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Effect of Nano-Pulsed Cold Laser (NPCL) Light on Calcium and Phosphate Loss from Dental Enamel During In-office Bleaching with High Concentrated Hydrogen Peroxide Using Atomic Absorption Spectophotometer.

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ABSTRACT

Aim & Objective: To assess calcium and phosphate loss from enamel using 37.5% hydrogen peroxide (HP) gel with additional application of light emitting diode (LED), Diode laser and Nano-Pulsed Cold laser (NPCL) light sources compared to that of 37.5% HP alone using atomic absorption spectrophotometer (AAS). **Methods**: Twenty teeth were sectioned to obtain mesiobuccal, distobuccal, mesiopalatal and distopalatal specimens. The specimens were randomly assigned to four equal groups. 37.5% HP of 1mm thickness was applied to the enamel surface of each specimen. Each group received three cycles of bleaching of 8 minute's duration. Group II, III & IV received additional application of LED, Diode laser & cold laser light respectively. **Results**: Data was analyzed by means of descriptive statistics. Group III showed the maximum loss of ions while Group IV showed the least loss and the differences were statistically significant ($P < .05$). **Conclusion**: Teeth treated with 37.5% hydrogen peroxide with application of Nano-Pulsed Cold Laser (NPCL) light presented with significantly minimal calcium and phosphate loss.

Keywords: Atomic absorption spectrophotometer, bleaching agent, dental enamel, hydrogen peroxide.

INTRODUCTION

Discolored teeth have been successfully whitened by using high concentration in-office beaching agents. Hydrogen peroxide (HP) is the main active chemical component for in-office bleaching which may be activated by heat or light with a chemical catalyst to accelerate the bleaching process.[1] Direct contact of concentrated HP with tooth surface may cause certain amount of enamel demineralization and matrix degradation.[2] These changes are directly proportional to the treatment time and peroxide concentration. So lower concentrations, faster decomposition with shortened treatment period of bleaching agent has been recommended.[3] Calcium (Ca^{2+}) and phosphate (PO_4^{3-}) are the main building blocks of dental hard tissue which are responsible for the hardness of the enamel. Changes in Ca^{2+}/ PO_4^{3-} ratio, during bleaching, indicate alterations in the inorganic components of hydroxyapatite with more mineral loss as the time of bleaching increases. Loss of mineral content from enamel, during bleaching, compromises its strength and resistance to fracture.[4] This mineral loss has been most commonly measured by an atomic absorption spectrophotometry (AAS).[5] Various light sources, ranging from LED, halogen lamps, plasma arc lamps to lasers, have been used with HP bleaching.[6] Most of the light sources decompose peroxide faster to form free radicals which whiten teeth, thereby, shortening the treatment time and possibly preventing the side effects of concentrated HP.[7] Laser light has been widely used to improving dental bleaching technique but it is difficult to establish which laser source is the most efficient because the literature is quite

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conflicting.[8] These light sources have a disadvantage of increasing the intrapulpal temperature above 5.5 degree centigrade which can damage teeth. Cold laser light in-office bleaching technique has been proven to be effective in bleaching discolored teeth.[9] Recently high concentration of 37.5% HP (polaoffice+) was introduced which can be also be used with light to decrease the treatment time. The aim of this study was to evaluate calcium and phosphate loss from enamel by high concentration HP (37.5%) in-office bleaching agent with and without additional application of three different light sources using AAS. The null hypothesis tested was that there will be no variation in calcium and phosphate loss among test groups.

MATERIALS AND METHODS

Twenty pumiced non carious premolar teeth, extracted for orthodontic reasons stored in 0.1% thymol solution at 40C, were randomly marked from 1 to 20 until preparation. Crown of each tooth was cross-split bucco-lingually and mesio-distally with a low speed diamond disc under water coolant. Four standard specimens were obtained (4mm long by 4mm wide by 3mm thick) from each crown. [Figure 1] The specimens were randomly assigned to four equal groups (n=20) to receive bleaching agent. The dentinal surface of each specimen was coated with varnish to prevent contact with the bleaching agent. The bleaching agent of 1mm thickness was evenly applied on to the enamel surface of each specimen using electronic micropipette. Each group received three cycles of bleaching of 8 minute's duration according to the manufacturer's instructions. The subsequent procedure differed between groups by additional application of a light source, as mentioned in individual groups. Group I: (n=20) 37.5% HP applied in three cycles of 8 minutes duration each. Group II: [Figure 2(a)] (n=20) 37.5% HP applied in three cycles of 8 minutes duration each with additional application LED light (C- Bright-I;COXO® Medical Instrument Co. Ltd, Foshan, China). Group III: Figure 2(b)] (n=20) 37.5% HP applied in three cycles of 8 minutes duration each with additional application of diode laser light (SIROLaser, Sirona Dental Systems GmbH, Bensheim, Germany). Group IV: [Figure 2(c)] (n=20) 37.5% HP applied in three cycles of 8 minutes duration each with additional application of cold laser light (MILTADENT, Physioquanta, Montpellier, France). Light application was done for 5 min in each cycle (after every 8 min of HP application). The light source was kept at a standardized distance of 10 mm from the specimen using a custom made jig. After each application the bleaching agent was removed using de-ionized water and rinsing water was collected in microcentrifuge tubes and homogenized in tube shaker [Figure 3] (Labnet Vortex International, Mayfield Ave, Edison, NJ). The sample solutions were then subjected to atomic absorption spectrophotometric analysis for evaluation of calcium and phosphate loss using an AAS (AAnalystTM 800, Bodenseewerk Perkin- Elmer GmbH, Uberlingen, Germany) [Figure 4] calibrated prior to evaluation for calcium and phosphate.

RESULTS

The calcium and phosphate concentrations of the samples were measured as shown in [Table 1], [Figure 5 & Table 2], [Figure 6], respectively. The loss of calcium and phosphate between test groups were compared. A statistically significant difference was observed among the groups ($P < .05$). The order of calcium and phosphate loss among groups was as follows: Group III>Group II>Group I>Group IV. The calcium and phosphate loss of Group II, and III [Table 2] was higher than that of the Group I. Group III showed the max loss of ions which was statistically significant than Group I($P<0.001$) and statistically insignificant than Group II. ($P < .056$) The loss of calcium and phosphate ions was statistically insignificant between Group I and Group II. ($P < .052$) Group IV showed the least loss and the differences were statistically significant as compared to all the three groups. ($P<0.001$)

Statistical Methods

Statistical software SPSS (version 20.0) and Microsoft Excel were used to carry out the statistical analysis of data. Continuous variables were expressed as Mean±SD and categorical variables were summarized as percentages. Analysis of variance (ANOVA) was employed for inter group analysis of data and for multiple comparisons, least significant difference (LSD) test was applied. Graphically the data was presented by bar diagrams. A P-value of less than 0.05 was considered statistically significant. All P-values were two tailed.

Table 1: Descriptive statistics of calcium and phosphate release (micrograms/ml) among various groups.

Group		Mean	SD	Min	Max
Calcium release (micrograms/ml)	Group I	1.19	0.103	1.0	1.4
	Group II	1.24	0.102	1.1	1.4
	Group III	1.30	0.082	1.1	1.45
	Group IV	1.07	0.066	1.0	1.2
Phosphate Release (micrograms/ml)	Group I	0.86	0.081	0.7	1
	Group II	0.89	0.093	0.75	1.1
	Group III	0.93	0.085	0.75	1.05
	Group IV	0.77	0.093	0.6	0.9

Table 2: Inter group comparison based on calcium and phosphate release (micrograms/ml) among various groups

S. No.	Calcium Release		Phosphate release	
	Group Comparison	P-value	Group Comparison	P-value
1	I vs II	0.052	I vs II	0.172
2	I vs III	<0.001*	I vs III	0.007*
3	I vs IV	<0.001*	I vs IV	0.002*
4	II vs III	0.056	II vs III	0.162
5	II vs IV	<0.001*	II vs IV	<0.001*
6	III vs IV	<0.001*	III vs IV	<0.001*

*Statistically Significant Difference (P-value<0.05)

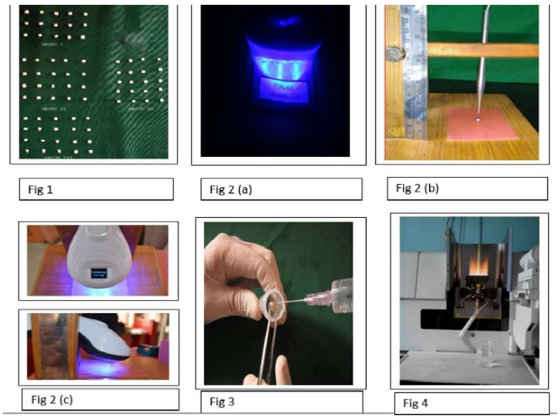

Figure 1: Eighty standardized specimens, Figure 2(a): Hydrogen peroxide gel with additional application of (1) LED light (C- Bright-I; COXO® Medical Instrument Co. Ltd, Foshan, China), Figure 2(b): diode laser light (SIROLaser, Sirona Dental Systems GmbH, Bensheim, Germany), Figure 2(c): cold laser light (MILTADENT, Physioquanta, Montpellier, France), Figure 3: The bleaching agent was removed using de-ionized water and rinsing water was collected in microcentrifuge tubes, Figure 4: Atomic absorption spectrophotometer.

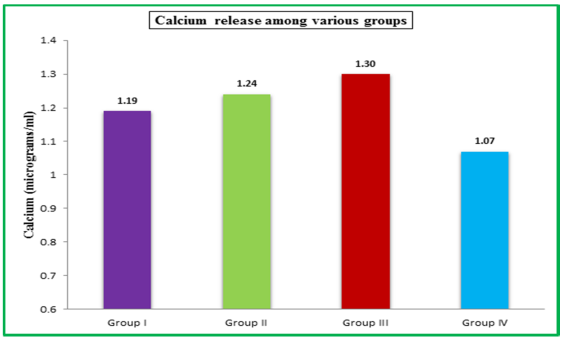

Figure 5: Calcium release among groups.

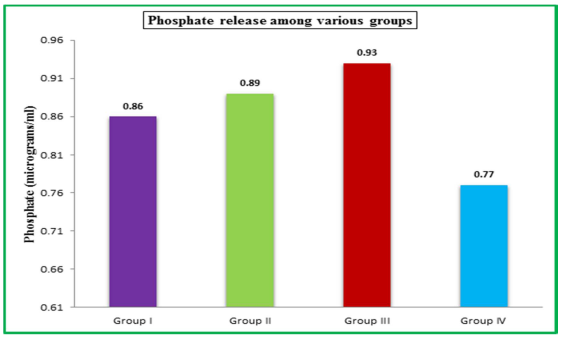

Figure 6: Phosphate release among groups.

DISCUSSION

In-office bleaching with hydrogen peroxide (HP) has been accepted as one of the most effective non-invasive methods of treating discolored teeth.[10] The application of high concentrations of HP should be cautious, attending not only to fast outcomes of the bleaching process but also to the protection of the dentin-pulp complex.. During this study polaoffice+ was applied for 3 cycles of 8 minutes each in activated groups too as we intended to evaluate the effect of light in a complete bleaching cycle (24minutes). Our results show that calcium loss was more as compared to the phosphate which is consistent with the previous study.[11] The reason for this may be that the calcium is bonded weakly to the hydroxyapatite and its loss could be due to the degradation of the enamel's organic component which release Ca^{2+} from the enamel apatite. Light-activated in-office whitening technique plays a major role on the time taken for bleaching as the light energy speeds the breakdown of hydrogen peroxide, accelerating the chemical redox reactions of the bleaching process and produce faster results.[12] Most of the bleaching treatments in combination with light irradiation therapy markedly reduce chairside time and the loss of mineral content from enamel surface. Activation with various light sources (LED/diode laser/ Nd:YAG laser) do not have additional deleterious effects on enamel and thus can be used safely to hasten the bleaching procedure.[13] Three different light sources were used to evaluate the effect of polaoffice+ on calcium and phosphate loss. The LED used in the present study was C-Bright-I belonging to Class II, Type B in continuous mode. The Light source is a 6 pcs high power LED with maximum light intensity of 6000mW/cm2 at 420nm - 490nm. When laser is applied on enamel, the carbonates get driven off, and the surface Ca^{2+} and PO_4^{3-} is decreased.[14] The diode laser used in the present study was a Class IV laser, type IIB device having wavelength of 970 nm±15 nm with optical power of approx. 0.5-7.0 W used in a continuous wave. Increase in the temperature during bleaching process is considered to be the main reason for accelerating the bleaching reaction. Statistically significant differences (P) were seen when calcium and phosphate loss of group III were compared with group I and insignificant difference between group II and group I. This may be due to formation of free radicals which is higher for diode laser than in the LED, causing more mineral loss. These results are in accordance with the previous studies.[11,15] Cold laser also referred to as ''Low level laser therapy" uses energy densities that are low without increasing the temperature and act by inducing a photochemical reaction in the cell, a process referred to as biostimulation or photobiomodulation.[16] The objective of low intensity laser bleaching is to achieve the bleaching process using most efficient energy source, while avoiding any adverse effect is weakening of enamel structure by oxidation of organic or inorganic elements.[17] In the present study cold laser, MILTA-DENT (Physioquanta/34000 Montpellier-France), a pan-spectral magneto infrared Nano-Pulsed Cold Laser (NPCL) device (Class 3R) belonging to the visible & infrared technology. The device can be programmed for wavelengths (from 470 to 905nm) to obtain the whole spectrum of light. The release of mineral ions (calcium and phosphate) was significantly less (P<0.001) in group IV as compared to all groups. This may be due to reason that cold lasers absorb water from the hydroxyapatite of tooth tissues and modify the crystalline structure, acid solubility, and permeability of the tooth surface to increase resistance against demineralization which in accordance to a recent study.[18] On the basis of these findings, the null hypothesis was rejected. The limitations of our study were that we did not study the actual times till the desired bleaching effect was achieved in each group. Although care was taken to standardize the sample of extracted teeth, as teeth from different subjects were selected, yet it's impossible to standardize the concentration of calcium and phosphate inter-specimen. Further in vivo studies should be done to generalize these findings.

CONCLUSION

Within the limitations of the present study it can be concluded that in-office bleaching of teeth done with 37.5% HP gel with additional application of Nano-Pulsed Cold Laser (NPCL) light presented with significantly minimal calcium and phosphate loss as compared to LED and Diode laser lights and even HP gel alone.

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