and fracture resistance.

2. Materials and Methods

Twenty six intact human first premolars extracted for orthodontic reasons were used in this study. Selected teeth were free from any clinical evidence of demineralization lesions, visible structural defects on enamel and restorations on surface. They were kept in distilled water (freshly prepared in lab of Khalil pharmacy, Alexandria, Egypt) until the beginning of study. The teeth were ultrasonically cleaned in distilled water for the removal of any tissue remnants and other debris, and then will be polished using polishing brushes with fine polishing paste in order to remove all organic and inorganic materials adherent to the crown surfaces.

The specimens collected were randomly divided into two equal groups (13 in each group) according to the bleaching techniques. Before the bleaching, the specimens in each group were sectioned buccolingually. Half of each crown were treated while the other half served as its control. Then, the specimens of the two studied groups were arranged in two sheets. (split-tooth in vitro study) (Figure 1)



Figure 1: Test halves (three blue marks) and control halves (single blue mark).

Group 1 (G1) in-office bleaching:

The specimens were exposed to UV light activated H₂O₂ 25% (zoom whitening system, Discus Dental, LLC, Los Angeles, USA) for 4 sessions – 15 minutes each. The bleaching gel was applied on the buccal surfaces of test specimens and then the time of the session was adjusted before application of UV light.

Group 2 (G2) at-home bleaching:

The specimens were treated with 20% carbamide peroxide 20% carbamide peroxide (opalescence, ultradent, South Jordan, UT, USA) 4 hours daily for 14 days. The bleaching gel was applied on the buccal surfaces of test specimens. At the end of session, the gel was removed by a clean cotton piece and distilled water. The specimens of the two studied groups were stored in artificial saliva (freshly prepared in lab of Khalil pharmacy, Alexandria, Egypt) at 37° C in a dark environment during the treatment period.

Measuring surface microhardness of enamel

The microhardness were measured through the use of Vickers Hardness Testing Machine (Model LM-100, Leco corporation, Michigan, USA). The middle third of buccal surface of each specimen was undergone three hardness tests with a load of 50 g for 15 seconds. Then, the average of the three values was recorded and used as the microhardness of each sample.

Scanning electron microscopic examination

The specimens were washed with distilled water to remove any adherents or deposits and immediately fixed in glutaraldehyde formaldehyde fixative. They were dehydrated in ascending grades of alcohol. Twenty minutes immersion in each solution were performed at concentrations of 30%, 50%, 70%. Critical point were dried in liquid CO₂, glued to copper stubs. (Figure 2)

They were sputter-coated with gold in a fine coat (Joel JF 1100 E ion sputtering device) and then examined under SEM at 25 kV and 10⁻⁶ A° beam current (Jeol JSM-5300, Tokyo- Japan). All specimens were observed at magnification (x 200).



Figure 2: The specimens were glued to copper stubs.

3. Results

Surface Microhardness

The results of surface microhardness test showed that the values were lower in the test subgroup than the control subgroup in both in-office and home bleaching groups. The difference was statistically significant. (Table 1 Figure 3). The comparison between the difference in surface microhardness of both in-office and home bleaching was statistically significant. (Table 2 Figure 4)

Table 1: Comparison between the control and test subgroups in each group according to surface microhardness

Surface microhardness	In-office bleaching		At-home bleaching	
	Control (n= 10)	Test (n= 10)	Control (n= 10)	Test (n= 10)
Min. – Max.	209.0 – 241.33	102.33 – 144.67	161.67 – 203.33	154.83 – 168.83
Mean ± SD.	223.23 ± 11.39	130.66 ± 11.44	185.07 ± 13.49	162.35 ± 5.63
Median	219.83	134.28	186.0	163.42
P	<0.001*		<0.001*	

p: p value for Paired t-test for comparing between control and test in each group.

*: Statistically significant at $p \leq 0.05$.

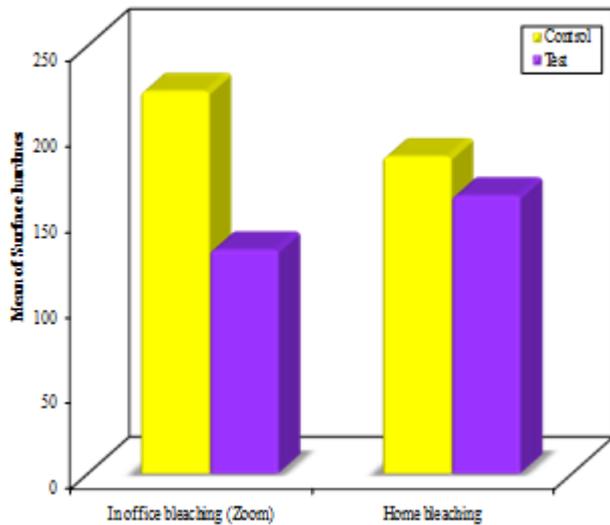


Figure 3: comparison between control and test subgroup in each group

Table 2: Comparison between the two studied groups according to the changes in surface microhardness after bleaching

The change in surface microhardness	In-office bleaching (n = 10)	At-home bleaching (n = 10)	P
Min. – Max.	-113.67 - -74.0	-39.96 - -6.0	<0.001*
Mean ± SD.	-92.58 ± 13.10	-22.71 ± 13.17	
Median	-90.72	-22.67	

p: p value for Mann Whitney test for comparing between in-office and at-home bleaching.

*: Statistically significant at $p \leq 0.05$.

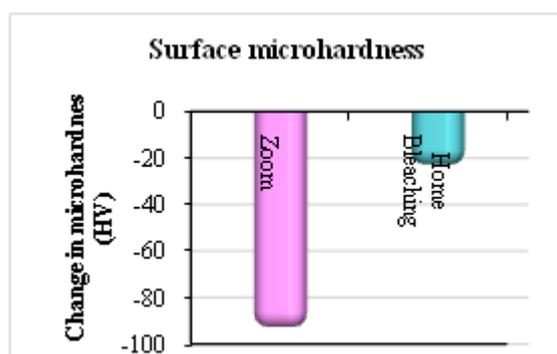


Figure 4: Comparison between the two studied groups according to the change in surface microhardness after bleaching.

Scanning Electron Microscopic Examination

- **Control subgroups:** The SEM images of the enamel surface showed the perikaymata in their normal appearance, in which they showed shallow furrows run in circumferentially horizontal lines across the surface. (Figure 5)
- **Test subgroup of in-office bleaching:** The SEM images of the test surface showed erosion of enamel surface which was characterized by areas of depressions. The normal appearance of perikaymata was affected. (Figure 6)
- **Test subgroup of at-home bleaching:** The SEM images of the enamel surface of the home bleaching specimens demonstrated that the perikaymata were similar to those of control surfaces. However, the test surfaces revealed slight enamel erosion. (Figure 7)

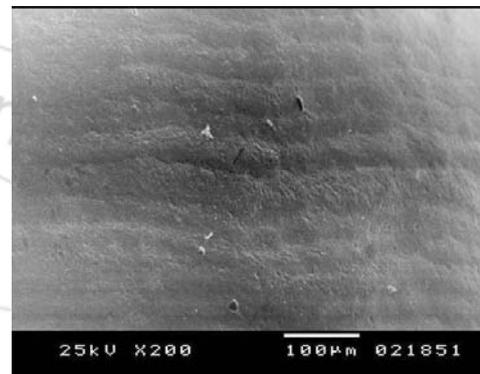


Figure 5: SEM photomicrographs: of control subgroups: normal wavy surface of enamel and the perikaymata in their normal appearance(x200 magnification)

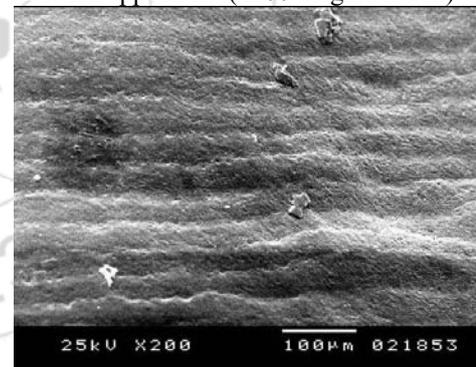


Figure 6: SEM photomicrographs of test subgroup of in-office bleaching: Areas of depressions (Arrows) and eroded enamel surface (x200magnification)

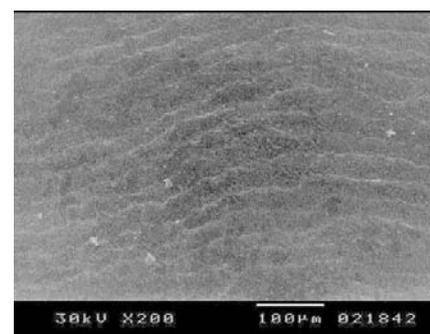


Figure 7: SEM photomicrographs of test subgroup of at-home bleaching: More flattened surface, indicating slight uniform erosion of enamel surface.(x200magnification)

4. Discussion

Teeth whitening or bleaching has gained popularity in recent years as an easy, affordable, and conservative way of treating discolored teeth.⁽⁹⁾ However, a great deal of controversy is reported concerning the short and long term effects of bleaching on dental hard tissue, particularly dental enamel.

The reason for the lack of consensus concerning the effects of bleaching on enamel may be due to a variety of factors; such as the use of non-standardized protocols in different studies, the origin of the enamel samples examined (human or nonhuman, erupted or extracted teeth, and their differing ages), the immediate remineralizing effect of saliva after removal of bleaching agent, and the pHs of the products and some foods, which alter the morphology of the enamel surface leading to alteration in their physical and chemical properties.⁽²⁵⁾

In this split-tooth in vitro study, specimens in each group were sectioned buccolingually. Half of each crown was treated and the other half served as its control. So, the net effects of bleaching (test surfaces versus control surfaces) were compared, giving more reliable results and better evaluation.

In general, the studies in the literature that do show an effect on enamel surface have some limitations in vitro methods used which do not reflect the in vivo situation.⁽²⁶⁾ It was suggested that the mineral content of the saliva might act a remineralizing agent for enamel. In order to simulate oral condition, the specimens were stored in artificial saliva during active period of treatment.^(27,28)

Furthermore, quantitative and qualitative examinations were used in this study. Regarding enamel hardness, the middle third of the buccal surfaces was only examined as the occlusal third is vulnerable to wear due to stresses and the cervical third is a retentive area to bacterial and food accumulation. Then, their values were statistically analyzed. On the other hand, microscopic pictures that give good idea about enamel surface morphology could be obtained using SEM.

The present study revealed that bleaching resulted in significant decrease in microhardness of enamel surface and alteration in enamel surface morphology. The key factors that affect the efficacy of bleaching agents are concentration and time. In general, higher concentrations are faster than lower concentrations in their effects and lower concentration can approach the efficacy of higher concentration with extended treatment time.⁽²⁹⁾

One of the best methods to study the enamel surface is SEM. Dudea et al. (2008)⁽³⁰⁾ examined by SEM the enamel surface after a regimen of repeated application of 15% CP bleaching agent for 14 days (which simulates the usual at-home bleaching). They found areas of depressions on the surface of enamel which indicated an increase in the enamel porosity. These findings are similar to our results.

In contrast, Cadenaro et al. (2010)⁽¹⁶⁾ conducted an in vivo study to test the effect of a hydrogen peroxide in-office whitening agent on enamel. Results demonstrated that the application of a 38% hydrogen peroxide in-office whitening agent did not change enamel surface roughness, even after multiple applications. This may be attributed to the protective effects of saliva, which provided dilution, buffering capacity, and a supply of Ca and P ions for tooth remineralization.

Concerning microhardness, the results of this study are in agreement with Azer et al. (2009)⁽²³⁾ who examined the nanohardness of human enamel after treatment with tray and strip bleaching systems. They exposed human enamel samples to five different bleaching agents. Their results showed that the nanohardness of human enamel were significantly decreased after the application of home-bleaching systems.

Contrary to our results, Araujo et al. (2010)⁽²¹⁾ investigated the effects of various light sources on the microhardness of human dental enamel following treatment with an in-office vital bleaching agent (35% hydrogen peroxide) using enamel slabs subjected to hardness testing after four time periods (baseline and after 1, 7 and 14 days). Results indicated that the different light sources tested did not significantly affect the microhardness of human enamel following treatment with 35% hydrogen peroxide.

There is evidence indicating that the reduced enamel translucency caused by bleaching agents is due to the oxidation of the organic matter, but not to any decrease in either organic or mineral contents.⁽³¹⁾ Considering that the amount of organic matter in enamel is much lower than that of dentine, it is not possible to explain the higher contribution of enamel in tooth bleaching compared to dentine's contribution when only the oxidation of organic matter is considered. Probably the mineral component, which is the most abundant in enamel, plays a role in tooth bleaching.⁽³²⁾

Some studies reported demineralization of enamel associated with tooth bleaching.⁽³³⁻³⁶⁾ This is consistent with the fact that hydrogen peroxide, even at neutral pH, breaks down producing hydrogen ions that might cause demineralization in enamel.⁽²⁰⁾

In summary, the present study included the lack of follow-up related to the mineral content of enamel for extended periods of time after the bleaching. Therefore, further studies are needed to evaluate the ultrastructural effects of different bleaching agents on dental hard tissues after extended periods of time. It would also be interesting to evaluate whether tooth remineralization would have any effect on bleached enamel.

5. Conclusions

Within the limitations of the present study the following conclusions were reached:

- 1) The high concentration of bleaching agents could affect intensively enamel hardness particularly in superficial layers.
- 2) SEM investigation demonstrated that the bleaching agents affected enamel surface morphology producing erosion of outer rodless layer, areas of depressions, and

exposure of enamel rod ends. These findings were pronounced in in-office bleaching more than in at-home bleaching.

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Author Profile



Abdelrahman Eid, received the MSc of Oral Biology 2016 and the B.D.S in Dental and Oral Surgery 2009 from Faculty of Dentistry, Alexandria University, Egypt. During 2011-2014, he worked as a demonstrator in Faculty of Dentistry, Pharos University, Egypt

