Effects of Bleaching and Remineralising Agents on the Surface Hardness of Enamel

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KEY WORDS
Bleaching;
Carbamide Peroxide;
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Enamel

ABSTRACT

Statement of Problem: Home bleaching is a common method for whitening the teeth. However, bleaching may lead to a decrease in the hardness of the enamel.

Purpose: The purpose of this study was to investigate the effects of two different concentrations of carbomide peroxide (CP) on the hardness of the enamel and also to evaluate the effects of the remineralising agents on the hardness of bleached enamel.

Materials and Method: Crowns of 100 intact extracted human anterior teeth were resected from their roots and mounted in acrylic resin in a way that the buccal surface was parallel to the floor (horizontal). The samples were then divided into 10 groups. The baseline hardness in the middle of the buccal surface was measured through Vickers Micro-hardness test and at a load of 500 gram per second. Then five groups were bleached with 10% carbomide peroxide and other five groups with 22% carbomide peroxide. The bleaching was performed for 21 days and was applied 4 hours each day. The samples were kept in distilled water in the interval time between the applications of bleaching agents. In the next phase the enamel micro-hardness was measured and different demineralising agents were used for each group. The Tooth Mousse (TM) paste; MI paste plus (MI); and Crest fluoridated toothpaste was applied for 4 hours to the surface of the enamels in three groups. In the forth group, samples were embedded in fresh cow milk for the same period and the fifth group was kept in distilled water as a control group. Then, the final hardness was measured and the collected data were analyzed by t-test, paired sample t-test and One-way ANOVA test.

Results: Bleaching with the aforementioned concentration of CP had no effects on enamel microhardness. In the groups with a 10% CP, none of the demineralising agents had any effect on the hardness value. However, the application of milk increased the hardness. In the groups with a 22% CP, TM paste reduced the enamel microhardness value while Crest, increased it. MI paste and milk didn't have any effect on it.

Conclusion: The use of TM paste results in lower hardness of the bleached enamel. It seems that the high concentration of fluoride in MI paste may be responsible for increased microhardness of enamel. Milk and fluoridated toothpaste have propensity to increase the enamel hardness.

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Introduction

Having white teeth increases self-confidence in the people and improves their social eminence. There are different ways to reach this aim, such as composite filling, ceramic coating, porcelain laminates, and so on. However, they lead to loss of tooth tissue and possible changes of normal contours. Furthermore, they are expensive and time consuming. Bleaching (whitening) is easier, more acceptable, economical, and conservative [1].

Hydrogen Peroxide, with low molecular weight, can easily pass through the organic matrix of the enamel. The whitening potential of hydrogen peroxide is related to its capability to make free radicals in most solutions including water. Hydrogen peroxide, H_2O_2, releases hydroxyl radicals (HO°) which are extremely active in the water solution. Free radicals have missed one electron and thus, they are electrophilic and instable. Therefore, they react with other organic molecules to regain their stability. They can easily react with most unsaturated bounds. Their discoloration removal process is described as when they react with the organic molecules of a compound; they disturb the continuity of electrons in these molecules and change the absorption energy of them. This disturbance further leads to change in absorption spectrum from a longer wavelength into a shorter wavelengths and consequently the compound loses its colour [2].

Since the introduction of home bleaching, there have always been a report of its success and the researchers have always predicted successful results after applying this method. However, the researchers have been worried about the possible removal of the mineral materials of the enamel and dentine and the occurrence of sensitivity after using it [3]. The findings of some studies have shown that the CP which is used in home bleaching has a negative effect as it removes calcium, phosphate, and fluoride elements from the mineral content of the enamel [4].

So the change in the physical and chemical structure of the enamel arouses the worrisome of any dentist. It is especially true about home bleaching because in this method the dentist has a limited control over the treatment phases. Therefore investigating the effects of different concentrations of CP on the tooth surface and morphology of the enamel is of great importance.

There are some substances whose presence leads to the acceleration of the remineralisation process. Some of them have been identified in dentistry and include fluoride, dairy products including milk, and in recent years, a complex composition derived from the existing proteins in the milk; Casein phosphopeptite-Amorphous calcium phosphate (CPP-ACP), which can be used between bleaching appointments and also after the time when bleaching is fully completed [5].

Milk is a cellular emulsion or a colloid of fatty acids in a water base solution that can provide a proper condition inside the mouth and accelerate the remineralisation [6-7]. The main structures in this liquid are casein milks which are composed of thousands of molecules of protein bounded with calcium and phosphate [8]. Casein phosphopeptite (CPP) is derived from the main protein in the milk and plays a role in the stabilization of calcium, phosphate and fluoride ions. CPP-ACP can be connected to the biofilm, plaque, bacteria, hydroxyl-appetite, and mucous tissues. Its role in delaying tooth caries and also its low aptitude to induce caries has been proved [9-10]. A systematic review has also proved the demineralising effect of it [11].

CPP-ACP is available as a commercial product of GC factory and is known as Tooth Mousse (Tooth Mousse, GC Corp.). Tooth Mousse is used to protect the enamel during orthodontic treatment. It also eliminates sensitivity of the dentine. The manufacture of this product has added sodium fluoride (900ppm) to it and offered MI paste plus.

Seghi et al. found out that the application of 10% CP will result in a decrease in the fracture toughness of the enamel and a decrease in the resistance to its abrasion. However, it had no effect on the enamel microhardness [2].

Attin et al. applied four types of 10% CP to the enamel surface and observed no changes in the enamel hardness in neither of the groups [12].

Based on the study done by Lewinstein et al., the application of 10% and 15 % CP led to a decrease in the enamel hardness [13].

Asefzadeh et al. applied 10% CP of two different factories and found that both brands decreased enamel hardness [14]. Many tests were introduced to investigate the changes which happen in the content of enamel. Some of them are scanning electron microscopy; nanoindentation; infrared spectroscopy; x-ray; atomic force microscopy, profilometric technique, microhardness and so on [15].

However, the application of most of these tests entails the application of fairly complex and expensive machines. From all those aforementioned tests, one which is both quick and cheap is the microhardness test. Furthermore, it is not destructive and it is possible to re-examine a case which has already been looked at [16].

To investigate Vickers hardness test (Vickers, Ltd),

the diameter of the square shaped indent on the sample surface is measured optically. The Vickers hardness value will be obtained through dividing the exerted load by the area of indention of the surface.

So, despite the abundant research on the effects of CP on the enamel hardness, the issue is still under discussion. Furthermore, the effects of different demineralising agents on the enamel surface were investigated in few studies and thus more studies should be established in this regard. This study benefited from Vickers test which is a common test in investigating the interfering effects of demineralization or remineralisation phases on the teeth tissues.

Materials and Method

This was an experimental study (analytic lab trial). To conduct this study, a hundred intact extracted human anterior teeth were used. The teeth were kept in normal saline until the samples were collected. They were without any caries, previous filling, fissure, and structural fault drawback. The crowns were resected from the roots and mounted in the self- curing acrylic resin (Acropars, Iran) in a way that the buccal surfaces were parallel to the floor. Then, the teeth were serially polished with diamond polishing disks (Brazil-FGM). Each disk was used for five cases.

The cases were then divided into 10 groups; each with equal number of 10 samples. The hardness of baselines was measured through the use of Vickers Hardness Testing Machine (Copa MHI, Iran) with a load of 500 g, in 15 seconds. Then, based on the standards and according to the plastic deformation resistance of enamel, each case underwent three hardness tests. Our preferred unit of measuring was kg/mm^2 (Figure 1).

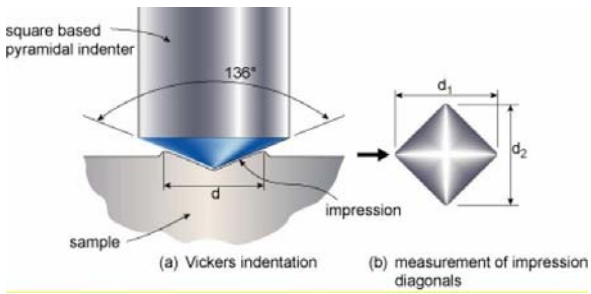

Figure 1 The image of Vickers test

Then, the average value recorded from these three tests was used as the hardness of each sample. Although it is stated that hardness testing has no destructive effects, it causes a permanent change in the form of the area which undergoes testing. So the area under the test should be different each time the test is performed. It is recommended to do these tests in the region where there is the least disparity in the hardness. It has been indicated that the middle area of incisal half of buccal surface has the least variation in microhardness [3]. Therefore, in this study, the central area of the buccal surface was used.

Then 5 groups were randomly selected for the application of 10% CP and 22% CP was applied to the other five groups. CP was applied for 21 days, 4 hours per day (84 hours in total) for each group. Usually bleaching agents are used from 2 to 6 weeks and because half of the activity of these agents occurs in the first two hours, its application for less than 4 hours is a waste of them [15]. The gel was applied in a way that covered the surface of the teeth with a thickness of 2 mm and after each phase, it was removed from the surface by water and air flows. The samples were kept in distilled water at the room temperature, in the interval time between the bleaching phases.

The enamel hardness was measured for the second time keeping the proper distance away from the areas in which the previous tests have been carried out. For each sample 3 tests with the same load and same load time, were performed and the average value of these tests was recorded as the hardness value. In the next phase, we used the following materials for groups 1, 2, 3, and 4, respectively: Tooth Mousse (Japan, CG); MI-paste plus (Japan, CG); Fluoridated toothpaste (Crest, Germany); and fresh milk (Mihan, Iran). Group 5 was selected as the control group and was kept in distilled water.

The samples were completely covered by the pastes for about 4 hours. The samples in group 4 were also dipped into the fresh milk. Total time of application was based on the findings of the previous studies to be matched with the time consumed in daily bleaching. Then the samples were washed and kept in distilled water. Finally, the hardness was measured three times for each case, keeping the proper distance away from the areas in which the previous tests had been accomplished. Then the average value was recorded as the hardness (Figure 2). The data was analyzed through running t-test, paired t-test, and One way ANOVA test.

Table 1 The comparison of the average values of enamel hardness before and after the application of different bleaching agents (T-paired test and T- test)

Bleaching agent	Hardness testing	Average	Standard Deviation	P.value	The Average difference	Standard deviation difference	P.value
10% Carbomide Peroxide	Before After	336.71 336.91	34.7 46.96	0.962	0.19	29.13	0.193
20% Carbomide Peroxide	Before After	338.67 346.67	35.59 44.86	0.07	0.19	29.13	

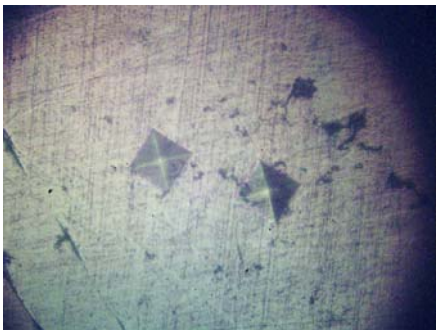

Figure 2 An example of two performed hardness testing

Results

Table 1 depicts the average values of enamel hardness before and after the application of different bleaching agents. Based on the findings of this study, the enamel hardness value which was obtained through Vickers test was 337.69 ± 34.97. The hardness values which were related to the first five groups (the groups with 10% CP applied to them) and the second five groups (with 22% CP applied to them) have been displayed in Tables 1 and 2. Table 3 depicts the comparison of the hardness values of each group before and after the application of demineralising agents.

Based on the findings of this study, the application of 10% CP and 22% CP didn't have any influence on the enamel hardness and the two different concentrations of this substance made no change to enamel (p= 0.193). In groups in which 10% CP was applied, only milk caused an increase in the enamel hardness (p= 0.01).

Among the groups in which 22% CP was applied; Tooth Mousse paste caused a decrease in the hardness (p< 0.001) and fluoridated toothpaste caused an increase in the hardness (p= 0.04). The application of the MI paste plus had no effects on the bleached enamel hardness (p> 0.05).

Discussion

Based on the findings of the current study, the average value of Vickers enamel microhardness was 337.69, which is similar to the findings of Panich [17], Justino [1] and Lopes et al. [18]. However, this finding is not in accordance with the findings of Ulukapi [19], Asefzadeh et al. [14], and Seghi et al. [2]. As it has been mentioned by Cuy et al. [20], enamel hardness depends on different factors such as degree of enamel mineralization, enamel prisms and enamel tufts variations in different areas of enamel, presence or absence of any structural defects in the enamel, type of the teeth (whether it is anterior or posterior), and procedures for preparing the samples to perform the hardness test [12]. Other factors influencing enamel hardness are the bio environmental factors, fluoridation of the drinking water, age of the teeth, and different eating habits in different societies [21]. We used random sampling in this study, thus these factors did not have any negative effects on our findings.

Table 2 The comparison of the average hardness values of the bleached enamel, with 10% carbomide peroxide, before and after the application of demineralising agents

Remineralising agents	Bleaching	Number	Average	Standard Deviation	P.value
Tooth Mousse	Before After	10 10	325.58 335.99	46.71 30.37	0.108
MI-Paste plus	Before After	10 10	346.47 346.13	39.72 48.22	0.936
toothpaste	Before After	10 10	350.79 341.75	53.77 23.44	0.396
Milk	Before After	10 10	317.56 355.14	54.76 31.18	0.001
Control	Initial hardness Final hardness		344.13 334.84	37.76 26.77	0.276

Table 3 The comparison of the average hardness values of bleached enamel (with 22% Carbamide Peroxide) before and after the application of demineralising agents (T-paired test)

Remineralising agents	Bleaching	Number	Average	Standard Deviation	P.value
Tooth Mousse	Before	10	365.91	37.86	0.001>
	After	10	323.38	34.82	
MI-Paste plus	Before	10	344.42	55.60	0.229
	After	10	353.75	41.35	
toothpaste	Before	10	346.77	53.96	0.04
	After	10	373.37	34.14	
Milk	Before	10	335.88	40.85	0.854
	After	10	337.24	18.87	
Control	Initial hardness		340.53	35.55	0.125
	Final hardness		332.92	27.18	

To investigate the direct effect of CP on the enamel hardness, samples should be kept in a solution without having any demineralising or remineralising effect and in this study the samples were kept in distilled water.

Based on the results of the current study, 10% and 22% CP raised the initial enamel hardness from 338.67 to 346.70, but was not statistically significant (p= 0.07) and didn't find any effect on the enamel hardness. This is in accordance with the findings of Seghi et al. [2], Attin et al. [12] and Ulukapi [19]. Seghi et al. applied 10% CP in their study and found out that it had no effect on the enamel hardness [2]. In another study, Attin et al. placed 4 types of 10% CP on the surface of the teeth for about 8 hours and could not find any differences between groups [12]. Moreover, Ulkapi applied 10% CP on the surface of enamel for 112 hours and observed no changes on the enamel hardness [19]. However, such findings could be due to the small number of the samples and the short time of the application of CP.

The findings of our study are different from those of Lewinstein et al. which stated that the 14 hours application of 10% and 15% CP instigated a decrease in the enamel hardness. This undesirable effect was related to the PH of the materials they used [13].

Asefzadeh et al. revealed in their study that the 56 hours application of 10% CP, from two different brands, led to a decrease in the enamel hardness [14]. Justino et al. also applied 10% CP for about 112 hours and observed a decrease in the hardness [1]. Perhaps the difference between the results reported by the Justino et al. and the results of the current study is due to the fact that former study suffered from having a few number of cases (12 cases).

The bleaching materials in our study had neutral PH. According to the previous researches, the enamel does not show demineralization with PHs lower than 5.5. On the other hand, it was observed in some researches that bleaching material containing a neutral PH will decrease the enamel hardness [1].

Some researchers believe that in the competition between the bleaching gel and the enamel structure (Hydroxyapatite), the presence of high concentrations of phosphate ions in the bleaching gel will hinder it from solving the calcium ions of the enamel structure and consequently no changes will occur in the enamel hardness [12].

The indenter may have also penetrated into an area which has not undergone bleaching, yet. In other words, it is possible that the depth of enamel which undergoes demineralization under the influence of CP is less than the depth of the penetration of the indenter in Vickers test (500 g load in about 15 seconds).

With regard to the above explanations, it is suggested that some studies be carried out on the power and penetration depth of CP on the enamel and also on the application of loads no more than the effective penetration depth of the bleaching materials.

In most previous studies which surveyed the effects of bleaching on the enamel hardness, samples were kept in the demineralising solutions, such as artificial saliva in the interval times between the bleaching applications to reconstruct the oral conditions.

As these solutions have the potential for remineralisation, the effects of CP on enamel hardness are influenced by their presence. Therefore, to estimate the direct effects of these remineralising agents on the bleached enamel, these remineralising agents should be applied to the bleached enamel surface after bleaching and the hardness value should be recorded again instead

of keeping them in the saliva which contains the demineralising agents.

Lewinstein et al. applied CP, recorded a decrease in the enamel hardness, and kept the cases in the 0.05% stannous fluoride and observed that samples of both groups (with 10% or 22% CP) regained their initial hardness [13].

In the current study, using toothpaste with 1450 ppm fluoride for the cases to which 22% CP was applied, led to an increase in the enamel hardness. The studies which showed an increase in the enamel hardness as a result of the application of fluoride are mostly performed on the samples which have undergone demineralization and the influence of bleaching materials have not been considered. The researches have stated that in comparison to the unbleached enamel, CP increases the bleached enamel potential to absorb fluoride. The bleaching material opens the path for diffusion of ions towards the enamel structure. So the existing ions can settle and penetrate into the surface more easily [19]. The porosities between and within the enamel prisms will be filled with mineral materials after application of fluoride and thus it causes an increase in the enamel hardness [19, 27].

Based on the findings of the present study, application of fluoridated toothpaste on the 10 % CP applied samples did not lead to an increase in the enamel hardness. It seems that CP has a limited erosive effect that could not decrease the average value of enamel hardness. Probably, the porosities in the surface of the enamel and the penetration of fluoride and mineral materials are found more often with application of 22% CP, compared to 10% CP which led to an increase in the enamel hardness. However, in the 10% CP groups, the effect of the bleaching gel is more limited and no changes occur in the enamel hardness. Hence, the application of fluoridated toothpaste after home bleaching with CP is recommended.

The findings of this study revealed that the use of Tooth Mousse paste in the 22% CP groups decreased the enamel hardness, but, did not change the enamel hardness in the 10% CP groups. The application of MI paste plus paste did not have any effect on the bleached enamel hardness in any group.

However, the application of Tooth Mousse paste with the high amounts of fluoride (900ppm) counteracts its decreasing effect on enamel hardness. The undesirable effect of Tooth Mousse paste might be related to the presence of phosphoric acid in its composition. Phosphoric acid in 0.2% to 0.3% concentration is added to the aforementioned pastes to neutralize their alkaline compositions and to increase their efficiency.

These findings are in disagreement with the findings of Panich and Tantbiroj [17, 28]. They observed that the pastes containing CPP-ACP increased the enamel hardness. It should be mentioned here that in their studies, the enamel samples underwent erosion with acidic solutions and the influence of bleaching material was not investigated.

The amount of bleaching demineralization is more in the 22% CP groups when compared to 10% CP groups. This effect is increased with the application of Tooth Mousse paste, and leads to a decrease in the enamel hardness. However, in the 10% CP groups, this effect is not enough to decrease the enamel hardness.

Thus, after bleaching, it is recommended to use those brands of CPP-ACP pastes which contain fluoride in their composition.

Embedding the teeth into the milk after application of 10%CP resulted in an increase in the enamel hardness. However; this was not observed in the 22% CP groups. Also it should be noted that with regard of these results, and to the best of authors' knowledge, no similar study was found. Some studies have indicated that milk had some demineralising effects on the enamel. Gadelia et al investigated the effects of milk on the hardness of enamel which was softened in cola drink solution (an acidic solution). They concluded that it could increase the enamel hardness [29]. This was due to the settlement of organic and mineral materials. Weigand et al, in their in situ study, placed the enamel samples, which were softened by acidic solution of cola drink in the mouth apparatus, in milk, fluoride and non-carbonate mineral water as mouth washes for about 60 second and found out that the application of this type of mouthwashes increased the enamel hardness. However, the increase in the hardness might be related to the saliva since the samples were washed after the application of mouth washes and remained in the mouth apparatus for about 4 hours and then, the hardness of the samples was measured [16]. Further studies are required to investigate why milk does not have any remineralisation

effects in the 22% CP groups. Maybe, our limited sample size, (N=10) play a part in this upshot and an increase in number of samples would result in more accurate conclusion.

Conclusion

With regard to the limitations of this experimental study, it could be concluded that the application of CP in the home bleaching method have no undesirable effects on the enamel hardness and two different concentrations of CP (10% and 22%) have no difference in this regard. The pastes containing CPP-ACP (known under the brand name of Tooth Mousse) have some demineralising effects on the bleached enamel whilst those types which contain fluoridated composition do not have this undesirable effect. Furthermore, milk and fluoridated toothpaste (1450ppm) have the potential to increase the enamel hardness.

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