Evaluation and comparison of the microhardness of enamel after bleaching with fluoride free and fluoride containing carbamide peroxide bleaching agents and post bleaching anticay application: An *in vitro* study

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Abstract

Aims and Objectives: The purpose of the study was to evaluate and compare the microhardness of enamel after the application of anticay on bleached enamel with fluoride containing and fluoride free bleaching agent. **Materials and Methods:** Twenty freshly extracted teeth decoronated and divided mesiodistally into two halves were randomly divided into five groups with 10 samples in each group. The enamel surface was treated as follows: Group 1 - no treatment, Group 2 - fluoride free bleaching agent, Group 3 - fluoride containing bleaching agent, and Group 4 - fluoride free bleaching agent followed by anticay application. The samples were subjected to indentation to test the microhardness using Vicker's hardness analyzer. **Conclusion:** Enamel microhardness significantly increased in samples where anticay was used after the application of bleaching agent.

Keywords: Anticay, bleaching, microhardness

Introduction

Bleaching although not new to dentistry has gained much popularity in recent years. It is the most preferred conservative method to lighten discolored teeth.[1] Bleaching agents contain either hydrogen peroxide or carbamide peroxide (CP). One of the popular bleaching techniques is night guard bleaching that utilizes 10% CP in a customized tray that is worn by the patient at night. CP is formed from hydrogen peroxide and urea and about one-third of CP is released as hydrogen peroxide.[2] The color change in enamel and dentin is the result of penetration of hydrogen peroxide through the enamel and break down of high molecular weight organic molecule into simpler low molecular weight molecules with lesser color reflectance.[3] However, its action is not restricted and could also contribute to protein damage and mineral loss.[4]

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The concentration of CP in night guard bleaching technique is less and its bleaching action is made effective by increasing its duration of contact with the teeth. Morphological changes have been observed in enamel with the use of CP bleaching agents.[5-11] Also, there can be alteration in microhardness of enamel following treatment with 10% CP bleaching material.[12]

The loss of mechanical properties of enamel after the use of bleaching agents could be regained by the incorporation of agents that can compensate for the mineral loss. Studies have reported that the use of agents such as fluoride and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) promote remineralization of tooth structure. CPP-ACP enhances remineralization by stabilizing the level of calcium and phosphate in saliva.[13-18] In a study conducted by Panich and Poolthong it was found that CPP-ACP increased the microhardness of enamel in eroded teeth.[19] Anticay (ToothMin) that contains calcium sucrose phosphate (SP) releases calcium and phosphate ions to remineralize the enamel.

The purpose of this study was to evaluate and compare the microhardness (Vicker's hardness analysis) of enamel after bleaching with fluoride free, fluoride containing CP

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bleaching agents, and also to compare the microhardness after the application of anticay (ToothMin) following the use of fluoride free CP bleaching agent. The null hypothesis states that the microhardness of enamel does not increase after the use of anticay.

Materials and Methods

Twenty non carious freshly extracted human premolars for orthodontic purpose were obtained as samples and visually inspected to exclude those with cracks or defects. After cleansing, the samples were stored in distilled water until use. The roots of the teeth were decoronated at the level of cemento-enamel junction using diamond disc (Horico, Berlin, Germany) with copious water spray. Crowns were vertically sectioned into two equal halves. Two halves from each tooth were embedded in acrylic resin of standard dimension of 10 mm × 10 mm × 5 mm such that the enamel surface faced upward. The samples were kept in cold water until complete curing of the resin to avoid thermal effects generated by the resin during curing process. The enamel surface of the teeth was ground flat using water-cooled abrasive wheel in a sequence of fresh 120, 240, and 400-grit silicon carbide papers to obtain a flat surface.

The samples were randomly divided into the following five treatment groups:

- Group 1 - Control group: Samples not treated with any bleaching agents ($n = 10$)
- Group 2: Samples treated with fluoride free bleaching agent (opalescence 10%) ($n = 10$)
- Group 3: Samples treated with fluoride containing bleaching agent (opalescence PF 10%) ($n = 10$)
- Group 4: Samples treated with fluoride free bleaching agent (opalescence 10%) and an additional ToothMin application for 1 min after each bleaching treatment ($n = 10$).

Bleaching agent [Table 1] was applied onto the exposed enamel surface of the samples 8 h a day for 7 consecutive days in the respective groups. The samples were rinsed with air water spray for 30 s and air dried after each treatment. In Group 4, samples were subjected to additional application of ToothMin paste for 1 min after each bleaching treatment. All the samples were immersed in distilled water for the remaining hours.

Determination of microhardness

The surface hardness of each sample was determined with a Vickers hardness tester (Matsuzawa MMT7, Matsuzawa SEIKI Co., Ltd., Tokyo, Japan) with 200 g load, which was used to make indentations on the enamel surface. The loaded diamond was allowed to sink and rest on the enamel surface for 15 s and the Vickers hardness number was determined and recorded.

Statistical analysis

The inter-group difference was statistically analyzed by one-way analysis of variance (ANOVA) test followed by Tukey's *post-hoc* test. Statistical significance was set at $P < 0.05$.

Results

The mean enamel microhardness was shown to be maximum in Group 4 (opalescence and ToothMin) followed by Group 1 (control), Group 3 (opalescence PF), and Group 2 (opalescence) [Table 2 and Graph 1]. The standard deviation of enamel microhardness was maximum in Group 1 followed by Group 4, Group 2, and Group 3 [Table 2].

From the ANOVA test [Table 3], it was observed that the mean enamel microhardness (F [3, 36] = 63.205, $P = 0.000$) for the four groups was significantly different. Tukey's *post-hoc*

Table 1: Bleaching agents and remineralization agents used in this study

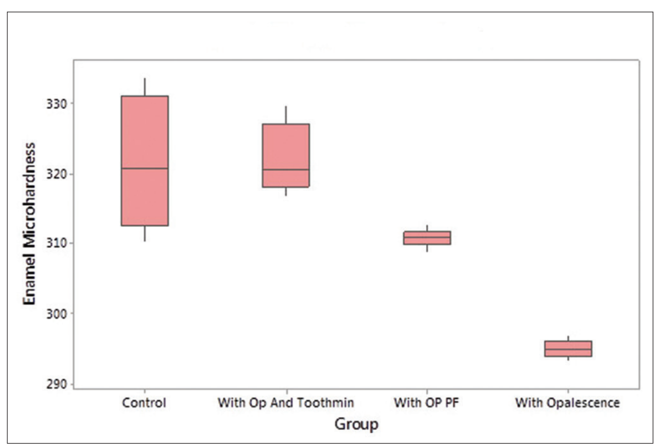
Product	Active ingredients	pH	Manufacturer
Opalescence 10%	10% CP	6.0-6.5	Ultradent Products, Salt Lake, UT, USA
Opalescence PF 10%	10% CP, 0.11 (w/w) fluoride, 3% (w/w) potassium nitrate	6.0-6.5	Ultradent Products, Salt Lake, UT, USA
ToothMin	Calcium SP, calcium orthophosphate	8.5	Abbott Healthcare Pvt., Ltd, Mumbai, Maharastra, India

CP: Carbamide peroxide; SP: Sucrose phosphate

Table 2: Descriptive statistics of enamel microhardness

Groups	*N*	Mean	SD	Minimum	Maximum
1	10	321.9980	8.91955	310.31	333.70
2	10	294.8830	1.29907	293.12	296.82
3	10	310.7030	1.16778	308.73	312.57
4	10	322.0500	4.66973	316.71	329.50
Total	40	312.4085	12.28866	293.12	333.70

SD: Standard deviation



Graph 1: Boxplot of enamel microhardness in different groups

analysis [Table 4] further showed that except Group 1 and Group 4 all other groups had statistically significant difference between them.

Discussion

Mechanical properties of enamel depend largely on the degree of mineralization[20] and alignment of enamel prisms with respect to the crack orientation.[21] Cuy *et al*. hypothesized that there is a strong correlation between degree of mineralization of enamel and its mechanical properties and that prism alignment and loading direction have limited influence.[22] Bleaching agents have been found to affect the mechanical properties of enamel by decreasing the microhardness. In a study done by Senghi and Denry human enamel microhardness decreased after application of 10% CP.[23] Asefzadeh and Hoseini, in their study found that 56 h application of 10% CP from two different brands, led to a decrease in the enamel hardness.[24] The microhardness decreased over a period of two weeks when enamel slabs were subjected to 10% CP bleaching agent as observed by Shannon *et al*.[6] In the current study also, opalescence containing 10% CP was found to decrease the enamel microhardness compared to the control group.

Factors such as concentration and time duration of the bleaching application has got a direct effect on the enamel hardness.[25] Other factors influencing the enamel hardness are the fluoridation of drinking water, age, etc. In this study random sampling was done to nullify such effects. All the samples were stored in distilled water to study the direct effect of CP without the effect of demineralization and remineralization.

Table 3: Level of significance of enamel microhardness using one-way ANOVA test

	Sum of squares	df	Mean square	*F*	*P*
Between groups	4949.689	3	1649.896	63.205	0.000
Within groups	939.745	36	26.104		
Total	5889.434	39			

ANOVA: Analysis of variance

Table 4: Level of significance of enamel microhardness using Tukey's *post-hoc* test

(I) Group	(J) Group	Mean difference (I-J)	SE	*P*
1	2	27.11500	2.28491	0.000
	3	11.29500	2.28491	0.000
	4	−0.05200	2.28491	1.000
2	1	−27.11500	2.28491	0.000
	3	−15.82000	2.28491	0.000
	4	−27.16700	2.28491	0.000
3	1	−11.29500	2.28491	0.000
	2	15.82000	2.28491	0.000
	4	−11.34700	2.28491	0.000
4	1	0.05200	2.28491	1.000
	2	27.16700	2.28491	0.000
	3	11.34700	2.28491	0.000

SE: Standard error

Incorporation of fluoride in dentrifice remineralizes artificial caries lesion by forming a layer of calcium fluoride.[26] Further, it has been shown that post bleaching application of fluoride in the form of fluoride varnish prevented loss of enamel hardness.[27] Addition of fluoride as a protective mechanism in a CP bleaching agent may raise concerns regarding its whitening efficiency. This may be attributed by the calcium fluoride layer formed on the surface which impairs the whitening efficiency of CP. However in a study done by Chen *et al*., scanning electron microscopy evaluation of the enamel specimens treated with fluoridated bleaching agents showed only minor erosive patterns without crystal deposition on the enamel surface and the whitening efficiency was not altered.[28] Fluoridated bleaching agents preserved the microhardness of enamel more effectively than post bleaching fluoride application.[28] Caries resistance further increased in teeth treated with fluoridated bleaching agents.[29] In the current study also, incorporation of fluoride in the bleaching agent increased the microhardness.

Anticay consists of calcium SP and inorganic calcium phosphate. It is completely soluble in water at all pH values and hence able to provide high concentrates of calcium and phosphate ions in the oral cavity. It has been proposed that one percent anticay solution contains about 30×10^{-3} M calcium (Ca) and 10×10^{-4} M SP ions. Saliva contains about 1.4×10^{-3} M calcium and 4×10^{-3} M phosphate (P) ions. High concentration of calcium and phosphate in the presence of anticay facilitates rapid remineralization of enamel. This has been demonstrated *in vitro* by rapid hardening of acid-softened enamel.[30] In the current study, application of anticay significantly improved the microhardness of enamel by helping to regain the minerals lost during the bleaching procedure.

Conclusion

Within the limitations of the study, it was concluded that:

- Fluoride containing bleaching agent increased the microhardness of enamel than when bleached with agents without the incorporation of fluoride
- The application of remineralizing agent containing calcium SP/anticay after bleaching increased the microhardness of enamel.

Further clinical trials supplemented with *in vitro* studies are needed to authenticate the results for future clinical applications.

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Conflicts of interest

There are no conflicts of interest.

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