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Effect of Delaying Toothbrushing During Bleaching on Enamel Surface Roughness: An *In Vitro* Study

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Clinical Relevance

Although daily toothbrushing immediately after bleaching increased enamel surface roughness, postponing the procedure for one or two hours after daily bleaching resulted in no changes in enamel surface roughness.

SUMMARY

This study aimed to evaluate the effect of toothbrushing on enamel surface roughness at three different intervals after daily bleaching treatment. Eighty enamel slabs were initially evaluated for surface roughness and then randomly divided into four groups. The bleaching procedure was carried out for 21 days, six hours daily. In the control group (group 1), the specimens were not brushed after bleaching, but in groups 2–4, they were brushed with toothpaste immediately, one hour, or two hours after bleaching, respectively. Then the specimens were stored in artificial saliva. Enamel surface roughness was reevaluated at the end of the period. Kruskal-Wallis and Mann-Whitney U tests showed statistically significant differences in the means of surface roughness values between the immediately brushed group and the three other groups ($p<0.001$). Daily toothbrushing immediately after bleaching increased enamel surface roughness; however, postponing the procedure for one or two hours after daily bleaching and exposing the specimens to artificial saliva during the study period resulted in enamel surface roughness comparable to that of the control group.

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INTRODUCTION

In recent years bleaching the teeth has gained utmost importance as a result of an increase in demand for esthetics and beautiful teeth in the community. Additional research is under way to achieve more appropriate results.

Carbamide peroxide, which is recommended for at-home bleaching procedures, is generally used in a tray for six to eight hours during the night.[1] One routine for oral hygiene procedures during the at-home bleaching period is to remove the tray after daily bleaching is finished and then brush the teeth with toothpaste. This way the bleaching agent is removed from tooth surfaces.[2] Considering the penetration of bleaching agents into tooth hard tissues, there is a possibility for changes in tooth structures. Some studies have demonstrated surface deterioration, formation of defects on the surface and porosity in electron microscope studies, and decreases in enamel hardness.[3–11] Therefore, the patient's oral hygiene procedures during the bleaching period can have a substantial role in creating subsequent complications, including tooth hypersensitivity. One study demonstrated that brushing teeth bleached with 10% carbamide peroxide using abrasive toothpastes increases enamel surface roughness.[2] On the other hand, using a fluoride varnish or mouthwash after exposing the teeth to carbamide peroxide can remineralize the enamel.[11–13]

Because use of saliva or artificial saliva in laboratory studies can also have a role in the remineralization of teeth,[2,7,14] the present study attempted to evaluate changes in enamel surface roughness subsequent to bleaching and brushing at three time intervals and storing the specimens in artificial saliva during the study period. The null hypothesis was that enamel surface roughness is not influenced by when toothbrushing is initiated after bleaching (immediately, at one hour, and at two hours after bleaching).

MATERIALS AND METHODS

In the present *in vitro* study, the specimens were prepared from impacted human third molars extracted surgically. The teeth were gathered after obtaining informed consent and approval from the deputy dean of research at Tabriz University of Medical Sciences. The teeth were then stored in 0.5% chloramine T solution (pH 8-11) until used. Impacted third molars were included in the study because there are no changes on enamel surface in such teeth. Teeth that had enamel surface abnormalities or cracks or fractures that occurred during surgical extraction were excluded from the study. The study design is illustrated in Figure 1.

Subsequent to cleaning the teeth the roots were cut away at the cementoenamel junction. Two 2-mm-thick enamel slabs measuring 6 × 4 mm were prepared from the middle third of the buccal and lingual aspects with the use of double-sided diamond disks (D&Z, Berlin, Germany). Eighty enamel slabs were prepared from 40 human third molars. The tooth sections were rechecked for any cracks or fractures under a stereomicroscope (Nikon, Tokyo-Japan) at ×20 and discarded if defective. Water spray was used during specimen preparation to avoid dehydrating the specimens. The specimens were stored in distilled water at 37°C after cutting and then placed, with the enamel surface on top, inside cold-cured acrylic resin in a cylindrical mold with a diameter of 1.5 cm. Subsequent to removal of the slabs from the mold, the side of a flat-end tapered diamond bur (Teezkavan, Tehran, Iran) in a high-speed handpiece under water spray was used to make the surface of the enamel slab horizontal so that it could be properly placed under the device that measures surface roughness. Then the enamel surface was smoothed using an enamel adjustment kit (Shofu Dental Corp, Kyoto, Japan) containing Dura White stones for adjusting and finishing and Ceramiste Points in standard, ultra, and ultra II grits for polishing. These stones and points were used in a low-speed handpiece under water spray. Finally, a disk composed of felt polishing cloth (Super-Snap Buff Disk, Shofu Dental Corp, Kyoto, Japan) was used in a low-speed handpiece along with first a 6- and then a 1-μm abrasive diamond paste (Microdent, São Paulo, Brazil) for the final polish of the enamel surfaces.

The specimens were placed in an ultrasonic device containing distilled water for 10 minutes to remove polish debris. Then the slabs were randomly divided into four groups of 20 specimens each, as follows:

Group A (control): No toothbrushing after bleaching
Group B: Toothbrushing immediately after bleaching
Group C: Toothbrushing one hour after bleaching
Group D: Toothbrushing two hours after bleaching

The initial surface roughness values of all the specimens were measured and recorded before the study using a stylus profilometer (MARSURF-PS1, Mahr, Göttingen, Germany) that uses the contact method. The measurements were randomly performed on the surface of enamel slabs. To this end,

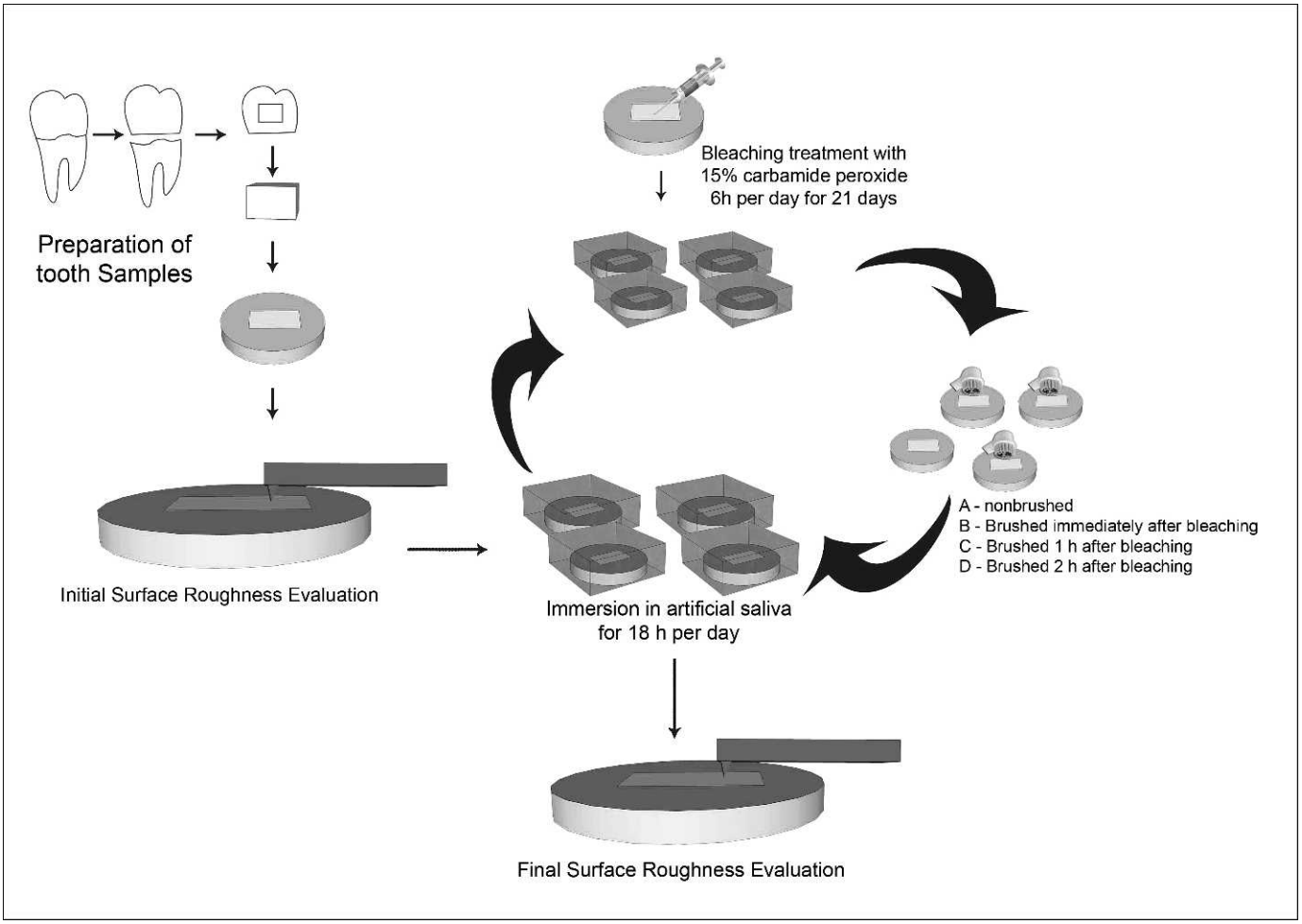

Figure 1. *Experimental design of the study.*

a 2-μm diamond bar scanned the surface at a rate of 0.1 mm/s and a force of 0.7 mN. Evaluation and cutoff lengths were 1.25 mm and 0.25 mm, respectively. The average roughness (Ra), which describes the overall surface roughness and can be defined as the arithmetic mean of all absolute distances of the roughness profile from the center line within the evaluation length,[15] was registered by profilometer in micrometers. Three tracings were made on each specimen at different locations. The mean roughness value, achieved after three measurements on each slab, was statistically analyzed. Because this system had an integrated calibration standard there was no need for external calibration.

After the initial surface roughness was measured, the bleaching process was instituted. To this end, a special tray was fabricated for each specimen in a vacuum apparatus; each tray was made of ethylvinyl acetate and was 1 mm thick. Then 0.02 mL of 15% carbamide peroxide gel (Opalescence PF, Ultradent Products, South Jordan, UT) was placed inside each tray, and the tray was placed on each specimen for six hours daily. During the process each specimen covered with the tray was placed in a separate vial containing artificial saliva, which was replaced daily. The composition of the artificial saliva was as follows: 1.0 mM $CaCl_2$, 3.0 mM KH_2PO_4, and 100 mM NaCl; the pH value was 6.30 and was adjusted with NaOH solution.[16]

After the daily bleaching procedure, the specimens were rinsed with deionized distilled water for five seconds. Then the subsequent steps for each group were carried out as follows.

Group A: The specimens in group A were placed in 1 mL of artificial saliva at 37°C in an incubator for 18 hours after bleaching and rinsing.
Group B: In group B the specimens were brushed immediately after they were bleached for six hours and rinsed for five seconds; the specimens were

Table 1. *Mean Differences in Surface Roughness Values Before and After Intervention (ΔRa = final value – baseline value)*

Group	Baseline Value		Final Value		Change in Roughness (ΔRa)				
	Mean	SD	Mean	SD	Mean	SD	Median	Lowest	Highest
A	0.63	0.18	0.48	0.18	–0.15	0.22	–0.13	–0.55	0.20
B	0.51	0.16	0.64	0.29	0.13	0.25	0.14	–0.45	0.86
C	0.52	0.13	0.32	0.06	–0.20	0.12	–0.20	–0.45	–0.02
D	0.55	0.19	0.44	0.12	–0.11	0.19	–0.05	–0.49	0.10

brushed with a powered brush (Oral-B Vitality Precision model, Oral-B Corp, Belmont, CA) inside a reservoir of freshly prepared toothpaste slurry (Opalescence whitening toothpaste, Ultradent Products) with one part (50 g) of toothpaste to three parts (150 g) of deionized distilled water. The brush was fixed on a bar with a clamp, and brushing was carried out once daily for three minutes with a typical force of 200 g. The amount of force applied was measured with an orthodontic gauge. The brush head was made of nylon and was multitufted. A separate and specific brush was used for each specimen. The specimen was placed inside the toothpaste slurry, which was agitated before use. The toothpaste slurry was replaced every two days so that a neutral pH was maintained. After daily brushing, the specimens were rinsed with distilled water and stored in special containers containing artificial saliva at 37°C for the rest of the day.

Group C: The same brushing procedure described for group B was repeated for group C, except that after bleaching and rinsing, the specimens were kept in artificial saliva for one hour, after which the brushing procedure was carried out. Then the specimens were once again stored in artificial saliva until the next day.

Group D: The procedure for group D was the same as for group C, but after bleaching there was a time interval of two hours before toothbrushing occurred.

The bleaching, brushing, and rinsing procedures continued for 21 days in all the groups. At the end of this period the surface roughness values of the specimens were once again measured, recorded, and compared with the initial values. Data were analyzed with a nonparametric Kruskal-Wallis test. A nonparametric Mann-Whitney U test was used for two-by-two comparison of the groups. Statistical significance was defined at $p<0.05$.

RESULTS

Table 1 demonstrates the descriptive statistics of mean differences in surface roughness values before and after intervention in the groups under study.

Before the study was initiated, the means of surface roughness values in the four groups were compared. The nonparametric Kruskal-Wallis test showed that there were no significant differences in the means of surface roughness values before intervention among the four groups ($p=0.12$).

Because data were widely dispersed, logarithmic transformation of the data was considered, and then a nonparametric Kruskal-Wallis test was used to evaluate the differences. The results showed that the differences in the means of surface roughness values before and after intervention were statistically significant among the groups under study ($p<0.001$). Mann-Whitney U test revealed significant differences in the means of surface roughness values between the immediately brushed group and the three other groups ($p<0.001$). There were no significant differences between the other groups ($p\geq0.06$).

The linear and bar graphs of the mean differences in enamel surface roughness values before and after intervention in the groups are presented in Figure 2.

DISCUSSION

Surface roughness, a measure of the texture of a surface, is one of the test methods used to evaluate the effects of different bleaching materials and oral hygiene procedures on tooth hard tissues.[2,9,10,17,18] The oral cavity is inhabited by many diverse microbial species, and most of these microorganisms, especially those responsible for caries or periodontitis, can only survive in the oral cavity when they

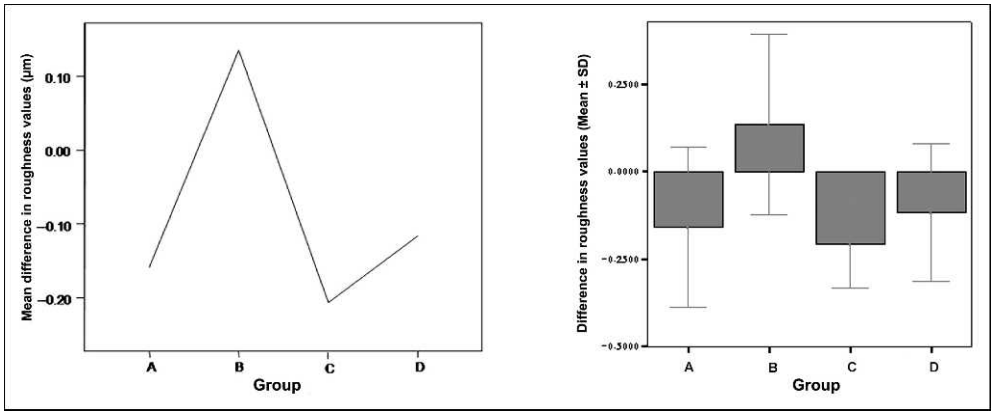

Figure 2. *The linear and bar graphs of differences in surface roughness values before and after intervention in the groups under study.*

adhere to rough surfaces. Therefore, the roughness of intraoral hard tissues can promote formation, maturation, and retention of plaque, resulting in increased risk of dental caries, periodontitis, or tooth discoloration.[19,20]

According to the results of the present study, the only group demonstrating significant increases in surface roughness after bleaching compared with the control group was group B (toothbrushing immediately after bleaching). In other words, postponement of toothbrushing for one or two hours after daily bleaching procedures and storing the specimens in artificial saliva during the wait period led to surface roughness values comparable to those in the control group; therefore, the null hypothesis was rejected.

Bleaching agents might exert negative influences on the integrity of organic structures of the tooth, including proteins and collagen. By-products of carbamide peroxide breakdown are urea and hydrogen peroxide. Urea can denature tooth structure proteins by penetrating the enamel structure and influencing prismatic and interprismatic structure and also by increasing permeability and ultrastructural changes. These processes result in pore formation and increases in the diameter of enamel and dentin surface pores. On the other hand, the free oxygen present in hydrogen peroxide increases surface porosity and reacts with the organic structure of dental tissues. Increases in the surface porosity facilitate the passage of oxygen radicals beyond enamel and dentin and the breakdown of stained macromolecules into smaller and light-colored molecules.[5,10]

In the present study, 15% carbamide peroxide gel (Opalescence PF, Ultradent Products) was used. This gel contains 3% potassium nitrate and 0.11 wt% (equal to 1100 ppm) fluoride ion. According to the manufacturer, the incorporation of fluoride ions and potassium nitrate into the structure of this gel has helped reduce the odds of caries and tooth hypersensitivity during bleaching and has improved enamel health and integrity. In addition, according to some studies, bleaching with these gels does not increase enamel susceptibility to caries or acidic erosion or increase the odds of demineralization.[21–24] However, some studies have reported significant increases in enamel surface roughness subsequent to the use of bleaching agents.[8,9] Some of the complications of applying bleaching agents in tooth hard tissues are changes in tooth chemical structure, demineralization and decreases in the mineral content including calcium and phosphate,[14,25,26] decreases in the fluoride content of enamel,[11] and changes in the micromorphology of enamel.[9,27,28] However, other studies have reported different results with no statistically significant differences in tooth hard tissue characteristics, including enamel and dentin surface roughness, after bleaching with 10% and 15% carbamide peroxide.[17,18,29–31]

The discrepancies in the results of various studies might be attributed to differences in the formulation and concentration of bleaching agents; the duration of daily applications or treatment protocols; differences in study designs, including the environments in which the specimens are stored (eg, artificial or human saliva); the use of remineralizing agents; continuous use of fluoridated toothpastes; and the technique of toothbrushing during the procedure and after bleaching.[2,14,30,32,33] In the present study, tooth structures underwent surface changes after bleaching, and toothbrushing immediately after bleaching had a substantial role in increasing enamel surface

roughness. In other words, under such conditions, bleaching might have a synergistic effect with toothbrushing, contributing to increases in enamel surface roughness. However, in our previous study, which measured enamel microhardness in the same experimental conditions, it was shown that toothbrushing immediately after bleaching has no detrimental effect on enamel microhardness.[34]

Another study has demonstrated that toothbrushing with abrasive toothpastes with or without fluoride after bleaching with 10% carbamide peroxide increases enamel surface roughness.[2] Therefore, in the present study a low-abrasive fluoridated toothpaste recommended by the bleaching agent manufacturer was used for toothbrushing in all the groups. According to some studies, despite the probable destructive role of toothbrushing, the fluoride present in toothpastes can play a balancing role between remineralization and demineralization when used daily after the bleaching procedure.[12,13] When fluoride is used, formation of calcium fluoride prevents enamel surface demineralization.[12,35,36] Because the toothbrushing regimen was the same in all the groups in the present study, improvements in enamel surface roughness cannot be attributed to the role of fluoride in the toothpaste.

Saliva can have a notable role in remineralization.[2,18] In most studies on bleaching, specimens have been stored in remineralizing solutions containing PO_4^{-3} and Ca^{+2} with concentrations similar to that of human saliva. According to some studies, artificial saliva can mimic oral saliva *in vitro* and plays a role in remineralization.[16] Considering the results of the present study, if teeth have the opportunity to be in contact with artificial saliva after bleaching and before toothbrushing, surface roughness will be comparable to that of the control group. It is likely that precipitation of minerals present in the artificial saliva on tooth surfaces can play a role in decreasing the surface roughness of bleached enamel[18]; however, further studies are necessary to evaluate the effects of different storage environments, such as artificial saliva and plain water, on changes in surface roughness of bleached enamel. In the present study the specimens were kept in artificial saliva for one or two hours before toothbrushing, which did not result in significant differences ($p \geq 0.06$).

One of the factors influencing changes in tooth surfaces during the bleaching period is the pH of the bleaching agent. More acidic pH values increase the likelihood of surface changes and demineralization.[37] The carbamide peroxide gel used in the present study had a pH value of 6.5. According to the results of several studies, two factors can neutralize this pH. The first factor is the urea produced by the breakdown of carbamide peroxide after its use, which is mainly responsible for an increase in the oral pH value to more than 8 for a few hours. The second factor is saliva, which is believed to have a role in neutralizing the acidity of the bleaching gel.[37] An *in vivo* study has shown that the low pH of bleaching agents in the first five minutes results in a decrease in the pH value of the patient's saliva. In 15 minutes, the pH value will be higher than the baseline value; this is attributed to the chemical reaction between carbamide peroxide and salivary bicarbonate ion as a result of the buffering capacity of saliva, which neutralizes the acidity of the bleaching agent.[38] Another positive role of saliva becomes evident when, similar to the present study, fluoridated bleaching agents are used. Fluoridated bleaching gels reinforce the fluoride-containing elements of tooth enamel. Although this influence is less effective than that of pure fluoride, fluoride-containing gel strengthens and restores microstructural defects on tooth surfaces by surface absorption and precipitating fluorapatite from calcium and phosphate ions of saliva.[21,39] However, further studies, especially clinical experiments, should be carried out to explore this further.

In the present study, enamel samples were smoothed and polished to allow standardized profilometric measurements with flat reference surfaces. Considering that the specimen preparation process in surface roughness tests might have influenced the results, it might not be entirely possible to extrapolate the conclusions of *in vitro* studies to the clinical setting.

Finally, it is suggested that in future studies other techniques, including microradiography and micro-computed tomography scanning, be used for more accurate evaluation of changes in the enamel surface.

CONCLUSION

Within the limitations of the present study it can be concluded that daily toothbrushing with low-abrasive fluoridated toothpaste, carried out immediately after bleaching with 15% carbamide peroxide, can increase enamel surface roughness; however, postponing the procedure for one or two hours after daily bleaching and exposing the teeth to saliva during the delay results in surface roughness comparable to that of the control group.

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Conflict of Interest Declaration

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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