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ORIGINAL ARTICLE

Bleaching effect of activation of hydrogen peroxide using photon-initiated photoacoustic streaming technique

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Abstract
Objectives This study aims to investigate the bleaching effectiveness of photon-initiated photoacoustic streaming (PIPS) using 35 % hydrogen peroxide on discolored teeth as compared with different devital bleaching techniques.
Materials and methods Fifty extracted human mandibular incisors were collected and artificially stained using sheep's blood. The teeth were then randomly divided into five groups according to the different bleaching procedures to be tested: walking bleach with sodium perborate and with 35 % hydrogen peroxide gel, both for 1 week; PIPS using 35 % hydrogen peroxide liquid for 30 min; and just 35 % hydrogen peroxide, as a liquid and as a gel (again, for 30 min). Spectrophotometric measurements were obtained on the buccal surfaces of the crowns, at the beginning, just after the bleaching procedures had been performed, and the following first, third, and seventh days. The ΔE values were calculated, and the data were analyzed with a two-way analysis of variance ($P=0.05$).
Results There were statistically significant differences between the PIPS technique using 35 % hydrogen peroxide liquid and the 35 % hydrogen peroxide liquid and gel without PIPS immediately after the procedures ($P<0.05$). On Days 1, 3, and 7, the PIPS technique further bleached specimens more than all of the other techniques ($P<0.05$).
Conclusions The PIPS technique using 35 % hydrogen peroxide was found to be more effective than all of the conventional techniques.
Clinical relevance Within limitations of this study, PIPS technique using hydrogen peroxide was superior to the conventional techniques. Further studies should be conducted to determine if the PIPS technique results in any complications, particularly cervical resorption.

Keywords Bleaching · Discoloration · Er/YAG · Hydrogen peroxide · Internal bleaching · Laser · Photon-initiated photoacoustic streaming · Sodium perborate

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Introduction

Systemic and local factors can cause intrinsic changes to the tooth surface [1]. The main intrinsic changes related to the endodontic process may result in a serious esthetic complaint. Internal bleaching is widely used to resolve this, as it is a minimally invasive, simple, and cost-effective intervention for discolored nonvital teeth [2].

Discolored nonvital teeth can be bleached with chemicals, such as sodium perborate, hydrogen peroxide, and carbamide peroxide. Hydrogen peroxide seems to be the optimum choice, as it produces free radicals, such as hydroperoxyl and hydroxyl. Moreover, it can penetrate to the enamel and dentin and release oxygen that breaks the double bonds of the organic and inorganic compounds [1, 3].

Bleaching agents can be applied in the pulp chamber followed by a heat source to catalyze and accelerate the breaking reaction. Photoxidation, the process for activating bleaching by light, causes the decomposition of the bleaching agents, thus releasing free radicals [4]. This mechanism may

be induced with halogen lamp appliances or lasers [3]. In order to accelerate the bleaching process, different laser devices have been used as light sources subsequent to the bleaching agent application [5, 6]. Laser devices also have been applied (internal bleaching) prior to the bleaching agent application, in order to increase the permeability of tubules and remove the smear layer (as a conditioner) [7]. However, laser energy has not yet been used at the same time as a bleaching agent for the purpose of agitating it.

A novel laser agitation technique, photon-induced photoacoustic streaming (PIPS), has been proposed. In this technique, an erbium/yttrium–aluminum–garnet (Er/YAG) laser with a radial and stripped tip of novel design is used at subablative power settings. This technique differs from other agitation techniques in the placement of only the tip into the coronal portion [8].

Cardoso et al. [9] evaluated the effect of the ultrasonic activation of bleaching agents on internal bleaching. To our knowledge, however, no studies have investigated the bleaching effect of laser agitation of bleaching agents on discolored teeth. Therefore, the aim of the present study was to investigate the bleaching effect of PIPS using 35 % hydrogen peroxide on discolored teeth as compared with walking bleaching techniques (sodium perborate and 35 % hydrogen peroxide gel),and 35 % hydrogen peroxide liquid and gel applications (without PIPS). The null hypothesis was that there is no difference between the PIPS using 35 % hydrogen peroxide and the other internal bleaching techniques.

Materials and methods

Specimen selection

Mandibular incisors were selected from a collection of teeth that had recently been extracted for reasons unrelated to this study. They were stored in distilled water until use. Fifty were selected from among those with similar mesiodistal (3.3±0.3) and buccolingual width (5.6±0.4) to obtain standard access cavity dimensions. Teeth with root canal treatment, restoration, immature root apices, and/or coronal defects were excluded from the study. The soft tissue and calculus were removed mechanically from the root surfaces with an ultrasonic scaler (Anthos u-PZ6; Imola, Italy), and then polished with pumice.

Preparation of specimens

A standard oval coronal access was performed, and the thickness of the buccal wall was gradually decreased with a size 6 number carbide bur and standardized at 2.6±0.3 by using an electronic digital caliper. Root canals were enlarged up to F3 using ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland). Coronal flaring was performed using size 4–5 Gates Glidden burs (Mani, Mani Inc., Takanezawa, Japan) to ease the placement of cement as a cervical plug. Specimens were irrigated to open dentinal tubules with combination of 5 mL of 17 % EDTA (Werax; Spot Dis Deposu A.S., Izmir, Turkey) and 5 mL of 5 % NaOCl, each for 60 s. Final rinse was performed using 5 mL of distilled water.

Artificial staining

The specimens were artificially stained as described by previous studies [9, 10], with a modified procedure based on that employed by Freccia and Peters [11]. First, the specimens were immersed in Eppendorf tubes containing sheep blood and centrifuged at 3,400 rpm for 20 min at 37 °C (Micro 220R; Hettich, Germany). After the plasma (supernatant) and precipitate were yielded, the plasma was removed. The Eppendorf tubes were then centrifuged for a further 20 min, followed by a centrifugation twice a day for a further 2 days. After each centrifugation, the teeth were irrigated with distilled water, reinserted into the Eppendorf tubes, and stored at 37 °C in 100 % humidity.

On the fourth day, the teeth were removed from the Eppendorf tubes and placed in clean ones. A total of 0.5 mL of distilled water was added to the original Eppendorf tubes (i.e., including blood), which were then centrifuged for a further 20 min to pioneer the hemolysis of erythrocytes. This resulted in a membranous precipitate and hemolysate, and the supernatant layer was removed. The teeth were then transferred back to these Eppendorf tubes, and the tubes were centrifuged again, for 20 min on three consecutive days. After 6 days, the blood was changed, and all the aforementioned procedures were repeated, over a further 6 days.

Baseline color measurement

After the artificial staining procedure, the teeth were cleaned in running water and dried with air spray, after which 2 mm thick glass-ionomer cement (Ketac Molar; 3M ESPE, Seefeld, Germany) was placed 1 mm apical to the cementoenamel junction (CEJ). A standardized circular strip with an internal diameter of 4 mm (external diameter=8 mm) was bonded to the buccal surface of the crown coronal to the CEJ to ensure that color measurement was performed on the same region at every turn with a vertical angle (Fig. 1) [12]. A baseline color measurement was performed using a noncontact-type spectrophotometer (Spectro Shade™ Micro; MHT, Milan, Italy) on the buccal surfaces of the crowns. The specimens were distributed equally according to the baseline color measurements across the five groups to be tested. Statistical analysis by one-way ANOVA confirmed no significant differences among the groups in terms of their baseline color (P=0.682).

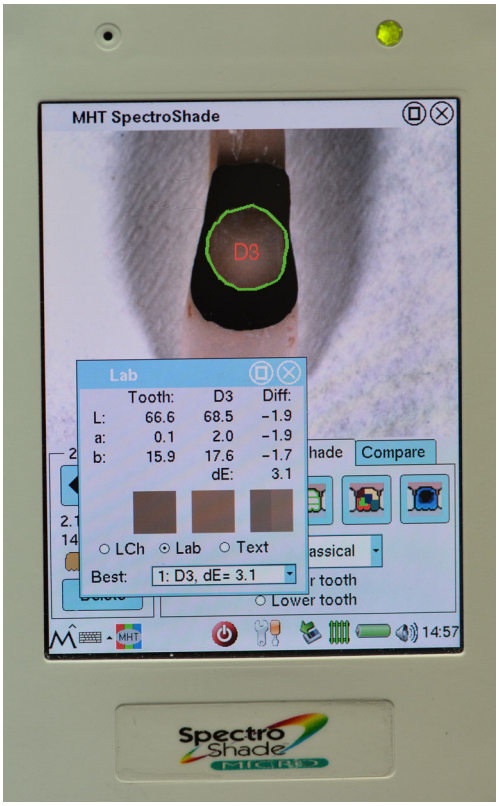

Fig. 1 Representative image of the analysis of crown color using a standardized circular strip

Conventional walking bleaching with sodium perborate The walking bleach paste was prepared by mixing tetrahydrate sodium perborate powder (Sultan Healthcare, Hackensack, NJ, USA) and distilled water, in a ratio of 2 g of powder to 1 mL of liquid, to a consistency of wet sand. With a plastic instrument, the pulp chamber was packed with the paste and tiny cotton pellet was placed over the paste. After the access cavity had been closed with temporary filling material (META Biomed Co. Ltd., Cheongju, Korea), the bleaching agent was left for 1 week and the specimens were stored in distilled water at 37 °C in 100 % humidity.

Conventional walking bleaching with 35 % hydrogen peroxide gel The pulp chamber was filled with 35 % hydrogen peroxide gel (Opalescence®Endo; Ultradent Products Inc., South Jordan, UT, USA) via its syringe, and tiny cotton pellet was placed over the gel. After the access cavity had been closed with temporary filling material, the bleaching gel was left for 1 week and the specimens were stored in distilled water at 37 °C in 100 % humidity.

PIPS using 35 % hydrogen peroxide liquid A band was agglutinated above the endodontic access cavity to hinder irrigant extrusion during the activation procedure. It was holed using a size 40 spreader to allow needle and fiber tip insertion. Then, 0.5 mL of 35 % hydrogen peroxide liquid (Merck, Darmstadt, Germany) was inserted into the access cavity and activated by laser for 1 min. When the irrigating solution in the coronal reservoir decreased, the 35 % hydrogen peroxide liquid was refreshed, resulting in a total of 3 mL. After, the activated 35 % hydrogen peroxide liquid was left in the access cavity without any activation for 10 min of total time. These procedures were repeated for three times, totaling for 30 min, including laser activation time of 3 min. Laser activation was performed with an Er/YAG laser at a wavelength of 2,940 nm (Fidelis AT, Fotona, Ljubljana, Slovenia). A 14-mm-long and 300-μm-diameter quartz tip was applied with 0.9 W, 30 Hz, and 30 mJ/pulse. The water and air on the laser system were turned off, and the optical fiber was placed into the endodontic access cavity.

At the end of the 30 min, the bleaching agent was removed using an air water jet. After the access cavity of the tooth had been filled with cotton pellet, and sealed with temporary filling material, the specimens were stored for 1 week at 37 °C in 100 % humidity.

Thirty-five percent hydrogen peroxide liquid without PIPS A band was agglutinated above the endodontic access cavity, as stated. A total of 3 mL of 35 % hydrogen peroxide liquid (Merck) was inserted into the access cavity during 1 min and left for 10 min of total time. This procedure was repeated for three times, totaling 30 min. Application of temporary filling material and storage were the same as the group of PIPS using 35 % hydrogen peroxide liquid.

Thirty-five percent hydrogen peroxide gel without PIPS Thirty-five percent hydrogen peroxide gel (Opalescence®Endo; Ultradent Products Inc., South Jordan, UT, USA) was placed into the access cavity via its syringe and stirred with bonding brush during 1 min and left for 10 min of total time. Again, this procedure was repeated for three times, totaling 30 min. Application of temporary filling material and storage were the same as the group of PIPS using 35 % hydrogen peroxide liquid.

Tooth color assessment

Color measurements were recorded just after the bleaching procedures, except for both conventional walking bleaching groups, had been performed and on Days 1, 3, and 7. For standardization, the temporary filling material was not removed from the teeth in all of the groups. The color of each specimen was assessed by the CIE-Lab system in *L*a*b** mode using a spectrophotometer (Spectro Shade™ Micro) on the buccal surface of the crown by means of circular strip that was stack at the baseline color measurement. The spectrophotometer was calibrated at the beginning of the test procedure according to the manufacturer's recommendations.

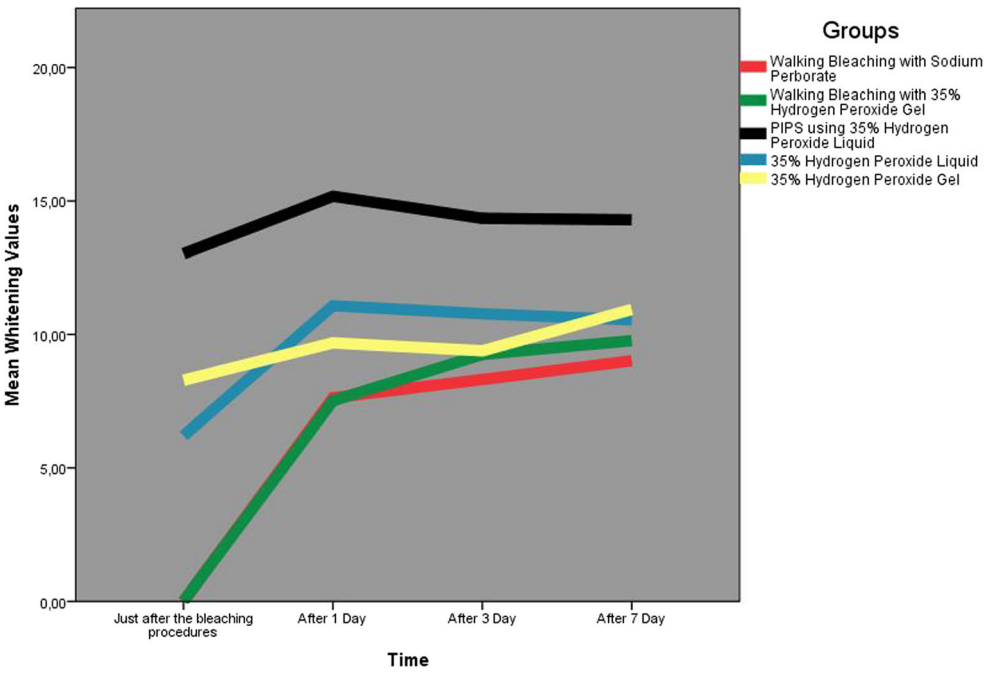

Fig. 2 Mean whitening values of the groups at the different times

The color measurements were performed thrice at each time point on a white background, and the mean of these measurements was calculated. The total color difference or distance between two colors (ΔE) was calculated using the formula below, where L^* represents the value of lightness/darkness, a^* represents the measurement along the red–green axis, and b^* represents the measurement along the yellow–blue axis:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Statistical analysis

All statistical analyses were performed using software (SigmaStat for Windows Version 3.5; Systat Software Inc., Erkrath, Germany) at a significance level of 0.05 and confidence interval of 95 %. The data were subjected to statistical analysis using a two-way analysis of variance (ANOVA) considering two factors (bleaching procedures and time intervals). Tukey post hoc test was used for multiple comparisons.

Results

The data of two-way ANOVA revealed that the bleaching of the discolored teeth was significantly affected by the bleaching procedures ($P<0.001$) but not the factor of time interval ($P>0.05$). In addition, there was no significant interaction between the bleaching procedure and the time interval ($P>0.05$).

Figure 2 and Table 1 show the mean whitening values of the five groups at the four time intervals.

Immediately after the bleaching procedures, regardless of walking bleach groups the bleaching effect of the PIPS technique using 35 % hydrogen peroxide liquid was found to be superior to that of 35 % hydrogen peroxide liquid ($P<0.001$) and gel ($P=0.022$) applied for 30 min.

On Days 1, 3, and 7, the PIPS technique bleached specimens more than the walking bleaching techniques and the (non-PIPS) 35 % hydrogen peroxide gel and liquid ($P<0.05$). There were no statistically significant differences between the other groups ($P>0.05$) (Fig. 3).

Discussion

Activation of irrigating solutions with laser tips has recently become popular in endodontics [13–15]. The mechanism of laser-activated irrigation is based on the formation of bubbles [16]. When the energy of erbium lasers is absorbed by water, it causes evaporation [17, 18]. The vapor bubble starts to expand and form a void in front of the laser light. Assuming that this activation process may enhance the efficacy of the irrigating solution, the aim of the present study was to investigate the bleaching effect of PIPS using 35 % hydrogen peroxide on discolored teeth as compared with other internal bleaching techniques.

The main finding of this study was that the PIPS technique using 35 % hydrogen peroxide bleached specimens after 7 days more than did the walking bleaching techniques. In

Table 1 The mean (standard deviation) ΔE values of the groups at the time intervals

Groups	Time interval			
	Just after the bleaching	After 1 day	After 3 days	After 7 days
Walking bleaching with sodium perborate	N/a	7.62 (2.8) a	8.31 (3.43) a	9.01 (3.62) a
Walking bleaching with 35 % hydrogen peroxide	N/a	7.51 (2.68) a	9.25 (2.43) a	9.77 (2.61) a
PIPS using 35 % hydrogen peroxide liquid	13.03 (4.24) a	15.18 (4.23) b	14.35 (3.97) b	14.3 (3.47) b
Without PIPS 35 % hydrogen peroxide liquid	6.2 (3.83) b	11.08 (4.11) a	10.78 (3.99) a	10.55 (3.43) a
Without PIPS 35 % hydrogen peroxide gel	8.28 (3.36) b	9.68 (4.02) a	9.38 (3.7) a	10.94 (4.07) a

Different letters in the same column indicate statistically significant difference

N/a not applicable

addition, there were no statistically significant differences between walking bleaching techniques applied for 1 week and 35 % hydrogen peroxide liquid or gel applied for 30 min. Therefore, the null hypothesis that there is no difference between the PIPS technique using 35 % hydrogen peroxide and the other internal bleaching techniques is rejected.

In the PIPS technique, an Er/YAG laser is used with a radial and stripped tip of novel design at subablative power settings. Er/YAG laser irradiation is highly absorbed by hydroxyapatite and water [19, 20]. In the PIPS technique at low power, each impulse interacts with the water molecules, creating expansion and successive shock waves that lead to the formation of a powerful streaming fluid [21]. An interesting finding in the present study was that even immediately after the bleaching procedures had been performed, the bleaching effect of the PIPS technique using 35 % hydrogen peroxide liquid was superior to that of 35 % hydrogen peroxide liquid and gel applied for 30 min (i.e., without PIPS). This finding indicates that PIPS enhances the dentinal permeability of hydrogen peroxide in the short-term. However, as there is limited data on this in the literature, our finding should be confirmed in further studies.

In the present study, the specimens were stained using sheep's blood to simulate the clinical tooth discoloration. The specimens and sheep blood were placed in an Eppendorf tube and centrifuged to separate the blood in order to remove the plasma and yield the precipitate containing hemoglobin. This method was used as described by previous studies [9–11].

Lim et al. [2] artificially stained teeth using whole blood and bleached them using different bleaching agents. These authors found 35 % hydrogen peroxide

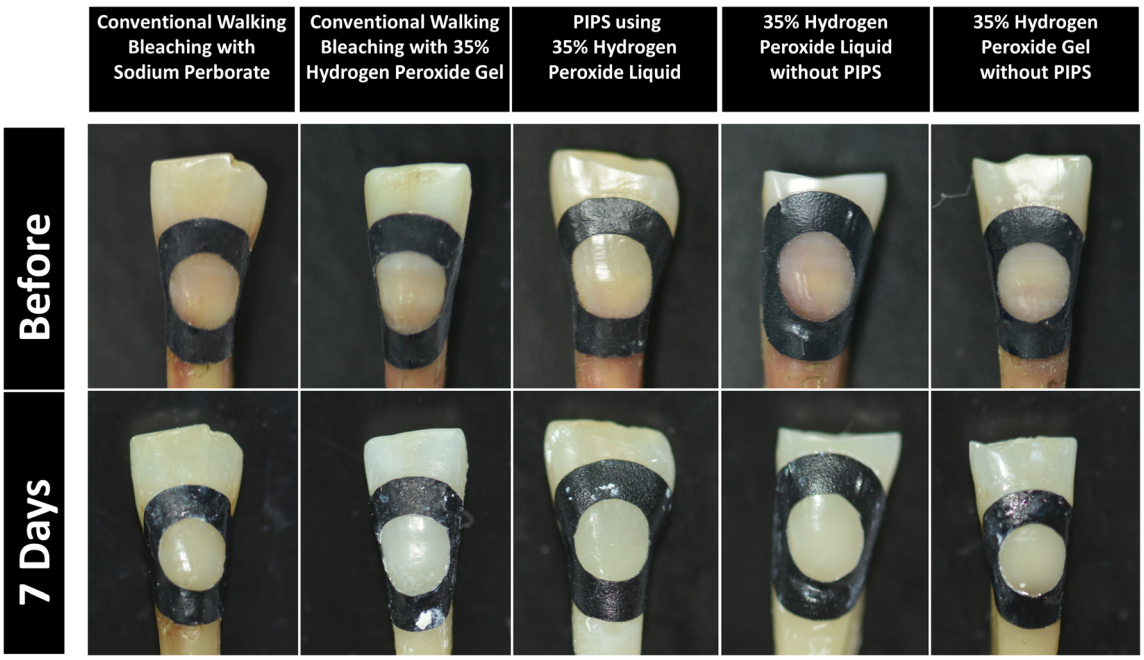

Fig. 3 Representative images of the specimens before and after the bleaching procedures, by group

to be more effective than sodium perborate for internal bleaching. In the present study, all of the hydrogen peroxide groups apart from the PIPS technique were not superior to the sodium perborate in terms of bleaching discolored teeth.

In in vitro bleaching efficacy studies, human maxillary incisors, human premolars, or bovine teeth were used [2, 7, 9, 10, 12]. In the present study, mandibular incisors were used to evaluate the bleaching effectiveness of PIPS using 35 % hydrogen peroxide. When compared with maxillary incisors, mandibular incisors are easy to obtain, and also, their diameters were suitable and easy to use for immersing in Eppendorf tubes for centrifugation. Previously, bleaching efficacy was evaluated using contact-type spectrophotometers whose measurement tips are suitable for wide teeth like maxillary incisors, premolars, or bovine teeth [7, 9]. However, the contact-type spectrophotometer is not suitable for mandibular incisors, which are smaller than the teeth aforementioned. In the present study, a noncontact-type spectrophotometer was used. The image was taken on the surface of tooth, and color was evaluated on this image (Fig. 1) [12]. The noncontact-type spectrophotometer could be beneficial for evaluating color changes in relatively smaller teeth.

It is well established that hydrogen peroxide is caustic, burning tissue on contact [1]. In the present study, therefore, a band was agglutinated above the endodontic access cavity to hinder hydrogen peroxide extrusion during the activation procedure. The PIPS technique in activating hydrogen peroxide was experimentally evaluated in the present study. Clinical usage cannot be suggested according to the present study, however, as further studies should be conducted to investigate further hindrance of hydrogen peroxide extrusion during the activation procedure.

Conclusions

Within the limitations of the in vitro study, the PIPS technique was more effective than both walking bleach techniques as well as both hydrogen peroxide groups. Bleaching materials have adverse effects, however, both are localized and systemic, such as cervical external resorption [22–24], reduction in microhardness of hard dental tissues [25], and toxicity [26]. Thus, further studies should be conducted to determine if the PIPS technique results in any complications, including cervical resorption.

Conflict of interest The authors declare that there are no conflicts of interests in writing this article.

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