ORIGINAL RESEARCH

Determination of Microhardness of Remineralized Bleached Surface Subjected to Erosion—An *In Vitro* Study

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Abstract

Objectives: The aim of this study is to evaluate the microhardness of remineralized bleached surface subjected to erosion.

Materials and methods: Fifteen samples were divided into three groups and subjected to bleaching and erosion treatment. Group I—treated with 30% hydrogen peroxide then treated with 1% citric acid solution; group II—treated with 30% hydrogen peroxide and a remineralizing agent followed by 1% citric acid solution; group III—without bleaching with 1% citric acid solution. Five samples per group, GI, GII, and GIII were subjected to the Vickers microhardness analysis. Loss of surface hardness loss (% SHL) was analyzed followed by one-way ANOVA test, post hoc multiple comparison test, Bonferroni test to compare the various group.

Result: Group II showed the lowest % SHL after the erosive phase when compared with group I.

Conclusion: Erosion which usually occurs as a consequence of bleaching can be minimized by the application of remineralizing agents after bleaching.

Keywords: Bleaching, Citric acid, Hydrogen peroxide, Remin pro, Vicker's microhardness.

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Introduction

Tooth bleaching is one of the most popular cosmetic dental treatments, which helps to improve the appearance of discolored teeth. Vital tooth bleaching is used for the removal of extrinsic stains caused by tea, coffee, and tobacco and for the reduction of intrinsic stains within the dentin.[1] The process of tooth whitening lightens the color of a tooth. Whitening of teeth can be achieved by physical removal of the stain, or a chemical reaction. The chemical degradation of the chromogens within or on the tooth is termed as bleaching.[1] Hydrogen peroxide (H_2O_2) is the active ingredient in the most commonly used whitening products and is delivered as either H_2O_2 or carbamide peroxide.[2] Increased intake of acidic food and beverages after bleaching can lead to increased erosion. Studies have proved that remineralization treatments with a remineralizing agent are capable of reestablishing lost enamel surface's hardness and alterations caused by dental bleaching.[3] In the present study, changes in microhardness were analyzed upon the application of a remineralizing agent after subjecting the enamel surfaces to bleaching and erosive challenge.[4]

Materials and Methods

The present *in vitro* study was approved by the ethical committee. Fifteen freshly extracted human upper premolars were selected after confirming the absence of dental imperfections.

Source of Data

Freshly extracted human upper premolars were selected after confirming the absence of dental imperfections. Enamel blocks ($n = 15$) were obtained from these teeth and submitted to two phases of treatment: a bleaching phase and an erosive phase.

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Selection and Preparation of Enamel Blocks

The crowns of healthy human premolars were fixed onto acrylic plates and sectioned at the level of cemento enamel junction (CEJ) and divided into two equal halves to obtain enamel specimens of 3 × 3 mm dimension of 15 enamel samples, 5 samples per group. Finally, the samples were washed in running water and stored in a humid environment until they were submitted to the initial surface microhardness analysis.

Materials Used

- 30% hydrogen peroxide (H_2O_2)—a bleaching agent.
- Remin Pro—a remineralizing agent (Fig. 1).
- 1% citric acid solution—an erosive agent.

Assessment of Initial Surface Microhardness

All the samples (*n* = 15) were submitted to an initial surface microhardness test. For this purpose, a microdurometer with a Vicker's type diamond penetrator was used (100 g load for 10 seconds). Two indentations spaced at a distance of 100 µm from one another were made in the center of each sample, thus, obtaining the hardness values (Vickers hardness number [VHN]) (Fig. 2). The mean of these two values represented the hardness of the samples. After obtaining the initial microhardness, the samples are randomly divided into three groups (*n* = 5) based on the concentration of a bleaching agent, the use of a remineralizing agent, and erosive treatment.

Bleaching Phase: After prophylaxis with pumice and water in GI and GII, one drop of bleaching gel (30% hydrogen peroxide) was deposited on the surface of samples for a period of 30 minutes according to the recommendation of the manufacturers. After this period, all the samples were washed with distilled water for 5 minutes and stored in artificial saliva for 6 hours.

Fig. 1: Remineralizing agent - Remin Pro(VOCO, Germany)

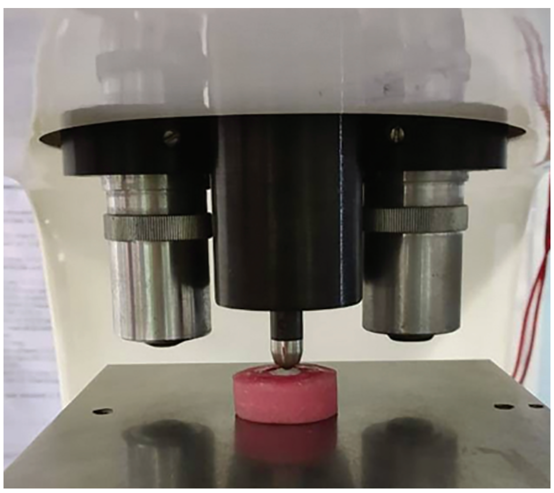

Fig. 2: Micro - Vickers Hardness tester Make: ESSWAY

- Group I: (*n* = 5) Treated with 30% hydrogen peroxide then treated with 1% citric acid solution.
- Group II: After bleaching treatment in GII remineralizing agent Remin Pro (VOCO, Germany) (Fig. 1) was applied to the samples during a 15-day period for 5 minutes, twice daily. Samples were then washed with distilled water and stored in an artificial saliva.
- Group III: Without bleaching (*n* = 5).

Erosive Phase: The samples of the group I, group II, and group III were immersed in a 1% citric acid solution with pH 3.6 under agitation, for 5 minutes. After this erosion protocol, they were stored in an artificial saliva solution for 120 minutes, which simulated the time of digestion. This sequence was performed twice a day for 3 consecutive days, totaling 30 minutes exposure to the acid solution. The samples were kept in the artificial saliva solution for 12 hours after each erosive protocol.

Surface Microhardness Analysis After Erosive Phase: The final microhardness was measured (Fig. 3) after two phases of treatment as the same pattern as that of the previous analyses. The percentages of surface hardness loss (% SHL) were obtained according to the following equation.

The Percentage of surface hardness loss(%SHL) = Micro hardness after erosion - Initial microhardness (baseline)

-- X 100

Initial Microhardness (baseline)

Statistical Analysis Used

The data will be statistically analyzed using one-way ANOVA test, post hoc multiple comparison test, Bonferroni test to compare the various group.

Results

Results of this *in vitro* study are given in Table 1 and are plotted graphically (Fig. 3). After the samples were submitted to bleaching and erosive challenge, the % SHL value was significantly higher in GI in comparison with the other groups of treatment. However, GII

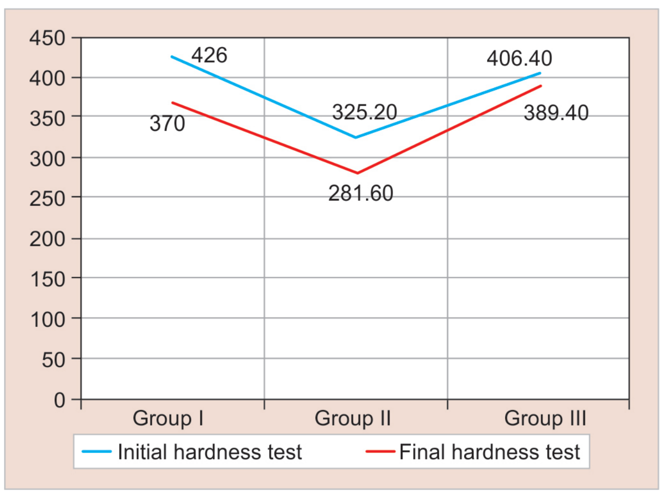

Fig. 3: Mean plot multiple comparison test of Group I to Group III

Table 1: Percentage of surface hardness loss value

		N	Mean	Std. Deviation	Std. Error	F Value	P Value
Initial hardness test	Group I	5	426.00	6.285	2.811	411.977	0.000<0.001
	Group II	5	325.20	6.760	3.023		
	Group III	5	406.40	4.336	1.939		
	Total	15	385.867	45.497	11.747		
Final hardness test	Group I	5	370.00	2.550	1.140	998.577	0.000<0.001
	Group II	5	281.60	5.505	2.462		
	Group III	5	389.40	3.578	1.600		
	Total	15	347.00	48.711	12.577		

showed the lowest % SHL after the erosive phase when compared with GI.

Discussion

The process of tooth whitening lightens the color of a tooth. The chemical degradation of the chromogens within or on the tooth is termed as bleaching. Vital tooth bleaching is used for the removal of extrinsic stains caused by tea, coffee, tobacco, and for the reduction of intrinsic stains within dentin.[1,2] Tooth bleaching procedures are conservative, effective, and cost-effective techniques have become very popular choices. However, like all other treatment modalities, they are associated with some side effects.[5] Bleaching agents have created microstructural changes on the enamel surface resulting in demineralization, degradation, and alteration on surface microhardness and roughness of sound enamel surface.[6] The breakdown of hydrogen peroxide into active oxygen is accelerated by application of heat, the addition of sodium hydroxide, or light. Hydrogen peroxide-releasing bleaching agents are therefore chemically unstable. Only fresh preparations should be used, which must be stored in a dark cool place.[7] H_2O_2 is the active ingredient most commonly used in whitening products and is delivered as either H_2O_2 or carbamide peroxide.[7] In essence, H_2O_2 is analogous to carbamide peroxide as it is ultimately released when the stable complex is in contact with water. This means that most tooth whitening is due to H_2O_2. When it diffuses into the tooth, H_2O_2 acts as an oxidizing agent that breaks down to produce unstable free radicals. In the spaces between the inorganic salts in tooth enamel, these unstable free radicals attach to organic pigmented molecules resulting in small, less heavily pigmented components. Reflecting less light, these smaller molecules create a “whitening effect”.[4] A study published in the *Journal of the American Dental Association* showed that while carbamide peroxide appeared to produce slightly more dramatic results at first, ultimately products containing equivalent amounts of carbamide peroxide and H_2O_2 produced exactly the same results.[8] Tooth bleaching procedures such as conservative, effective, and cost-effective techniques have become very popular choices. However, like all other treatment modalities, they are associated with some side effects. A recent study comparing neutral and acidic hydrogen peroxide (HP) on the surface morphology showed that neutral HP did not affect the surface morphology whereas acidic HP resulted in significant enamel surface changes.[8] Bleaching agents have created microstructural changes on the enamel surface resulting in demineralization, degradation, and alteration on surface microhardness and roughness of sound enamel surface.[7] Morphological changes have been observed in the enamel, with erosion, craters, and porosity being reported by various authors.[10] A bleached tooth treated with remineralization agents has produced a positive effect on enamel morphology and microhardness recovery. Remin Pro (VOCO, Germany) remineralizing paste contains calcium, phosphate in the hydroxyapatite form along with fluoride, and xylitol.[11] It promotes remineralization by filling porous areas, forming a protective film on the tooth surface and thereby impeding the adhesion of bacterial plaque. In this study, a bleached tooth treated with a remineralizing agent resulted in the lowest percentage of surface hardness loss after erosion when compared to bleached tooth treated without application of a remineralizing agent. By the application of a remineralizing agent, we can reduce the effect of erosion occurring after bleaching.[12] Mohammed Q. Alqahtani et al., studied the effects of low and highly concentrated bleaching agents on microhardness and surface roughness of bovine enamel and root dentin and proved that while bleaching did not change enamel microhardness and surface roughness, it affected root dentin in terms of microhardness, which seems to be dependent on the bleaching agent used. Moreover, Lewinstein et al. showed a decrease in the microhardness of dentin following exposure to a 30% solution of hydrogen peroxide at pH 3,[9] while Tam et al. found that *in vitro* fracture resistance of dentin was reduced after the prolonged use of bleaching products applied directly to dentin.[13] An increased bleaching effect was observed for HP, mainly due to an improved reduction of redness and yellowness. Participants perceived improved tooth bleaching for HP and reduced sensitivity for CP, but no differences regarding the comfort of the techniques were noted.[14] The authors conclude that the 35% hydrogen peroxide solution had a major demineralization effect on enamel surface and the cold-light had no significant increased or decreased effect on the demineralization or color change. The mechanism suggested for the surface demineralization is that during the bleaching procedure the pH of the whitening agent becomes more acidic and the hydrogen ions attack the enamel crystals, freeing calcium and phosphate ions from the enamel surface.[15] In a study, sweet potato extract was used as an additive into a hydrogen peroxide-based gel and showed that the addition of such extract maintained the whitening potential of the hydrogen peroxide system, while the deleterious alterations in enamel morphology caused by the use of hydrogen peroxide alone were reduced.[16] Sodium perborate with hydrogen peroxide was associated with higher values of penetration which could be explained by the nature of 30% hydrogen peroxide compared to water. Similar results were seen among the SPCW and SPCHP groups. This finding is in concordance with another radicular peroxide penetration study.[17] The outcomes of microhardness test and AFM observation demonstrated that acidic 30% HP could result in a significant microhardness loss and morphological change of enamel, which were in accordance with the previous studies.[6] Since neutral HP had little effects on the microhardness and morphology of enamel, it suggested that acidic HP could cause microhardness loss and morphological change of enamel due to the demineralization effect of acidic solution.[18]

A possible explanation for these different bleaching results might be the different shapes of the light sources as well as HP concentrations ranging from 0–6%. Philips ZOOM®! 25% (PZ 25HP), Opalescence® Boost 40% (OP 40HP), and Philips Dash 30% (PD 30HP), all three products of the in-office group containing more than 6% HP, resulted in significant tooth whitening. These results are in agreement with those obtained by Park et al., who achieved solid whitening effects using PZ25 with light activation.[19]

Conclusion

Erosion which usually occurs as a consequence of bleaching can be minimized by the application of remineralizing agents after bleaching.

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