Evaluation of Mineral Loss from Enamel by 37.5% Hydrogen Peroxide in-office Bleaching Agent, with and without Additional Activation by Light Emitting Diode and Diode Laser – An Atomic Absorption Spectrophotometric Study.

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ABSTRACT

Background: Bleaching of vital teeth is a very common practice nowadays prompted by high esthetic demands of patients. But bleaching agents are known to cause some transient damage to enamel microstructure, so the need arises to determine the effect of bleaching agents on the mineral content of enamel. Aim: To evaluate mineral loss from enamel using in-office bleaching agent Hydrogen Peroxide and the effect of LED and Laser activated bleaching agent on mineral loss from enamel. **Methods:** Forty human premolar crowns were divided into buccal and lingual specimens and 80 blocks of 4x4x3mm each were obtained. Samples were randomly divided into four groups of twenty each depending upon the beaching treatment and activation method. Group I – Hydrogen Peroxide without additional activation, Group II – Hydrogen Peroxide with additional activation by Diode Laser, Group III – Hydrogen Peroxide with additional activation by LED Light and Group IV – Control group. Amount of calcium and Phosphate loss was calculated using atomic absorption spectrophotometry. **Results:** Results showed that bleaching with hydrogen peroxide leads to calcium and phosphate loss from enamel with additional activation resulting in higher amounts of mineral loss especially with Laser activation. **Conclusion:** Bleaching agents result in calcium and phosphate loss from enamel with significantly high loss of minerals due to additional activation by Laser and LED Light. Therefore bleaching procedures must be followed by re-mineralization treatments to prevent damage to enamel.

Keywords: Diode Laser, Hydrogen Peroxide, In-Office bleaching, LED Light.

INTRODUCTION

Tooth whitening techniques are commonly used to treat discolored teeth. Most whitening products are based on hydrogen peroxide (HP). Tooth bleaching is not considered as generating macroscopically visible defects but there are many studies that show micro structural changes of dental hard tissue by application of bleaching agents,[1,2] especially when peroxides are used in high concentrations. However, a range of other studies exhibited little or no topographic changes on bleached dental hard tissues.[3,4] While the effects of bleaching on morphological changes to tooth tissue are contradictory, it is generally agreed that peroxides can modify mineral content of enamel and dentin.

Nowadays various bleaching modalities are available which include over – the–counter bleaching, in-office bleaching and dentist supervised take-home bleaching. Modern society desires to see the effect of bleaching immediately, resulting in higher concentrations of chemicals used in the composition of the whiteners with different light sources believed to accelerate the bleaching process.[5,6] Today in-office bleaching mainly uses carbamide peroxide (CP) or hydrogen peroxide (HP) which may be activated by heat or light (with a chemical catalyst) to catalyze the tooth bleaching process.[7] It is believed that most light sources decompose peroxide faster (by increasing the temperature) to form free radicals which whiten teeth.[8,9] Various light sources are available for activating bleaching agents for example: light-emitting diodes (LED's), lasers,

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halogen lamps and plasma arc lamps (PAC), activation by heat, light or laser should not increase the intra-pulpal temperature with more than 5.5°C to avoid tooth damage.[4]

In-office bleaching is done by applying higher concentrations of bleaching gels (30–38% HP or 20–37% CP) directly onto the tooth surface in the dental chair.[10,11] The decomposition of hydrogen peroxide results in oxygen and per-hydroxyl free radicals, which then oxidize the stained macromolecules and breaks them down into smaller fragments that diffuse across the tooth surface, resulting in the bleaching effect.[10,12] To accelerate this reaction, heat, lights and lasers have been used but today lights and lasers are the preferred activation methods. A shortened treatment period may eradicate the side effects of highly concentrated bleaching agents.[13,14]

Because several studies have reported surface alterations in enamel after 35% hydrogen peroxide bleaching, concerns have been expressed regarding mineral loss in bleached surfaces.[15-18] While tooth sensitivity, chemical burns and gingival or mucosal irritation side effects are well described in dental literature,[19,20] the enamel composition and its structural adverse effects that occur after 35% hydrogen peroxide in-office bleaching agent using light (LED) and laser irradiation is not well documented and needs to be further investigated The aim of this study was to evaluate calcium and phosphate loss from enamel by 37.5% Hydrogen Peroxide bleaching agent with and without additional activation by light emitting diode and diode laser using atomic absorption spectrophotometer (AAS).

MATERIALS & METHODS

Forty human premolars extracted for orthodontic reasons and without any enamel defects or caries were decoronated at cementoenamel junction. The buccal and lingual surfaces were separated so as to obtain 80 blocks of enamel supported by dentin, each measuring 4x4x3mm. The dentinal surfaces of each specimen were coated with a layer of varnish to prevent contact of bleaching agent. The specimens were then randomly divided into four groups (n=20) according to the bleaching treatment applied.

Group I:

37.5% Hydrogen Peroxide gel (Pola office plus, SDI, Australia) was applied in two cycles of 20 minutes duration with a rest period of 10 minutes without any additional activation

Group II:

37.5% Hydrogen Peroxide gel was applied as in group I with additional activation by Diode Laser (SIRO Laser Advance, Sirona Dental Systems GmbH, Bensheim, Germany) using wavelength = 970±15nm and power =7w. Diode laser activation was done for 180 seconds in each cycle (18 seconds after every 2 minutes). The Laser tip was kept at a distance of 10mm from each sample using customized jig.

Group III:

37.5% Hydrogen Peroxide gel was used as in Group I with additional activation by LED (C-Bright-I), wavelength = 420-490nm, 100-240V, 50/60Hz and 2.5A. LED activation was done for 180 seconds in each cycle (18 seconds after every 2 minutes). The LED light was kept at a distance of 10mm from the sample which was standardized by using a scale.

Group IV:

No bleaching agent was applied. The samples were stored for 15 days in polyethylene tubes with deionised water.

The bleaching agent was evenly applied in 1mm thickness to the enamel surface using electronic micropipette except in Group IV. After each application the bleaching agent was removed using deionized water and collected in polyethylene tubes and homogenized in tube shaker. After that the samples were stored at 100% relative humidity at 37°C using a humidifier for 24 hours. The collected samples were subjected to Atomic Absorption Spectrophotometric analysis (Perkin Elmer USA, AAS 800). Calcium and Phosphate content was measured in micrograms per milliliter. Mean, standard deviation and range were calculated. The data was analyzed using one way ANOVA test. A P-value of <0.05 was considered as statistically significant.

RESULTS

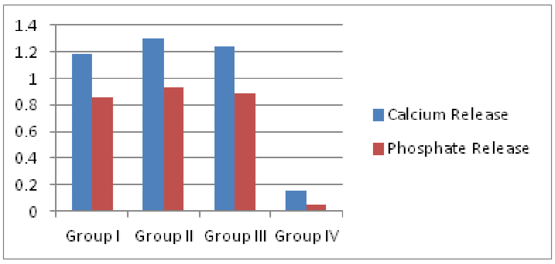

Figure 1: Comparison of Calcium and Phosphate release from enamel in four different groups.

The mean and standard deviation values of calcium and phosphate loss from enamel are shown in [Table1]. Results showed that activation by Diode Laser resulted in highest calcium loss from enamel followed by activation by LED Group both of which resulted in more calcium loss than use of un-activated bleaching agent. Also Phosphate loss from enamel was highest when Diode Laser was used for activation of bleaching agent followed by activation by LED Diode. Overall, results showed that activation of bleaching agents results in more amounts of calcium and Phosphate loss from enamel surface. No statistically significant differences were found between Group II and Group III [Table 2], Group I and Group III although Group II showed statistically significant mineral loss when compared with Group I and control Group IV.

Table 1: Mean and Standard deviation (SD) of Calcium and Phosphate release (µg/ml) from enamel treated with 37.5% Hydrogen Peroxide.

Group	Calcium Release (µg/ml)				Phosphate Release (µg/ml)			
Activation Mode	**Mean**	**SD**	**Range**	**P - Value**	**Mean**	**SD**	**Range**	**P - Value**
Group I No Activation	1.19	0.103	1.0-1.4	<0.001	0.86	0.081	0.7-1.0	<0.001
Group II Diode Laser Activation	1.30	0.082	1.1–1.45		0.93	0.085	0.75-1.05	
Group III LED Activation	1.24	0.102	1.1–1.4		0.89	0.093	0.75-1.1	
Group IV Control Group	0.15	0.084	0.07–0.3		0.05	0.028	0.01-0.09	

Table 2: Intergroup comparison of Calcium and Phosphate release (µg/ml) from enamel treated with 37.5% Hydrogen Peroxide.

Calcium Release (µg/ml)		Phosphate Release (µg/ml)	
Comparison	**P - Value**	**Comparison**	**P - Value**
Group I vs Group II	<0.001	Group I vs Group II	0.002
Group I vs Group III	0.061	Group I vs Group III	0.115
Group I vs Group IV	<0.001	Group I vs Group IV	<0.001
Group II vs Group III	0.066	Group II vs Group III	0.106
Group II vs Group IV	<0.001	Group II vs Group IV	<0.001
Group III vs Group IV	<0.001	Group III vs Group IV	<0.001

DISCUSSION

Bleaching has been accepted as one of the most effective methods of treating discolored teeth and considered to be a conservative approach towards obtaining esthetic or cosmetic results rather than other methods such as veneering or crowning. The efficacy of bleaching is influenced by many factors like the type, concentration of the bleaching agent, time of application, activation method used (heat, light, laser, etc.), cause of the stain and the condition of teeth. The use of hydrogen peroxide for conventional bleaching was introduced way back in 1884. The decomposition of hydrogen peroxide results in the formation of oxygen and per-hydroxyl free radicals that oxidize the stained macromolecules and break down them into smaller fragments which are lighter in color, the fragments diffuse across the tooth surface resulting in the bleaching effect but the oxidation reaction should not exceed the saturation point in which the organic and inorganic elements of enamel and dentin are damaged.[10] Many concentrations of bleaching agents are used with and without activation for bleaching of teeth. The purpose of light is to minimize the time required for tooth bleaching by activating or accelerating the effect of bleaching agents. The objective of laser bleaching is to achieve the ultimate power bleaching process using most efficient energy source, while avoiding any adverse effect.

Calcium and phosphate are present in the hydroxyapatite crystals, the main building block of dental hard tissue. Changes in calcium phosphate ratio indicate alterations in the inorganic components of hydroxyapatite. It has been shown that the bleaching agents cause calcium loss in hard dental tissues, change calcium phosphate ratio and surface alterations depending on their concentration. The linear relationship between decrease in enamel hardness and calcium and phosphate loss shows that hardness measurements can be used as an indication of the degree of enamel mineralization which relates to enamel caries. Ingram and Ferjerskov observed that macroscopically the degree of chemical attack roughly correlated with the appearance of discrete white spot lesions where approximately 7µg or more calcium had been removed from the experimental area ($1.77mm^2$).[21] This means that when approximately $3.95\mu g/mm^2$ of calcium loss is observed in a surface, the surface cannot be re-mineralized.

In the present study on making the intra-group comparison, statistically significant difference was found when mineral ion (calcium and phosphate) release of groups treated with 37.5% HP activated by diode laser (group II) was compared with control group (group IV) and the group in which no-additional mode of activation was used (group I). The above results are comparable with studies done by Shafie et al,[22] Lopes et al,[23] and Justino et al.[24] They also concluded that the decrease in enamel micro-hardness may be due to morphological alterations in mineralized structures caused by bleaching agents which considerably reduces the amount of calcium and phosphate ions, in addition to modifying the morphology of a large quantity of crystals in the superficial layer, when compared with non-treated enamel.[25] It has been noted that alterations in the mineral content of dental enamel are directly related to its micro-hardness. Re-mineralization increases and demineralization decreases enamel micro-hardness.

It is obvious from the results of the present study that release of minerals (calcium and phosphates) is more in case of 37.5% of HP activated by diode laser group (group II) as compared with 37.5% of HP activated with LED (group III) and 37.5% HP group with no additional activation (group I). This also agrees with study of Pinto C et al,[26] who showed that after treatment with high concentration of hydrogen peroxide demineralization (loss of minerals) results in decrease in enamel micro-hardness. This may be due to high concentration of hydrogen peroxide and formed free radicals are higher in laser activated group than in the LED activated group, so causes more demineralization to the enamel. Also the means of release of calcium ions is more than phosphate ion release and can be attributed to high concentration of calcium ions than the phosphate ions in the enamel surface of permanent teeth as was also observed in the research done by Justino et al.[24]

Overall it can be determined that every bleaching treatment must be followed by re-mineralization treatment to prevent irreversible damage to enamel and formation of carious lesions although in the oral cavity good amount of mineral loss is compensated from salivary constituents. Also power bleaching using Laser activation must be taken with caution due to higher amount of mineral loss from Laser activated bleaching.

CONCLUSION

Tooth bleaching leads to significant changes in the mineral content of enamel. Laser-activated tooth bleaching has been shown to be especially damaging so bleaching procedures should be followed by the application of re-mineralizing toothpastes, especially those containing calcium and phosphate.

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