In-vitro Evaluation of Color Change and Surface Roughness of Human Enamel submitted to Different Bleaching Regimens

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Abstract: Objectives: The purpose of the present study was to evaluate the color change and surface roughness of human enamel treated with different bleaching materials and techniques after different storage periods. **Materials and methods:** 36 freshly sound human anterior teeth (shade A3 or darker) were extracted due to periodontal problems. The specimens were randomly divided into four groups (n = 9 samples per group) according to bleaching technique and desensitizer used. **GP I:** The specimens received Over-The Counter bleaching procedures followed by desensitizer application. **GP II:** The specimens received Over-The Counter bleaching procedures only. **GP III:** The specimens received In-Office bleaching treatment followed by using desensitizer. **GP IV:** Teeth received In-Office bleaching procedures only. The teeth color was measured using the same spectrophotometer and this was done along the evaluation periods at baseline (before any treatment), directly after bleaching and in 3 months intervals for a year postoperatively). Measurements of surface roughness were carried out using an optical interferometer (ZYGO). **Results:** Data regarding color change and surface roughness were analyzed using analysis of variance and Tukey's test at different evaluation periods (base line, after treatment, 3, 6, 9 and 12 month). One way repeated measure ANOVA test was revealed that no significant difference between all tested groups at any evaluation periods since P value > 0.05. In addition the effect of time was tested among each group and a highly statistically significant difference was shown within all groups where P values =0.000. In addition the effect of time was tested among each group and statistically analyzed using a Post hock (Tukey's test). A highly statistically significant difference was shown within all groups where P values =0.000. **Conclusion:** Bleaching techniques resulted in identical tooth whitening but promote superficial changes in enamel structure surface, so faster color regression was recorded.
[Ashraf M. Nassar, Hussein Y. Elsayed, Wedad M. Etman and Ali I. Abdalla. **In-vitro Evaluation of Color Change and Surface Roughness of Human Enamel submitted to Different Bleaching Regimens.** *N Y Sci J* 2018;11(7):36-50]. ISSN 1554-0200 (print); ISSN 2375-723X (online). http://www.sciencepub.net/newyork. 6. doi:10.7537/marsnys110718.06.

Keywords: in-office bleaching, over the counter bleaching, desensitizing agent, spectrophotometer, surface roughness.

1. Introduction

The widely known popular saying "The smile is our business card" must always be respected, highlights and considered, since there is scientific evidence evincing the smile as the most important element in the context of dentofacial esthetics[1]. Dental esthetics has a considerable importance to the general population and the social; also it has psychological impact on quality of life and may be one of the driving forces behind the current demand for noninvasive procedures to improve tooth color[2]. In addition tooth whitening procedure employed by professionals and patients is considered the least invasive way and the most cosmetic dental procedures requested by patients who want a more pleasing smile[3]. Vital tooth bleaching can be accomplished by a variety of methods and techniques, which can be generally categorized as In-office (professionally administered), At-home (professionally dispensed) or Over-the-counter (self-administered) [4]. In-office bleaching is a popular option available to patients desiring a whiter, more attractive smile, as outcomes can already be seen in a single clinical appointment with a dental professional[5]. While, At-home whitening include the application of low concentrations of whitening agent (10-20% carbamide peroxide) placed in a custom-made mouth guard and administered daily over a 2-6 week period (This should be supervised by a dental professional)[6].

Moreover, different over-the-counter products are available in supermarkets, drug stores or on the Internet, including mouthwashes, toothpastes, chewing gums, paint-on brushes, dental floss, and whitening strips without the need for a prescription or professional supervision[7-9]. Regression of tooth whitening is a phenomenon that was reported to occur following bleaching procedures[10]. It was thought that the initial whitening of the tooth color may be due to enamel dehydration[11].

Many researches denoting that, post-treatment sensitivity is usually related to small microscopic enamel defects and subsurface pores, which allow the whitening agent to penetrate into the dentinal tubules and ultimately the pulp, causing reversible pulpitis and

consequent teeth thermal sensitivity[12,13]. Using desensitizing agent containing a 5% potassium nitrate and 2% sodium fluoride was found to be useful to treat post-operative sensitivity following vital bleaching regimen[14]. It also does not interfere with the bleaching efficacy of in-office or at-home bleaching [15,16].

Many other alterations in dental tissue, such diminished enamel microhardness[17,18], surface roughness[19,20] and porosity have also been observed following the application of bleaching materials[21]. There exists a significant and positive correlation between surface roughness and bacterial adhesion[22]. Roughness is considered a predisposing factor for bacterial adhesion and extrinsic stain. It has been reported to play a distinguished function in biofilm development of oral bacteria[23]. The special effects of surface roughness on biofilm development can be demonstrated by the reality that a rough surface can act as a buffer against shear forces and can increase the area available for biofilm formation[24]. So, rough enamel surface encourages adhesion of Streptococcus mutans which is the major causative microorganism in the pathogenesis of dental caries as the subsistence of S. mutans in the oral environment be based on their ability to adhere to a tooth surface [25]. After bleaching procedures, coloring pigments may adhere to the rough bleached enamel surface with the micropores or superficial defects and lead to more discoloration than the original tooth color[26]. To overcome the adverse effects of bleaching procedures, enamel surface defects could be managed by saliva, artificial saliva, or remineralizing agents[27]. Therefore, the current study was performed to determine the effect of WHITEsmile. LIGHT WHITENING AC and Crest 3D White Luxe Supreme FlexFit Whitestrips bleaching procedures in changing surface roughness of human anterior teeth.

2. Materials and Methods:

2.1 Study setting:

This study was performed in the Restorative department laboratory, Faculty of Dentistry, Tanta University.

2.2 Study design:

It was conducted for a year.

2.3 Collection of Teeth:

36 freshly sound human anterior teeth shade A3 or darker (age range 18- 25 years) were extracted due to periodontal problems. These were collected from the Oral and Maxillofacial Surgery Department at the Faculty of Dentistry, Tanta University and were randomly distributed into four tested groups. The teeth were cleaned from residual periodontal fibers, debris and blood under running tap water by using sharp hand scaler (Prima-Dent, International, Frankfurt, Germany). They were cleaned with pumice and water and stored in 0.9% NaCl plus solution in a refrigerator at 4ºC in order to avoid dehydration, changed regularly until the experiment time which was scheduled within three months after extraction.

2.4 Patient's rights:

Approval for this study was obtained from Faculty of Dentistry, Tanta University Research Ethics Committee (REC). All steps and procedures of this research were informed in details to participants. The purpose of the present study was explained to the patients and informed consents were obtained to use their extracted teeth on the research according to the guidelines on human research published by the Research Ethics Committee at Faculty of Dentistry, Tanta University.

2.5 Specimen Preparation:

The root of each tooth was cut off (1mm) below Cemento Enamel Junction (CEJ) using a double sided diamond disc (Edental Golden S.A.W., Switzerland) mounted to low speed contra angled handpiece under water cooling system, then coronal pulpal tissue of the tested teeth was carefully extirpated using barbed nerve broaches (Dentsply/Maillefer, Ballaigues, Switzerland) and properly irrigated using copious amount of sodium hypochlorite solution (5.2%).

The crowns were embedded in an auto polymerizing resin cylinders (Acrostone-cold cure, Egypt). The experimental labial surfaces were left uncovered by acrylic resin, and were cleaned and polished (Ultrapro® Prophy PASTE, Ultradent Dental, Medizinische Geräte, GmbH, Germany). Then the specimens were stored in artificial saliva specifically formulated for the re-mineralization of the dental hard tissues (Nacl 0.381(g), Kcl 1.114(g), $Cacl_2$ 0.231(g), KH_2po_4 0.738(g), NaN_3 2.2(g), Gastro Mucin 2.2(g), Deionized Water 1000(g)). The storage was done within light proof container at an incubator at 37±1°C through the time of experiment for one year. The storage media (artificial saliva) was changed daily to avoid bacterial growth. Then silk adhesive tape (CALDENOR-S, Alexandria Co. for pharmaceuticals, Alexandria, Egypt) was placed on the labial surfaces of the samples, and a square shape window 2×2mm was cut off by mean of scalpel blade at the middle part of the exposed facial surface of the specimens. The window was made by using a metallic device with well-formed borders at a radius of 2 mm. This perforation was compatible with the size of the spectrophotometer tip.

2.6 Grouping system:

Specimens were divided into four groups according to bleaching materials and/or desensitizer used as shown in table 1.

Table 1: Grouping system

Groups	Treatment	Central incisor	Lateral incisor	Canine
GP I	The specimens received Over-The Counter bleaching procedures followed by desensitizer application.	**3**	**3**	**3**
GP II	The specimens received Over-The Counter bleaching procedures only.	**3**	**3**	**3**
GP III	The specimens received In-Office bleaching treatment followed by using desensitizer.	**3**	**3**	**3**
GP IV	Teeth received In-Office bleaching procedures only.	**3**	**3**	**3**

2.7 Colorimetric Evaluation (Measurements):

The teeth color was measured using a contact-type intra-oral spectrophotometer (Vita Easy shade V, VITA Zahnfabrik, Bad Säckingen Germany, S\N:H50000 V503i) (Figure IV-4) based on the CIE L*a*b* color space system. This system was defined by the International Commission on Illumination in 1967 and is referred to as CIELab (Commission Internationale de L'Eclairage, 1978).

- The (L*) represents the value (lightness or darkness) of an object,
- The (a*) value is a measure of red (positive a*) or green (negative a*),
- The (b*) value is a measure of yellow (positive b*) or blue (negative b*).

Total color differences or distances between two colors (ΔE) was calculated using the formula: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

The color shade match was performed in standardized conditions using the same light source, the same time of day and spectrophotometer along the evaluation periods at baseline, before any treatment, directly after bleaching and in 3 months intervals for a year postoperatively. These measurements were taken two times consecutively. When these values were equal, they were registered. When the values were not equal, additional measurements were taken until equal measurements were obtained, and only one measurement for each tooth was recorded.

2.8 3D Optical Surface Profile Measurements:

The samples were washed with distilled water for 5 minutes, shaking them gently and left to dry for 48 hours. The surface roughness metrology of the samples of each group was examined using an optical interferometer (ZYGO) as the average of four measurements. Mean surface roughness values (Ra) were calculated for each specimen. (Ra) describes the arithmetic mean of all values of the roughness profile (R) over the evaluated length. Three measurements were performed on surface of each sample at the same evaluation period in different directions with a distance of 0.5 mm between them

The 3D surface profile measurements were repeated close to the initial measurements points for the same periods of evaluation as done for the colorimetric evaluation.

2.9 Bleaching procedures:

Gp I: 5.3% hydrogen peroxide thin, flexible disposable polyethylene strips (Crest 3D White Luxe Supreme Flex Fit White, WHITEsmile, GmbH, Birkenau, Germany) were applied on labial surface of anterior teeth for one hour per day for one week. Then a desensitizing gel (WHITEsmile After Whitening Mousse, WHITEsmile, GmbH, Birkenau, Germany) was applied and was left undisturbed for 10 minutes. **Gp II:** the same protocol of gp I was followed but without desensitizer usage. **Gp III:** The 32% hydrogen peroxide in-office bleaching protocols (WHITEsmile. LIGHT WHITENING AC, WHITEsmile, GmbH, Birkenau, Germany) was was applied directly over the labial surfaces of the anterior teeth with a thickness about1-2 mm, The whitening lamp of the LED bleaching device (BT Cool Plus, Taiwan) was used with a wavelength of 430-490 nm and irradiance of 350 mW/cm^2. The lamp was placed close to teeth during the gel application, then it turned on for 15 minutes (this process was repeated three times and each time the bleaching gel was refreshed with a new one) for total application time 45 minutes. Then a desensitizing gel was applied to the labial teeth surfaces of bleached teeth and was left undisturbed for 10 minutes. **Gp IV:** following the same protocol of gp III except no desensitizer was applied.

3. Results:

All the data concerning the color change and surface roughness tests was collected, recorded and tabulated at different evaluation periods (base line, after treatment, 3, 6, 9 and 12 month) thus the mean values± standard deviations of all groups were expressed to be statistically analyzed.

To statistically analyze the color change values of the 4 different tested groups at each tested period, One way repeated measure ANOVA test was performed. The comparison was done at a level of 95% significance, and revealed that no significant difference between all tested groups at any evaluation periods since P value > 0.05 (table 2). In addition the effect of time was tested among each group and

statistically analyzed using the same previous test (table 3). A highly statistically significant difference was shown within all groups where P values =0.000.

Table 2: The result of color change value (ΔE) at different evaluation periods within each tested group.

Duration	Group	(ΔE) Mean ±S.D	F	p-value
Baseline (before any treatment)	Group I	20.79 ± 4.87	0.090	0.965
	Group II	20.82 ± 13.80		
	Group III	21.84 ± 5.70		
	Group IV	22.49 ± 5.25		
After treatment	Group I	10.94 ± 5.29	2.082	0.122
	Group II	10.90 ± 5.47		
	Group III	7.92 ± 4.97		
	Group IV	6.44 ± 2.04		
After 3 months	Group I	10.21 ± 5.93	0.394	0.758
	Group II	8.36 ± 2.54		
	Group III	8.43 ± 4.36		
	Group IV	10.36 ± 6.95		
After 6 months	Group I	5.58 ± 5.32	1.120	0.355
	Group II	9.13 ± 3.16		
	Group III	7.43 ± 3.19		
	Group IV	8.30 ± 5.11		
After 9 months	Group I	3.99 ± 2.67	2.047	0.127
	Group II	7.70 ± 5.38		
	Group III	4.41 ± 3.11		
	Group IV	7.63 ± 5.02		
After 12 months	Group I	6.20 ± 3.98	1.298	0.292
	Group II	10.03 ± 4.36		
	Group III	7.34 ± 5.21		
	Group IV	9.39 ± 5.08		

Table 3: Mean ± SD color change values of ΔE for tested groups at different evaluation periods.

Duration	**Group I**	**Group II**	**Group III**	**Group IV**
Baseline	20.79 ± 4.87	20.82 ± 13.80	21.84±5.70	22.49 ± 5.25
After treatment	10.94 ± 5.29	10.90 ± 5.47	7.92 ± 4.97	6.44 ± 2.04
After 3 months	10.21 ± 5.93	8.36 ± 2.54	8.43 ± 4.36	10.36 ± 6.95
After 6 months	5.58 ± 5.32	9.13 ± 3.16	7.43 ± 3.19	8.30 ± 5.11
After 9 months	3.99 ± 2.67	7.70 ± 5.38	4.41 ± 3.11	7.63 ± 5.02
After 12 months	6.20 ± 3.98	10.03 ± 4.36	7.34 ± 5.21	9.39 ± 5.08
F	60.658	59.688	118.536	97.048
p-value	0.000**	0.000**	0.000**	0.000**

By analyzing the color change values (ΔE) for group I, it was found that the mean and ± standard deviation readings at base line, after treatment, 3,6,9 and 12 month recorded 20.79 ± 4.87, 10.94 ± 5.29, 10.21 ± 5.93, 5.58 ± 5.32, 3.99 ± 2.67, 6.20 ± 3.98 respectively. To find out which period of time is responsible for the significant difference, Post hock (Tukey' test) was used. This analysis showed that original base line data was mainly responsible for this significant difference showing a significant difference between base line and each evaluation period. However the data representing color change just after treatment was also significantly different compared to those after 6,9,12 months sharing the responsibility with base line data (table 4). It was observed also that there was a significant change in the color recorded at 9 month evaluation periods compared with that at baseline, after treatment and after 3 months.

Table 4: Color Lab Post hock (Tukey's test) for group I.

Comparing mean values of ΔE for Group I

Duration	Baseline	After treatment	After 3 months	After 6 months	After 9 months	After 12 months
Baseline	--------	**0.001***	**0.000****	**0.000****	**0.000****	**0.000****
After treatment	**0.001***	-------	**0.576**	**0.018***	**0.004***	**0.027***
After 3 months	**0.000****	**0.576**	------	**0.012***	**0.017***	**0.084**
After 6 months	**0.000****	**0.018***	**0.012***	-------	**0.351**	**0.677**
After 9 months	**0.000****	**0.004***	**0.017***	**0.351**	--------	**0.015***
After 12 months	**0.000****	**0.027***	**0.084**	**0.677**	**0.015***	--------

To analyze the color change values (ΔE) for group II, the mean and± standared deviation at base line, after treatment, 3,6,9 and 12 month recorded 20.82 ± 13.80, 10.90 ± 5.47, 8.36 ± 2.54, 9.13 ± 3.16, 7.70 ± 5.38, 10.03 ± 4.36 respectively. The base line data showed a great chance to be responsible for the significant difference comparing to 3,6,9 and 12 month with p value < 0.05. However the color change after treatment evaluation period was not statistically significant compared to other time periods with p value > 0.05 (table 5).

Table 5: Color Lab Post hock (Tukey's test) for group II.

Comparing mean values of ΔE for Group II

Duration	Baseline	After treatment	After 3 months	After 6 months	After 9 months	After 12 months
Baseline	--------	**0.053**	**0.015***	**0.029***	**0.010***	**0.021***
After treatment	**0.053**	---------	**0.235**	**0.321**	**0.255**	**0.708**
After 3 months	**0.015***	**0.235**	-------	**0.491**	**0.646**	**0.21**
After 6 months	**0.029***	**0.321**	**0.491**	--------	**0.332**	**0.608**
After 9 months	**0.010***	**0.255**	**0.646**	**0.332**	---------	**0.235**
After 12 months	**0.021***	**0.708**	**0.21**	**0.608**	**0.235**	----------

To analyze the color change values (ΔE) for group III the mean and ± standard deviation at base line, after treatment, 3,6,9 and 12 recorded 21.84±5.70, 7.92 ± 4.97, 8.43 ± 4.36, 7.43 ± 3.19, 4.41 ± 3.11, 7.34 ± 5.21 respectively. The base line time was responsible for that significant difference. The data for color change after 9 months was also highly significantly different compared to those after 3,6 months (p value < 0.05) (table 6).

Table 6: Color Lab Post hock (Tukey's test) for group III.

Comparing mean values of ΔE for Group III

Duration	Baseline	After treatment	After 3 months	After 6 months	After 9 months	After 12 months
Baseline	----------	**0.000****	**0.001***	**0.000****	**0.000****	**0.000****
After treatment	**0.000****	----------	**0.814**	**0.729**	**0.059**	**0.827**
After 3 months	**0.001***	**0.814**	----------	**0.379**	**0.032***	**0.646**
After 6 months	**0.000****	**0.729**	**0.379**	----------	**0.019***	**0.958**
After 9 months	**0.000****	**0.059**	**0.032***	**0.019***	----------	**0.120**
After 12 months	**0.000****	**0.827**	**0.646**	**0.958**	**0.120**	---------

To analyze the color change values (ΔE) for group IV, the mean and ± standard deviation at base line, after treatment, 3 month,6 month,9 month and 12 months recorded 22.49 ± 5.25, 6.44 ± 2.04, 10.36 ± 6.95, 8.30 ± 5.11, 7.63 ± 5.02, 9.39 ± 5.08 respectively. The base line data was also the period responsible for the significance recorded showing a highly significant difference with each evaluation period. In addition the color change was significantly different comparing data collected at 9 months versus those of 12 months (p value < 0.05) (table 7).

Table 7: Color Lab Post hock (Tukey's test) for group IV.

Comparing mean values of ΔE for Group IV

Duration	Baseline	After treatment	After 3 months	After 6 months	After 9 months	After 12 months
Baseline	----------	**0.000****	**0.001***	**0.000****	**0.000****	**0.001***
After treatment	**0.000****	---------	**0.096**	**0.374**	**0.542**	**0.148**
After 3 months	**0.001***	**0.096**	---------	**0.361**	**0.242**	**0.650**
After 6 months	**0.000****	**0.374**	**0.361**	----------	**0.760**	**0.579**
After 9 months	**0.000****	**0.542**	**0.242**	**0.760**	----------	**0.012***
After 12 months	**0.000****	**0.148**	**0.650**	**0.579**	**0.012***	--------

The numerical data for surface roughness tests were collected and tabulated. F test was used to compare the data of different tested groups at each evaluation periods (base line, after treatment, 3,6,9 and 12 month) thus the mean values± standard deviations of all groups were expressed to be statistically analyzed (table 8).

Table 8: different rough surface values of different groups at each time period.

Duration	Group	Mean ±S.D	F	p-value
Baseline	**Group I**	0.382 ± 0.205	0.282	0.838
	Group II	0.394 ± 0.093		
	Group III	0.364 ± 0.092		
	Group IV	0.446 ± 0.309		
After treatment	**Group I**	0.463 ± 0.129	1.563	0.217
	Group II	0.367 ± 0.196		
	Group III	0.292 ± 0.099		
	Group IV	0.389 ± 0.222		
After 3 months	**Group I**	0.383 ± 0.134	1.908	0.148
	Group II	0.329 ± 0.065		
	Group III	0.292 ± 0.059		
	Group IV	0.274 ± 0.134		
After 6 months	**Group I**	0.347 ± 0.099	0.819	0.493
	Group II	0.351 ± 0.097		
	Group III	0.313 ± 0.051		
	Group IV	0.387 ± 0.133		
After 9 months	**Group I**	0.624 ± 0.196	1.118	0.356
	Group II	0.624 ± 0.300		
	Group III	0.454 ± 0.138		
	Group IV	0.532 ± 0.264		
After 12 months	**Group I**	1.014 ± 0.459	4.760	0.007*
	Group II	0.658 ± 0.283		
	Group III	0.473 ± 0.189		
	Group IV	0.642 ± 0.254		

To statistically analyze the surface roughness values (Ra) of the 4 different tested groups at each tested period was performed using One way repeated measure ANOVA test. The comparison was done at a level of 95% significance, and revealed that no significant difference between all tested groups at any evaluation periods since P value > 0.05 (table 9). In addition the effect of time was tested among each group and statistically analyzed using a Post hock (Tukey's test). This was presented in. A highly statistically significant difference was shown within all groups where P values =0.000.

Table 9: Statistical analysis of Mean ± SD Ra values (μm) of ΔE by micrometer for tested groups at different evaluation periods.

Duration	Group I	Group II	Group III	Group IV
Baseline	0.382 ± 0.205	0.394 ± 0.093	0.364 ± 0.092	0.446 ± 0.309
After treatment	0.463 ± 0.129	0.367 ± 0.196	0.292 ± 0.099	0.389 ± 0.222
After 3 months	0.383 ± 0.134	0.329 ± 0.065	0.292 ± 0.059	0.274 ± 0.134
After 6 months	0.347 ± 0.099	0.351 ± 0.097	0.313 ± 0.051	0.387 ± 0.133
After 9 months	0.624 ± 0.196	0.624 ± 0.300	0.454 ± 0.138	0.532 ± 0.264
After 12 months	1.014 ± 0.459	0.658 ± 0.283	0.473 ± 0.189	0.642 ± 0.254
F	**202.346**	**123.861**	**280.474**	**147.605**
p-value	**0.000****	**0.000****	**0.000****	**0.000****

To analyze the surface roughness values (Ra value) for group I the mean and ± standard (μm) deviation was calculated at base line, after treatment, 3,6,9 and 12 month recording 0.382 ± 0.205, 0.463 ± 0.129, 0.383 ± 0.134, 0.347 ± 0.099, 0.624 ± 0.196, 1.014 ± 0.459 respectively. As shown in the table 10, One year data and the period of 9 month were both responsible for this significant difference since there was a significant different between both 9 and 12 month and each evaluation period in group I. However the data representing Ra value at 9 month was also significantly different compared to those at baseline, 3, 6 month. Thus recording that the period of 9 month was also sharing a part of this responsibility.

For group II, to analyze the surface roughness values (Ra value), the mean and ± standard deviation was calculated at base line, after treatment, 3,6,9 and 12 months recording 0.394 ± 0.093, 0.367 ± 0.196, 0.329 ± 0.065, 0.351 ± 0.097, 0.624 ± 0.300, 0.658 ± 0.283 respectively. 0ne year data was also the main factor responsible for that difference even though no significant difference was recorded between period 9 month versus 12 month. Moreover, a highly significant different was recorded between 9 months versus after treatment, 3 and 6 month evaluation periods (table 11). This translates a part of its responsibility for the recorded significant effect of time.

Table 10: surface roughness Lab Post hock (Tukey's test) for group I.

Comparing Ra values for Group I

Duration	Baseline	After treatment	After 3 months	After 6 months	After 9 months	After 12 months
Baseline	-------	**0.220**	**0.990**	**0.704**	**0.047***	**0.005***
After treatment	**0.220**	-------	**0.343**	**0.138**	**0.120**	**0.013***
After 3 months	**0.990**	**0.343**	-------	**0.140**	**0.006***	**0.002***
After 6 months	**0.704**	**0.138**	**0.140**	--------	**0.003***	**0.002***
After 9 months	**0.047***	**0.120**	**0.006***	**0.003***	---------	**0.005***
After 12 months	**0.005***	**0.013***	**0.002***	**0.002***	**0.005***	--------

Table 11: surface roughness Lab Post hock (Tukey's test) for group II.

Comparing Ra values for Group II

Duration	Baseline	After treatment	After 3 months	After 6 months	After 9 months	After 12 months
Baseline	-----	**0.703**	**0.056**	**0.273**	**0.054**	**0.022***
After treatment	**0.703**	-------	**0.578**	**0.825**	**0.026***	**0.001***
After 3 months	**0.056**	**0.578**	-------	**0.376**	**0.023***	**0.012***
After 6 months	**0.273**	**0.825**	**0.376**	-------	**0.018***	**0.013***
After 9 months	**0.054**	**0.026***	**0.023***	**0.018***	-------	**0.081**
After 12 months	**0.022***	**0.001***	**0.012***	**0.013***	**0.081**	--------

Regarding group III, to analyze the surface roughness values (Ra value), the mean and ± standard deviation was calculated at base line, after treatment, 3,6,9 and 12 month recording 0.364 ± 0.092, 0.292 ± 0.099, 0.292 ± 0.059, 0.313 ± 0.051, 0.454 ± 0.138, 0.473 ± 0.189 respectively. The responsibility for the significant difference fell on both 9 and 12 months evaluation periods since there were significantly different versus after treatment, 3 and 6 month evaluation periods (table 12).

Table 12: surface roughness Lab Post hock (Tukey's test) for group III.

Comparing Ra values for Group III

Duration	Baseline	After treatment	After 3 months	After 6 months	After 9 months	After 12 months
Baseline	-------	**0.145**	**0.057**	**0.114**	**0.156**	**0.114**
After treatment	**0.145**	--------	**1.000**	**0.551**	**0.017***	**0.016***
After 3 months	**0.057**	**1.000**	--------	**0.085**	**0.025***	**0.019***
After 6 months	**0.114**	**0.551**	**0.085**	--------	**0.023***	**0.024***
After 9 months	**0.156**	**0.017***	**0.025***	**0.023***	--------	**0.736**
After 12 months	**0.114**	**0.016***	**0.019***	**0.024***	**0.736**	-------

For group IV, to analyze the surface roughness values (Ra value), the mean and ± standard deviation was calculated at base line, after treatment, 3,6,9 and 12 month recording0.446 ± 0.309, 0.389 ± 0.222, 0.274 ± 0.134, 0.387 ± 0.133, 0.532 ± 0.264, 0.642 ± 0.254 respectively. The data after 12 month sharing a highly significant difference with that after treatment, 3 and 6 months evaluation period, in addition to Ra values at 9months compared to 3 months (table 13).

Table 13: surface roughness Lab Post hock (Tukey's test) for group IV.

Comparing Ra values for Group IV

Duration	Baseline	After treatment	After 3 months	After 6 months	After 9 months	After 12 months
Baseline	--------	**0.607**	**0.204**	**0.550**	**0.554**	**0.191**
After treatment	**0.607**	--------	**0.315**	**0.977**	**0.303**	**0.038***
After 3 months	**0.204**	**0.315**	-------	**0.094**	**0.020***	**0.008***
After 6 months	**0.550**	**0.977**	**0.094**	-------	**0.163**	**0.018***
After 9 months	**0.554**	**0.303**	**0.020***	**0.163**	-------	**0.177**
After 12 months	**0.191**	**0.038***	**0.008***	**0.018***	**0.177**	-------

Pearson's coefficient of coordination test was used to find the correlation between color change and Ra values within each group. This was presented in figure (1-4) which showed a negative relation through follow up periods between color change and surface roughness, whenever ΔE increases a decrease in Ra was recorded.

Finally, a Pearson's coefficient of coordination test was used to find out the relationship between all the data regarding the total color changes and surface roughness values throughout the study as collectively presented in table 14. The statistical analysis of this correlation was illustrated in figures 5.

(r) Value resembles the correlation coefficient. R test was used to compare the data of Ra values and ΔE values for different tested groups. When (r) is negatively recorded (-) means there is inverse relationship between two groups (one increases while the other decreases), when (r) is positively recorded (+) means there is positive relationship between two groups (the 2 groups increase together or decrease together).

A statistically inverse correlation was present between the surface roughness and total color change values for all tested groups which recorded -0.421, -0.228, -0.159 and -0.020 for gp I, II, III and IV respectively.

Table 14: Pearson's coefficient of coordination test to find out the relationship between all the data regarding the total color changes and surface roughness values throughout the study.

Surface roughness values (Ra)

	Groups		**Group I**	**Group II**	**Group III**	**Group IV**
Total color change values (ΔE)	**Group I**	**r**	-0.421	-0.467	-0.327	-0.279
		p-value	0.406	0.350	0.527	0.592
	Group II	**r**	-0.242	-0.228	-0.056	0.031
		p-value	0.644	0.664	0.916	0.953
	Group III	**r**	-0.345	-0.344	-0.159	-0.117
		p-value	0.504	0.504	0.764	0.962
	Group IV	**r**	-0.252	-0.193	0.014	-0.020
		p-value	0.630	0.714	0.979	0.969

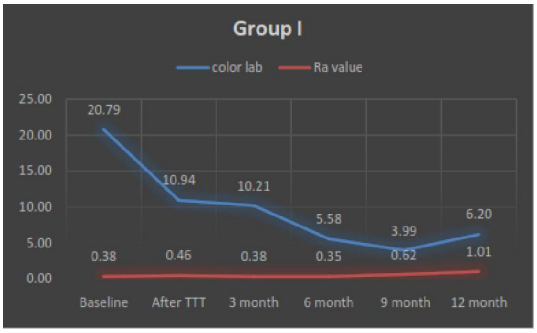

Figure 1: Barchart showing the correlation between the color change values and surface roughness values for gp I throughout the study.

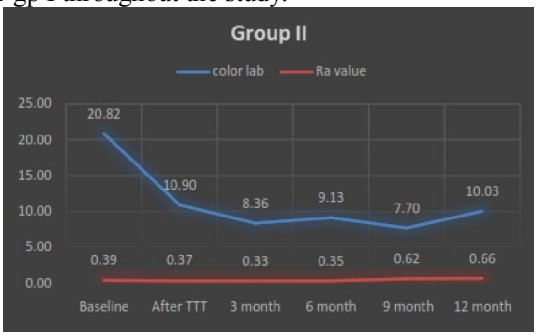

Figure 2: Barchart showing the correlation between the color change values and surface roughness values for gp II throughout the study.

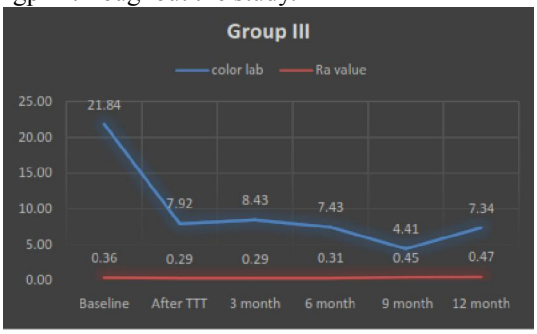

Figure 3: Barchart showing the correlation between the color change values and surface roughness values for gp III throughout the study.

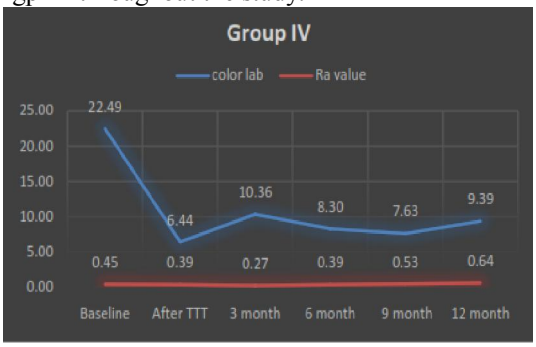

Figure 4: Barchart showing the correlation between the color change values and surface roughness values for gp IV throughout the study.

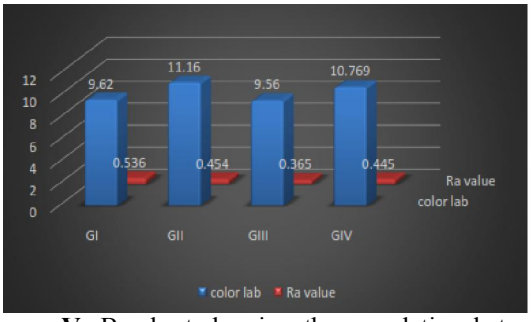

Figure V: Barchart showing the correlation between the color change values and surface roughness values throughout the study.

4. Discussion:

Selection of artificial saliva as a storage medium in the current study was based on rejecting any chemicals that can be absorbed by, and/ or alter tooth substance which may lead to negative effects on the color or surface roughness [28].

In addition, the buffering capacity and the remineralization potential of saliva (saliva can reverse some mineral loss caused by bleaching treatment) might overcome detrimental bleaching effects. Also it induces increasing mineral uptake, which replaces the mineral lost during treatment. Storing the specimens at room temperature in the artificial saliva keeps it hydrated during all steps of the experiment which is important [29].

Moreover, in the present study, the artificial saliva was changed daily to minimize deterioration, dehydration and bacterial growth [30].

32% WHITEsmile LIGHT WHITENING AC) as an in-office bleaching material was chosen, according to **Meng et al.,** [31], since it exhibits a 'milder' or even 'non-invasive' bleaching therapy. The pH =8,0 - 9,7, in the mixture may help to prevent the irreversible alterations of enamel surface. A WHITEsmile After Whitening Mousse was used as a desensitizing agent following bleaching techniques which contain 30% Xylitol, 4.2% Potassium nitrate and 1450 ppm Sodium Fluoride.

In the present investigation, the instrumental evaluation was done using spectrophotometer under controlled clinical conditions which has been preferred and sensible over the visual evaluation because it makes the process more practical, reproducible for quantitative evaluation of tooth color change, statistically more reliable, more precise in obtaining the color alteration (ΔE) in numeric values (within the LCH color space system) and provides normal distribution results. In a study done by **Kim-Pusateri, et al.,** [32] they confirmed that this method gives more confidence and standard results, with 96% accuracy.

In the current study, coronal pulp tissues were intentionally extirpated according to **Ahmed et al.,** [33]

who found that extracted human teeth showed change in color with time. They explained that after tooth extraction, the pulp degenerates producing various iron compounds which can be converted into black ferric sulphide. These disseminate into the dentinal tubules leading to intrinsic discoloration.

In the present study, specimens were polished prior to application of bleaching materials. This procedure was necessary for effective plaque and stains removal and also to keep the surface highly polished with nearly similar surface roughness of all specimens which was used as a baseline data. The use of a prophylactic paste (Ultrapro Tx pure Prophy PASTE) in this study with medium grit and unique sphere particles achieved good cleaning ability with simultaneous polishing [34].

The surface roughness measurements were performed. Zygo has an advantage of accurate and precise measurement of the surface roughness without the need for additional measurements. The profilometric method was considered by many studies as an effective quantitative evaluation [35]. Thus it was chosen as a method for measuring surface roughness.

According to the statistical analysis of the current collected data for color change values (ΔE) concerning gp I and II, it was founded that the color was effectively changed, since the mean readings at base line and after treatment recorded 20.79, 10.94 respectively for gp I and 20.82, 10.90 respectively for gp II. i.e. the shade differ completely from baseline to after treatment evaluation periods.

In agreement with our findings, **Matis et al.,** [36] who concluded that over the counter therapy significantly lightened teeth.

Confirming the findings of group II, some investogators [37-39] found that 5.3 and 6% hydrogen peroxide strips results a highly significant color improvement.

This may be attributed to the protective effects of low fluoride concentrations applied on enamel after tooth bleaching to prevent, or at least reduce, the trans-enamel ingress of chromogen from daily food [40]. The hypothesis is that the fluorapatite precipitation can reduce tooth permeability to HP without affecting the oxidizing potential of the active bleaching agent [41].

Another explanation might be concerned with the treatment times and extension of OTC bleaching technique. Whitening effects depend on how much time per day the patient spends applying the technique. Also the active ingredients in whitening agents would be important, which includes polyvinylpyrrolidone (a water soluble homopolymer). This polymer is thought to bind and remove stains in several oral care applications and prevent stain redeposition [42].

One of the important observations in this in-vitro study was the time factor which had a significant effect on the color of the tested bleached materials since a high significant difference was recorded between the baseline data and other evaluation periods for both gp I and II. Color regression was considered along the follow up periods.

This was found to confirm the results of **Annette eta l.,** [43] who quantified the color regression of enamel bleached specimens over a period of 12 months in vitro. They concluded that bleaching resulted in a significant color change (ΔE) of specimens. However, color change of in vitro bleached samples was not stable over time with regard to lightness.

Also, **Lima et al.,** [44] recorded color regression of Over the Counter products after 45 days of treatment.

In addition to **de Vasconcelos et al.,** [45] who found that Tooth Mousse application after bleaching using 7.5% hydrogen peroxide produce the highest values of ΔE immediately after completion of the bleaching regimen. Regression in color was demonstrated 7 days after bleaching.

Color regression which might be the result of the previously oxidized substance that become chemically reduced and causes the samples to reflect the old coloration of enamel or dentine. Furthermore, **Al–Tarakemah and Darvell** [46] explained the color regression to be related to the permeability of the enamel is not reduced by the treatment and it remains susceptible to precisely the same sources of discoloration as before.

On the other hand, **Pinto et al.,** [47] found that whitening strips demonstrated color stability after 12 months of follow-up.

By using Over-The Counter bleaching followed by desensitizer (gpI), a significant relation was recorded regarding the surface roughness values (Ra) as compared with Over-The Counter bleaching therapy without desensitizer (gpII).

A reasonable explanation might be the remineralization ions which could decrease the surface roughness. This could explain the reduction of stains gain ability of bleached specimens of gp I due to the remineralizing ability of desensitizing agents. This was confirmed by the results of **Cadenaro et al.,** [48] who stated that topical application of fluoride has also been reported effective in reducing roughness after bleaching.

The current results also confirmed those of **Agnieszka et al.,** [49] who concluded that Crest Whitestrips produced significant tooth lightening with respect to increase surface roughness.

One of the important observations in this in-vitro study is the time factor which had a significant effect on the surface roughness of the tested bleached materials since a significant difference was recorded

between the baseline data and one year evaluation period for both gp I and II.

According to the statistical analysis of the current collected data for surface roughness values (Ra) concerning gp I and II, it was founded that there were an increase in the roughness since the mean readings at base line and 12 month recorded 0.382, 1.014 µm respectively for gp I and 0.394, 0.658 µm respectively for gp II.

The results of surface roughness for group I showed the highest Ra value (mean = 1.014 µm) after one year. Moreover, there was a significant different between both 9 and 12 months and each evaluation period.

This significant increase of surface roughness after one year might be explained by a lot of study's findings which investigated the effects of bleaching on enamel morphology and the surface texture morphological alteration of the enamel surface [50]. Increased porosity of the superficial enamel structure, increased depth of enamel grooves, demineralization and decreased protein concentration, organic matrix degradation, modification in the calcium: phosphate ratio, and calcium loss were recorded, thereby supporting the hypothesis that bleaching agents are chemically active components potentially able to induce substantial structural alterations in human dental enamel [51-57].

This confirmed the findings of **Charles et al.,** [58] who stated that the roughness of the enamel is increased with whitening, which could also contribute to staining after bleaching.

In addition, Ludmila etal, [59] found that 6% hydrogen peroxide group showed statistically higher Ra values using SEM with 50% silver nitrate solution.

On the other hand, some studies have reported that bleaching did not significantly affect the enamel surface [52-54].

By using In-Office bleaching followed by desensitizer (gpIII), a significant relation was recorded regarding the color changes values (ΔE) as compared with In-Office therapy without desensitizer (gpIV).

The great majority of the bleaching gels are delivered in a low pH in a way to increase the product's shelf life. The disadvantage of such low pH is that it can promote enamel demineralization and changes in chemical composition, morphology, and mechanical properties of the tooth structure [57].

Hydrogen peroxide brought about minor alterations in enamel surface in the form of expansion of the prism sheath and narrow gaps resembling cracks between crystals. These gaps were consistent with the organic components in enamel. **Yiming et al.,** [60] attributed to HP for an increase in porosity as well as extensive fattening in enamel describing an enamel pattern similar to type II etching pattern.

According to the statistical analysis of the current collected data for color change values (ΔE) concerning gp III and IV, it was found that there were an effective treatment since the mean readings at base line, after treatment and after 12 month recorded 21.84, 7.92, 7.34 respectively for gp III and 22.49, 6.44, 9.39 respectively for gp IV. i.e. color enhancement was recorded immediately after treatment and remain stable over one year evaluation period for both groups.

Similar to our results, **Gonzalo et al.,** [61] found that a significant teeth whitening was achieved by the end of treatment. Lightness remained significantly high when treatment was finished and one week after in hydrogen peroxide group.

In addition, **Bacaksiz et al.,** [62] found that in-office bleaching technique demonstrated significant tooth color enhancement.

One of the important observations in this in-vitro study is the time factor which had a significant effect on the color of tested bleached materials since a highly significant difference was recorded between the baseline data and other evaluation periods for both gp III and IV. Color was stable over time.

Previous studies evaluated the influence of high peroxide concentration on enamel surface roughness and color changes, it was found that high concentrations produce a great improvement in color especially in deep discoloration but such improvement will affect enamel surface roughness [36].

According to the statistical analysis of the current collected data for surface roughness (Ra values) concerning gp III, it was recorded that both 9 and 12 months evaluation periods were significantly different versus after treatment, 3 and 6 month evaluation periods. These recorded the least Ra values (mean = 0.454 and 0.473) respectively.

This might be explained by the findings of **Chen et al.,** [64] who reported that fluoridated bleaching gel resulted in less marked demineralization changes, without affecting whitening efficiency. Fluoride forms a calcium fluoride layer on the enamel surface, inhibiting further demineralization. Acidulated fluoride gel results in more fluoride deposition in bleached enamel than neutral gel. Frequent use of low concentration fluoride gel after bleaching may benefit patients with a high risk of demineralization.

In addition, several studies have reported that fluoride application combined with bleaching gels might prevent mineral loss during tooth bleaching procedures and reduce tooth permeability [65-66].

Moreover, other researchers used desensitizing agents after vital tooth bleaching is associated with many unwanted side effects, which include enamel surface alterations which can be reduced with remineralizing agents [67].

The results of the present study were compatible with **Latha et al.,** [68] the bleaching systems used in this study increased the surface roughness.

In the current study a 'milder' or even 'non-invasive' in-office bleaching therapy (pH =8,0 - 9,7, in the mixture) brought by in office bleaching material with hydrogen peroxide concentration of 32% may help to prevent the irreversible alterations of enamel surface [31].

This may be explained as some manufacturers have released in-office bleaching gels with alkaline and neutral pH, which are less aggressive to tooth structure. Additionally, the efficacy of hydrogen peroxide bleaching is directly proportional to the increase of the pH of the bleaching gel, which is explained by the fact that the dissociation constant of the hydrogen peroxide is about 11.5 in a pH of 9, the dissociation rate of the hydrogen peroxide was 2.7 times higher than that in an acidic solution (pH = 4.4)[69].

According to the statistical analysis of the current collected data for surface roughness (Ra values) concerning gp IV (In-Office bleaching only), it was recorded that the data after 12 month sharing a highly significant difference with that after treatment, 3 and 6 months evaluation period

Similar to our findings for surface roughness in gp IV, many other studies reported that bleaching procedures led to increased surface porosity of the enamel. This was explained by demineralization with decreased protein concentration, organic matrix degradation, modifications in the calcium: phosphate ratio with calcium loss and modifications in enamel crystal distribution [52,57].

In addition, the results of the current study were supported by the results of previous studies [53, 70]; where they demonstrated that there was alteration on enamel surface and increase in surface roughness after application of 35% hydrogen peroxide bleaching.

Similar to our findings, **El Halim et al.,** [71] found that in-office bleaching agents (25% HP Zoom 2) produced a significant increase in the mean surface roughness (Ra, um) values of enamel, while the color change values decreased.

The current results were in a line with that of other studies such as a study conducted by **Amr et al.,** [72] who observed the surface roughness and color measurements immediately after bleaching using 30% and 38% hydrogen peroxide and found a significant increase in surface roughness of the bleached samples compared to the baseline measurements. However, color evaluation both bleaching agents showed a significant whitening effect (lowest mean ΔE) compared to the baseline where the 30% hydrogen peroxide was more significant on immediate measurement.

Also, **Rodrigues et al.,** [73] found that bleaching with 6, 15 and 35% hydrogen peroxide, activated with UV radiation, improve the teeth color, they alter the properties of the enamel, inducing morphological changes, increase its roughness and wettability and decrease the hardness and wear resistance. It was found that the 15% hydrogen peroxide was the solution that less damaged enamel.

Moreover, **Anaraki et al.,** [74] concluded that the use of Crest White Strip supreme (14% H_2O_2) bleaching agent for 21 day produced color enhancement of the enamel blocks. Also result in unwanted side effects such as increased the enamel surface roughness. Surface roughness at baseline in white strip group was 2.22 ± 0.67, while after treatment was 4.06±1.42 which indicated a significant increase in comparison to baseline ($p<0.05$).

On the other hand, **Pelin et al.,** [75] found that 10% hydrogen peroxide bleaching agents induced noticeable color improvement of human enamel. The bleaching was performed for 6 hour a day for 4 weeks. A profilometer was used to measure average roughness values of the initial surface roughness and at each 7-day-interval. The bleaching with 10% hydrogen peroxide did not alter the enamel surface roughness.

Conclusion:

Under limitations of this study it can be concluded that:

1- The degree of whitening is superior in the power bleaching over the OTC bleaching technique.

2- Both types of bleaching agents with different concentration of hydrogen peroxide have a significant influence on the surface roughness of human enamel.

3- This investigation showed that in vitro use of different bleaching regemins resulted in significant increased surface roughness which is prominant at 12 month evaluation period for all groups but more obvious for over the counter technique (gP I & II).

4- Desensitizer application has no rule in surface roughness or rebound of color.

5- Further studies are required to assess the effect of increasing the storage time in different storage media on the chemistry and surface topography of sound and bleached enamel.

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