

***In Vitro* Evaluation of the Effect of Delaying Toothbrushing With Toothpaste on Enamel Microhardness Subsequent to Bleaching the Teeth With 15% Carbamide Peroxide**

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Clinical Relevance

Delaying oral hygiene procedures during bleaching does not seem to cause any change in enamel microhardness.

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SUMMARY

Changes in enamel surface microhardness as a result of bleaching with carbamide peroxide in various *in vitro* conditions have been reported. The present study evaluated the effect of oral hygiene procedures on enamel microhardness at three time intervals following bleaching with 15% carbamide peroxide. Although this was an *in vitro* study, the purpose was to address whether or not a patient's toothbrushing following at-home bleaching might affect surface changes in tooth enamel. Eighty enamel slabs were prepared from impacted human third molars that had been extracted surgically.

Subsequent to placing the specimens in acrylic resin, their surfaces were smoothed, and they were randomly divided into four equal groups. The specimens were initially evaluated for microhardness by Vickers test. The bleaching procedure was carried out for 21 days for 6 hours daily. In each group, the surfaces of specimens were brushed with toothpaste immediately, 1 hour, and 2 hours after bleaching except for the control group. The specimens were stored in artificial saliva. Enamel microhardness was again measured at the end of the bleaching period. Then the differences in enamel microhardness between the two periods were calculated. Data were analyzed with a nonparametric Kruskal-Wallis test at a significance level of $p < 0.05$. The differences in the microhardness values before and after intervention between the groups were not significant ($p = 0.59$). Daily oral hygiene procedures either immediately or 1 or 2 hours after daily bleaching procedures and exposing the specimens to artificial saliva during the study period produced no significant differences in enamel microhardness values.

INTRODUCTION

One of the techniques available for whitening the teeth is "at-home vital bleaching technique," with all the steps carried out by the patients at home. Except for the fabrication of the bleaching trays, the dental practitioner has nearly no control over the procedure.

Bleaching can result in structural changes on tooth surfaces. However, there is controversy over the effect of carbamide peroxide-containing bleaching agents on enamel microhardness. Some studies have demonstrated surface deterioration, formation of defects on the surface, and porosity in electron microscope studies and decreases in enamel hardness in microhardness studies.¹⁻⁵ Daily oral hygiene procedures (toothbrushing with toothpaste) are carried out by the patient during the bleaching period, which may intensify the destructive effects of the procedure on enamel surfaces.⁶ On the other hand, saliva or other remineralizing agents can create an environment to help remineralize the teeth following bleaching.⁷⁻¹⁰ Enamel microhardness is a property that can be influenced by a combination of the above-mentioned factors.

In the present study we evaluate changes in enamel microhardness 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 microhardness is not influenced by the time the oral hygiene procedures are initiated after bleaching (immediately, and at 1-hour and 2-hour intervals after bleaching).

MATERIALS AND METHODS

In the present study the specimens were prepared from impacted human third molars that had been surgically extracted. The teeth were stored in a 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. The teeth that had any enamel surface abnormalities or had undergone cracks or fractures during surgical extraction were excluded from the study.

Subsequent to cleaning the teeth the roots were cut away at the cemento-enamel junction. Two enamel slabs measuring $6 \times 4 \times 2$ mm were prepared from the middle third of the buccal and lingual aspects with the use of bilateral diamond disks (D&Z, Berlin, Germany). Eighty enamel slabs were prepared from 40 human third molars. The slabs were checked for any cracks or fractures. Water spray was used during specimen preparation to avoid dehydrating the specimens. The specimens were stored in distilled water at 37°C after cutting. Then the slabs were placed inside cold-cured acrylic resin in a cylindrical mold with a diameter of 1.5 cm, with the enamel surface on top. Subsequent to removal of the slabs from the mold, the surface of the enamel slab was prepared to a horizontal surface to be properly placed under the device which measures the microhardness; a flat-end tapered diamond bur (Teezkavan, Tehran, Iran) was used to this end. Then the enamel surface was smoothed using white aluminum oxide stones (Dura White, Shofu Dental Corp, Kyoto, Japan) under water spray. Finally, a piece of felt cloth along with 1- and 6- μ m abrasive diamond pastes (Microdent, Sao Paulo, Brazil) was used for polishing the enamel surfaces.

The specimens were placed in an ultrasonic device containing distilled water for 10 minutes to remove polish debris. Then the 80 slabs were randomly divided into four groups of 20 specimens each, as follows:

- Group A (control): No hygiene procedures after bleaching
- Group B: Hygiene procedures immediately after bleaching

- Group C: Hygiene procedures 1 hour after bleaching
- Group D: Hygiene procedures 2 hours after bleaching

Microhardness values of all the specimens were measured and recorded before the study. In order to measure microhardness a 10-g force was applied for 30 seconds on the specimens by Vickers microhardness indenter (FM-700, Future Tech Corp, Tokyo, Japan). Microhardness of each specimen was measured at three separate locations 500 μm apart from each other and a mean was reported for each specimen.

Subsequent to measuring the initial microhardness, 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 Inc., South Jordan, UT, USA) was placed inside each tray; the tray was placed on each specimen for 6 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: CaCl_2 1.0 mM, KH_2PO_4 3.0 mM, and NaCl 100 mM; the pH was 6.30 and was adjusted with NaOH solution.⁸ After the bleaching procedure every day, the specimens were rinsed with deionized distilled water for 5 seconds. Then the subsequent steps for each group were carried out as follows:

- Group A: The specimens in this group were placed in 1 mL of artificial saliva at 37°C in an incubator for 18 hours after the bleaching and rinsing procedure.
- Group B: In this group the specimens were brushed immediately after they were bleached for 6 hours and rinsed for 5 seconds; the specimens were brushed with an electric brush (Oral-B Vitality Precision model, Oral-B Corp, Belmont, CA, USA) inside a reservoir of freshly prepared toothpaste (Opalescence whitening toothpaste, Ultradent) with one part (50 g) of toothpaste in 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 3 minutes with a typical force of 200 g. The amount of the 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 solution which was agitated before use.

The toothpaste specimen was replaced every 2 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 as in group B was repeated in this group except that after bleaching and rinsing, the specimens were kept in artificial saliva for 1 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 was the same as that in group C, but there was a time interval of 2 hours after bleaching for the brushing technique to begin.

The bleaching and cleaning procedures continued for 21 days in all the groups. At the end of this period the enamel microhardness values of the specimens were once again measured, recorded, and compared with the initial values. Data were analyzed with a nonparametric Kruskal-Wallis test. Statistical significance was defined at $p < 0.05$.

RESULTS

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

Before the study was initiated the means of microhardness values in the four groups were compared. As such, the nonparametric Kruskal-Wallis test showed that there were no significant differences in the means of microhardness values before intervention between the four groups ($p = 0.89$).

Since data were widely distributed, logarithmic transformation of the data was considered and then a nonparametric Kruskal-Wallis test was used to evaluate the differences. The nonparametric Kruskal-Wallis test did not demonstrate any significant differences in the means of microhardness values before and after intervention between the groups under study ($p = 0.59$). The linear graph and error bar of the mean differences in microhardness values before and after intervention in the groups are presented in Figure 1.

DISCUSSION

In the present *in vitro* study, an attempt was made to simulate a clinical course of an at-home bleaching procedure as exactly as possible. The procedure

Table 1: Descriptive Statistics of Mean Differences in Microhardness Values Before and After Intervention in the Groups Under Study ($\Delta MH = \text{Final Value} - \text{Baseline Value}$)

Group	Mean (ΔMH)	95% Confidence Interval		Median	Lowest Value	Highest Value
A	-8.36	-41.94	25.21	-23.66	-139.33	125.33
B	-13.58	-43.00	15.83	-9.16	-176.33	79.00
C	-18.80	-34.09	-3.50	-13.16	-95.33	20.00
D	8.00	-21.32	37.32	-0.83	-116.00	152.33

lasted 21 days, 6 hours daily, with 15% carbamide peroxide. In addition, daily routine tooth brushing was carried out with the low-abrasive fluoridated toothpaste suggested by the manufacturer after each daily bleaching procedure. Artificial saliva was used to store the specimens during the bleaching period to simulate the physiologic conditions of the oral cavity. According to the results of the present study, although the changes in the enamel microhardness after 2 hours of storage of the bleached specimens in artificial saliva before brushing had an ascending trend, differences among the groups were not statistically significant.

Some studies have reported that bleaching agents can significantly decrease microhardness.⁹⁻¹² In addition, some of the complications of the use of bleaching agents on enamel include changes in the chemical composition of teeth, changes in the mineral content of dental structures such as calcium and phosphate,¹³ changes in enamel fluoride con-

tent,¹¹ topographic changes and increase in enamel porosity, open enamel prisms, and an appearance similar to etched enamel.⁹ According to some reports, the acidity of carbamide peroxide and the presence of glycerin and Carbapol influence the physicochemical structure of teeth, factors that are believed to be responsible for tooth hypersensitivity during bleaching.¹⁴

Despite what was previously mentioned, some studies have reported no significant differences in dental hard tissue microhardness values and other properties after bleaching with 10% and 15% carbamide peroxide.¹⁵⁻¹⁸ The discrepancies in the results of various studies might be attributed to differing study designs, including different dental substrates (human vs bovine teeth); differences in microhardness testing procedures and equipment (eg, Vickers, Knoop); differences in storage conditions of the specimens between bleaching procedures (in solutions without remineralizing properties,

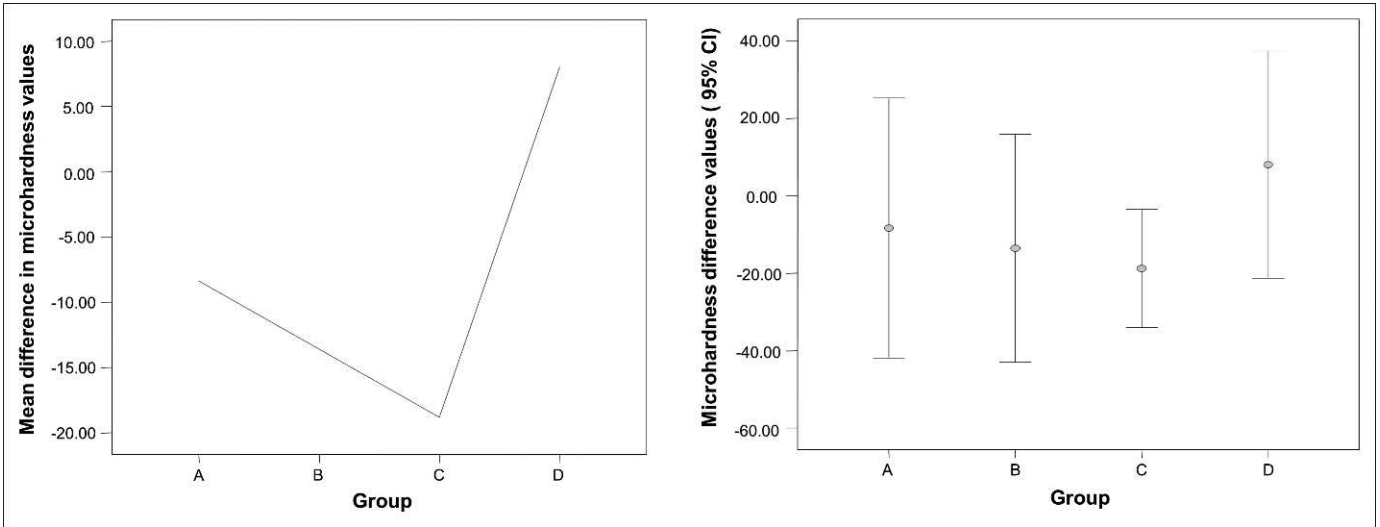


Figure 1. The linear graph (left) and error bar (right) of differences in microhardness values before and after intervention in the groups under study.

artificial saliva, or human saliva); fluoride use or lack thereof; the type of the study (*in vitro* or *in vivo*); the time of testing the specimens (immediately after daily bleaching sessions or after completion of the bleaching procedure); concentration and composition of the bleaching agent; and the duration of the study and the duration of each treatment session.^{9,10,19}

In studies in which the duration of the bleaching procedure is long, significant decreases in enamel microhardness values have generally been reported.¹¹ Another factor which contributes to a decrease in microhardness is lack of a remineralization period, including lack of specimen storage in artificial saliva or the short duration of this storage process.^{12,18} On the other hand, the effect of different concentrations of carbamide peroxide on enamel microhardness is dependent on the amount of hydrogen peroxide released, its pH level, and the proportion of bleached enamel organic and mineral content.¹¹ In the present study 15% carbamide peroxide gel (Opalescence PF) was used. This gel contains 3% potassium nitrate and 0.11 wt% (equal to 1100 ppm) fluoride ion. According to the manufacturer, incorporation of fluoride ions and potassium nitrate into the structure of this gel has aimed at reducing the odds of caries and tooth hypersensitivity during bleaching and also at improving enamel health and integrity, including improvements in its microhardness.

Changes in the mineral content of enamel surface are directly related to changes in microhardness. Remineralization increases and demineralization decreases enamel microhardness.⁹ Given the fact that in the present study no differences were observed in microhardness values of the groups, it seems the probable enamel demineralization has been compensated by factors involved in remineralization. One of the factors influencing the retention of microhardness in the present study is the use of fluoridated toothpaste in the oral hygiene procedures in all groups. According to some studies, despite the probable destructive role of toothbrushing, fluoride present in the toothpaste can create a balance between remineralization and demineralization on a daily basis after the completion of the bleaching procedure.^{9,20} On the other hand, the fluoride ion can prevent demineralization and microhardness decrease by forming a layer of calcium fluoride on the enamel surface.^{9,21,22}

Apart from the effect of fluoridated toothpaste, artificial saliva was used in the present study as an environment to store the specimens. Saliva can have a role in remineralization and can change the oral

cavity conditions after bleaching in favor of the improvements in tooth structure properties.⁶ Potential agents in the saliva which serve as remineralizing agents are calcium and phosphate ions.¹¹ In the present study, hygiene procedures in the bleached specimens after storing them in artificial saliva for 2 hours resulted in an ascending trend in microhardness, but the change was not significant. It is probable that a longer time is necessary to produce noticeable changes.

According to a review article on the effect of bleaching agents on enamel microhardness, it has been shown that in studies in which the oral cavity conditions have been simulated, including the use of human saliva, fluoride, and fluoridated toothpastes, enamel microhardness has exhibited lower decreases during the postbleaching period compared to other studies.¹⁹

Another factor in the present study, which probably led to the lack of significant differences between the groups, was the type of test used to evaluate microhardness. Vickers hardness test was used in the present study. The tip of the diamond indenter of Vickers equipment penetrates into the deeper layers of enamel, which are probably not influenced by the bleaching procedure, and the enamel hardness in these layers is probably not comparable to that of the bleached enamel.⁹ The pH of the bleaching agent, too, is another influential factor in the bleaching process. In the present study the pH of the bleaching agent was around 6.5. Considering that the critical pH for enamel demineralization is around 5.5,²³ and the pH of the agent in this study was higher than the critical pH, it is probable that this fact has influenced the lack of changes in microhardness in the present study. In a study in which the effect of Opalescence bleaching agent with a pH value of 6.5 was compared with that of Rembrandt having a pH value of 4.9 on enamel microhardness, Opalescence increased and Rembrandt decreased microhardness.¹⁷

Within the limitations of this study, postponement of daily oral hygiene procedures subsequent to bleaching with 15% carbamide peroxide does not influence enamel microhardness. It is suggested that in future studies other hardness evaluation test methods, such as Knoop, be used.

CONCLUSION

Daily oral hygiene procedures either immediately or 1 or 2 hours after daily bleaching procedures and exposing the specimens to artificial saliva during the

study period produced no significant differences in enamel microhardness values.

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REFERENCES

1. Basting RT, Rodrigues Junior AL & Serra MC (2001) The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentine *in situ* *Operative Dentistry* **26**(6) 531-539.
2. Pinto CF, Oliveira R, Cavalli V & Giannini M (2004) Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology *Brazilian Oral Research* **18**(4) 306-311.
3. Cavalli V, Arrais CA, Giannini M & Ambrosano GM (2004) High-concentrated carbamide peroxide bleaching agents effects on enamel surface *Journal of Oral Rehabilitation* **31**(2) 155-159.
4. Gultz J, Kaim J, Scherer W & Gupta H (1999) Two in-office bleaching systems: a scanning electron microscope study *Compendium of Continuing Education in Dentistry* **20**(10) 965-968.
5. McCracken MS & Haywood VB (1996) Demineralization effects of 10 percent carbamide peroxide *Journal of Dentistry* **24**(6) 395-398.
6. Worschech CC, Rodrigues JA, Martins LR & Ambrosano GM (2006) Brushing effect of abrasive dentifrices during at-home bleaching with 10% carbamide peroxide on enamel surface roughness *Journal of Contemporary Dental Practice* **7**(1) 25-34.
7. Attin T, Knöfel S, Buchalla W & Tütüncü R (2001) *In situ* evaluation of different remineralization periods to decrease brushing abrasion of demineralized enamel *Caries Research* **35**(3) 216-222.
8. Nakashima S, Yoshie M, Sano H & Bahar A (2009) Effect of a test dentifrice containing nano-sized calcium carbonate on remineralization of enamel lesions *in vitro* *Journal of Oral Science* **51**(1) 69-77.
9. Attin T, Kielbassa AM, Schwanenberg M & Hellwig E (1997) Effect of fluoride treatment on remineralization of bleached enamel *Journal of Oral Rehabilitation* **24**(4) 282-286.
10. da Costa JB & Mazur RF (2007) Effects of new formulas of bleaching gel and fluoride application on enamel microhardness: an *in vitro* study *Operative Dentistry* **32**(6) 589-594.
11. Basting RT, Rodrigues AL Jr & Serra MC (2003) The effects of seven carbamide peroxide bleaching agents on enamel microhardness over time *Journal of the American Dental Association* **134**(10) 1335-1342.
12. Attin T, Müller T, Patyk A & Lennon AM (2004) Influence of different bleaching systems on fracture toughness and hardness of enamel *Operative Dentistry* **29**(2) 188-195.
13. de Menezes M, Turssi CP, Faraoni-Romano JJ & Serra MC (2007) Susceptibility of bleached enamel and root dentin to artificially formed caries-like lesions *American Journal of Dentistry* **20**(3) 173-176.
14. de Oliveira R, Basting RT, Rodrigues JA, Rodrigues AL Jr & Serra MC (2003) Effects of a carbamide peroxide agent and desensitizing dentifrices on enamel microhardness *American Journal of Dentistry* **16**(1) 42-46.
15. Faraoni-Romano JJ, Turssi CP & Serra MC (2007) Concentration-dependent effect of bleaching agents on microhardness and roughness of enamel and dentin *American Journal of Dentistry* **20**(1) 31-34.
16. Murchison DF, Charlton DG & Moore BK (1992) Carbamide peroxide bleaching: effects on enamel surface hardness and bonding *Operative Dentistry* **17**(5) 181-185.
17. Rodrigues JA, Basting RT, Serra MC & Rodrigues AL Jr (2001) Effects of 10% carbamide peroxide bleaching materials on enamel microhardness *American Journal of Dentistry* **14**(2) 67-71.
18. Justino LM, Tames DR & Demarco FF (2004) *In situ* and *in vitro* effects of bleaching with carbamide peroxide on human enamel *Operative Dentistry* **29**(2) 219-225.
19. Attin T, Schmidlin PR, Wegehaupt F & Wiegand A (2009) Influence of study design on the impact of bleaching agents on dental enamel microhardness: a review *Dental Materials* **25**(2) 143-157.
20. Bizhang M, Seemann R, Duve G, Römhild G, Altenburger JM, Jahn KR & Zimmer S (2006) Demineralization effects of 2 bleaching procedures on enamel surface with and without post-treatment fluoride application *Operative Dentistry* **31**(6) 705-709.
21. Featherstone JD, Cutress TW, Rodgers BE & Dennison PJ (1982) Remineralization of artificial caries-like lesions *in vivo* by a self-administered mouthrinse or paste *Caries Research* **16**(3) 235-242.
22. TenCate JM & Arends J (1977) Remineralization of artificial enamel lesions *in vitro* *Caries Research* **11**(5) 277-286.
23. Larsen MJ & Pearce EI (2003) Saturation of human saliva with respect to calcium salts *Archives of Oral Biology* **48**(4) 317-322.