A Novel Method of Tooth Whitening Using Cold Plasma Microjet Driven by Direct Current in Atmospheric-Pressure Air

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***Abstract*—We reported in this paper the tooth-whitening effect by a direct-current cold atmospheric-pressure air plasma microjet (PMJ) and saline solution (0.9%). All teeth were randomly divided into three groups, treated for 20 min with an air blow and saline solution, a PMJ and saline solution, and 35% hydrogen peroxide gel, respectively. Compared with the other groups, the teeth treated with the PMJ and saline solution showed the best tooth-whitening effect. The microhardness of the tooth enamel measurement showed no apparent differences among the three groups. A minor enamel surface morphological change was observed via SEM but was considered acceptable. Singlet oxygen ($^1O_2$) and hydroxyl ($^\bullet OH$) radicals were detected by electron spin resonance spectroscopy. Atomic oxygen (O) was observed in the optical emission spectra of the PMJ. $^1O_2$, $^\bullet OH$, and O were considered to be the key agents during the tooth-whitening process. This novel method of tooth whitening has the potential to revolutionize the clinical tooth-whitening procedure.**

***Index Terms*—Electron spin resonance (ESR), optical emission spectra, plasma microjet (PMJ), tooth whitening.**

I. Introduction

TOOTH whitening has become an indispensable part of esthetic dentistry. The discoloration of a tooth is a complex process of physical and chemical interactions between stain-causing materials and the teeth. Tooth stains are usually classified as either intrinsic or extrinsic [1].

Extrinsic tooth stains are mainly composed of single- or double-bond organic compound chromophores. Nowadays, tooth-whitening systems are based primarily on hydrogen peroxide or one of its precursors, notably carbamide peroxide [2], [3]. They are often used in combination with an activating agent such as a laser or an incoherent light source [4], [5], although the exact mechanism is not yet fully understood [6], [7].

Plasma is the fourth state of matter, aside from solid, liquid, and gas states, which comprises over 99.9% of the universe. Plasma has long been used in fusion research studies, the lighting industry, and microchip fabrication, as well as in cutting and welding processes. Nonthermal plasmas (where the temperature of the plasma neutrals and ions stays at or around the room temperature while the electrons are energetically hot) have recently attracted much interest in biology and biomedicine due to their applications in bacteria inactivation [8], [9], tissue sterilization [10], blood coagulation [11], [12], wound healing [13], treatment of corneal infections [14], [15], cancer therapy [16], [17], and dental applications [18], [19]. Its potential in tooth whitening has also been explored in a few recent studies: Lee *et al.* [20] reported the enhanced bleaching of extracted teeth by hydrogen peroxide with the assistance of a nonthermal atmospheric-pressure helium plasma jet. A hydroxyl radical ($^\bullet OH$) formed at the tooth surface was considered to contribute significantly to the bleaching process. More interestingly, the tooth was found to serve as a catalyst for the production of $^\bullet OH$. Sun *et al.* [21] also observed the enhanced bleaching of teeth by H_2O_2 but with the assistance of a direct-current atmospheric-pressure air plasma source. They evaluated the tooth morphology as well as the microhardness. No considerable change has been observed. Nevertheless, in both cases, H_2O_2 [either the liquid phase (28%) or a commercially available gel (35%)] has to be used as the intermediate agent between the teeth and the plasma. In this paper, for the first time, H_2O_2 is eliminated as an external agent from the plasma tooth-whitening process. However, a saline solution (0.9% NaCl) is added to keep the tooth surface constantly moist. The surface morphology and microhardness of enamel are evaluated. The results are compared to the cases where the teeth were treated with an air blow plus the saline solution and simply with a H_2O_2 gel. The safety and mechanism of the method are also briefly analyzed.

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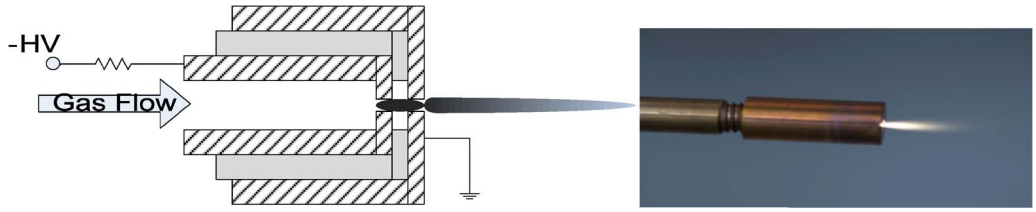

Fig. 1. (a) Schematic diagram. (b) Picture of PMJ.

II. Method

A. Plasma Device

The plasma device used in this paper is consisted of two coaxial copper cylinders as electrodes, which are separated by a dielectric layer with a thickness of around 0.5 mm, as shown schematically in Fig. 1(a). The inner electrode is powered by a dc high-voltage power supply (negatively biased) while the outer electrode is grounded for safety considerations. The nozzle opening of the plasma device has a diameter of around 0.8 mm. The details of the plasma device and the electrical circuitry can be found in [9], [21], and [22]. Compressed air is used as the working gas and is forced through the inner electrode. The sustaining voltage of the plasma microjet (PMJ) is in the range of 400–600 V, with an operating current of 20–35 mA. Fig. 1(b) shows a picture of the PMJ working in air with a typical length of the plasma plume of about 10 mm.

B. Tooth-Whitening Procedure

Sixty intact caries-free human teeth were chosen from premolars extracted for orthodontic reasons and stored in a 0.1% thymol solution. They were randomly divided into three groups: Group A, Group B, and Group C. In Group A, the teeth were exposed to an air blow and the saline solution for 20 min. In Group B, the teeth were exposed to plasma (a 30-mA current and a 5-slm air flow) and the saline solution for the same time duration. The teeth were placed at a distance of 10 mm away from the exit nozzle of the PMJ device, where the temperature was measured with a thermal couple to be approximately 40 °C [21]. In Group C, the teeth were exposed to a H_2O_2 gel (35%, Beyond Technology Corporation, USA) at room temperature for 20 min. The saline solution was applied every 30 s to the surface of the teeth in Group A and Group B to avoid dehydration. A schematic diagram of the PMJ treatment for an extracted tooth is shown in Fig. 2.

C. Tooth-Whitening Evaluation

The color of the teeth was evaluated before and after the treatment using a Crystaleye spectrophotometer (Olympus Corporation, Tokyo, Japan) [21]. In order to quantify the results, the images of the tooth specimens were transformed using the CIE$(L^*a^*b^*)$ system, a standard system for evaluating the tooth whitening in a clinic which allows for a color specification in 3-D space. The L^*-axis represents the degree of brightness within a sample, ranging from 0 (black) to 100 (white). The a^*-plane denotes the degree of green/red while the b^*-plane represents the degree of blue/yellow in the sample. Δ denotes the value difference before and after the treatment in each group. The overall color change (ΔE^*) of the specimen can be calculated from the following expression [23]:

$$\Delta E^* = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2}.$$

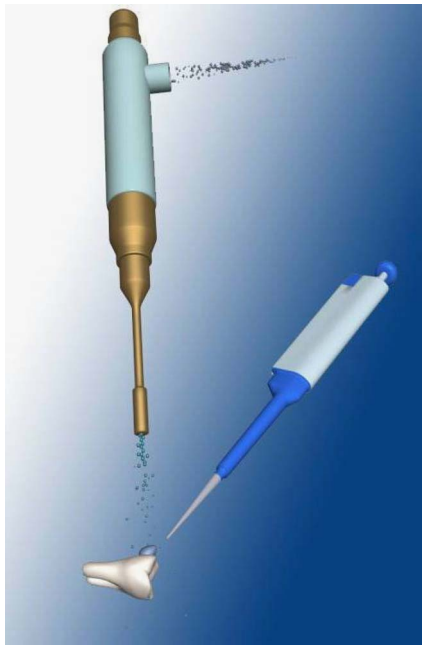

Fig. 2. Schematic diagram of the PMJ treatment of extracted tooth (saline solution (0.9% NaCl) was applied to tooth surface every 30 s).

D. Microhardness

To assess the enamel hardness, another 12 premolars without cracks and defects were cut into halves. The specimens were embedded in acrylic resin with the enamel surface parallel to the horizontal plane. The enamel surfaces were then polished with a water-proof abrasive paper (grade 500-800-1000) to create a flat surface of approximately 5 mm × 5 mm. The specimens were randomly divided into three groups (eight samples per group) and treated following the procedure in Groups A, B, and C (described in Section II-B), respectively. The microhardness of the specimen was determined with a microhardness tester (Shimadzu Corporation, Kyoto, Japan). It was measured three times with a Knoop indenter at a load of 0.9807 N for 10 s. The microhardness value for each group was recorded before and after the treatment.

E. SEM

Three specimens from each group were examined for changes in their enamel surface morphology after different treatments. All the specimens were cleaned, desiccated, and

coated with gold before being evaluated with a scanning electron microscope (SEM) (JSM-5600LV) at magnifications of 5000 and 10 000.

F. ROS

Reactive oxygen species (ROS) generated at the plasma–liquid interface are believed to be essential for the tooth-whitening process. Electron spin resonance (ESR) spectroscopy was used to detect the reactive species generated by the PMJ. ESR spectroscopy is a common method used for the detection of species possessing an unpaired electron at a certain orbit. Many reactive species are rather short lived, making them extremely difficult to be detected through an ESR spectrum directly. These species, however, can be spin trapped by particular reagents, form new radical adducts, and present a characteristic ESR spectrum. Three types of ROS were monitored in particular, namely hydroxyl radical ($^{\bullet}OH$), superoxide anion radical ($^{\bullet}O_2^-$), and singlet oxygen ($O_2^*(^1\Delta_g)$ and $O_2^*(^1\Sigma_g^+)$, collectively written as $^1O_2$ hereinafter). 5,5-dimethyl-1-pyrroline-N-oxide (DMPO, Sigma Aldrich Company, Ltd.) was used to trap $^{\bullet}OH$ and $^{\bullet}O_2^-$, with spin-trapped adducts DMPO-OH and DMPO-OOH, respectively. When water was used as the solution, it was difficult to obtain a clear signal for either adduct in the ESR spectrum. A prominent signal from 5,5-dimethyl-1-pyrrolidone-2-oxyl (the three-electron oxidation product of the spin trap) was rather observed due to a high oxygen stress [24]. Dimethyl sulfoxide (DMSO), a polar aprotic organic solvent, was used instead of water as the solution. Twenty-μL DMPO (0.8 mol/L) was added into 1-mL DMSO (AR, Sinopharm Chemical Reagent Company, Ltd.) and treated by air plasma for 20 s. The spin-trapped adduct DMPO-OOH of $^{\bullet}O_2^-$ is rather difficult to be detected directly possibly due to the smaller reaction rate constant of $^{\bullet}O_2^-$ with DMPO and the shorter lifetime of the adduct DMPO-OOH. The DMPO-OH signal was used as an indication of the production of $^{\bullet}O_2^-$ in the system while superoxide dismutase (SOD, S4636, Sigma Aldrich Company, Ltd.) was added into the system prior to the PMJ treatment to scavenge any $^{\bullet}O_2^-$ produced in the system. The measurement of the ESR signal was carried out with an ESP-300 ESR spectrometer (Bruker Ltd., Germany) operated at room temperature under the following conditions: magnetic field, 3455.00 G; sweep width, 100.0 G; frequency, 9.70 GHz; modulation frequency, 100 kHz; and power, 20 mW. 2,2,6,6-Tetramethylpiperidine (TEMP, Sigma Aldrich Company, Ltd.) was used to trap $^1O_2$ with TEMPO as the spin-trapped adduct. Twenty-μL TEMP (99.9%) was added into 1-mL distilled water and then treated by an air PMJ for 20 s. The measurements of the ESR signals were carried out on an ER-200D-SRC ESR spectrometer (Bruker Ltd., Germany) operated at room temperature under following conditions: central magnetic field, 3420.00 G; sweep width, 200.0 G; frequency, 9.54 GHz; modulation frequency, 100 kHz; and power, 20 mW.

G. OES

The optical emission spectra (OESs) of the air PMJ were recorded in the UV–visible–near infrared range along the axial direction. The light was collected by a fiber optics cable and imaged onto the entrance slit of a 0.75-m spectrometer (Princeton Instrument/Acton Spectra Pro 2750) equipped with a 1800-groove/mm blazed holographic grating. The entrance slit was set at 100 μm in order to obtain a reasonable signal-to-noise ratio with a spectral resolution sufficient to observe major emission lines. The dispersed plasma emission spectra were then recorded by an intensified CCD camera (Princeton Instrument I-Max-512) in the exit plane of the spectrometer. A Roper Scientific ST-133 controller was used for the data acquisition.

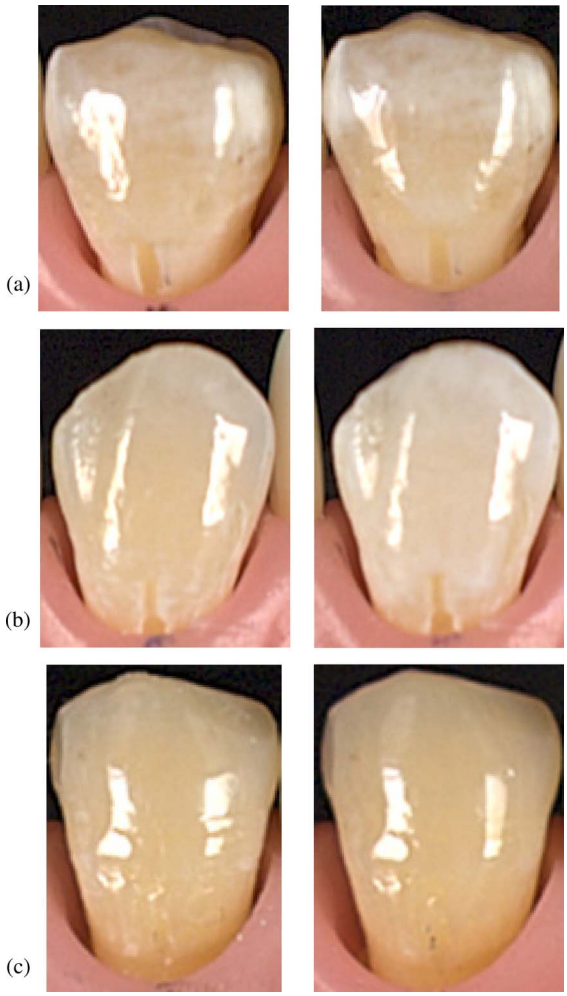

Fig. 3. Photographs of typical tooth (left) before and (right) after 20-min treatment. (a) Group A: Saline solution and air blow. (b) Group B: Saline and PMJ. (c) Group C: H_2O_2 gel only (at room temperature).

H. Statistical Analysis

The differences in the color and microhardness changes among the three groups were analyzed with a T-test and ANOVA in MATLAB (version 2009a).

III. Results

A. Tooth-Whitening Efficacy

The pictures of a typical tooth from Group B and Group C before (left column) and after (right column) the treatment are shown in Fig. 3(b) and (c), respectively. Compared to Group A

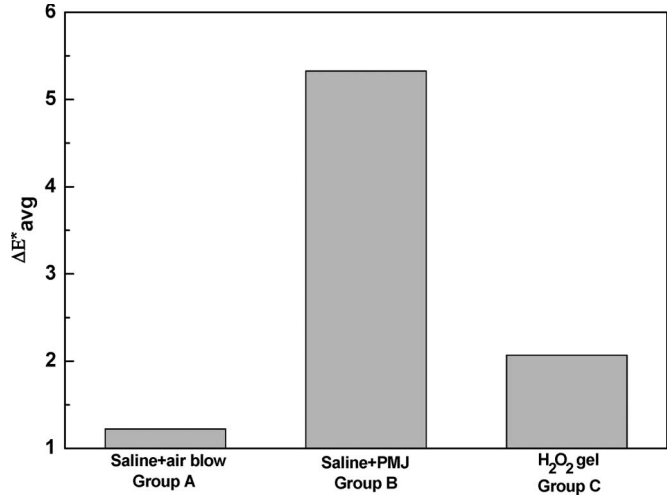

Fig. 4. Average colorimetric value $\Delta E^*_{\rm avg}$ of the teeth in the following: Group A (treated with saline solution and air blow at room temperature), Group B (treated with saline solution and PMJ), and Group C (treated with 35% H_2O_2 gel at room temperature). Teeth were treated for 20 min in all groups.

TABLE I
MEAN VALUES AND STANDARD DEVIATION OF COLOR DIFFERENCE (ΔL^*, Δa^*, Δb^*, AND ΔE^*) IN GROUP A, GROUP B, AND GROUP C

	△L*	△a*	△b*	△E*
Group A	0.381±0.709	-0.138±0.331	-0.189±1.149	1.222±1.016
Group B	3.128±1.817#	-0.249±0.420	-3.715±2.680#	5.325±2.388#
Group C	1.391±0.575	-0.209±0.248	-1.339±0.811	2.067±0.719

#Statistically (P_{BA}=0.0043< 0.05, P_{BC}=1.9699e-008< 0.05) significant difference among the three groups. T-test is used between each two groups.

and Group C, the whiteness of the tooth in Group B is improved significantly after the treatment.

The average colorimetric values $\Delta E^*_{\rm avg}$ of the teeth from these three groups after the treatment are shown in the bar diagram in Fig. 4. $\Delta E^*_{\rm avg}$ of the teeth in Group A is slightly above one. $\Delta E^*_{\rm avg}$ of the teeth in Group C is around two while $\Delta E^*_{\rm avg}$ of the teeth in Group B reached approximately 5.4.

The changes in the colorimetric values of the teeth were obtained by determining the differences in L^*, a^*, and b^* among the three groups (see Table I). In Group B, the overall color change (ΔE^*) exceeded five units; a statistic analysis showed that there were significant differences between Groups A and B $(P_{BA} = 0.0043 < 0.05)$ and Groups B and C $(P_{BC} = 1.9699e - 008 < 0.05)$, which means a significant whitening effect. The improved ΔE^* was attributed to a significant increase in the whiteness (ΔL^*) and a reduction in the yellow shades (Δb^*) of the teeth.

B. Microhardness Results

The enamel microhardness of the specimens after the treatment is shown in Table II. The microhardness of the teeth in Group A showed a little change before and after the treatment while that of the teeth in Group B and Group C slightly decreased. However, a statistical analysis suggests no significant difference among the three groups $(P = 0.4594 > 0.05)$. This indicates that the PMJ treatment does not affect the hardness of the tooth enamel significantly.

TABLE II
MICROHARDNESS OF THREE GROUPS

	before treatment	after treatment	△MH
Group A	301.6±31.33	312.4±36.51	-4.104±13.77
Group B	324.4±15.50	316.6±10.29	10.29±2.497
Group C	341.6±18.12	337.2±19.46	1.239±4.249

#Statistically (P=0.4594 > 0.05) insignificant difference between the teeth in the three groups. ANOVA is used among the groups.

C. Surface Morphology Evaluation

The SEM photographs of the teeth treated with the different methods are shown in Fig. 5. It can be clearly seen that the enamel surface condition of the teeth in Group A has a little change. The teeth in Group C show a slightly rougher surface condition after the treatment of 20 min. The teeth in Group B show a morphological change that is comparable to that in Group C and, therefore, is considered acceptable.

D. Measurement of ROS

When DMPO is used as the spin-trap reagent, the resulting spin-trapped adduct DMPO-OH usually shows a 1 : 2 :2 : 1 quartet pattern in the ESR spectrum [25], as seen in Fig. 6. SOD is used to indirectly detect the existence of $^{\bullet}O_2^-$ as described in Section II-F. When an increased amount of SOD is added into the system, the DMPO-OH signal decreases accordingly (as shown in Fig. 6). This indicates that $^{\bullet}O_2^-$ not only exists in the system but also very likely serves as one of the precursors of $^{\bullet}$OH. It has to be noted that Cu_2^+ and DMPO can essentially form an intermediate product that shows up in the ESR spectrum with the same quartet pattern [26]. We cannot yet, at this stage, differentiate one from the other.

When TEMP was used as the spin-trap reagent for $^1O_2$, the resulting spin-trapped adduct TEMPO showing a triplet pattern with a line intensity ratio of 1 : 1 : 1 [27] was detected directly in the PMJ-treated distilled water (as shown in Fig. 7). To further verify the existence of $^1O_2$, we added L-histidine (L-His, a $^1O_2$ quencher) into the system before the PMJ treatment. After the plasma treatment, the TEMPO signal decreased with the increase of L-His and completely disappeared when 20-mg L-His was added into the system.

E. OESs

Fig. 8 shows an end-on OES of the PMJ from 200 to 850 nm. The spectrum is dominated by N_2 and N_2^+ emission bands in the near UV region. These emissions are attributed to the excessive concentration of N_2 in air. A considerable amount of emission from NO can also be seen in the UV-C region from 215 to 280 nm. A near-infrared emission spectrum is shown in the inset of Fig. 8. Atomic oxygen emission at a wavelength of 777.2 nm, although weak, is still detectable.

IV. DISCUSSION

This paper was designed to investigate the efficiency of tooth bleaching by a direct-current nonthermal PMJ. Unlike previous investigations [21], no hydrogen peroxide was used as the

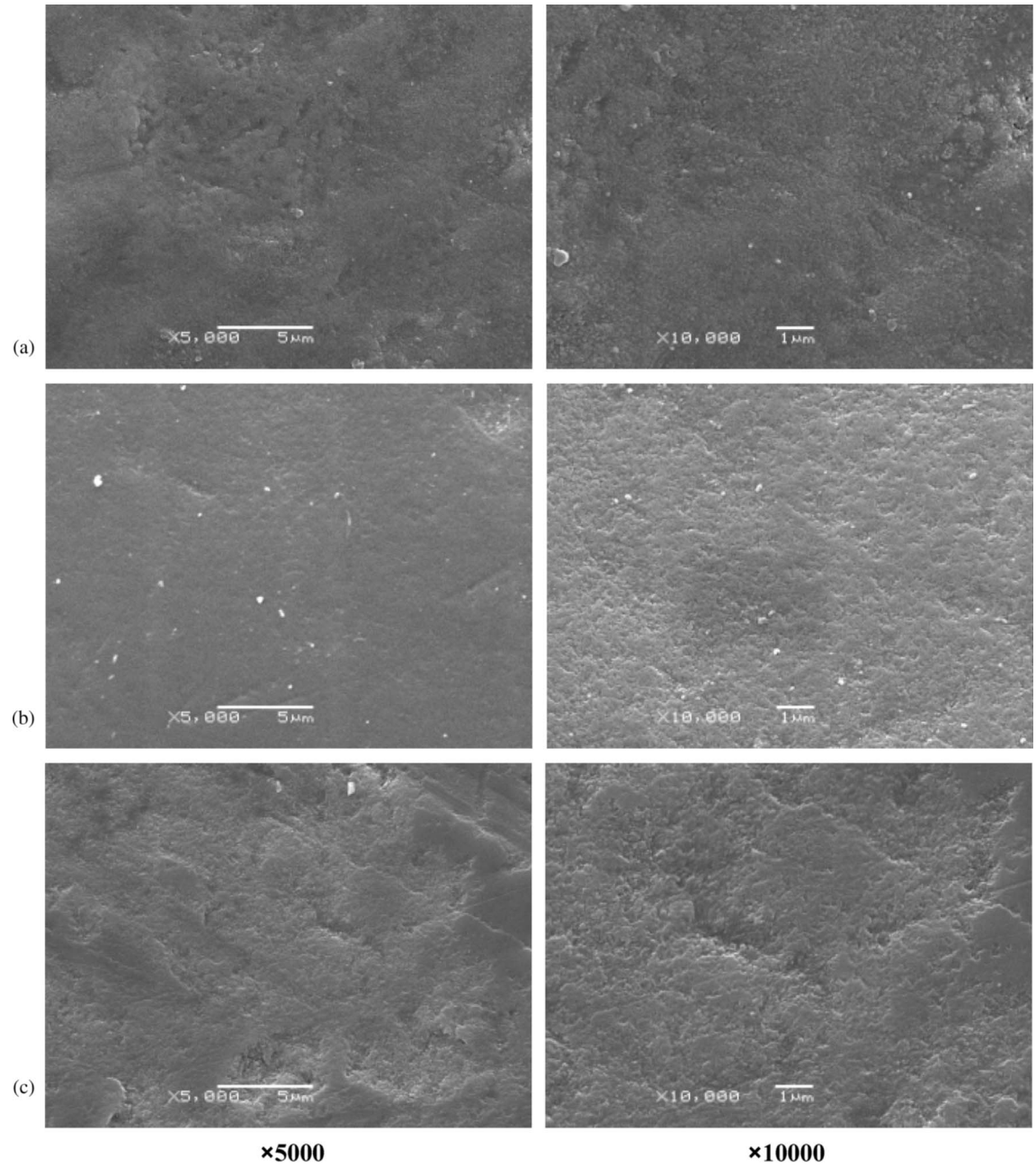

Fig. 5. SEM photographs of teeth (a) treated with saline solution and air blow, (b) treated with saline solution and PMJ, and (c) treated with H_2O_2 gel.

assisting agent in the process. The 20-min treatment with the PMJ resulted in a significant increase in the whiteness and a reduction in the yellow shades of the teeth. The average ΔE^* increased by about five units after the PMJ treatment in the presence of the saline solution.

In a traditional tooth-whitening process, hydrogen peroxide and carbamide peroxide are the common choices in tooth bleaching. Although the exact mechanism of this process is not fully understood, it is believed that the formation of perhydroxyl anions (HO_2^-) and $^\bullet$OH is critical [6], [7]. The effectiveness of the PMJ in tooth whitening is most likely due to the production of ROS at the plasma–liquid–tooth interface, which is similar to what occurs at the whitening gel–tooth interface in the traditional process. It has been reported in another study [27] that a trace amount of ozone can be detected in the downstream of the PMJ. Atomic oxygen and ozone produced in the plasma interact with water produce $^\bullet$OH and $^\bullet O_2^-$, its protonated conjugate $^\bullet$OOH and $^1O_2$, and other intermediate ROS [22], [27], [28]. Although no quantitative evaluations of the complex reaction sequence have been carried out so far, we believe that the improved whitening efficacy of the PMJ with the saline solution is attributed to the following mechanisms, whose respective importance is difficult to ascertain.

1) ROS ($^\bullet$OH, $^1O_2$, $^\bullet O_2^-$, etc.) yielded from plasma–water interactions directly or indirectly react with the stain molecules on the tooth surface by breaking the bonds of the long carbon chain, thus achieving the whitening effect.
2) ROS generated at the plasma–liquid interface are probably more easily and effectively accessible to the tooth than those produced by the H_2O_2 gel.

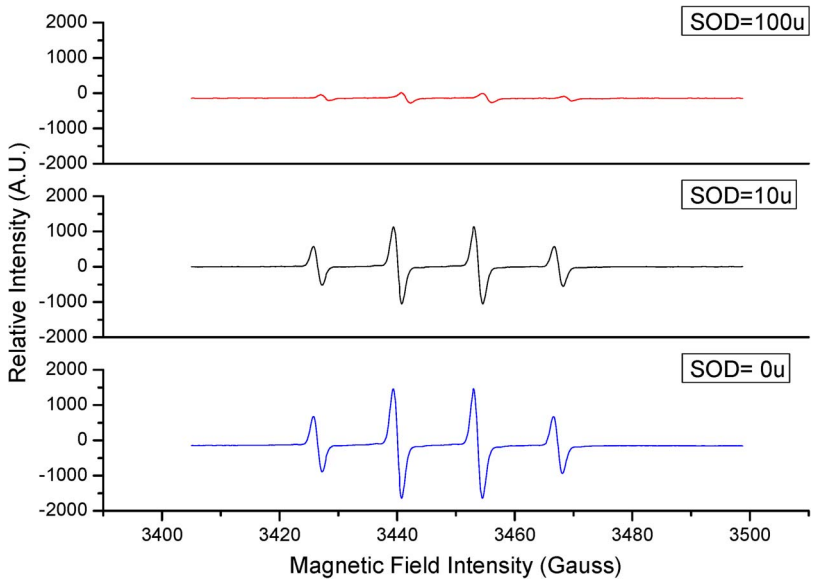

Fig. 6. DMPO-OH signals at different SOD concentrations.

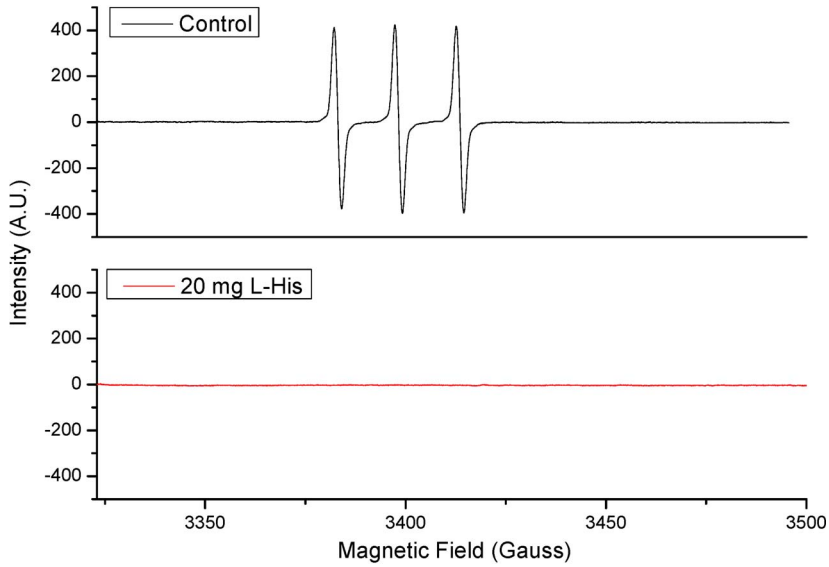

Fig. 7. TEMPO signal and the influence of L-His.

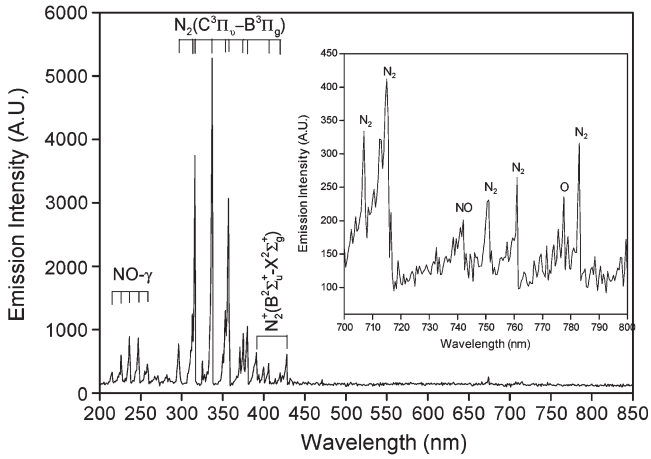

Fig. 8. OES (from 200 to 850 nm) of the PMJ in air. Inset shows the emissions in the near-infrared section from 700 to 800 nm.

3) With NO present in the PMJ and aqueous environment, a trace amount of nitric acid was found in our previous work [22]. As a result, localized acid pickling on the surface of the teeth may also contribute to the increased whitening efficacy.

It is also conceivable that the PMJ reacts with NaCl in the saline solution to produce chlorine-based ions and radicals. However, since the concentration of NaCl in the saline solution is very low (0.9%), only trace amounts of the chlorine-based ions and radicals are expected to be produced so that they are not expected to contribute significantly to the whitening effect.

The teeth treated by the PMJ and saline solution have slightly rougher enamel surfaces compared to the control sample but are similar to those treated with H_2O_2 gel. Some studies have also shown that tooth whitening by H_2O_2 or carbamide peroxide may cause mild changes in the enamel surface morphology [29], [30]. Therefore, we consider this morphological change acceptable.

Although nonthermal plasmas have already shown promise in cell adhesion [31], tissue sterilization [16], and skin regeneration [32] with little known harmful side effects to humans, some safety concerns need to be addressed in future studies. For instance, ROS produced in the system may be a key player in the tooth-whitening process, but they can also be harmful for human oral tissues and the respiratory system if the dosage is not properly controlled. It is necessary to carefully quantify the reactive species (including harmful species such as NO_x and O_3) produced in the plasma–liquid system as well as in open air and to compare them to known standards before any *in vivo* experiments are carried out.

V. Conclusion

In conclusion, this paper has investigated the tooth-whitening effect of a direct-current atmospheric-pressure cold air PMJ. A saline solution (0.9% NaCl) was used to keep a moist environment. The whitening efficacy in 20 min well exceeds that of a traditional H_2O_2 gel treatment situation. The increased value of ΔE^* was attributed to an increase in the whiteness and a decrease in the yellow color saturation. The tooth surface morphology was slightly modified but is considered to be within acceptable limits. The ESR spectra showed the existence of $^{\bullet}OH$, $^{\bullet}O_2^-$, and $^1O_2$ while the OESs showed the existence of atomic oxygen and the presence of nitric oxide. These reactive species directly or indirectly contribute to the whitening process. The evident whitening effect and the replacement of the whitening gel may eventually revolutionize tooth whitening.

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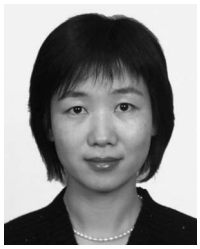

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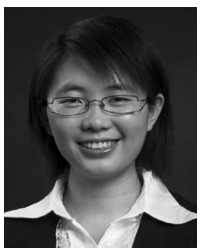

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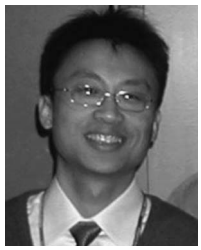

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