Tooth Bleaching with Nonthermal Atmospheric Pressure Plasma

Hyun Woo Lee, MSD, Gon Jun Kim, MSD,* Jae Moon Kim, DDS, MSD,† Jeong Kil Park, DDS, PhD,‡ Jae Koo Lee, PhD,* and Gyoo Cheon Kim, PhD§*

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 μL every 30 seconds) plus plasma (5 W) for 10 minutes; the control group was treated with H_2O_2 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_2O_2 alone. Tooth surface proteins were noticeably removed by plasma treatment. When a piece of tooth was added to a solution of H_2O_2 as a catalyst, production of ·OH after plasma treatment was 1.9 times greater than when using H_2O_2 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

From the *Department of Electronic and Electrical Engineering, Pohang University of Science and Technology, Pohang; and †Department of Pediatric Dentistry, ‡Department of Conservative Dentistry, and §Department of Oral Anatomy, School of Dentistry, Pusan National University, Busan, Republic of Korea.

Address requests for reprints to Dr Gyoo Cheon Kim, Pusan National University, Department of Oral Anatomy, School of Dentistry, 1-10 Ami-Dong, Seo-Gu, Busan 602-739, Republic of Korea. E-mail address: ki91000m@pusan.ac.kr.

0099-2399/$0 - see front matter

doi:10.1016/j.joen.2009.01.008

Tooth 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 (·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 ·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

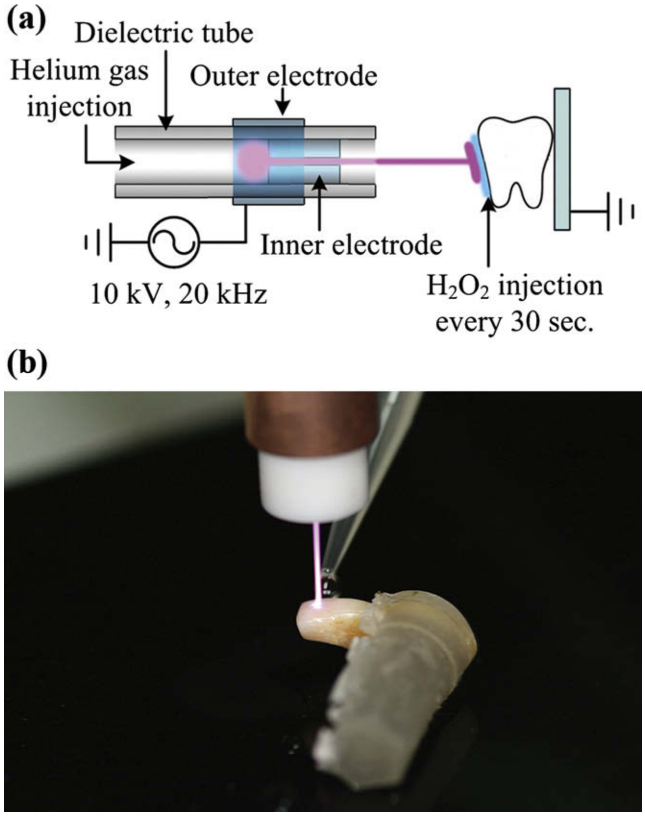

Figure 1. Configuration (*a*) of the tooth bleaching experiment and schematic of the plasma device and (*b*) of the process.

(external bleaching) and dentin (internal bleaching) were treated as follows: the experimental group was treated by using H_2O_2 (28%, 20 μL 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 (Δ) in the value of L^*, a^*, and b^* between before and after treatment in each group were measured, and the overall color changes (ΔE) were calculated according to the following formula:

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

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 ·OH generated from H_2O_2 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 μL) 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 ·OH (17). We added a piece of a tooth ($3 \times 3 \times 2$ mm) as a catalyst to some solutions (group T, 100 μL) and not to others (group N, 100 μL). 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 μL 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 ΔE 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°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

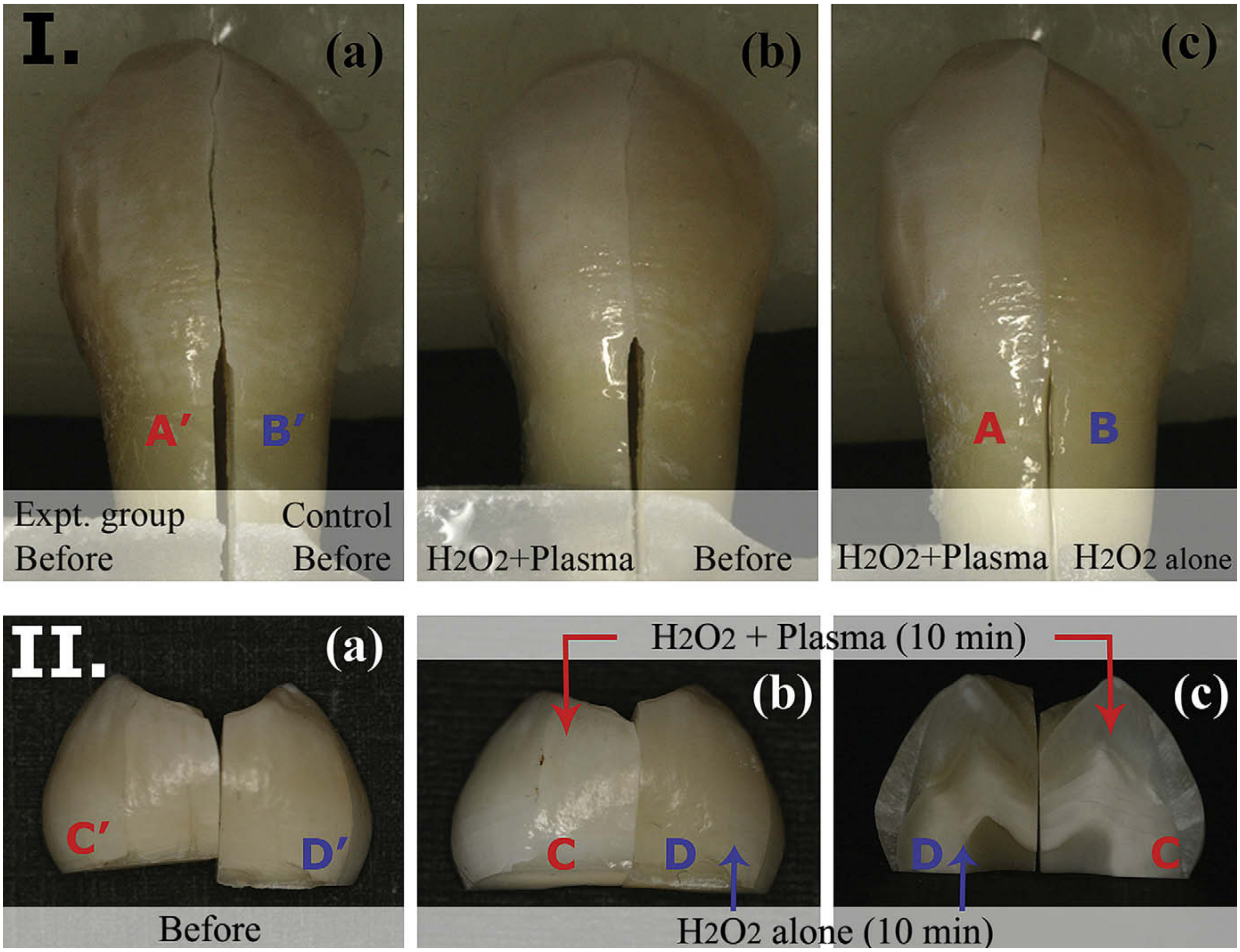

Figure 2. *I,* The external bleaching effect of plasma treatment. Photographs of canine tooth used (*a*) before, (*b*) after treatment for the experimental group only, (*c*) followed by treatment for control groups, demonstrating typical results in the experimental group (A' → A) and the control group (B' → B). *II,* The internal bleaching effect of plasma treatment. Photographs of molar tooth used (*a*) before, (*b, c*) after treatment for both groups, (*b*) outer surface and (*c*) inner surface, demonstrating typical results in the experimental group (C' → C) and the control group (D' → D). The experimental group was treated by using H_2O_2 (28%, 20 μL every 30 seconds) plus plasma (5 W) for 10 minutes; the control group was treated by using H_2O_2 alone for the same duration.

the surfaces of the teeth; these were much more prominent in the control group (Fig. 4*a, c*) than in the experimental group (Fig. 4*b, d*).

Quantification of ·OH

Signals of DMPO-spin adducts of ·OH (DMPO-OH), quartet lines with intensity ratio of 1:2:2:1 and hyperfine coupling constants of a_N = a_H = 1.49 mT (17–19), did not show any signal of ·OH in group N, irrespective of the plasma treatment (Fig. 5*a, b*). However, in group T a clear ·OH signal was detected after treating the tooth for 1 minute (Fig. 5*c, d*); this signal was 1.9 times greater when using plasma plus H_2O_2 than when using H_2O_2 alone (Fig. 5*e*).

Discussion

This study was conducted to investigate the potential of plasma as a tool for tooth bleaching. Bleaching can occur when pigments on the tooth surface are destroyed (1, 3, 21). H_2O_2 is used routinely for bleaching because it can generate different radicals or ions that interact with colored organic molecules that are adsorbed to the enamel surface, making teeth clearer (1, 3, 21–23). In-office bleaching uses relatively high concentrations of H_2O_2 (up to 35%) and various light or laser sources (1, 3, 5). Light or laser heats H_2O_2, thereby speeding decomposition to form free radicals such as ·OH, consequently reducing total bleaching treatment time and giving immediate results (1–3, 5, 24–27).

·OH is crucial in tooth bleaching (17, 19–21). ·OH possesses an unpaired electron, so it is unstable and very reactive (1). It captures an electron from surrounding molecules; when these molecules are pigments, their structures are disrupted, and bleaching occurs (1). Generally, ·OH is generated from H_2O_2 by interaction with a catalyst that contains divalent cations (4, 17, 19, 20). We observed significant enhancement of ·OH concentration when including a piece of tooth in a solution of H_2O_2 and DMPO; this result demonstrates that the tooth itself includes a catalyst required for ·OH generation from H_2O_2 (Fig. 5).

In group T, the amount of ·OH nearly doubled after plasma treatment for 1 minute, compared with samples treated with only H_2O_2. This result is strong evidence that plasma enhanced ·OH generation.

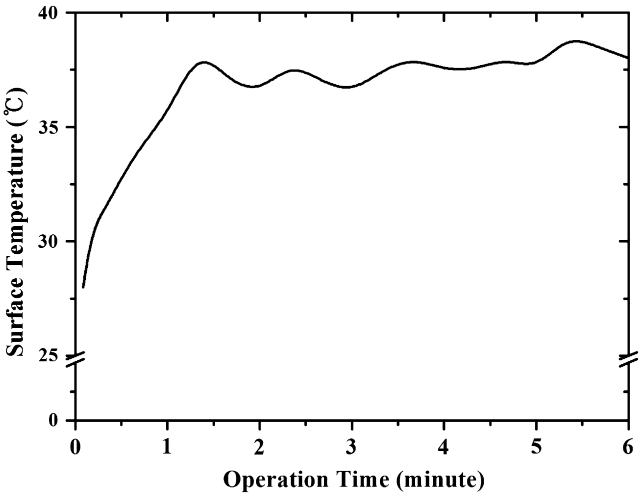

Figure 3. Surface temperature of teeth during bleaching experiment (H_2O_2 plus plasma treatment).

Considering that the plasma is a rich source of reactive oxygen species and high energy electrons (9–11, 13, 14), we suggest that the plasma caused ·OH formation by cleaving the O–O bond in H_2O_2 (21).

Free radicals can cause oxidation of lipids, proteins, and nucleic acids (23). The color-producing materials on a tooth surface are typically organic compounds that possess extended conjugated chains of alternating single or double bonds (21). In this study, SEM images showed remarkable reduction in the quantity of extraneous substances on the tooth surface. Ponceau staining, which reveals proteins, revealed a weak response after plasma treatment. These results indicate that the tooth surface proteins are removed or denatured by plasma treatment. Therefore, we speculate that capability of plasma to remove surface proteins contributes to this accelerated bleaching effect.

The temperatures of the tooth specimens and of the plasma should be kept low during plasma bleaching because excessive heating can damage pulp irreversibly (28). In our study, the surface temperatures of the teeth did not exceed 40°C during the bleaching treatment (Fig. 3). In addition, spectroscopic analysis showed that the plasma gas temperature was near room temperature (300 K, data not shown).

In conclusion, the tooth bleaching method with plasma that we suggest in this study can be complementary to the conventional method because it provides effective bleaching without thermal damage. The tooth bleaching efficacy in the experimental group (H_2O_2 with the plasma treatment for 10 minutes) was 3 times better than that of the control group (H_2O_2 alone for the same duration). During plasma treatment, production of ·OH, which is crucial in tooth bleaching, was observed in samples that included a tooth as a catalyst; concentrations of this radical were about double those observed in the absence of plasma treatment. The combination of plasma treatment with H_2O_2 gels might become an effective method of tooth bleaching.

Acknowledgments

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (no. R01-2007-000-10730-0) and the Korea Ministry of Education through its Brain Korea 21 program. The authors thank Dongkyu Lee and Sang Min Jeon for their technical assistance with SEM. Helpful support by Dr Mun-Ki Song, Young-Jun Kim, and Sang-Hun Oh is gratefully acknowledged.

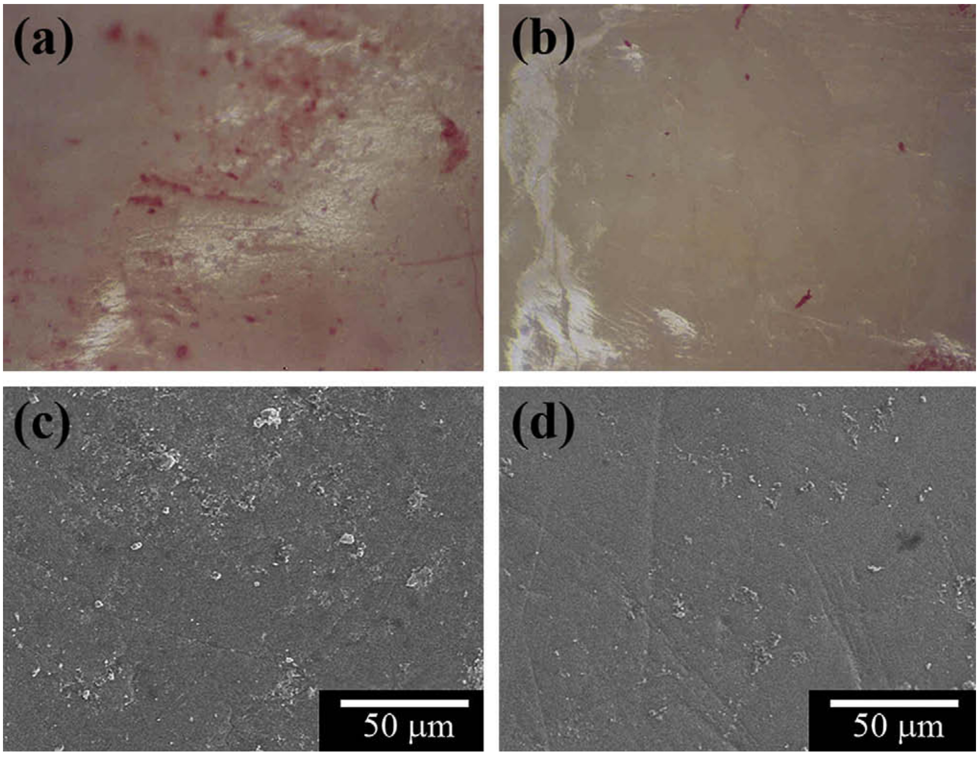

Figure 4. (*a, b*) Ponceau staining and (*c, d*) SEM images of the (*a, c*) control group (H_2O_2 alone) and the (*b, d*) experimental group (H_2O_2 plus plasma treatment).

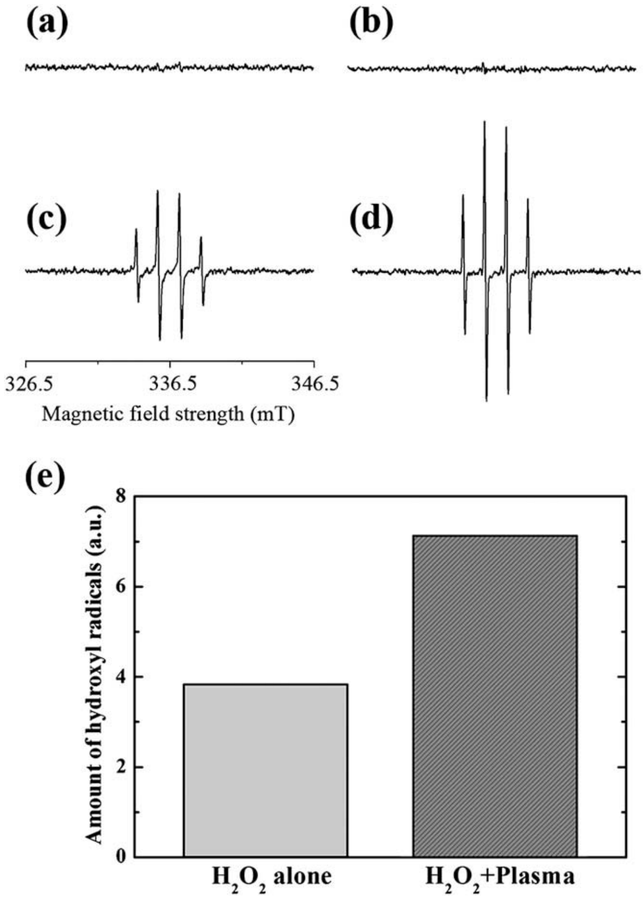

Figure 5. Typical ESR spectra measured from the mixture composed of H_2O_2 (28%, 50 μL) and DMPO (0.3 mol/L, 50 μL) with *(a, b)* no additional reagent (group N) or *(c, d)* a piece of a tooth (group T): *(a, c)* before and *(b, d)* after plasma treatment for 1 minute. *(e)* Quantity of ·OH in group T before and after plasma treatment.

References

1. Coutinho DS, Silveira L Jr, Nicolau RA, Zanin F, Brugnera A Jr. Comparison of temperature increase in *in vitro* human tooth pulp by different light sources in the dental whitening process. Lasers Med Sci 2008 (online first).
2. Christensen GJ. The tooth-whitening revolution. J Am Dent Assoc 2002;133:1277–9.
3. Yazici AR, Khanbodaghi A, Kugel G. Effects of an in-office bleaching system (ZOOM) on pulp chamber temperature *in vitro*. J Contemp Dent Pract 2007;8:19–26.
4. Naik S, Tredwin CJ, Scully C. Hydrogen peroxide tooth-whitening (bleaching): review of safety in relation to possible carcinogenesis. Oral Oncol 2006;42: 668–74.
5. Luk K, Tam L, Hubert M. Effect of light energy on peroxide tooth bleaching. J Am Dent Assoc 2004;135:194–201.
6. Tavares M, Stultz J, Newman M, et al. Light augments tooth whitening with peroxide. J Am Dent Assoc 2003;134:167–75.
7. Jones AH, Diaz-Arnold AM, Vargas MA, Cobb DS. Colorimetric assessment of laser and home bleaching techniques. J Esthet Dent 1999;11:87–94.
8. Clinical Research Associates. Why resin curing lights do not increase tooth lightening. CRA Newsletter 2000;24:3.
9. Iza F, Lee JK, Kong MG. Electron kinetics in radio-frequency atmospheric-pressure microplasmas. Phys Rev Lett 2007;99:075004.
10. Iza F, Kim GJ, Lee SM, et al. Microplasmas: sources, particle kinetics, and biomedical applications. Plasma Process Polym 2008;5:322–44.
11. Kieft IE, Broers JL, Caubet-Hilloutou V, Slaaf DW, Ramaekers FCS, Stoffels E. Electric discharge plasmas influence attachment of cultured CHO K1 cells. Bioelectromagnetics 2004;25:362–8.
12. Deng XT, Shi JJ, Chen HL, Kong MG. Protein destruction by atmospheric pressure glow discharges. Appl Phys Lett 2007;90:013903.
13. Lu X, Jiang Z, Xiong Q, Tang Z, Hu X, Pan Y. An 11 cm long atmospheric pressure cold plasma plume for applications of plasma medicine. Appl Phys Lett 2008;92: 081502.
14. Park GY, Lee HW, Kim GC, Lee JK. Global model of He/O_2 and Ar/O_2 atmospheric pressure glow discharges. Plasma Process Polym 2008;5:569–76.
15. Canay S, Cehreli MC. The effect of current bleaching agents on the color of light-polymerized composites *in vitro*. J Prosthet Dent 2003;89:474–8.
16. Antharavally BS, Carter B, Bell PA, Mallia K. A high-affinity reversible protein stain for Western blots. Anal Biochem 2004;329:276–80.
17. Kawamoto K, Tsujimoto Y. Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching. J Endod 2004;30:45–50.
18. Noda Y, Mankura M. Inhibitory effect of antioxidants on hydroxyl radical generation from methylquanidine: an ESR study. Neurochem Res 2008 (online first).
19. Kashima-Tanaka M, Tsujimoto Y, Kawamoto K, Senda N, Ito K, Yamazaki M. Generation of free radicals and/or active oxygen by light or laser irradiation of hydrogen peroxide or sodium hypochlorite. J Endod 2003;29:141–3.
20. Sakai K, Kato J, Kurata H, et al. The amounts of hydroxyl radicals generated by titanium dioxide and 3.5% hydrogen peroxide under 405-nm diode laser irradiation. Laser Phys 2007;17:1062–6.
21. Joiner A. The bleaching of teeth: a review of the literature. J Dent 2006;34: 412–9.
22. Kugel G, Petkevis J, Gurgan S, Doberty E. Separate whitening effects on enamel and dentin after fourteen days. J Endod 2007;33:34–7.
23. Attin T, Paqué F, Ajam F, Lennon ÁM. Review of the current status of tooth whitening with the walking bleach technique. Int Endod J 2003;36:313–29.
24. Hein DK, Ploeger BJ, Hartup JK, Wagstaff RS, Palmer TM, Hansen LD. In-office vital tooth bleaching: what do light add? Compend Contin Educ Dent 2003;24: 340–52.
25. Sun G. The role of lasers in cosmetic dentistry. Dent Clin North Am 2000;44:831–50.
26. Reyto R. Laser tooth whitening. Dent Clin North Am 1998;42:755–62.
27. Rosenstiel SF, Gegauff AG, Johnston WM. Duration of tooth color change after bleaching. J Am Dent Assoc 1991;122:54–9.
28. Zach L, Cohen G. Pulp response to externally applied heat. Oral Surg Oral Med Oral Pathol 1965;19:515–30.