

# Effects of Delayed Tooth Brushing on Enamel Surface Roughness after Home Bleaching

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Received: 10 Nov, 2020 | Accepted: 16 Nov, 2020 | Published: 23 Nov, 2020

**Citation:** Al Shamrani A (2020) Effects of Delayed Tooth Brushing on Enamel Surface Roughness after Home Bleaching. Int J Dent Oral Health 7(1): dx.doi.org/10.16966/2378-7090.343

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## Abstract

**Objective:** Few studies have evaluated the effects of daily oral hygiene procedures on enamel surface roughness after bleaching. This study aimed to further assess the changes in enamel surface roughness after bleaching with 15% carbamide peroxide, followed by tooth brushing at three different time intervals (immediately, 1 h, and 2 h after bleaching). We hypothesized that the enamel surface roughness of the bleached teeth would not be affected by delayed tooth brushing after bleaching.

**Materials and Methods:** In this *in vitro* study, 72 enamel specimens were prepared from human premolars extracted for periodontal or orthodontic reasons. The specimens were divided into four groups (n=18): group 1, no brushing (control group); group 2, brushing immediately after bleaching; group 3, brushing 1 h after bleaching; and group 4, brushing 2 h after bleaching. A profilometer was used to evaluate the surface roughness at baseline, and after bleaching and/or brushing. The specimens were bleached with 15% carbamide peroxide, followed by brushing with a low-abrasive tooth paste at different time intervals. The process of bleaching and brushing was performed in each group for 21 days. The final enamel surface roughness was measured at the end of the bleaching treatment period.

**Results:** There was a significant increase in enamel surface roughness after bleaching in each group (P-value <0.05). The highest increase in surface roughness was observed in group 2; however, the differences in enamel surface roughness among all groups were not significant.

**Conclusions:** Bleaching with 15% carbamide peroxide altered the enamel surface roughness. No brushing, and immediate or delayed tooth brushing after bleaching had comparable effects on the enamel surface roughness. Further *in vitro* and *in vivo* studies are necessary to assess other adverse effects of bleaching on the enamel surface.

**Keywords:** Bleaching; Carbamide peroxide; Enamel roughness; Profilometer; Tooth brushing

## Introduction

Tooth color is an important esthetic factor in dental treatments [1]. Personal satisfaction with tooth color decreases with increased discoloration [2]. Depending on the cause and severity of discoloration, dentists can treat discolored teeth using various methods, such as removal of surface stains, tooth bleaching, veneers, and crowns. Vital tooth bleaching, when performed correctly, is an effective, conservative, and predictable technique for lightening discolored teeth. The popularity of tooth bleaching has increased over the last decade because it is a simple, effective, and the least destructive method for treating tooth discoloration [3]. Since the introduction of dental bleaching agents, several methods and approaches using different concentrations of bleaching agents, application times, and modes have been reportedly used to lighten teeth [4]. Vital tooth bleaching treatments include in-office bleaching, professionally-prescribed at-home bleaching, and non-prescription over-the-counter bleaching. The main active chemical component of most dental bleaching agents is hydrogen peroxide (HP) [5]. HP can be used either in its pure form

or as a product of the chemical reaction of carbamide peroxide (CP) and sodium perborate [6]. While CP is considered safe, studies have shown that changes in the mineral content, chemical composition and surface roughness of the teeth may occur with the use of CP [7,8]. Furthermore, increased enamel surface roughness after bleaching has been demonstrated [9-11]. This roughness might occur due to surface alterations such as erosion and surface irregularities of the enamel due to loss of mineral content, which may be associated with the pH and concentration of the bleaching agent, frequency of bleaching, and duration of exposure to the bleaching agent [12]. A rough enamel surface can lead to the accumulation of food, biofilm formation, and increased risk of periodontal disease [13]. Moreover, adherence of *Streptococcus mutans* to rough enamel surfaces after bleaching has been confirmed as well [14]. However, other studies have shown that no changes were observed in the surface roughness of enamel treated with 10%, 15%, or even 37% CP [15-18]. Therefore, further studies are needed to clarify the relationship between the surface roughness of enamel and tooth bleaching.

After at-home bleaching, patients typically remove the bleaching tray and brush their teeth with toothpaste to remove the bleaching gel. Bruzell EM, et al. reported that scratches from cleaning procedures were observed on the bleached enamel surfaces more frequently because of their reduced resistance to abrasion [19]. Further, Worschech et al. showed that brushing bleached teeth using abrasive toothpaste increased the enamel surface roughness [20]. However, Navimipour, et al. reported that no changes in the enamel surface roughness were observed when tooth brushing was delayed for 1 or 2 h after daily bleaching and that allowing for remineralization between bleaching and tooth brushing could minimize the potential surface loss [21]. These findings suggest that the patients' oral hygiene routines could affect the enamel surface roughness during tooth bleaching treatment. Accordingly, this study aimed to further assess the changes in enamel surface roughness after bleaching with 15% CP, followed by brushing at three different time intervals (immediately, 1 h, and 2 h after bleaching). We hypothesized that the enamel surface roughness of the bleached teeth would not be affected by delayed tooth brushing after bleaching.

## Materials and Methods

### Enamel specimen preparation

In this *in vitro* study, tooth specimens were prepared from human premolars extracted for periodontal or orthodontic reasons. Ethical approval was obtained from the Institutional Review Board of King Saud University, College of Medicine, and University Hospital (E-19-4038).

An ultrasonic scaler was used to clean all the teeth. Subsequently, a rubber cup mounted on a slow-speed hand piece (No. 6412500, Kavo EWL, Biberach, West Germany) was used to polish the teeth with non-fluoridated pumice to remove any surface debris or contaminants. After cleaning, all the teeth were stored in distilled water with a 0.05% thymol solution. All the teeth were examined under a digital microscope (KH-7700; Hirox, Tokyo, Japan) at 50 × magnification to exclude any teeth with caries, restorations, or structural enamel defects or cracks. Each tooth was horizontally sectioned 1-mm apical to the cemento-enamel junction using a slow-speed diamond saw (Isomet low-speed Buehler, Lake Bluff, IL, USA) and water-coolant spray to remove the root. Further sectioning was performed to obtain the buccal and lingual surfaces of each crown. A total of 72 specimens were finally obtained. The teeth were mounted on a polyvinyl mold with an external diameter and height of 20 mm and 5 mm, respectively, and filled with self-cure acrylic resin. Each tooth section was placed in the resin with either the buccal or lingual surface facing upwards. All specimens were placed in cold water until the resin had completely cured to prevent the thermal effects of the resin-curing process. The surface of each tooth specimen was then polished with a series of silicon carbide sandpaper of 120, 400, and 600 grits (Buehler, São Paulo, Brazil), and re checked for any cracks or fractures under the digital microscope at 50 × magnification.

### Experimental groups

The obtained tooth specimens were randomly assigned to one of the following four groups (n=18 per group), according to the interval between bleaching and the tooth brushing procedure: no tooth brushing after bleaching (group 1 [control]); tooth brushing immediately after bleaching (group 2); tooth brushing 1 h after bleaching (group 3); and tooth brushing 2 h after bleaching (group 4).

### Surface roughness measurements

Three-dimensional (3D) surface roughness was examined using an optical non-contact profiling system (Bruker Contour GT-K; Bruker Nano GMBH, Berlin, Germany), which uses 3D non-contact metrology with interferometry. A 5 × Michelson magnification lens was used with a 1.5 × 1.5mm field-of-view, a scan speed of 1× with a Gaussian regression filter, and a threshold value of 4. For a precise scan-based area determination at baseline and after treatment, the samples were placed on the stage of the device with a customized Teflon guide so that the tooth surface to be scanned was exposed. Then, the focus was manually adjusted to obtain an image on the monitor. The Vision 64 software (Bruker Corporation, San Jose, CA, USA) was used to control the settings of the microscope, analyze the data, and provide a graphical output. The measurements were obtained using vertical scanning interferometry, which employs broad band (normally white) light source. Vertical scanning interferometry is effective for measuring objects with rough surfaces and those with adjacent pixel-height differences exceeding 135 nm; the resolution is in the nanometer range, and the maximum height is 10 mm. As each point on the surface comes into focus, the modulation on that point reaches a maximum and then tapers off as the objective passes through the focus. By recording the height at maximum modulation, the system can determine the height of each pixel. Each wire sample was scanned at three selected points. Each point underwent three interval interferometric scans and the average was used to determine the roughness value. A mean roughness profile (Ra) was determined for each specimen to describe the overall roughness of the surface (µm). Surface roughness measurements were performed twice for each specimen: at baseline (Ra<sub>1</sub>) and at the end of the bleaching procedure (expressed as the final value [Ra<sub>2</sub>]).

### Bleaching treatment and brushing procedures

After measuring the baseline surface roughness, the enamel specimens were bleached with 15% CP (Opalescence PF, Ultradent Products, South Jordan, UT, USA). The composition of the bleaching gel is shown in table 1. According to the manufacturer's instruction, a 1-mm layer of gel was applied to the specimens using a syringe with a nozzle included in the bleaching kit and was left on the specimens for 6 h per day, for 21 days. The specimens were placed in an incubator at 37°C during bleaching, and at the end of each bleaching session, the specimens were rinsed under a water faucet for 5 seconds to remove the bleaching gel. The following procedures were then performed in each group. In group 1, after bleaching and rinsing, the specimens were placed in artificial saliva for 18 h in an incubator at 37°C. In group 2, the specimens were brushed immediately after bleaching using a toothbrush simulator (ZM-3.12, SD Mechatronik GMBH, Feldkirchen-Westerham, Germany), rinsed with distilled water, and stored in artificial saliva at 37°C until the next day to simulate clinical oral conditions. In group 3, the specimens were rinsed after bleaching and placed in artificial saliva for 1 h at 37°C, followed by the brushing procedure performed as described above. The specimens were then rinsed with distilled water and stored in artificial saliva at 37°C until the next day to simulate clinical oral conditions. In group 4, the specimens were rinsed after bleaching and placed in artificial saliva for 2 h at 37°C, and the brushing procedure was then performed. Subsequently, the specimens were rinsed with distilled water and stored in artificial saliva at 37°C until the next day to simulate clinical oral conditions. The artificial saliva was replaced daily for each group. The composition of the artificial saliva is shown in table 1.

**Table 1:** Materials used in the study and their compositions.

Materials	Manufacturers	Compositions
Opalescence™ PF 15%	Ultradent Products, Inc., South Jordan, UT, USA	15% Carbamide Peroxide, Potassium Nitrate, Sodium Hydroxide, Oils, Peppermint, Glycerin, Sodium Fluoride, and Flavoring
Opalescence™ Whitening Toothpaste	Ultradent Products, Inc., South Jordan, UT, USA	Glycerin, Synthetic Amorphous Silica, Methyl Salicylate, Sodium Lauryl Sulfate, Oils, Peppermint, Sodium Fluoride, and Sodium Hydroxide
Artificial Saliva for Medical and Dental Research	Pickering Laboratories, Inc. Space Park Way, Mountain View, CA, USA	Water, Distilled Water, De-Ionized Water, Potassium Phosphate, Potassium Chloride, Magnesium Chloride, Carboxymethyl Cellulose Sodium, Methyl 4-Hydroxybenzoate, Sodium Phosphate Dibasic, and calcium Chloride Dihydrate
		pH value, 6.75

### Simulated tooth brushing

A toothbrush simulator (ZM-3.12, SD Mechatronik GMBH, Feldkirchen-Westerham, Germany) was used in this study. The simulator had 12 separate slots in which 12 soft toothbrushes with straight bristles were attached (Trisa AG, Triengen, Switzerland). The specimens were positioned inside the containers and secured using vinyl polysiloxane putty impression material (Express STD, 3M Dental Products Division, St. Paul, MN, USA). The container was filled with freshly prepared toothpaste slurry (Opalescence whitening toothpaste, Ultradent Products, Inc.) in distilled water, with the tooth paste/water ratio of 1:2 by weight. The technical ISO specifications for tooth brushing in abrasion studies are limited to a force range of 50-250 g; in this study, a 200-g force was applied [22]. Brushing was performed once daily under a load of 200 g in a circular movement at a speed of 20 mm/s to achieve 250 cycles in 3 min. After the daily brushing procedure, the samples were placed in artificial saliva.

### Final surface roughness measurements

Daily procedures of bleaching, brushing, and rinsing were performed for 21 days in each of the four groups. At the end of these procedures, the final surface roughness measurements ( $R_a$ ) of the specimens were recorded.

### Statistical Analysis

Data obtained from the optical profilometer tests were compared using SPSS v.17 (SPSS Inc., Chicago, IL, USA). Differences with a P-value of <0.05 were considered statistically significant. The enamel surface roughness data were statistically analyzed by descriptive statistics using mean values and standard deviations. A paired-sample t-test was used to compare the mean values of surface roughness at baseline with the final values for each of the four groups, followed by an analysis of variance (ANOVA) for statistical comparisons.

### Results

After preparing the initial 80 specimens (20 per group), outlier detection was performed, and two specimens from each group were excluded. Hence, the final sample size in each group was 18. Figure 1 and table 2 presents the baseline values, final values, and differences in the mean surface roughness between  $R_{a2}$  and  $R_{a1}$ . Compared with their respective baselines, group 2, in which the tooth specimens were brushed immediately after bleaching, showed the largest increase in surface roughness after 3 weeks, followed by groups 3, 4, and 1, respectively.

The results of the paired-sample t-test for the study groups are summarized in table 3. There were significant differences in the mean surface roughness ( $\mu\text{m}$ ) between the final and baseline periods in each group (P-value < 0.05). A positive sign of the mean difference indicates an increase in enamel surface roughness (mean difference) at the end of the experiment compared to the baseline. The highest mean difference was seen in group 2.

ANOVA was used to assess whether the average final enamel surface roughness scores differed significantly among the four groups (Table 4). There were no significant differences in average final enamel surface roughness across the study groups (P-value=0.075). The assumption of the equality of variances among study groups was valid at  $\alpha=0.05$ , with a P-value of 0.350. In other words, compared with no brushing after bleaching, brushing at different times did not affect the average final enamel surface roughness of the teeth in this study.

Table 5 summarizes the results of the two independent-samples t-tests for groups 2, 3, and 4. Levene's test showed that the assumptions of the equality of variances between groups 2, 3, and 4, and the control group, respectively, were valid. There were no significant differences in the average final enamel surface roughness between groups 2,3, or 4, and the control group (Table 5). These findings suggest that compared with no brushing, daily brushing at different times after bleaching did not affect the average final enamel surface roughness of the teeth.

### Discussion

Tooth specimens in groups 2,3, and 4 showed significantly greater changes in enamel roughness than their respective baselines. Notably, group 1 (non-brushed specimens) showed a significant increase in enamel roughness, even though the specimens were kept in artificial saliva and not brushed after bleaching. There were no significant differences in mean final enamel surface roughness among groups 2, 3, and 4, and each of these three groups demonstrated surface roughness values comparable to those of the control group after bleaching. The findings suggest that compared with no brushing, brushing at different times after bleaching does not affect the average final enamel surface roughness of the teeth.

Surface roughness is a clinically important property that can influence both esthetics and health of the teeth [23,24]. A rough tooth surface stains easily [25,26], and has increased bacterial adhesion [14] and plaque maturation, which can lead to periodontal disease [27,28]. Several methods are currently available for measuring and assessing the texture of the tooth surface, such as scanning electron microscopy, profilometers, and compressed air measuring. Scanning electron microscopy lacks in the quantitative assessment of the tooth surface

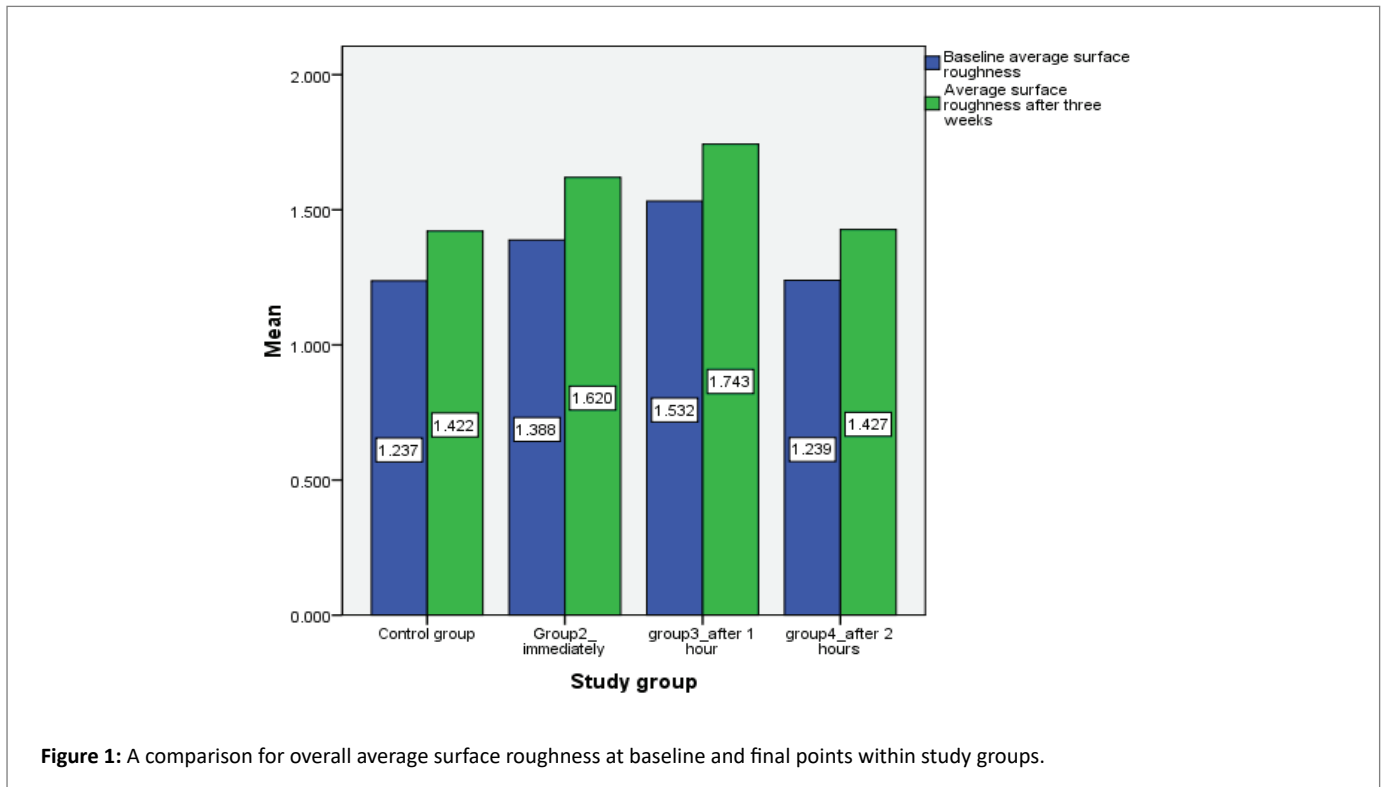


Figure 1: A comparison for overall average surface roughness at baseline and final points within study groups.

Table 2: Mean differences in surface roughness values between before and after bleaching ( $\Delta Ra$  Final value-Baseline value).

Groups	Baseline values		Final values		Changes in Roughness ( $\Delta Ra$ )				
	Mean	SD	Mean	SD	Mean	SD	Minimum	Maximum	Median
1	1.237	0.436	1.422	0.452	0.185	0.233	-0.313	0.67	0.179
2	1.388	0.302	1.62	0.344	0.232	0.21	-0.166	0.565	0.222
3	1.532	0.489	1.743	0.488	0.211	0.171	0.006	0.595	0.179
4	1.239	0.432	1.427	0.418	0.188	0.157	0.037	0.593	0.154

and only allows visualization of the surface morphology [29]. Notably, a profilometer provides a quantitative assessment of the tooth surface. Surface roughness is often quantified by variations in the height of the surface relative to a reference plane. If the variations are large, the surface is considered rough; if they are small, the surface is considered smooth [23]. The Ra parameter (average roughness) obtained using the profilometer is used to describe the surface texture and overall roughness of the surface [23]. Therefore, in this study, we used this methodology to compare surface roughness among the different groups.

At-home bleaching has become a popular, inexpensive, and efficient treatment for removing tooth discoloration [30]. It is an easy procedure that can be performed at home using a tray with 15% CP for 4-6 h per day. However, the bleaching agent might affect the hardness and surface roughness of the dental hard tissues [31], and might cause irregularities and erosion of the enamel surfaces. Although some studies on tooth bleaching did not observe any change in enamel surface roughness [15,16], we found that the enamel surface

roughness values increased after bleaching. Our result is corroborated by other studies that have also reported increased surface roughness after the application of 10% or 15% CP [9-11]. The discrepancy among the forementioned studies may be due to differences in the bleaching procedures (e.g., the pH and concentration of the bleaching agents, exposure time, and the storage medium of the specimens), and the methods used to assess surface roughness.

Enamel surface roughness may occur due to surface alterations such as erosion and irregularities caused by loss of minerals in the enamel, which might be associated with the pH and concentration of the bleaching agent, the frequency of application, and duration of exposure to these agents [12]. For example, studies have shown that the oxidative process that occurs on enamel surfaces during bleaching and the pH of tooth bleaching products is the primary causes of adverse effects of bleaching on mineralized tissues [9]. However, even a bleaching agent with an almost neutral pH can cause surface alterations similar to those resulting from pH values much lower than 7.0 [32,33]. Additionally, Carbopol, which is added to the bleaching



**Table 3:** Comparison of average surface roughness between the study groups by paired t-test.

Group	Pair	Paired differences		Test statistics		
		Mean	SD	t	d.f.	P-values
1	Ra <sub>2</sub> - Ra <sub>1</sub>	0.185	0.233	3.356	17	0.004
2	Ra <sub>2</sub> - Ra <sub>1</sub>	0.232	0.21	4.694	17	<0.001
3	Ra <sub>2</sub> - Ra <sub>1</sub>	0.211	0.171	5.246	17	<0.001
4	Ra <sub>2</sub> - Ra <sub>1</sub>	0.188	0.157	5.061	17	<0.001

SD: standard deviation; t: Paired-samples t-test value; d.f: degree of freedom

**Table 4:** Comparison of average final surface roughness among the study groups by analysis of variance.

Sources of variation	The sum of squares	d.f.	Mean Square	F	p-values
Between Groups	1.33	3	0.443	2.409	0.075
Within Groups	12.511	68	0.184		
Total	13.841	71			

d.f: degrees of freedom; F: F-statistic

**Table 5:** Comparison of average final surface roughness between the brushing groups and the control group by the 2-independent-samples t-test.

Study groups	Levene's test		t-test for the equality of means			
	F	P-values	t	d.f.	P-values	Mean difference
Group 2 vs. Control	2.472	0.13	1.48	34	0.15	0.198
Group 3 vs. Control	0.029	0.87	2.051	34	0.05	0.322
Group 4 vs. Control	0.166	0.69	0.036	34	0.97	0.005

F: test statistic of Levene's test; t: test statistic; d.f: degree of freedom

agent as a thickening agent, can alter the mineral content of enamel and lead to demineralization of the surface [34]. Even though low concentrations of CP promote various degrees of structural changes, surface porosity, and mineral loss in the enamel, these changes might not be clinically perceptible.

The changes in the dental hard tissue mentioned earlier remained unaffected by the tooth brushing procedure. Studies on the effects of brushing on enamel surface roughness of bleached teeth have yielded controversial results. Some studies found no change in the enamel surface roughness, whereas other studies demonstrated that tooth brushing after bleaching procedures altered the surface of the enamel [20,35]. In this study, increased roughness was observed after simulated tooth brushing. After 3 weeks of bleaching and brushing procedures, the highest increase in surface roughness was observed in group 2 (specimens brushed immediately after bleaching), followed by group 3 (specimens brushed 1 h after bleaching). Group 4 specimens, who were brushed 2 h after bleaching, ranked third in terms of the

increase in surface roughness. The control group (non-brushed specimens) showed the lowest increase in surface roughness. During bleaching, oral hygiene procedures may cause surface changes on the teeth, particularly when using highly abrasive dentifrices, such as a whitening dentifrice [36-38]. A bleached enamel is vulnerable to mechanical tooth wear (e.g., toothbrush abrasion), and daily tooth brushing of bleached teeth decreases enamel hardness and increases enamel surface roughness [39]. However, some studies have shown that enamel surface loss is lower when bleaching gels are supplemented with calcium or fluoride [40], that the abrasive surface loss can be reduced by allowing remineralization between bleaching and tooth brushing [38], and that bleaching with low peroxide concentrations does not make the tooth enamel surface more vulnerable to erosion or brushing abrasion [20,41]. In this *in vitro* study, excessive bleaching was avoided by following the manufacturer's instructions. Notably, we found no statistically significant differences in surface roughness between the different brushing groups and the control group after bleaching. This finding is in contrast to that in the study by Navimipour, EJ, et al. in which the enamel surface roughness increased if the specimens were brushed immediately after bleaching, and that postponing brushing for 1 or 2 h after bleaching resulted in surface roughness comparable to that of the non-brushed specimens [21]. Furthermore, after brushing, the bleached specimens in this study showed enamel surface changes comparable to those in the control group, possibly due to the use of low-abrasive fluoridated toothpaste. The fluoride present in the toothpaste may help balance the remineralization and demineralization processes when used daily after bleaching [42,43]. The harmful effects of bleaching, combined with brushing, may be aggravated by an abrasive-whitening dentifrice, such as that used in the current study.

An ideal dentifrice should provide optimum cleaning with minimum abrasion of the dental hard tissues. Toothpaste abrasiveness is evaluated using the Relative Dental Abrasion (RDA) index, based on dentin abrasion. Toothpaste with high RDA values is more abrasive, can cause enamel damage, and decrease the luster of restorations; therefore, it may not be safe for everyday use. The ISO standard 11609 and the American Dental Association recommend tooth paste with RDA values less than 250 [44]; most commercial toothpaste has RDA values between 90 and 150. The toothpaste used in this study had a low RDA value of 90. Our findings indicate that this toothpaste can be used as a daily toothpaste, as claimed by the manufacturer.

Fluoridated bleaching gels help reinforce the fluoride-containing elements of tooth enamel; the incorporation of fluoride and potassium nitrate ions in these bleaching gels improves enamel health and integrity and reduces tooth hypersensitivity during bleaching. Moreover, the alteration in the microstructure of bleached enamel can be reversed by the remineralization action of fluoride present in the bleaching gels, further strengthening the enamel [45]. Furthermore, because the specimens in the current study were maintained in artificial saliva, it can be assumed that there was continuous mineral uptake due to the presence of calcium and phosphate ions (PO<sub>4</sub><sup>3-</sup> and Ca<sup>2+</sup>) in the artificial saliva. These factors might have contributed to the lack of differences in enamel surface roughness between brushed and non-brushed specimens, as the precipitation of minerals from the artificial saliva onto the tooth surfaces might have decreased the surface roughness of bleached enamel *in vitro* [46].

This study has some limitations. The enamel specimens were smoothed and polished to produce a flat surface and standardize the profilometric measurements; this procedure might have influenced

the results. Additionally, it might not be entirely possible to extrapolate the conclusions of this *in vitro* study to clinical settings.

## Conclusions

Tooth specimens that were brushed immediately after bleaching showed the highest increase in surface roughness after 3 weeks, compared to the baseline. However, the surface roughness did not differ between delayed tooth brushing and no brushing groups. Our findings suggest that the enamel surface roughness after bleaching is not affected by delayed tooth brushing after bleaching. Further *in vitro* and *in vivo* studies are necessary to assess other adverse effects of tooth bleaching on the enamel surface.

## Conflict of Interest

The author declares no potential conflicts of interest concerning the authorship and publication of this article.

## Funding Sources

The author received no financial support for the research, authorship, and publication of this article.

## Data Availability Statement

The author confirms that the data supporting the findings of this study are available within the article.

## Acknowledgments

None

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