

Hydrogen peroxide penetration into the pulp chamber during conventional in-office bleaching and diode laser-assisted bleaching with three different wavelengths

Mahdi Abbasi ¹, Edris Pordel ², Nasim chiniforush ³,
Sattar Gorgani Firuzjaee ⁴, Ladan Ranjbar Omrani ⁵

1: DDS, MSc's, assistant professor, Restorative Dentistry Department, School of Dentistry, Tebran University of Medical Sciences, Tebran, Iran

2: Department of Pediatric Dentistry, Borujerd Branch, Islamic Azad University Boroujerd, Iran.

3: Dentist, PhD of laser dentistry, Laser Research Canter of Dentistry, Dental Research Institute, Tebran University of Medical Sciences, Tebran, Iran

4: DDS, Department of Medical Laboratory Sciences, School of Allied Health Medicine, Aja University of Medical Sciences, Tebran, Iran

5: DDS, MSc's, associate professor, Restorative Dentistry Department, School of Dentistry, Tebran University of Medical Sciences, Tebran, Iran

Background and aims: Penetration of hydrogen peroxide into the pulp chamber and subsequent tooth hypersensitivity is a common concern in dental bleaching. The aim of this study was to assess the penetration of hydrogen peroxide (H_2O_2) into the pulp chamber in diode-laser activated bleaching with different laser wavelengths.

Materials and methods: Fifty extracted human maxillary anterior teeth were collected and divided into five groups ($n = 10$). Group 1: conventional in-office bleaching using Opalescence Boost gel. Group 2: Bleaching with Biolase Laser White 20 gel activated by 980 nm diode laser. Group 3: Bleaching with Biolase Laser White 20 gel activated by 810 nm diode laser. Group 4: Bleaching with Biolase Laser White 20 gel activated by 940 nm diode laser. Group 5: No bleaching control group.

After bleaching, the solution into the pulp chamber was collected and analyzed using a spectrophotometer. The recorded data were compared with a standard sample and the results were analyzed and compared using one-way ANOVA and Tukey's HSD tests.

Results: In all bleached groups, H_2O_2 had infiltrated into the pulp chamber. The highest level of penetration was noted in group 2 ($2.32 \pm 0.25 \mu g$), while the lowest level was noted in group 3 ($1.85 \pm 0.33 \mu g$). The difference in this regard was significant between groups 2 and 3 ($P = 0.024$), but the differences between other groups were not statistically significant ($P \geq 0.42$).

Conclusion: Considering the results of this study, it can be stated that hydroge peroxide penetration in to pulp chamber in diode laser activation of bleaching agent according to manufactures instruction is not higher than in-office bleaching. The wavelength of diode laser had significant effect on penetration of hydrogen peroxide into pulp chamber.

Key words: Bleaching, Laser • Diode • Hydrogen peroxide

Introduction

Tooth whitening has gained increased popularity in aesthetic dentistry ¹⁾. At present tooth whitening, is performed using different concentrations of bleaching agents

at home or in dental office to correct discolorations caused by aging or trauma, and even inherent discolorations of the tooth. Bleaching can be performed for both vital and endodontically treated teeth ^{1, 2)}. Bleaching has lower costs and, easier application and more conservative, than other aesthetic dental treatments ^{1, 3)}.

Bleaching gels mainly consist of water and an oxi-

Addressee for Correspondence:

Ladan Ranjbar Omrani

Tel: +989122132110

North Amir Abad, Dental School of Tehran University of Medical Sciences

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bleaching agent with whitening effect, such as hydrogen peroxide (H_2O_2), sodium perborate, or carbamide peroxide. Sodium perborate and carbamide peroxide release hydrogen peroxide upon activation⁴⁾.

Hydrogen peroxide releases free radicals which react with saturated bonds and oxidize the pigments, and organic compounds and discolouring agents conferring lighter appearance to the tooth⁵⁾.

Several factors may affect the efficacy of the bleaching procedure such as, cleaning of the tooth surface, duration of exposure of tooth structure to the bleaching agent, concentration of hydrogen peroxide, rate of chemical reactions and the energy source used to enhance the chemical reactions. All the above-mentioned factors can affect the efficacy, and safety of the bleaching procedure⁶⁾.

In order to increase the efficacy of the bleaching treatment, it is often coupled with an energy source to accelerate the breakdown of hydrogen peroxide and oxidation reaction. Halogen lamps, light emitting diodes (LED), diode lasers, argon laser and plasma arc lamps are used for this purpose^{7, 8)}.

In laser-assisted bleaching, titanium dioxide or pigments are added to the gel to absorb laser energy from the laser radiation and convert it into thermal energy⁹⁾. These compounds are dispersed throughout the bleaching product, thus when the laser light reaches the surface of the tooth, these compounds absorb part of the laser's energy and transform it into thermal energy, consequently the efficacy of the bleaching agent might increase^{4, 9)}.

There are some concerns about tooth whitening such as swallowing the bleaching gel and its local effects on the enamel, dentin, and dental pulp^{10, 11, 12, 13)}. Hydrogen peroxide penetration into the pulp chamber, is a major concern in tooth bleaching, which may exacerbate by use of an energy source during the bleaching process and damage the odontoblastic layer beneath the dentin and interfere with the pulp's cellular metabolism¹⁴⁾.

Changing the wavelength of laser, changes its absorption spectrum. This study aimed to quantify the level of penetration of hydrogen peroxide into the pulp chamber during bleaching treatment assisted with 810, 940, 980 nm diode laser with different wavelengths of 810, 940 and 980 nm in comparison with the conventional in-office bleaching to determine the safest and most efficient of bleaching technique.

Materials and Methods

The sample size was calculated to be 10 according to a study by Camargo et al⁵⁾. Assuming ($\alpha = 0.05$, $\beta = 0.2$), mean standard deviation of 0.82 and minimum mean difference of 1.15 using Minitab software.

The study approved in the ethics committee of Tehran University of Medical Sciences (No. 94-01-69-27354).

Human maxillary central incisors with equal dimension that had been extracted due to hopeless periodontal prognosis were collected for this study. All the teeth had been extracted within the past 3 months. After removal of soft tissue residues and scaling of the teeth using a U15/33 scaler (Hu-Friedy, Pearson, USA), the teeth were immersed in 0.5 % chloramine T solution for one week and then stored in saline until the experiment. The teeth were observed under a stereomicroscope (SMZ800, Nikon, Japan) x 20 magnification to ensure absence of cracks, fractures, abrasion, white spots and other defects. A total of 50 sound teeth were chosen as such.

The roots were cut 2 mm apical to the cementoenamel junction using a high speed disc (Shofu, Japan). The pulpal tissue inside the pulp chamber was removed using Hedstrom files (Maillefer, MI, USA). The pulp chamber was then rinsed with saline and widened with a # 2 round bur and high-speed handpiece to create space for the micro-syringe to enter into the pulp chamber, and the thickness of the labial tooth surface remained around 2 ± 0.1 mm.

Standard-sized teeth were chosen in order to have standard-sized pulp chambers. A standard round area with 4 mm diameter at the middle of the buccal surface was outlined and the remaining surfaces was covered with two layers of nail varnish.

The teeth were fixed vertically on a wax sheet. The pulp chamber was dried using paper points, next 50 μl of acetate buffer solution was injected into the pulp chamber using a micro-syringe. Acetate buffer stabilizes the unstable hydrogen peroxide. Bleaching was performed as follow:

Group 1: Conventional in-office bleaching

The Opalescence Boost (Ultradent Products, Inc. South Jordan, UT, USA) syringes containing 40% hydrogen peroxide were connected and mixed rapidly. The red mixture was applied on the tooth surface in 1 mm thickness for 15 minutes, the gel was agitated every 5 minutes.

Group 2: Diode laser (980 nm) assisted bleaching

Two syringes of Biolase Laserwhite 20 gel containing 45% hydrogen peroxide (Irvine, CA, USA) bleaching gels were connected and their contents were mixed until a homogenous mixture was obtained. The gel was applied on the tooth surface with a 2 mm thickness. Diode laser (Dr Smile, Wiser, Italy) with a 980nm wavelength 1.5 W power was irradiated in a continuous wave mode for 3 x 30 s with 1-minute interval between radiations using a single-tooth bleaching handpiece with a 600 μm diameter, held perpendicularly at 1 mm distance from the gel and tooth surface. Five minutes after the final irradiation, the bleaching gel was removed 5 minutes after the last radiation. Total bleaching time was about 7.5

minutes, and acetate buffer was collected from the pulp chamber.

Group 3: Diode laser (810 nm) assisted bleaching

Laserwhite 20 bleaching gel was placed on the tooth similar to group B. Diode laser (Wuhan, Gigga model: DE-N7A, China) with a wavelength of 810 nm and an intensity of 1.5 W was radiated with a constant beam for 3 x 30 s with 1-minute interval between radiations using a single tooth bleaching handpiece with a diameter of 400 μm held perpendicularly and 1 mm away from the gel and tooth surface. Five minutes after the last radiation, the bleaching gel was removed, and the acetate buffer was collected from the pulp chamber.

Group 4: Diode laser (940 nm)-assisted bleaching

The Laser white 20 bleaching gel was applied on the tooth surface, Diode laser (Epic, Biolase, USA) was radiated with a 940 nm wavelength and 1.5 W power in continuous wave mode for 3 x 30 seconds using a single-tooth bleaching handpiece with a 500 μm diameter. Five minutes after the last radiation, the bleaching gel was removed, and the acetate buffer was collected from the pulp chamber.

Group 5: control group in this group no intervention was performed, after applying the acetate buffer solution, was collected after 20 minutes.

The collected acetate buffer solution was transferred into a microtube using a micro-syringe. The pulp chamber was then rinsed with 50 μL of distilled water twice and it was then transferred to a microtube.

A total 100 μL of 0.5mg/ml leuco-crystal violet solu-

tion (Aldrich: Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 50 μL of 1 mg/mL enzyme horseradish peroxidase solution (Sigma; Sigma Chemical Co. St. Louis, MO, USA) were added to the microtube. Then 2,700 μL of distilled water was added to each solution such that the total volume of each microtube reaches 3 mL.

This process was repeated for each tooth. The resultant blue solution was analyzed by a UV-visible spectrophotometer (Novaspec 2, Pharmacia) at 596 nm wavelength (at room temperature) and the optical density (OD) of the solution was read.

The acquired OD from spectrophotometric analysis was converted to micrograms using the spectrophotometric calibration curve. To plot a standard graph, different concentrations of hydrogen peroxide were obtained and analyzed using a spectrophotometer (**Figure 1**).

One-way ANOVA was used to compare the OD of the five study groups. The Tukey's HSD test was used for pairwise comparison of the groups. The level of significance was set at 0.05.

Results

The results showed that hydrogen peroxide had penetrated the pulp chamber in all groups. The highest level of penetration was $2.32 \pm 0.25 \mu\text{g}$ in the diode laser (980 nm)-assisted bleaching group (group 2) while the lowest level was $1.85 \pm 0.33 \mu\text{g}$ in the diode laser (810 nm)-assisted bleaching group (group 3).

The level of hydrogen peroxide levels in the acetate buffer in the pulp chamber of the control group was very low and negligible, and only caused minute changes in the absorption spectrum (0.001) which was below the sensitivity levels of this test. This value was considered

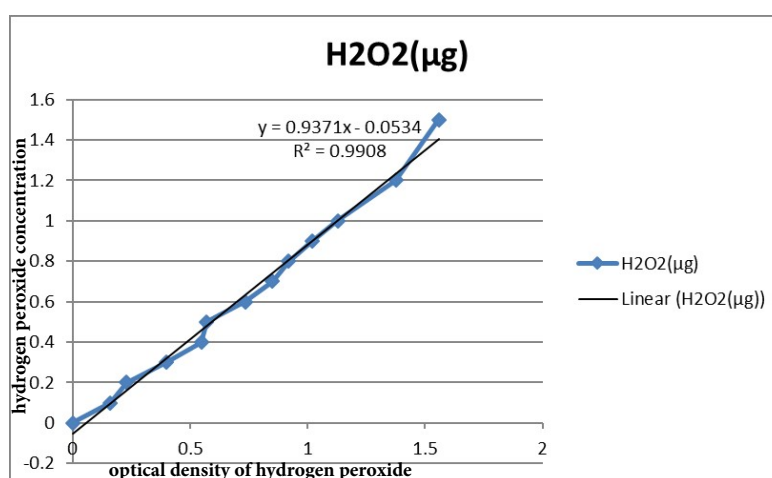


Figure 1: Spectrophotometric calibration curve of hydrogen peroxide with different concentration (the vertical line shows hydrogen peroxide concentration, and the horizontal line shows optical density of hydrogen peroxide)

zero for the control group as shown in **Table 1**.

One-way ANOVA revealed a statistically significant difference among the groups ($P = 0.02$). The results of the Tukey HSD test showed a statistically significant difference between 810 nm and 980 nm groups ($P = 0.24$). However, the difference between other groups was not statistically significant ($P > 0.42$).

Discussion

This study evaluated the effect of laser-bleaching with 980 nm, 940 nm and 810 nm wavelengths of laser on the level of penetration of hydrogen peroxide into the pulp chamber in comparison with the conventional in-office bleaching. The lasers had wavelengths within the infrared spectrum and had photothermal effects. The Laser White gel 20 contains 45% hydrogen peroxide as its active ingredient; after mixing, a bleaching gel containing 35% hydrogen peroxide is obtained. This gel contains special activators and the manufacturer claims that they can be activated with 940 nm diode laser. In the conventional in office bleaching group, Opalescence Boost bleaching gel was used which contains 40% hydrogen peroxide and does not require light activation.

There are various methods to quantify the penetration level of hydrogen peroxide in microgram scale. A spectrophotometer was used for this purpose in the present study, which is reliable, easy and accurate^{15, 16}. It is widely used in the pharmaceutical industry and allows estimation of the concentration of active ingredients of products, based on the oxidation reaction of the leuco-crystal violet buffer solution and hydrogen peroxide, which is catalyzed by horse radish peroxidase. The colour change of the solution as the result of oxidation reaction to indicates the presence of hydrogen peroxide¹⁷.

Evidence shows that dental hard tissue is highly permeable¹⁸. Wataha et al. stated that dental pulp injury caused by dental material depends on their ability to penetrate through the enamel and dentin and reach the pulp chamber¹⁹. Hydrogen peroxide penetrates the pulp

due to its low molecular weight and its ability to degrade proteins in dental hard tissues²⁰. The level of hydrogen peroxide that penetrates the tooth depends on the thickness of enamel and dentin, concentration of the bleaching agent, contact time, positive pulp pressure, thermal or light exposure and presence of restoration²¹.

This study was carried out on extracted human anterior teeth. The thickness of dentin and the surface of the tooth exposed to the bleaching agent were standardized as much as possible. The bleaching time was determined according to the minimum time recommended by the manufacturer.

All groups showed penetration of hydrogen peroxide into the pulp chamber. This result was in accordance with the results of previous studies^{22, 23}. Diode laser with 980 nm wavelength caused the highest level of penetration while Diode laser with 810 nm wavelength resulted in the lowest level of penetration. The result in the conventional in-office bleaching group was not statistically significant different from that in other groups.

Lack of the significant difference between the conventional group and other groups (despite longer exposure time in the conventional group), is probably due to the photothermal effect of laser. When the light energy reaches the bleaching gel, a portion of it is absorbed by the gel, causing it to temperature rise and subsequently increasing its molecular mobility. Leading to greater penetration of hydrogen peroxide²¹. In laser-activated groups, the hydrogen peroxide penetration level increased in use of higher wavelengths of laser. This was because of the fact that lower wavelengths of laser such as 810 nm wavelength are not completely absorbed by the bleaching gel and pass through the gel, whereas wavelengths such as 980 nm wavelengths are mainly absorbed by the bleaching gel causing its temperature rise and increasing its molecular mobility and subsequently enhancing the release and penetration of hydrogen peroxide in to the pulp chamber. Additionally, 980 nm wavelength is absorbed more readily by water causing its evaporation and increasing the temperature of the gel and accelerating the

Table 1: Mean and standard deviation of penetration level of hydrogen peroxide into the pulp chamber. Groups with non-similar superscripted letter have a significant difference with each other.

Groups	N	Mean penetration level of H ₂ O ₂ (µg ± SD)	Minimum	Maximum
1 (conventional)	10	2.23 (0.39) ^{ab}	1.60	2.76
2 (Diode 980)	10	2.32(0.25) ^a	1.79	2.59
3 (Diode 810)	10	1.85 (0.33) ^b	1.31	2.19
4 (Diode 940)	10	2.08(0.40) ^{ab}	1.54	2.68
5 (control group)	10	0	0	0

chemical reactions.

Camargo et al. reported that the depth of penetration of hydrogen peroxide would be higher when the bleaching agent is activated with Nd: YAG laser or LED, compared with in-office bleaching⁵⁾. Similarly, Park et al. concluded application of zoom chairside whitening system, increased the penetration level of hydrogen peroxide²⁴⁾. This increase is probably due to the use of lights for a time period like that in in-office bleaching that does not correlate with clinical conditions. Omrani et al. used diode laser in the recommended time duration recommended by the manufacturer with Opalescence Boost bleaching gel and found that it did not increase the penetration of hydrogen peroxide into the pulp chamber which was in accordance with the current results²⁵⁾.

In this study, we tried our best to simulate the *in vivo* conditions, however the generalizability of our results to the clinical setting must be done with caution since absorption of hydrogen peroxide into the pulp chamber in vivo may be different from that in vitro²⁶⁾. There are at least three factors in a vital tooth that, may prevent the penetration of hydrogen peroxide into the pulp chamber namely the positive pulpal pressure, dentinal fluid, and extension of odontoblastic processes into the dentinal tubules. Moreover, there are defence mechanisms in the pulp that can protect against hydrogen peroxide such as the enzymatic breakdown of hydrogen per-

oxide by peroxidase and catalase²⁶⁾. Additionally, the pulp chamber has been opened to analyze the penetration of hydrogen peroxide to the pulp chamber²⁴⁾.

Walsh et al. carried out a study in 2003 and reported that pulp cells can produce oxygenase 1 enzymes that are important in oxidative conditions²⁷⁾ and are especially seen in odontoblasts and epithelial cells closed to the bleached enamel. Based on the opinions of the authors, the pulp reacts to oxidative stress at a molecular level. Researches have shown that concentrations as high as 50,000 µg of hydrogen peroxide are required to exert negative effects on the pulpal enzymes²⁸⁾. Although the toxicity of hydrogen peroxide is dose-dependent, it can still cause pathological changes and decrease the count of viable cells and MDPC-23 odontoblast-like cells even in small concentrations²⁹⁾. Overall, dental pulp has defensive mechanisms that can neutralize the adverse effects of bleaching agents and only reversible side effects such as tooth hypersensitivity have been reported after tooth whitening procedures²⁹⁾.

Considering the results of this study, it can be stated that there is no difference in hydrogen peroxide level penetration in to pulp chamber in laser assisted bleaching with protocols recommended by the manufacturers in comparison to conventional in-office bleaching. In this study, by increasing the wavelength of the Diode laser the penetration of hydrogen peroxide into the pulp chamber significantly increased.

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