

Original Research Article

The Effects of remineralizing agents on Microhardness of Bleached Enamel with 40% Hydrogen Peroxide-An Invitro Study

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Abstract: Teeth whitening have been accepted as one of the most conservative treatment methods of discolored teeth; however, these materials have also some complications for teeth. The aim of this study was to investigate the effects of remineralizing agents on microhardness of bleached enamel with 40% hydrogen peroxide. Twenty extracted intact human anterior teeth were selected. As the initial preparation of the teeth, they were divided into two groups randomly. Primary microhardness of enamel was measured by Vickers test. Then both groups separately were bleached with 40% hydrogen peroxide, and microhardness was measured again. In the next step, first group was stored in artificial saliva (AS) and CPP-ACP was used in second group. The microhardness was measured again. The data was analyzed with Paired T- test at a significant level of $P < 0.05$. In both groups, enamel microhardness was decreased after bleaching, however, this reduction was not significant ($P = 0.3$ in the AS group, $P = 0.059$ in the CPP-ACP groups). As well as, the artificial saliva ($P = 0.05$) and CPP-ACP ($P = 0.049$) increased the enamel microhardness after bleaching. Based on this study, artificial saliva and CPP-ACP can increase microhardness of the enamel.

Keywords: Hydrogen peroxide- Microhardness - CPP_ACP

INTRODUCTION:

Smiling is one of the most important factors in social relationships [1]. White teeth increase self-confidence and improve social eminence of people [2]. Hence, now a days, patients' demands for esthetic dentistry treatments have been increased [1]. There are different ways to achieve this goal, including: composite restorations, ceramic crowns or laminates. However, these treatments can cause loss of tooth structure and change the normal contour of the tooth. Tooth bleaching treatments are easier, more acceptable, economical and more conservative in modifying the dental discolorations [3, 4]. There are three basic methods for whitening of vital teeth: In office, At home and bleaching with over the counter products (OTC). Bleaching can be performed with different materials such as hydrogen peroxide, carbamide peroxide and sodium perborate which these materials can be used with different concentrations [5]. These materials diffuse into the organic matrix of tooth

structure due to low molecular weight [1 & 6]. The attack of hydrogen peroxide in organic matrix leads to breaking of the long organic chains into colorless short chains during the oxidation process [1].

Some studies reported the complications of bleaching agents including: Changes in the chemical composition of teeth, changes in the mineral content of dental structures such as calcium and phosphate, changes in enamel fluoride content, topographic changes, increase in enamel porosity, opening enamel prisms, some effects similar to etched enamel [3, 7, 8], reducing the enamel microhardness [6, 9, 10], opening the dentinal tubules and increasing dentin sensitivity [6], as well as, in some studies, changes in the morphology and composition of the enamel were observed, which were also not limited to the surface and they can be seen in the subsurface layers of enamel [8]. Moreover, some studies have shown that the use of (CPP-ACP) in combination with hydrogen peroxide can

reduce side effects of bleaching treatment and increase the hardness of enamel [2, 6, 9, 11, 12]. The role of CPP-ACP was explained in localizing the ACP on the tooth surface, that absorbs the free calcium and phosphate [13] and in reducing the demineralization and increasing the remineralization in super saturation state [6, 14, 15]. In addition, the presence of CPP-ACP can lead to the rapid accumulation of free calcium and remineralization in the enamel substrates [15]. Some studies have shown that CPP-ACP significantly enhances calcium and inorganic phosphate in plaque and the CPP bonds with salivary pellicles and surface bacteria in plaque biofilm [11, 16]. As well as, a study, in which the enamel specimens treated with CCP-ACP topical paste showed smoother surfaces compare to the use of fluoride dentifrice [17]. On the other hand, there is some disagreement about the use of CPP-ACP after bleaching [1, 2, 9, 11, 12, 18]. Hence, this experimental study was carried out to evaluate the effects of remineralizing agents on microhardness of bleached enamel with 40% hydrogen peroxide.

MATERIAL AND METHODS:

Twenty extracted intact human anterior teeth were selected based on inclusion and exclusion criteria. They were extracted due to the orthodontics, periodontal diseases or missing reasons, so that they were without any cracks, caries, filling and buccal, lingual, incisal wears and without discolorations with internal or external origins of congenital imperfections such as fluorosis in visual examination. The teeth were washed thoroughly after being extracted and were kept in distilled water at room temperature until the tests. The specimens were cleaned by scaling instrument and rubber cap and fluoride-free pumice powder. Teeth were mounted in the self-curing acrylic resin (Acropars, Iran), so that the lingual side of the teeth was into the acryl and the labial side was out of it. The crowns of teeth were polished with diamond polishing disks (Brazil-FGM). Each disk was used for five cases.

The specimens were divided into two groups randomly. The microhardness of baselines was measured by Vickers Hardness Testing Machine, in such that, the area of load entering was the central part of the buccal surface of the tooth. The load was 100 gram per 10 seconds. Three Indents of microhardness were measured in each specimen and the average indent was recorded.

Both groups were treated with 40% hydrogen peroxide (Opalescence Boost / ultra-dent / USA) separately according to the manufacturer's instructions. The buccal surface of each tooth was covered with this material with thickness of 2 mm for 15 minutes separately and then was cleaned with cotton and this stage was repeated twice, each for 15 - 45 minutes. The gel was removed from the teeth by cotton and washed with distilled water. At this stage, enamel microhardness was measured again, in the same conditions. In the next step, the first group was stored in artificial saliva and CPP-ACP MI Paste plus (GC / Tokyo / Japan) was used for the second group for 4 hours, in both of them. Then the microhardness of both groups was measured again. SPSS 20 was used to analyze the data and Paired-T test was used to compare the mean microhardness of enamel in the AS and CPP-ACP groups, before and after bleaching and after expose to CPP-ACP and AS.

RESULTS:

This study aimed to evaluate the effect of remineralizing agents on microhardness of bleached enamel with 40% hydrogen peroxide 40%. According to the results presented in Table 1, microhardness of enamel (before bleaching) in the AS group was 400.1 ± 47.3 and it was 366.5 ± 65 after bleaching, in fact, comparing of the microhardness of both groups was not significant ($P= 0.3$). As well as, the average of primary microhardness in CPP-ACP group was 351.7 ± 41.9 and it was 272.6 ± 99.7 after bleaching.

Table-1: Comparison of enamel microhardness in group, before and after bleaching

Micro Hardness Group	Before bleaching	After Bleaching	P-Value
	Mean±SD	Mean±SD	
Artificial Saliva (control group)	400.1 ± 47.3	366.5 ± 65	0.3
CPP-ACP (Experimental group)	272.6 ± 99.7	351.7 ± 41.9	0.059

Then the mean microhardness after bleaching and after immersion in remineralized solutions on the basis of $P \leq 0.05$ were compared (Table II).

Table-2: Comparison of enamel microhardness in group, after bleaching and expose to remineralizing material

Group	Microhardness	After Bleaching	Remineralized Material	P-Value
		Mean±SD	Mean±SD	
Artificial Saliva (control Group)		366.5 ± 65	398 ± 53	0.05
CPP-ACP (Experimental Group)		272.6 ± 99.7	359.2 ± 81.1	0.059

According to the results presented in Table II, the average microhardness of enamel in the AS group after bleaching was 366.5 ± 65 and it was 398 ± 53 after immersion in the re-mineralized material, which the comparison of microhardness in two stages of both groups showed considerable difference ($P = 0.05$). Moreover, the average of enamel microhardness after bleaching treatments was 272.6 ± 99.7 , and it was 359.2 ± 81.1 after exposure to the remineralized material, which comparison of two stages of both groups showed significant difference ($P = 0.049$).

DISCUSSION:

In this study, the mean microhardness of the enamel in the artificial saliva was 400.1 ± 47.3 and it was 351.7 ± 41.9 in CPP-ACP. Difference in the initial microhardness is resulted from different levels of enamel mineralization with local variations of enamel tissue and enhancement in the porosity near DEJ area. In addition, the microhardness is reduced from the outer surface to the DEJ [11]. As well as, the microhardness of enamel is different in other studies, so that consistency in measuring technique scan is referred along with pre-mentioned factors. The use of Vickers hardness test or knoop hardness, the amount and duration of load in enamel microhardness measurement, microhardness measurement of surface or subsurface enamel, using of human or bovine teeth specimens, anterior or posterior teeth, can cause discrepancies in these studies [4]. In the present work, microhardness of human teeth was measured by Vickers test, under the force of 100 g per 10 seconds. As well as the factors such as environmental reasons, the fluoridation of drinking water, dental age and different habits in some countries could also affect the primary microhardness of enamel [19]. Results showed that the use of 40% hydrogen peroxide reduces the enamel microhardness however it was not significant (control group $P = 0.3$ and experimental group $P = 0.059$). These results were consistent with the results of Shannon *et al.*; (CP carbamide peroxide 10%) [20], Seghi *et al.*;

(CP 10%) [21], Cadenaro *et al.*; (HP 38%) [22], Suleiman *et al.*; (HP 35%) [23], Smidt *et al.*; (CP 15-16%) [24], and Davari *et al.*; study [2] (10 and 22% CP). While, it was inconsistent with the results of Soldani *et al.* study [25], study of Basting *et al.*; [26], Efeoglua *et al.*; [27], Tezel *et al.*; [28] and a part of Borges study [9]. It should be noted that in our study enamel microhardness was decreased, but not significantly, in other words, enamel mineral content was somewhat decreased, because hydrogen peroxide had oxidation effect on enamel organic matrix [5,11], however, the presence of fluoride and desensitizing agents in bleaching materials, compensate the reduction of enamel mineral contents(5). So that Potocnik *et al.*; [19] showed that the application of 10% carbamide peroxide causes micro structural and mineral content changes in enamel while it is ineffective on enamel hardness, it's probably the same thing which has been happened in this study. On the other hand, the presence of 1.1% fluoride and 3% potassium nitrate (desensitizing agents) in combination with this bleaching material and PH of 7, cause to balance the side effects. Hence, based on the study of Attin *et al.*; [29].

The reasons for conflicting with other studies include as the following: different bleaching materials, differences in PH level [24], the frequency and duration of the bleaching material usage, oxidative effects of bleaching agents, the amount of bleaching thickeners for stability of the material [30], the presence of fluoride and desensitizing agents [31], the variation in the morphology of the enamel [22], different experimental tests and dental specimens (human / bovine) [4] and the less number of specimens [2].

The results showed that the use of the CPP-ACP and artificial saliva after bleaching of teeth, lead to increase the enamel microhardness, which was significant (AS $P = 0.05$ and CPP-ACP $p = 0.049$). These results were consistent with the results of

Vasconcelos *et al.*; [12], Borges *et al.*; [9], Cai *et al.*; [13], Burlamaqui Pinheiro *et al.*; [6], Oshiro *et al.*; [14], Borges *et al.*; [10] and Panich *et al.*; [11] studies. Since, CPP includes Ser-Ser-Ser-Glu-Glu amino acid clusters which stabilize the ACP in solution with low stability, hence, CPP results in progressing toward the core and promoting of the transformation phase toward the remineralization by capturing the ACP [32]. However, the results of this study were inconsistent with the results of Davari *et al.*; study [2]. So that in the study of Davari, using Tooth mousse (a kind of CPP-ACP) after bleaching with 22% carbamide peroxide causes a reduction in microhardness and no changes in microhardness after bleaching with 10% carbamide peroxide, due to presence of 0.3- 0.2 % phosphoric acid in Tooth mousse.

Besides in the study of Davari, the use of MI paste after bleaching with 22% and 10 % carbamide peroxide was ineffective. The reasons for the different results, can be related to the different time, load and bleaching material. While the changes of the microhardness after bleaching was not significant for both works. In this study, 40% HP was used and Davari used 10 % and 22% CP, respectively. Davari used 500 gr forces per 15 seconds (g/s) and evaluated the microhardness of sub surface. Results showed that the areas between porous enamel prisms resulted from bleaching materials and inside of them were filled by minerals which led to an increased hardness. So that the absorption of minerals in the bleached tooth is more, compared to unbleached tooth. In the study of Davari, the porosity resulted from combining of CP due to the presence of low peroxide, was probably less than of that in this study (with 40% HP, bleaching material). However, given that $P = 0.049$, with more specimens, the results might also change. The storage of the specimens in the artificial saliva led to enhance of microhardness. Artificial saliva formulation used in this study was according to the study of Amaechi *et al.*; [33] with PH of 7. This study was consistent with Amaechi *et al.*; [33], and Gelhard *et al.*; [34] studies. Artificial saliva contains chemical compounds including potassium chloride, magnesium chloride, calcium chloride, di potassium hydrogen phosphate, potassium dihydrogen phosphate, which may improve the enamel remineralization [11]. While it was inconsistent with some other studies, including Borges *et al.*; [10] (in their studies the artificial saliva had no effects on enamel hardness), which the conflict was associated with the difference in artificial saliva formula, immersion time, the type of teeth and different study designs. It should be noted that with $P = 0.05$ in this

work, the results would be also altered, with more samples testing.

CONCLUSION AND RECOMMENDATIONS:

Although the present study couldn't completely mimic oral environment, the results showed that bleaching agents used in this research containing fluoride and other desensitizing agents, have reduced the microhardness of enamel, but this reduction was not significant statistically. Moreover, the CPP-ACP and artificial saliva had remineralization effects on the bleached enamel in vitro. However, further studies using demineralized agents, associated with the effect of oral environment would be required.

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