### Tooth Bleaching with Nonthermal Atmospheric Pressure Plasma

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#### Abstract

We demonstrated that room temperature plasma could be used for tooth bleaching. A nonthermal, atmospheric pressure, helium plasma jet device was developed to enhance the tooth bleaching effect of hydrogen peroxide  $(H_2O_2)$ . All teeth were sectioned sagittally into halves, which were assigned randomly to either the experimental group or the control group. The experimental group was treated with  $H_2O_2$  (28%, 20  $\mu$ L every 30 seconds) plus plasma (5 W) for 10 minutes; the control group was treated with H<sub>2</sub>O<sub>2</sub> alone for the same duration. Removal of the tooth surface protein was demonstrated by scanning electron microscopy images and Ponceau staining. Production of hydroxyl radicals (·OH) was measured by using electron spin resonance spin-trapping. Combining plasma and  $H_2O_2$  improved the bleaching efficacy by a factor of 3 compared with using H<sub>2</sub>O<sub>2</sub> alone. Tooth surface proteins were noticeably removed by plasma treatment. When a piece of tooth was added to a solution of H<sub>2</sub>O<sub>2</sub> as a catalyst, production of ·OH after plasma treatment was 1.9 times greater than when using H<sub>2</sub>O<sub>2</sub> alone. We suggest that the improvement in tooth bleaching induced by plasma is due to the removal of tooth surface proteins and to increased ·OH production. (J Endod 2009;35:587-591)

#### **Key Words**

Hydrogen peroxide, hydroxyl radical, nonthermal atmospheric pressure plasma jet, tooth bleaching

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**T** ooth bleaching has become a popular esthetic service in dentistry. Hydrogen peroxide  $(H_2O_2)$  is a widely used bleaching material that is effective and safe (1-4). However, the exact mechanism of bleaching action by  $H_2O_2$  is not completely understood. One possible mechanism is that  $H_2O_2$  breaks down to produce oxygen radicals, which attack organic pigment molecules, causing a bleaching effect (1, 3, 4). In-office bleaching systems use a 30%-44%  $H_2O_2$  bleaching gel and a high-intensity light source (3, 5). The light source might enhance bleaching by heating the  $H_2O_2$  and consequently accelerating bleaching, but this mechanism is yet to be confirmed (2, 3). Application of light might (5, 6) or might not (7, 8) significantly improve the efficacy of bleaching materials.

Plasma is the fourth state of matter; it consists of charged particles, radicals, and a strong electric field. In this study, we demonstrate a tooth bleaching procedure that uses room temperature plasma instead of a light source in an in-office  $H_2O_2$  bleaching system. Plasma has potential biomedical applications because it is nonthermal and nontoxic and can be realized in a simple hand-held device (9–13). Furthermore, plasma generates energetic ions, free electrons, and hydroxyl radicals ( $\cdot$ OH) that contribute significantly to tooth bleaching, so that plasma might have a synergic effect on tooth bleaching by  $H_2O_2$  (10, 11, 13, 14). In this article, we demonstrate enhanced bleaching of extracted teeth when combining plasma with  $H_2O_2$ . The efficacy, safety, and mechanism of the method are demonstrated by image analysis and measurements of temperature and of  $\cdot$ OH enrichment.

#### **Materials and Methods**

#### **Plasma Device**

The nonthermal atmospheric pressure plasma jet (Fig. 1*a*) consists of a tube constructed of a dielectric material (Teflon,  $\varepsilon_r = 2.6$ ) and 1 inner and 1 outer electrode (both aluminum). The Teflon tube has outer and inner diameters of 10 and 6.4 mm, respectively. The outer electrode surrounds the Teflon tube; it is 1 mm thick and is connected to a sinusoidal voltage power source that has a frequency of 20 kHz and a peak voltage of 10 kV. The inner electrode, which is not connected to any external power source, has capillary hole of 1 mm diameter. To prevent electrical or physical damage to teeth or gums, the outer and inner electrodes are set back 5 and 10 mm, respectively, from the outlet of the Teflon tube. Helium gas with a flow rate of 2 L/min was used as feeding gas at atmospheric pressure in air. The plasma source is less than 10 cm long, and the device can be hand-held.

The plasma generation occurs inside the Teflon tube near the powered outer electrode. Sufficient voltage applied to helium gas ionizes helium atoms by driving off electrons. Free electrons can trigger further ionization of neighboring helium species by collision. This series of reactions converts the helium gas to the plasma state, which is distinctly different from solid, liquid, and gas states. The device generates a room temperature plasma jet that passes through the capillary hole of the inner electrode (Fig. 1*b*) and extends up to 3 cm beyond the end of the Teflon tube. The plasma jet can be touched without discomfort.

#### **Tooth Bleaching Experiments**

Twenty-eight extracted human teeth were used in this experiment. Because individual teeth respond differently to the same bleaching treatments (5), all teeth tested were cut in half longitudinally, and the pieces were placed in 2 groups. Tooth surface

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### **Case Report/Clinical Techniques**







**Figure 1.** Configuration (*a*) of the tooth bleaching experiment and schematic of the plasma device and (*b*) of the process.

(external bleaching) and dentin (internal bleaching) were treated as follows: the experimental group was treated by using  $H_2O_2$  (28%, 20  $\mu$ L every 30 seconds) plus plasma (5 W) for 10 minutes, and the control group was treated by using  $H_2O_2$  alone for the same duration. The tooth surface temperature during bleaching was measured by using a fiber optic temperature measurement system (FTI-10 fiber optic signal conditioner, FOT-L-SD fiber optic temperature sensor; FISO Technologies Inc, Quebec, Canada). We controlled plasma conditions to maintain the tooth surface temperature at <40°C during the treatments.

#### Analysis of Bleaching Efficacy

We analyzed bleaching results by comparing the overall color changes in the teeth by using photos taken before and after treatments with a Canon EOS Kiss Digital X (Tokyo, Japan) with Canon MR-14EX Ring Flash and a 100-mm Canon Macro Lens EF. Adobe Photoshop CS2 (Adobe Systems, San Jose, CA) was used to measure the color change of each group on the basis of the Commission Internationale de L'Eclairage (CIE) Lab Color System. According to this system, all the colors can be expressed as a combination of 3 values,  $L^*$ ,  $a^*$ , and  $b^*$ , which represent lightness, redness-greenness, and yellowness-blueness, respectively. The differences ( $\Delta$ ) in the value of  $L^*$ ,  $a^*$ , and  $b^*$  between before and after treatment in each group were measured, and the overall color changes ( $\Delta E$ ) were calculated according to the following formula:

$$\Delta E = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}$$

This formula provides numeric data that represent the differences in the perceived color of 2 objects (15).

#### **Demonstration of Protein Removal from Tooth Surface**

After treatment, protein removal from tooth surfaces was demonstrated by Ponceau S staining (16) and scanning electron microscopy (SEM). Ponceau S is a sodium salt of a diazo dye that is used as a stain for rapid reversible detection of protein bands on nitrocellulose or polyvinylidine fluoride membranes. Teeth were rinsed with deionized water and stained with 0.1% Ponceau S in acetic acid for 5 minutes. Protein stained in red was observed with a stereomicroscope (SZ-PT; Olympus, Tokyo, Japan), and the surface of tooth was observed with SEM (S-4200 SEM; Hitachi, Tokyo, Japan) at 8 kV.

#### Measurement of ·OH

The amounts of  $\cdot$  OH generated from H<sub>2</sub>O<sub>2</sub> before and after the plasma treatment were measured by using electron spin resonance (ESR) spin-trapping method (17–20). Solutions composed of  $H_2O_2$ (28%, 50 µL) and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) (0.3 mol/L, 50  $\mu$ L) were prepared. We related the concentration of DMPO-OH with the amounts of the ·OH directly because DMPO is a spin-trapping agent that traps almost 100% of  $\cdot$  OH (17). We added a piece of a tooth  $(3 \times 3 \times 2 \text{ mm})$  as a catalyst to some solutions (group T, 100  $\mu$ L) and not to others (group N, 100  $\mu$ L). Samples from each group were placed individually in cylindrical quartz cells (8 mm diameter  $\times$  12 mm tall) and exposed to the plasma for 1 minute at a distance of 1 cm from the outlet of the plasma source. Solutions from each group were transferred into 100  $\mu$ L quartz capillary tubes either immediately after plasma treatment or after 1 minute in air after the mixture was prepared. The ESR spectrum was recorded by using an ESR spectrometer (JES-PX 2300; JEOL Ltd, Tokyo, Japan) under the following conditions: magnetic field,  $336.5 \pm 10$  millitesla (mT); power, 1 mW; modulation frequency, 9.41 GHz; amplitude,  $1 \times 300$ ; sweep time, 30 seconds. ESR measurements began 1 minute after completion of the plasma treatment. The amounts of ·OH generated were determined by comparing the intensity of the peaks of the DMPO-OH signals.

#### **Statistical Analysis**

The difference in color changes between 2 groups was tested with paired t test. The level of statistical significance was set at .05 of type I error.

#### Results Bleaching Efficacy

Photographs showed increased brightness of teeth in the experimental group but not in the control group (Fig. 2). We evaluated both external and internal bleaching. In both cases, the differences in brightness and color tone between the experimental and the control groups did not differ before treatment (Fig. 2*Ia*, *IIa*), but the teeth in the experimental group were clearly brighter than those in the control group after treatment (Fig. 2*Ic*, *IIb*). For external bleaching, the average (n = 28) of the overall color change  $\Delta E$  was 19.7 (standard deviation, 6.6) for the experimental group and 6.1 (standard deviation, 4.6) for the control group. For internal bleaching, bleached dentin was observed after plasma treatment (Fig. 2*IIc*).

#### Tooth Surface Temperature and Protein Removal From Tooth Surface

The tooth surface temperature increased from room temperature ( $\sim 25^{\circ}$ C) and stabilized near 38°C after 1.5 minutes of operation (Fig. 3). After treatment, uniformly distributed red color indicating the presence of proteins and many dust-like materials were observed on

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