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EFFECT OF VARIOUS BLEACHING METHODS ON COLOR CHANGE AND SURFACE ROUGHNESS OF HUMAN ENAMEL

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ABSTRACT

Purpose: This study investigated the effect of different bleaching methods on color change and surface roughness of human enamel. **Materials &Methods:** Fifty recently extracted caries free human upper anterior (central) teeth were collected, prepared and stained in a staining solution. Samples were divided randomly into 5 groups GP (n=10) according to bleaching methods; GP1: 3D Crest White Strips, GP2: Phillips Zoom Whitening Pen, GP3: Rembrandt Deeply White + Peroxide Mouthwash, GP4: Opalescence PF 15% CP, GP5: Opalescence Boost 40% HP (as control group). Bleaching was performed according to manufacturers' instructions. Color change and enamel surface roughness measurements were performed by spectrophotometer and Atomic Force Microscope respectively at pre and post-bleaching intervals. Data were collected and statistically analyzed using ANOVA followed by Tukey's HSD Post Hoc test ($p \leq 0.05$). **Results:** For color changes; mean ΔL^* values were: 3.92(0.23), 2.44(0.15), 2.5(0.2), 2.6(0.17) and 4.1(0.25) for GP1, GP2, GP3, GP4, and GP5 respectively, Δa^* means were: 0.6(0.04), 0.1(0.01), 0.15(0.01), 0.2(0.03) and 0.79(0.05) for GP1, GP2, GP3, GP4, and GP5 respectively, while means for Δb^* were: 1.18(0.03), 0.33(0.01), 0.52(0.02), 0.54(0.01) and 1.27(0.07) for GP1, GP2, GP3, GP4, and GP5 respectively. The color change values (ΔE) were: 4.13, 2.46, 2.55, 2.66 and 4.36 for GP1, GP2, GP3, GP4, and GP5 respectively. A statistical significant difference ($p \leq 0.05$) is present between Gp1 versus Gp2, Gp3 and Gp4, also between Gp5 versus Gp2, Gp3 and Gp4. No statistical significant difference ($p \leq 0.05$) between Gp1 & Gp5 and also between Gp2, Gp3 and Gp4. For surface roughness; mean (Ra) values for pre-bleaching tested groups were: 106.3(8.5), 109.1(10.2), 108(12.2), 110(11.9) and 107(9.7) for GP1, GP2, GP3, GP4, and GP5 respectively. While, post-bleaching values were: 90.3(6.3), 92.3(5.6), 93.6(5.4), 94.7(6.1) and 93.1(3.6) for GP1, GP2, GP3, GP4, and GP5 respectively. However, none of the tested methods showed statistically significant changes in surface roughness ($p \leq 0.05$). **Conclusions:** In-office bleaching may still achieve best whitening effect compared to whitening pen, whitening mouthwash, and home bleaching, while Whitestrips could have a comparable effect. All tested bleaching methods had no effect on enamel surface roughness.

KEYWORDS: Bleaching methods, Color change, Surface roughness, Teeth whitening.

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INTRODUCTION

Public esthetic demands are growing dramatically in recent years. Attractive smile has become the sake of the great majority of population rather than only meeting the basic dental needs.[1] One important component of an attractive smile is the color of anterior teeth. Dental treatment usually attains higher patient appreciation when accompanied by teeth whitening procedures. This can explain why the most popular esthetic procedure is bleaching.[2] Bleaching or whitening of teeth was first described in 1877.[3]

Tooth whitening is usually done through different methods with different mechanism of action. Tooth discoloration is a crucial factor in determining the efficiency of such different methods for tooth whitening. Tooth discoloration may be extrinsic or intrinsic. Extrinsic staining is caused by environmental factors such as stains in foods and beverages, smoking, metals such as iron or copper, and antibiotics. Intrinsic staining may be as a result of factors such as antibiotics, age (aging enamel wear which leads to yellowish color of dentin), genetics, increased fluoride levels, and developmental disorders. Tooth whitening is any process that lightens the color of a tooth. It could be attained by stain removal physically or lighten the color of the tooth by chemically. Chemical degradation of the chromogens is defined as bleaching. Hydrogen peroxide (HP) is considered the active ingredient in most whitening products; it is presented as HP or carbamide peroxide (CP), which is a stable complex that releases HP in contact with water.[4-8]

Different types of bleaching methods are present nowadays; in-office bleaching, home bleaching and Over the Counter (OTC) bleaching like, whitening strips, whitening pen, whitening mouth wash and home bleaching. In-office bleaching, which is performed by a dentist in the dental office, is the treatment of choice if rapid result is desired as HP is used with high concentration reaching (30-35%).[9]

Home bleaching can be performed either under dentist supervision or individually. It usually utilizes 10% CP gel as bleaching agent which is loaded in bleaching trays.[10]

Over the last decades, different types of OTC bleaching products have become available in the market. These can be purchased from pharmacies, online stores and markets without a prescription. They represent a cheaper substitute if patients cannot afford the cost of in-office bleaching. They are available in different formulas and each has a particular technique of application. The main advantages of OTC products are reduction of chair time, lower sensitivity and gingival irritation, but it is limited to removal of external stains.[11][12]

Measuring color shade of teeth is usually performed by different tools like visual inspection, digital camera and Spectrophotometer. Spectrophotometer is divided into two systems: contact and non-contact mode which both have relatively equivalent accuracy.[13-15]

Alteration of enamel surface is one of the drawbacks of bleaching, as the bleaching agent can affect morphological, roughness and mechanical properties of enamel surface.[16] There are many different methods reported in the literature for measuring morphological, roughness or mechanical changes of the enamel. Some of these methods are, Profilometric Analysis like Atomic Force Microscope (AFM), Computerized Roughness Tester, Scanning Electron Microscope SEM, and Knoop Microhardness (KHN).[1-2,13,17-24]

New products are continuously being introduced to the market for whitening purposes which makes it more difficult for clinicians and patients to choose among them and to know which is more effective and long lasting.[10]Up to date, sufficient scientific evidences concerning new products like whitening strips and whitening pen are scarce. Therefore, the aim of this in-vitro study was to investigate the effect of whitening strips, whitening pen, bleaching mouth wash and home bleaching on color change

and surface roughness and compare them to in-office bleaching. The hypothesis of this study was that whitening strips, whitening pen, bleaching mouth wash and home bleaching produce equivalent whitening effect and surface roughness changes to in-office bleaching.

MATERIALS AND METHODS

Specimens' preparation:

A total of 50 recently extracted caries free human upper anterior (central) teeth were collected. Restored teeth or teeth with gross surface defects were excluded. All teeth were inspected for closed apices to verify equal enamel maturation.[3] They were preserved in distilled water all time of experiment to avoid dehydration. Roots were sectioned using high speed diamond bur with copious water irrigation at level of CEJ. Cinguli were slightly prepared into flatter surfaces with high speed diamond bur in order to mount the teeth with the labial surface as flat as possible. Small pieces of utility wax were placed below specimens (at cingulum) to stabilize the flat position. Then, transparent self-cure acrylic resin was poured all around the teeth in silicon molds to match the size of measuring slots of spectrophotometer. All exposed dentinal tubules in CEJ and cinguli areas were sealed with acrylic. After that, acrylic molds were placed in pressure pots for 10 minutes to obtain smooth and free of irregularities in external surface. Afterwards, molds were painted with transparent nail varnish to avoid stain uptake when placed in staining solution. Finally, each specimen was labelled with a number from 1-50.

Staining procedures

All specimens were immersed in tea solution. This solution was prepared by boiling 3.5 gm in 100 ml of distilled water for 10-12 min. It was left in room temperature for 30 minutes to cool down. Subsequently, specimens were placed in it and transferred to the incubator at 44°C for 14 days and the solution was renewed every day.[11]

Assessment of color changes:

Each specimen color was assessed pre and post-bleaching at the center of each specimen by Spectrophotometer. Spectrophotometer operates by using CIELAB system defined by International Commission on Illumination; where L* axis represents the value (lightness or darkness) which ranges from black (0) to white (100), a* axis expresses redness (+ a*) or greenness (- a*), and b* axis expresses yellowness (+ b*) or blueness (- b*). Values for L*, a* and b* were recorded for each specimen prior to application of the bleaching agent (pre-bleaching) and at the end of the bleaching process (post-bleaching). The difference between L*, a* and b* at pre-bleaching and post-bleaching were expressed as ΔL^*; Δa^* and Δb^*.[3] The total color difference (ΔE) was calculated by the following equation: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} = [(L_1-L_0)^2 + (a_1-a_0)^2 + (b_1-b_0)^2]^{1/2}$, where the subscripts $_0$ and $_1$ denote pre-bleaching and post-bleaching values, respectively.[10,11,15,25,26,27]

Assessment of surface roughness:

AFM was used for measurement of surface roughness in contact mode for 2 times (pre and post-bleaching). One area at the center of specimen was scanned with an area of 10 X 10 micrometers and resolution of 514 X 514 pixels to obtain surface roughness average (Ra) values. Ra analysis was done using Nano Scop Analysis software. Three dimensional (3D) Images were obtained from height and deflection signals by Nano Drive software.[23]

Bleaching procedures

After pre bleaching measurements of color change and surface roughness, Samples were randomly divided into 5 groups GP (n=10). Each group was assigned to a bleaching method; GP1: 3D Crest White Strips, GP2: Phillips Zoom Whitening Pen, GP3: Rembrandt Deeply White + Peroxide Mouthwash, GP4: Opalescence PF 15% CP, GP5: Opalescence Boost 40% HP (as control group). Specimens were removed from distilled water and

TABLE (1) Technical specifications and bleaching procedures of bleaching agents tested in the study.

Material	Manufacturer	Active ingredient %	Duration	Frequency
3D Crest White Whitestrips Professional Effect (GP1)	Procter & Gamble, Cincinnati, OH, USA	6.5 % HP	30 min	Once daily for 20 days.
Phillips Zoom Whitening Pen (GP2)	Philips products	5.25 % HP	10 min	Twice daily for 14 days.
Rembrandt Deeply white + peroxide Mouthwash (GP3)	Johnson &Johnson Healthcare products, USA	Sodium fluoride 0.02% HP	1 min	Twice daily for 14 days.
Opalescence PF (Potassium nitrate & Fluoride ions) (GP4)	Ultradent Products, Inc., USA	15% CP	4-6 hours	Once for 14 days
Opalescence BOOST 40% HP (GP5)	Ultradent Products, Inc., USA	40% HP	45 min	Three applications in different 3 days.

left for 3 minutes to dry. Then bleaching agents were applied according to manufacturer instructions. At the end of each application, bleaching agents were wiped with wet gauze several times till all material washed away then stored in distilled water again till the following application of bleaching agent. Details of bleaching procedures, according to manufacturers' instructions are illustrated in table (1).

Statistical analysis

Data were collected and statistically analyzed using ANOVA followed by Tukey's HSD Post Hoc test to determine the effect of different bleaching methods on color change and on surface roughness of human maxillary central teeth separately. The level of significance was set at $p \leq 0.05$.

RESULTS

Color Change

The mean ΔL^* values were: 3.92(0.23), 2.44(0.15), 2.5(0.2), 2.6(0.17) and 4.1(0.25) for GP1, GP2, GP3, GP4, and GP5 respectively and Δa^* means were: 0.6(0.04), 0.1(0.01), 0.15(0.01), 0.2(0.03) and 0.79(0.05) for GP1, GP2, GP3, GP4, and GP5 respectively, while means for Δb^* were: 1.18(0.03), 0.33(0.01), 0.52(0.02), 0.54(0.01) and 1.27(0.07) for GP1, GP2, GP3, GP4, and GP5 respectively. The color change values (ΔE) were: 4.13, 2.46, 2.55, 2.66 and 4.36 for GP1, GP2, GP3, GP4, and GP5 respectively. The highest ΔE was recorded by GP5, while the lowest mean value was recorded by GP2. ANOVA followed by Tukey's HSD Post Hoc test, revealed a statistical significant difference ($p \leq 0.05$) between Gp1 versus Gp2, Gp3 and Gp4, also there was a statistical significance difference ($p \leq 0.05$) between Gp5 versus Gp2, Gp3 and Gp4. Moreover, there was no statistical significant difference ($p \leq 0.05$) between Gp1 & Gp5 and also between Gp2, Gp3 and Gp4. (Table 2) (Figure 1).

Surface roughness change

The mean (Ra) values for pre-bleaching tested groups were: 106.3(8.5), 109.1(10.2), 108(12.2), 110(11.9) and 107(9.7) for GP1, GP2, GP3, GP4, and GP5 respectively. While, post-bleaching values for tested groups were: 90.3(6.3), 92.3(5.6), 93.6(5.4), 94.7(6.1) and 93.1(3.6) for GP1, GP2, GP3, GP4, and GP5 respectively. ANOVA followed by Tukey's HSD Post Hoc test, revealed no statistical significant difference ($p \leq 0.05$) between all the tested groups. (Table 3)

Representative AFM 3D photomicrographs of enamel surface for different tested groups, both pre and post-bleaching, were shown in figures (2-6).

TABLE (2) Descriptive statistics and test of significance for color changes mean values and standard deviations (S.D.) of all tested groups.

Groups	ΔL*	S.D.	Δa*	S.D.	Δb*	S.D.	ΔE	P*
Gp 1	3.92	0.23	0.6	0.04	1.18	0.03	4.13	a
Gp 2	2.44	0.15	0.1	0.01	0.33	0.01	2.46	b
Gp 3	2.5	0.2	0.15	0.01	0.52	0.02	2.55	b
Gp 4	2.6	0.17	0.2	0.03	0.54	0.01	2.66	b
Gp 5	4.1	0.25	0.79	0.05	1.27	0.07	4.36	a

Means with the same letter within each column are not significantly different at P≤.05.

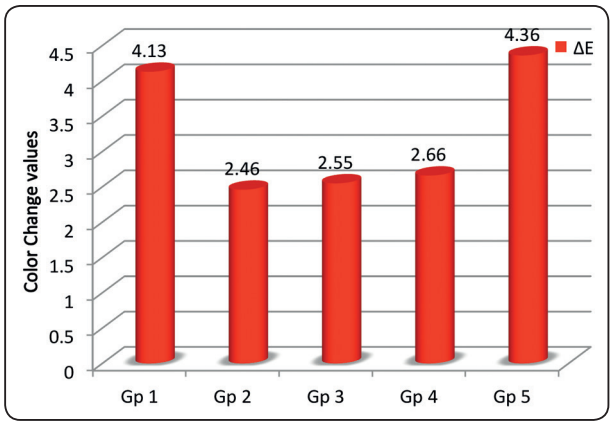

Fig. (1): Colour change values (ΔE) for the different tested groups.

TABLE (3) Descriptive statistics and test of significance for surface roughness (Ra) in nm mean values and standard deviations (S.D.) of all tested groups.

Groups	Mean (Ra) Pre-bleaching	S.D.	Mean (Ra) Post-bleaching	S.D.	Difference (Ra) values	P*
Gp 1	106.3 [a]	8.5	90.3 [a]	6.3	16	a
Gp 2	109.1 [a]	10.2	92.3 [a]	5.6	16.8	a
Gp 3	108 [a]	12.2	93.6 [a]	5.4	14.4	a
Gp 4	110 [a]	11.9	94.7 [a]	6.1	15.3	a
Gp 5	107 [a]	9.7	93.1 [a]	3.6	13.9	a

Means with the same letter within each column are not significantly different at P≤.05.

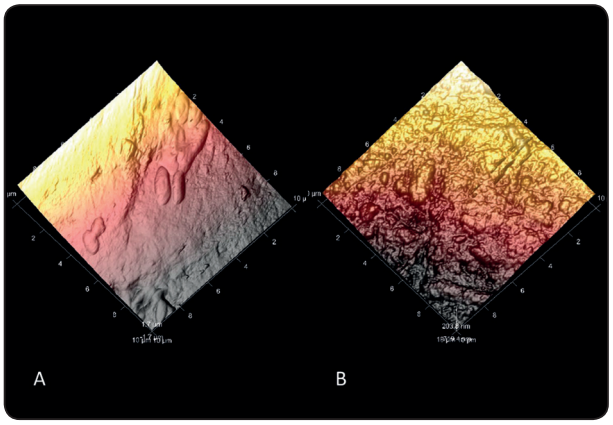

Fig. (2): AFM 3D photomicrographs of enamel surface pre-bleaching (A) and post-bleaching (B) for GP1

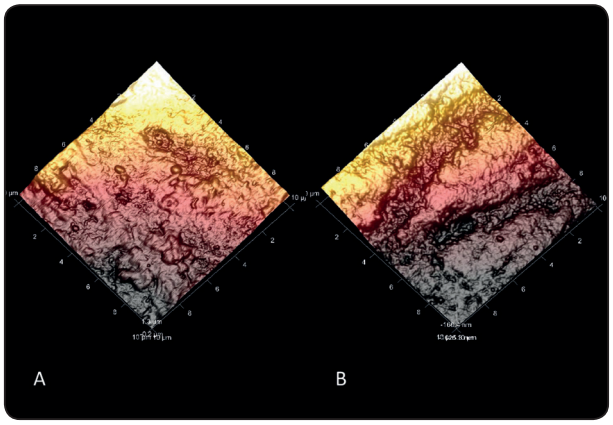

Fig. (3): AFM 3D photomicrographs of enamel surface pre-bleaching (A) and post-bleaching (B) for GP2

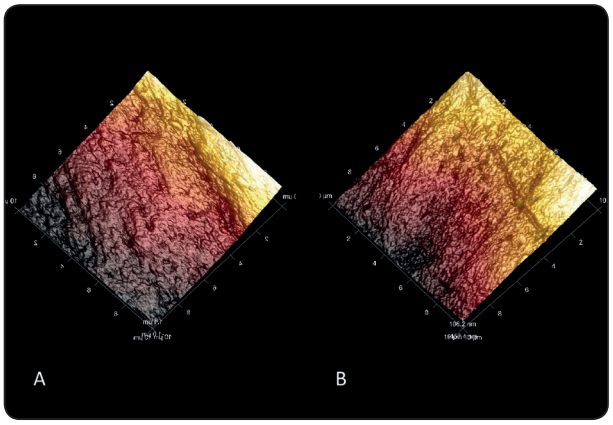

Fig. (4): AFM 3D photomicrographs of enamel surface pre-bleaching (A) and post-bleaching (B) for GP3

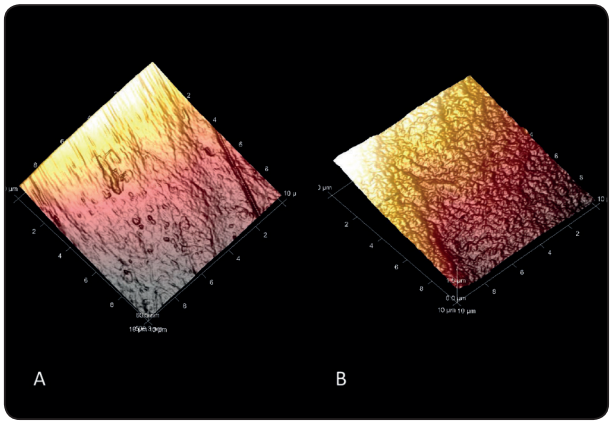

Fig. (5): AFM 3D photomicrographs of enamel surface pre-bleaching (A) and post-bleaching (B) for GP4

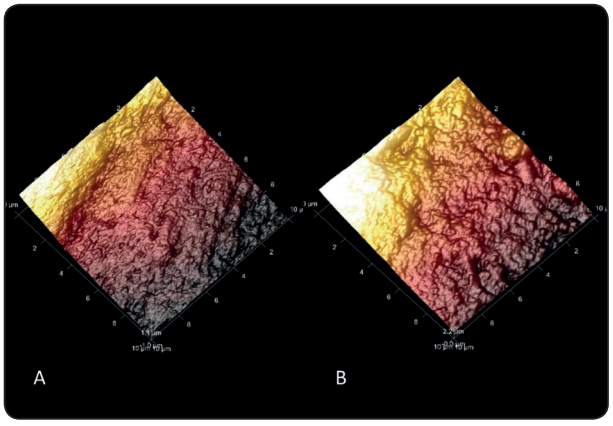

Fig. (6): AFM 3D photomicrographs of enamel surface pre-bleaching (A) and post-bleaching (B) for GP5

DISCUSSION

The aim of the present study was to investigate the effects of new OTC products; white strips, whitening pen, bleaching mouth wash and home bleaching on color change and surface roughness and compare them to in-office bleaching.

There are different ways of assessing color or shade of teeth. A very common and easy way to assess color of teeth is visual inspection accompanied by using shade guide.[15]Despite easiness and feasibility of this method, it has many limitations such as lack of consistency and reproducibility as it is a subjective method prone to variation from one observer to another.[15][28] Spectrophotometer

is an instrument that measures spectral reflectance from 360-740 nm at 10 nm intervals.[14] It solves previously mentioned issues since it evaluates shades quantitavely.[15] In other words, it is highly sensitive, and precise which make many studies use it as reference.[13] It is further divided into contact and non-contact systems. This study has used the contact system which has the privilege of being convenient and transportable.[15]On the other hand, spectrophotometer underestimates the color changes in comparison with digital imaging system in curved and translucent surface but has high accuracy in flat non translucent surface. Therefore, the labial surfaces of teeth were mounted as flat as possible to have more accurate readings with spectrophotometer in this study.[14][29]

Our hypothesis was accepted as one of tested OTC products (Crest 3D White Whitestrips) has comparable whitening effect to in-office bleaching. Whitening strips were first introduced in 2000. They are flexible plastic polyethylene strips coated with HP that conform to the top surface, around edges, and into cervices between teeth.[30] One clinical study conducted by Perry et al in 2013, supported same conclusion that strips result in equivalent whitening as in-office bleaching.[31] Another clinical trial confirmed the considerable color improvement of professional 6.5% HP white strips after 3 weeks of usage.[32] The present study demonstrated another important result in which Whitestrips produced significantly higher color change than home bleaching (CP 15%). Ferrari et al in 2007, had same result in which strips containing 6%HP has superior whitening effect to 10% CP although both exhibited efficient color improvement,[33] this might refer to the type of active agent, which is HP in Crest WhiteStrips. HP containing whitestrips may outweigh CP home bleaching tray not only in results but also in the duration of application which might extend to 8 hours per night for 6 weeks in home bleaching. This prolonged application might result in higher sensitivity and patient discomfort.[34]On the other hand, another study revealed that no significant difference in tooth color change between home bleaching with 35% CP in a tray and 14% hydrogen peroxide in whitestrips.[35] There are few factors might be responsible of this conflict like concentration of HP, mode of application, intimate contact to teeth, and duration of application.[33] A very good example of importance of factors variation is Xu et al study in 2011, as they compared two products of nearly equal peroxide concentrations but with different methods of application and the whitening outcome just was statistically different between the two methods.[36]

In 2001, paint-on bleaching gel has been launched to the market. It has a mode of application that is similar to a nail polish and it is a carrier-free.[37]Some manufacturers name this product as bleaching or whitening pen. Whitening pen is among the least tested bleaching methods in literature. Few studies reported using a carrier-free bleaching method that is similar to whitening pen method and designated by paint on gel. Our results indicated that the whitening pen used had significantly lower whitening effect in comparison to Whitestrip. A clinical trial comparing 6% HP Whitestrips and 5.9% HP paint on gel had a result in accordance with this result.[36] However, Karadas et al in 2015, reported no significant difference in whitening effect between 3D white strip and Dazzling White (paint on gel).[11]This contradiction might be caused by the diversity of manufacturer's instruction or duration of application of bleaching products. Karadas et al used Dazzling white (paint on gel) twice a day for 28 days of duration while duration of application for whitening pen in this study is exactly half that time.[11]

Mouthwashes are useful and common oral hygiene agents. There are many different manufactures of mouthwashes with variable active ingredients for teeth whitening. Whitening mouthwash is easiest and least demanding whitening method

to be performed at home. In contrast, it might not whiten shade of teeth significantly due to short time of application. Whitening mouthwash used in this study increased the lightness of teeth after bleaching and produced a positive ΔL*. Despite that increase in lightness, it ranked the last among the five groups in color change. That could be explained by the short duration of teeth contact in regard to the rest of groups which is 1 minute only.[11]In addition, two reviewed studies reported significant teeth color change resulted by different mouth washes.[4]

In Home bleaching, trays are placed in the mouth for approximately 2-8 hours per day for 2-6 weeks. Duration of daily application depends on CP concentration. In the present study, the use of 10% CP duration followed the manufacturer's guidelines once 4- 6hrs for 14 days, this may be the cause of the low color changes results in comparison to other groups as subsequent studies demonstrated that after the application of the product for 2 hrs, 50% of the active ingredient only is available and most treatments may be completed within 3 weeks.[34]

Various tools are used for studying surface roughness. Two tools among these are SEM and AFM. AFM use might be favoured as it provides numerical data (roughness average (Ra)) and qualitative data simultaneously compared to SEM which produces qualitative data only (electronic scans).[38] Qualitative data of AFM are represented by three-dimensional 3D morphological images at atomic level.[38] The most remarkable advantage of AFM that it lacks the need for specimen preparation.[38,39] The variations in different methods of surface roughness measurement might be one reason behind inconsistent conclusions drawn by the studies assessing enamel surface changes after bleaching. In other words, there is no solid agreement between these studies that bleaching changes enamel surface significantly. Some of reviewed studies stated no significant changes yielded by bleaching agents[21][18]or transient changes directly after bleaching only[1,2], but other reported significant alterations.[17,22,40] In addition, one important factor for this inconsistency is type of storage media of teeth after bleaching and how close the simulation is to oral environment. In other words, the closer intraoral conditions are simulated, the less reduction in enamel micro hardness due to mineralization action of saliva.[41] Although storage media for teeth was distilled water and not a remineralizing solution like artificial saliva in the current study, statistical analysis revealed no significant difference of all bleaching methods on surface roughness of enamel. The lack of bleaching effect on surface roughness in similar circumstances was explained by two studies.[18,20] Both studies relatively simulate this study in the absence of saliva in which Ozkan in-vitro study in 2013, used distilled water at all time of experiment and Cadenaro in-vivo study in 2008, implemented a rubber dam isolating the effect of saliva. They both suggested a common probable justification which is the neutral PH of the bleaching agent.[18,20]

CONCLUSIONS

Under the limitations of this study, it is safe to conclude that:

- In-office bleaching may still achieve best whitening effect compared to whitening pen, whitening mouthwash, and home bleaching.
- Whitestrips could have a promising result and could be an effective alternative to in-office bleaching.
- All tested bleaching methods had no effect on enamel surface roughness.

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The authors declare that they have no conflict of interest**.**

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