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Effect of Short- and Long-term Use of Home and In-Office Bleaching with Carbamide Peroxide and Hydrogen Peroxide on Enamel Microhardness

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ORIGINAL ARTICLE

Effect of Short- and Long-term Use of Home and In-Office Bleaching with Carbamide Peroxide and Hydrogen Peroxide on Enamel Microhardness

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ABSTRACT

Bleaching is a well-known esthetic dental treatment performed to lighten the tooth shade. Among different cosmetic dental procedures, including direct and indirect tooth-colored restorations, bleaching is likely the most conservative approach. Bleaching has been accepted well by patients and proven as a safe and effective approach.

Objective: Tooth bleaching can be conducted at home or in offices. This study was performed to compare the enamel microhardness before bleaching and one and eight weeks after the application of different bleaching agents.

Methods: Forty human premolars were randomly divided into four groups, and their buccal surface underwent a Vickers microhardness test before and after bleaching. Bleaching was performed with 40% hydrogen peroxide (HP) in-office bleaching agent (n = 10), 15% HP at-home bleaching agent (n = 10), and 15% carbamide peroxide (CP) at-home bleaching agent (n = 10) for two weeks. Ten samples were stored in artificial saliva and set as the control group. The microhardness of the samples was measured again one and eight weeks after bleaching. **Results:** The microhardness significantly decreased in the four groups one week after bleaching. **Conclusion:** The enamel microhardness was temporarily decreased by in-office bleaching. However, the microhardness increased with time and returned to the baseline value. Although the enamel microhardness initially reduced, it increased eight weeks after 15% CP at-home bleaching agent was applied.

Key words: bleaching, carbamide peroxide, hydrogen peroxide, microhardness

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INTRODUCTION

Bleaching is a well-known esthetic dental treatment for lightening the tooth shade. Among different cosmetic dental procedures, including direct and indirect tooth-colored restorations, bleaching is likely the most conservative approach that has been accepted well by patients and proven safe and effective.^{1,2} Bleaching can be performed at home, in offices, or both for vital teeth.^{3,4}

In in-office bleaching, a high concentration of bleaching agent is used to shorten the treatment time; conversely, in at-home bleaching, bleaching gels with a low concentration are used for a longer treatment period, e.g., 4–8 h a day for two weeks or longer.⁵ Although at-home bleaching is the most commonly used technique to bleach vital teeth, some patients are not interested in its daily application in a tray for a couple of weeks and even prefer in-office bleaching, which yields results faster.^{3,6} Although the optimal

efficacy of bleaching agents has been well documented in vital and nonvital teeth, some concerns are associated with the extensive use of bleaching techniques and their effects on bleached surfaces.⁷

Tooth whitening is defined as any process that lightens the color of a tooth. It can be accomplished by physically removing stains or stimulating a chemical reaction to lighten the tooth color. The active component of most whitening products is hydrogen peroxide (H_2O_2), which may be delivered in the form of hydrogen peroxide (HP) or carbamide peroxide (CP). CP is a stable complex that breaks down when it comes in contact with water to release HP. The chemical component of most tooth whitening agents is HP because CP releases HP. They are the most commonly used bleaching agents, whose mechanism of action is based on their oxidation capacity on the molecules of pigments responsible for discoloration.^{8,9} Aside from favorable esthetic results, unfavorable biological reactions can be triggered by free radicals.¹⁰ Some unfavorable effects, such as tooth hypersensitivity, and structural enamel changes, such as alteration in microhardness and roughness, have also been reported.¹⁰

The microhardness of the tooth structure is important because the hardness number indicates the strength of the tooth structure and its susceptibility to wear or cause wear in the opposing teeth. Thus, any reduction in hardness due to chemical reactions that may occur after the application of bleaching agents can adversely affect the longevity and clinical service of the teeth.¹¹ Hardness refers to the resistance of a solid hard object against permanent deformation after the application of compressive load, which is expressed by the hardness number. The lower the hardness number is, the softer the material will be. The hardness of materials is measured by several methods, including the Rockwell scale, the Brinell scale, the Knoop hardness test, and the Vickers hardness test.¹² The Vickers hardness test is suitable for the small-scale measurement of the hardness of brittle substances and dental materials.^{13,14}

The information about the effects of different bleaching agents on dental hard tissue is limited. Some observations have failed to show a significant change in the mechanical properties of the enamel after different concentrations of bleaching agents are applied.¹⁵ Other studies have reported that microhardness increases after bleaching.^{16,17} Furthermore, the application of different CP or HP concentrations causes a reduction in the enamel microhardness.^{18,19} A recent systematic review concluded that at-home and in-office bleaching do not significantly vary in terms of the risk of postoperative tooth hypersensitivity or treatment outcome. However, this comparison does not consider the variations in application protocols (daily use, number of bleaching sessions, and concentration of product).³ Thus, the present study aims to assess the enamel microhardness

of human permanent teeth after in-office and at-home bleaching agents are applied.

METHODS

In this *in vitro* experimental study, 40 sound human premolars extracted for orthodontic treatment were selected, stored in artificial saliva, and collected by random allocation. They were placed in a container and randomly selected. Four groups were prepared with 10 randomly selected samples in each group. The buccal surface of all the teeth was free from caries or cracks, and the debris and gingival tissue residues were removed. The roots were cut, and the samples were stored in artificial saliva until the hardness test was conducted. The teeth were longitudinally sectioned into buccal and lingual halves by using a diamond disc (Horico-horico-PFINGST, New Jersey, USA), diced into small rectangular samples, and mounted in acrylic resin such that their surface was parallel to the ground. This procedure was conducted to enhance the conduction of the microhardness test. The microhardness of the samples was measured under the light microscope of a Vickers microhardness tester (Zwick Roell) at 40× magnification by applying 10 g of load for 15 seconds. The applied load created a diamond-shape indentation on the enamel surface. The two diameters of this indentation were measured, and the mean value indicating the Vickers hardness number was calculated. For each sample, each test was repeated three times before bleaching, one week after bleaching, and eight weeks after bleaching.

The ingredients of artificial saliva (pH of 7.2) were as follows: 10 g/L CMC (sodium salt), 0.052 g/L $MgCl_2$, 1.2 g/L KCl, 0.146 g/L $CaCl_2$, 0.844 g/L NaCl, 0.342 g/L (K_2HPO_4), and 30 g/L sorbitol. The teeth in the four groups underwent the following interventions. In group 1, the samples were bleached with in-office 40% HP (Opalescence Boost) for 20 min in accordance with the manufacturer's instructions. This procedure was repeated thrice. In group 2, the samples were bleached with at-home 15% HP (15% Opalescence) in accordance with the manufacturer's instructions. It was applied to the samples for 15–20 min, and this procedure was repeated for two weeks. During the time interval between the bleaching procedures, the teeth were stored in artificial saliva. In Group 3, the samples were bleached with at-home 15% CP (Opalescence PF) in accordance with the manufacturer's instructions. It was applied for 4–6 h daily and repeated for two weeks. In Group 4, which served as the control group, the samples were stored in artificial saliva without intervention. The microhardness of the samples was measured again after one and eight weeks.

Data were analyzed using the Shapiro–Wilk, Kruskal–Wallis, Wilcoxon, and multiple comparison tests via SPSS version 23 at 5% significance level.

RESULTS

Table 1 shows the mean, standard error, median, and interquartile range of microhardness of the samples in the four groups before and after bleaching. Bleaching with in-office 40% HP (P = 0.005), at-home 15% HP (P = 0.005), and at-home 15% CP (P = 0.013) significantly decreased the enamel microhardness.

The mean (± std. error) reduction was 60.4±11.7 in the in-office 40% HP group, 35.4±8.3 in the at-home 15% HP, 11.7±4.6 in the at-home 15% CP, and 1.2±2.8 in the control group. The reduction in microhardness was significantly different among the four groups (P < 0.001). In particular, the reduction in microhardness in the in-office 40% HP group was higher than that in the at-home 15% HP (P = 0.021) and control (P < 0.001) groups, but they did not significantly differ from that in the at-home HP group (P = 1.00). The reduction in microhardness in the at-home 15% HP group and the at-home 15% CP group was not significantly different (P = 0.306). The reduction in microhardness in the 15% HP group was significantly different from that in the control group (P = 0.004). However, the 15% CP group did not significantly vary from the control group (P = 0.908). The difference in microhardness was not significant before and after bleaching in the control group (P = 0.355). However, this change was significant in the other groups (P < 0.05). The baseline

microhardness of the groups before bleaching was not significantly different (P = 0.41).

Table 2 shows the mean, standard error, median, and interquartile range of enamel microhardness eight weeks after bleaching compared with that at baseline and one week after bleaching. The increase in enamel microhardness after one week was significantly different among the four groups (P < 0.001). In particular, the increase in the in-office 40% HP (P < 0.001) and at-home 15% HP groups was greater than that in the control group. However, this increase in the control group did not significantly differ from that in the at-home 15% CP group (P = 0.171). The increase in the enamel microhardness the in-office 40% HP group significantly varied from that in the at-home 15% CP group (P = 0.003).

The increase in the enamel microhardness was not significantly different from that at baseline in the control (P = 0.5), in-office 40% HP (P = 0.799), and at-home 15% HP (P = 0.139) groups. However, enamel microhardness significantly changed in the at-home 15% CP group (P = 0.021).

In Table 2, the increase in microhardness in the control group eight weeks after bleaching was not significant compared with that one week after bleaching; however, this increase was significant in the other groups (P < 0.05).

Table 1. Mean, standard error (SE), median, and interquartile (IQR; gF/mm²) of microhardness before and after tooth bleaching in the groups

Type of bleaching agent	Microhardness at Baseline				Microhardness after 1 week				Change in microhardness after 1 week				P
	Mean	SE	Median	IQR	Mean	SE	Median	IQR	Mean	SE	Median	IQR	
15% HP	278.3	27.1	268.3	143.1	242.9	23.4	220.8	101	35.4	8.3	36.7	36.1	0.005
15% CP	294.3	32.0	290.5	185.7	282.5	30.7	282.5	172.1	11.7	4.6	8.2	13.2	0.013
40% HP	335.6	32.1	361	127.2	275.2	27.2	298	70.4	60.4	11.7	36.5	60.8	0.005
Control	276.8	28.9	262.8	181.7	275.6	29.0	261	183.2	1.2	2.8	0.7	3.2	0.355
P	0.410				0.603				<0.001				

Table 2. Increase in enamel microhardness in the groups eight weeks after bleaching compared with baseline and one week after bleaching

Bleaching agent	Increase in enamel microhardness after 8 weeks compared with that at baseline				P	Increase in enamel microhardness after 8 weeks compared with that after 1 week				P
	Mean	SE ^a	Median	IQR ^b		Mean	SE ^a	Median	IQR ^b	
15% HP	6.13	9.41	6.33	18.33	0.139	41.50	26.43	44.67	45.09	0.005
15% CP	5.20	6.64	3.33	8.83	0.021	16.93	13.76	15.67	14.67	0.007
40% HP	3.43	10.13	0.5-	17.08	0.799	63.80	33.28	54.84	58.50	0.005
Control	0.17	14.75	2.50	16.59	0.5	1.30	16.53	2.00	21.5	0.646
P	0.646				-	0.001 >				-

^aStandard Error ^bInterquartile Range

DISCUSSION

This study assessed the enamel microhardness of human permanent teeth before bleaching and one and eight weeks after the application of in-office and at-home bleaching agents. We found that the reduction in microhardness one week after bleaching significantly differed among the four groups ($P < 0.001$). In-office or at-home bleaching with HP could temporarily decrease the enamel microhardness; however, the enamel microhardness increased with time after the teeth were immersed in a medium containing calcium and phosphorous and reached the baseline state after eight weeks. The results showed that at-home bleaching with CP caused an increase in the enamel microhardness eight weeks after bleaching compared with that at baseline.

The passage of time and the presence of natural or artificial saliva can lead to enamel remineralization and positively affect the enamel microhardness. These observations can be attributed to the reduction in enamel microhardness because of changes in the mineral composition of the tooth structure, which can be repaired over time after it is placed in an environment that can replace the lost minerals. This phenomenon can increase the enamel microhardness to the normal baseline state even after two weeks.^{20,21} Similarly, Chen et al. stated that the superficial enamel demineralizes after the application of bleaching agents, so the microhardness decreases. However, after 7 days, the calcium-to-phosphorous ratio and the enamel microhardness gradually increase, and the microhardness of the teeth before bleaching does not differ from that 14 days after bleaching.²² Our study showed that the enamel microhardness decreased after the bleaching agents were applied and then increased after 7 days. After eight weeks, the remineralization of the tooth surface eventually resulted in the return of microhardness to the baseline value. Remineralization occurred and subsequently increased the microhardness because of the presence of calcium and phosphorous in artificial saliva; at first, the reduction in microhardness immediately and one week after the application of all three bleaching agents was significant.

Over time, immersing the teeth in artificial saliva resulted in the return of microhardness to the normal level in 40% HP and 15% HP groups. The microhardness also increased even after eight weeks in the 15% CP group. The difference between the change in the enamel microhardness after the application of CP and HP could be explained by various mechanisms of action because CP breaks down into urea and hydrogen peroxide. Urea has an alkaline pH and provides a suitable environment for the further remineralization of the teeth. It also increases the uptake of calcium and phosphorous and enhances the surface microhardness.

Furlan et al. stated that the composition, concentration, and method of bleaching agent application affect the enamel microhardness and showed that enamel microhardness decreases over time regardless of the presence or absence of fluoride or calcium in a bleaching agent.²³ However, they assessed the microhardness two weeks after bleaching, so the time for remineralization by calcium and fluoride is probably insufficient. Attin et al. noted in their review article a reduction in microhardness after bleaching in 51% of the cases ($n = 84$), but they found no reduction in 49% ($n = 82$). In regular follow-ups after bleaching, the microhardness was reduced in 29% ($n = 20$) but did not decrease in 71% ($n = 49$). They also added that microhardness can be affected by several parameters, such as type of material and technique of bleaching, human or animal study, in vitro or in vivo design, concentration of bleaching agent, method of measuring the microhardness, duration of bleaching, time lapse after bleaching, storage medium of teeth, and use or no use of fluoride. They concluded that the risk of reduction in microhardness is lower in studies that better simulated oral clinical settings.²⁴

The authors explained that results can also be influenced by the presence/absence of the control teeth, the assessment of the buccal or lingual surface, the type of tooth (incisor, premolar, or molar), the method of tooth preparation, and the type of microhardness tester. The absence of a control group can also affect the final result because of the differences in the enamel microhardness of the teeth. The enamel thickness varies in different surfaces of a tooth and in different teeth, thereby affecting the microhardness test results. Furthermore, the accuracy of measurements and final results can be influenced by the type of tooth preparation and the accuracy of devices.²⁴

We found that a high HP concentration would result in a significantly greater degree of decrease in the enamel microhardness after one week and a lower degree of the subsequent increase after eight weeks. Similarly, Imani et al. reported that a high percentage of HP results in a higher level of reduction in physical properties such as shear bond strength.²⁵ Moreover, Lilaj et al. reported that a high concentration of HP induces cytotoxicity and suggested that a low concentration of HP is effective and likely to cause less damaging effects on the enamel and tested cells.²⁶ Other studies have shown that the application of bleaching agents decreases enamel microhardness. The difference between their results and ours might be due to various factors, such as the storage of teeth in 100% humidity or their long-term exposure to bleaching agents.^{27,28} Conversely, some studies have demonstrated that the application of bleaching agents does not increase or decrease enamel microhardness possibly because of different factors, such as variations in pH and composition of bleaching

agents or artificial saliva^{29,30} for the storage of teeth. The preparation of premolar teeth for the microhardness test was difficult because of the need to make the buccal surface of the teeth parallel to the ground; thus, the test should be performed on flat surfaces, such as incisors.

CONCLUSION

The short-term application of HP (15% and 40%) and CP would result in a significant reduction in enamel microhardness after eight weeks of keeping the teeth in a solution containing calcium and phosphorus. Microhardness would increase, and this increase was more significant in the CP group. Thus, CP could be recommended as the preferred bleaching agent.

CONFLICT OF INTEREST

The authors of this article declared no conflict of interests related to the study.

REFERENCES

1. Meireles SS, Fontes ST, Coimbra LA, Della Bona A, Demarco FF. Effectiveness of different carbamide peroxide concentrations used for tooth bleaching: an in vitro study. *J Appl Oral Sci: Revista FOB*. 2012; 20(2):186–91.
2. Sun L, Liang S, Sa Y, Wang Z, Ma X, Jiang T, et al. Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide. *J Dent*. 2011; 39(10):686–92.
3. de Geus JL, Wambier LM, Kossatz S, Loguercio AD, Reis A. At-home vs in-office bleaching: a systematic review and meta-analysis. *Oper Dent*. 2016; 41:341–56.
4. Mushashe AM, Coelho BS, Garcia PP, Rechia BN, da Cunha LF, Correr GM, et al. Effect of different bleaching protocols on whitening efficiency and enamel superficial microhardness. *J Clin Exp Dent* 2018; 10(8):e772–5.
5. Ghanbarzadeh M, Ahrari F, Akbari M, Hamzei H. Microhardness of demineralized enamel following home bleaching and laser-assisted in office bleaching. *J Clin Exp Dent*. 2015; 7(3):e405–9.
6. Gallagher A, Maggio B, Bowman J, Borden L, Mason S, Felix H. Clinical study to compare two in-office (chairside) whitening systems. *J Clin Dent*. 2003; 13(6):219–24.
7. Giannini M, Silva AP, Cavalli V, Paes Leme AF. Effect of carbamide peroxide-based bleaching agents containing fluoride or calcium on tensile strength of human enamel. *J Appl Oral Sci: Revista FOB*. 2006; 14(2):82–7.
8. Faus-Matoses V, Palau-Martinez I, Amengual-Lorenzo J, Faus-Matoses I, Faus-Liacer VJ. Bleaching in vital teeth: combined treatment vs in-office treatment. *J Clin Exp Dent*. 2019; 11(8):e754–8
9. Carey CM. Tooth whitening: what we now know. *J Evid Based Dent Pract*. 2014;14 suppl 70–6.
10. Dantas AA, Bortolatto JF, Roncolato A, Merchan H, Floros MC, Kuga MC, et al. Can a bleaching toothpaste containing Blue Covarine demonstrate the same bleaching as conventional techniques? An in vitro, randomized and blinded study. *J Appl Oral Sci: Revista FOB*. 2015; 23(6):609–13.
11. Khamverdi Z, Kasraee S. Invitro comparison between the effects of two tooth bleaching agents on surface micro hardness of microhybrid composite. *Eur J Dent*. 2007; 31(1–2):37–46.
12. Yost F. On the definition of microhardness. *Metall Mater Trans A*. 1983; 14(4):947–52.
13. Lewinstein I, Rotstein I. Effect of trichloroacetic acid on the microhardness and surface morphology of human dentin and enamel. *Endod Dent Traumatol*. 1992; 8(1):16–20.
14. Lewinstein I, Ofek L, Gedalia I. Enamel rehardening by soft cheeses. *Am J Dent*. 1993; 6(1):46–8.
15. Polydorou O, Scheitza S, Spraul M, Vach K, Hellwig E. The effect of long-term use of tooth bleaching products on the human enamel surface. *Odontology*. 2018; 106(1):64–72.
16. Cakir FY, Korkmaz Y, Firat E, Oztas SS, Gurgan S. Chemical analysis of enamel and dentin following the application of three different at-home bleaching systems. *Oper Dent*. 2011; 36(5):529–36.
17. Toteda M, Philpotts CJ, Cox TF, Joiner A. Evaluation of a 6% hydrogen peroxide tooth-whitening gel on enamel microhardness after extended use. *Quintessence Int. (Berlin, Germany: 1985)*. 2008; 39(10):853–8.
18. Elfallah HM, Bertassoni LE, Charadram N, Rathsam C, Swain MV. Effect of tooth bleaching agents on protein content and mechanical properties of dental enamel. *Acta Biomater*. 2015; 20:120–8.
19. Vilhena KFB, Nogueira BCL, Fagundes NCF, Loretto SC, Angelica RS, Lima RR, et al. Dental enamel bleached for a prolonged and excessive time: Morphological changes. *PLoS One*. 2019; 14(4):e0214948.
20. Kutuk ZB, Ergin ECakir FY, Gurgan S. Effects of in-office bleaching agent combined with different desensitizing agents on enamel. *J Appl Oral Sci*. 2018; 27:e20180233.
21. Torres C, Zanatta RF, Silva TJ, Borges AB. Effect of calcium and fluoride addition to hydrogen peroxide bleaching gel on tooth diffusion, color, and microhardness. *Oper Dent*. 2019; 44(4):424–32.

22. Chen X, Chen Z, Lin Y, Shao J, Yin L. Effects of tooth whitening agents and acidic drinks on the surface properties of dental enamel. *Hua xi kouqiangyixue za zhi*. 2013; 31(5):530–2.
23. Furlan IS, Bridi EC, Amaral F, Franca FMG, Turssi CP, Basting RT. Effect of high- or low-concentration bleaching agents containing calcium and/or fluoride on enamel microhardness. *Gen Dent*. 2017; 65(3):66–70.
24. Attin T, Schmidlin PR, Wegehaupt F, Wiegand A. Influence of study design on the impact of bleaching agents on dental enamel microhardness: a review. *Dent Mater*. 2009; 25(2):143–57.
25. Imani MM, Azizi F, Bharami K, Golshah A, Safari-Faramani R. In vitro bleaching effect of hydrogen peroxide with different time of exposition and concentration on shear bond strength of orthodontic brackets to human enamel: a meta-analysis of in vitro studies. *Int Orthod*. 2020; 18:22–31.
26. Lilaj B, Dauti R, Agis H, Schmid-Schwab M, Franz A, Kanz F, et al. Comparison of bleaching products with up to 6% and with more than 6% hydrogen peroxide: whitening efficacy using BI and WI_D and side effects- An in vitro study. *Front Physiol*. 2019; 10:919.
27. Lewinstein I, Fuhrer N, Churaru N, Cardash H. Effect of different peroxide bleaching regimens and subsequent fluoridation on the hardness of human enamel and dentin. *J Prosthet Dent*. 2004; 92:337–42.
28. Al-Salehi SK, Wood DJ, Hatton PV. The effect of 24h non-stop hydrogen peroxide concentration on bovine enamel and dentine mineral content and microhardness. *J Dent*. 2007; 35:45–50.
29. Ulukapi H. Effect of different bleaching techniques on enamel surface microhardness. *Quintessence Int* 2007; 38:e201–5.
30. Joiner A, Thakker G, Cooper Y. Evaluation of a 6% hydrogen peroxide tooth whitening gel on enamel and dentine microhardness in vitro. *J Dent* 2004; 32:27–34.

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