Reactive hydroxyl radical-driven oral bacterial inactivation by radio frequency atmospheric plasma

Sung Kil Kang,[1] Myeong Yeol Choi,[2] Il Gyo Koo,[2] Paul Y. Kim,[2] Yoonsun Kim,[2] Gon Jun Kim,[2] Abdel-Aleam H. Mohamed,[1,3] George J. Collins,[2,a)] and Jae Koo Lee[1,b)]
[1]*Department of Electronic and Electrical Engineering, Pohang University of Science and Technology, Pohang 790-784, South Korea*
[2]*Department of Electrical and Computer Engineering, Colorado State University, Fort Collins, Colorado 80523, USA*
[3]*Department of Physics, Faculty of Science, Taibah University, Almadinah Almunawwarah, Saudi Arabia*

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We demonstrated bacterial (*Streptococcus mutans*) inactivation by a radio frequency power driven atmospheric pressure plasma torch with H_2O_2 entrained in the feedstock gas. Optical emission spectroscopy identified substantial excited state •OH generation inside the plasma and relative •OH formation was verified by optical absorption. The bacterial inactivation rate increased with increasing •OH generation and reached a maximum 5-$\log_{10}$ reduction with 0.6% H_2O_2 vapor. Generation of large amounts of toxic ozone is drawback of plasma bacterial inactivation, thus it is significant that the ozone concentration falls within recommended safe allowable levels with addition of H_2O_2 vapor to the plasma. © *2011 American Institute of Physics*.
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Bacterial inactivation in clinical dental practice is widely carried out using chlorhexidine (CHX), a chemical antiseptic with well-known antimicrobial efficacy. However, irritation of oral mucosa and staining of teeth have been reported with the use of CHX.[1] Consequently, non-thermal atmospheric pressure plasmas have been investigated as alternative tools for inactivation of oral bacteria.[2–4] Ozone and UV (Ref. 5) can play dominant roles in plasma sterilization at atmospheric pressure, however, breathing ozone is known to cause chest pain, impair respiratory function, and damage lung tissue. Our present work was motivated by a search for a hydroxyl radical-driven bacterial inactivation method using low temperature atmospheric pressure plasma with low-level ozone generation. In this letter we present our approach and the evidence of bacterial inactivation.

The plasma jet consists of a ceramic tube surrounded by two metal electrodes; one powered by a 13.56 MHz power (Cesar-133, Advanced Energy) and the other grounded [Fig. 1(a)]. Argon (Ar) carrier gas, regulated by a mass flow controller, flows into two lines; one directly through the ceramic tube and the other through a bubbler containing 30% hydrogen peroxide (H_2O_2) with water to form a gas mixture of Ar, H_2O, and 0%–1% final concentration (v/v) of H_2O_2. A 10 W effective power was sufficient to sustain a cold plasma jet with up to 1% entrained H_2O_2 vapor. Photos of the plasma jet operating with 0%–1% H_2O_2 vapor concentrations are presented in Fig. 2(a). The plasma jet is ~10 mm in length without H_2O_2 vapor and gradually shortens to ~3 mm as H_2O_2 vapor concentration reaches 1%. Above 1% H_2O_2 vapor, plasma is only sustained inside the ceramic tube.

Average plasma gas temperature were measured by thermosensor (Fluke 54 II) as 41.3 ± 2 °C and 34.9 ± 2 °C at the end of the plasma plume and 3 mm below the end, respectively. In comparison, sterilization by dry heat is typically carried out at 160–170 °C for up to 1 h. There was no significant change in gas temperature with addition of H_2O_2 vapor. The amount of plasma-generated ozone, measured 5 cm below the plume termination (Eco Sensors A-22), decreased with the concentration of H_2O_2 vapor as depicted in Fig. 3. Addition of 0.4% H_2O_2 vapor reduced the ozone concentration to less than 0.1 ppm, the exposure limit recommended by occupational safety and health organizations.[5] At 1% H_2O_2 vapor concentration, the level of ozone decreased by >83% compared to pure Ar plasma.

The spatial distribution of hydroxyl radical (•OH) was determined by means of optical emission spectroscopy with an intensified charge coupled device (ICCD) camera (Princeton Instruments PI-MAX) and 308 nm bandpass filter. ICCD images presented in Fig. 2(b) were acquired with a fixed integration time of 20 ms and gain of 150. The highest concentration of excited state of OH is observed at 0.6% H_2O_2 vapor concentration and most abundant at the end of jet nozzle, it appears to travel over a millimeter range during its

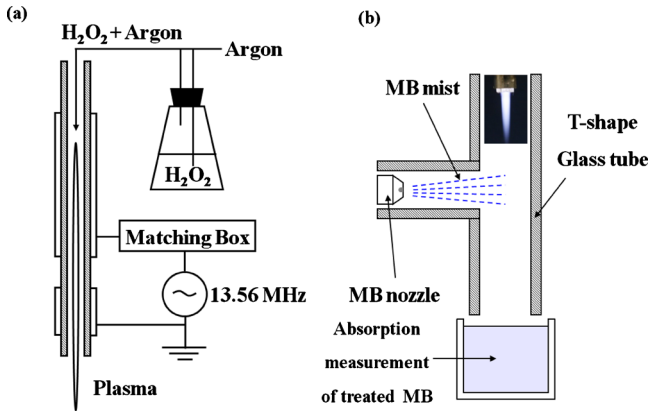

FIG. 1. (Color online) Schematic diagrams of (a) rf-driven atmospheric plasma jet (b) setup for measuring relative •OH concentration using MB dye degradation.

a)Electronic mail: gcollins@engr.colostate.edu. FAX: 1-970-491-8671.
b)Electronic mail: jkl@postech.ac.kr. FAX: 82-54-279-2903.

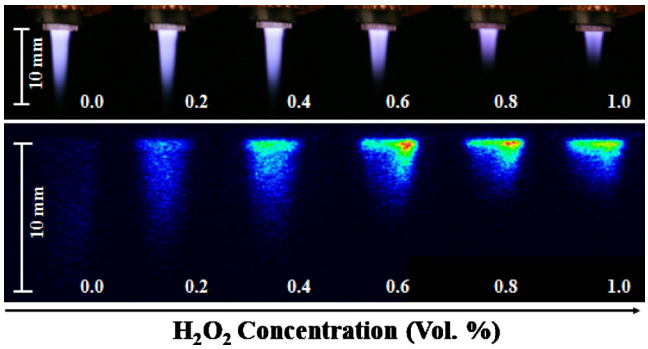

FIG. 2. (Color online) (a) Photos and (b) ICCD images (308 nm filter) of the plasma jet plume with entrained H_2O_2 vapor concentration ranging from 0 to 1%.

lifetime. Reactive oxygen species (ROS) is also determined with 777.1 nm bandpass filter (not presented). ROS is monotonically decreased as increasing concentration of H_2O_2 vapor because of high reactivity of ROS with H_2O_2 and H_2O vapor.[6]

The presence of •OH was verified using an aerated aqueous methylene blue (MB) solution[7] (0.3 mM, 0.8 ml/s) as depicted in Fig. 1(b). The absorbance at 665 nm of MB decreased as a function of H_2O_2 vapor concentration up to 0.6% (Fig. 3). This absorption data agrees qualitatively with optical emission spectra measurements and also confirms that the plasma-generated •OH can travel over a millimeter to the target.

Particle-in-cell Monte Carlo collision and fluid simulation results suggest that rf plasma produces enough high-energy electrons to break both the HO-OH bond (2.2 eV) in H_2O_2 and the O_2-H bond (3.8 eV) in H_2O (Ref. 8) to generate •OH.[6] In these reactions, •OH production increases linearly with the concentrations of H_2O_2 and H_2O. On the other hand, total plasma emission is observed to weaken with H_2O_2 concentration, signifying decreased electron density [Fig. 2(a)]. Therefore, •OH production reaches a maximum value at a specific H_2O_2 concentration, which our measurements indicate occurs near 0.6% H_2O_2. Ozone can be dissociated by H_2O in the feedstock and OH and H reaction products (from H_2O_2 and H_2O dissociation) by the following secondary reactions.[6]

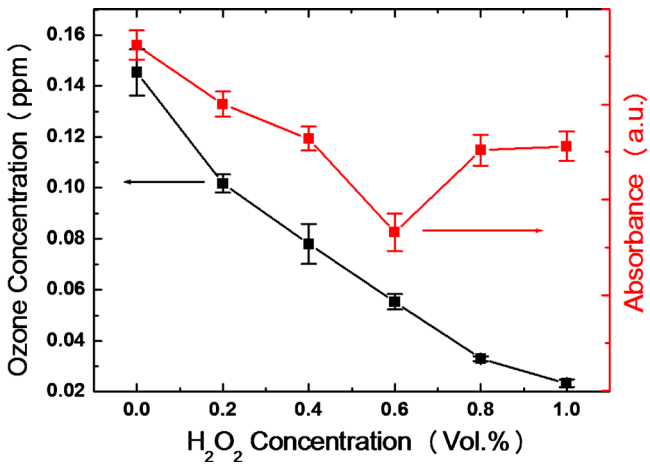

FIG. 3. (Color online) Saturated ozone concentration (black squares) measured 5 cm below the end of plasma plume and absorbance of MB at 655 nm (red squares) after plasma treatment with entrained H_2O_2 vapor concentration ranging from 0 to 1%.

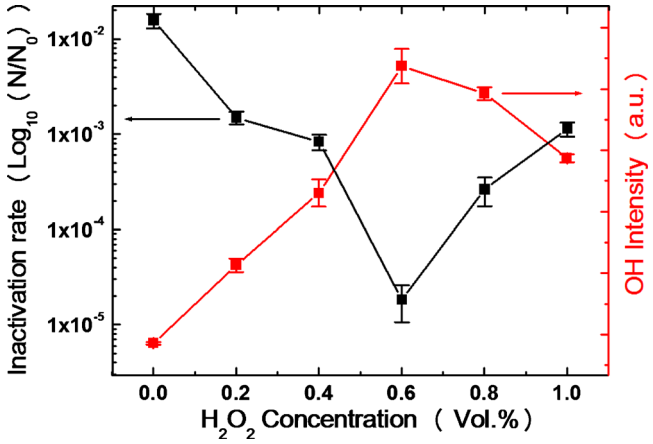

FIG. 4. (Color online) Inactivation rate $[\log_{10}(N/N_0)]$ of *S. mutans* (black squares) and integrated OH intensity (red squares) as a function of H_2O_2 vapor concentration. $N_0 \approx 8 \times 10^9$ CFU/ml, treatment time: 3 min.

$$\bullet OH + O_3 \rightarrow HO_2\bullet + O_2, \quad \text{Rate:} 7.55 \times 10^{-14}\ \text{cm}^3\ \text{s}^{-1}, \tag{1}$$

$$\bullet H + O_3 \rightarrow \bullet OH + O_2, \quad \text{Rate:} 1.4 \times 10^{-10}\ \text{cm}^3\ \text{s}^{-1}, \tag{2}$$

$$HO_2\bullet + O_3 \rightarrow \bullet OH + 2O_2, \quad \text{Rate:} 1.1 \times 10^{-14}\ \text{cm}^3\ \text{s}^{-1}. \tag{3}$$

We conducted a sterilization experiment with the dental pathogen *Streptococcus mutans* (*S. mutans*, strain: KCTC 3065/ATCC 25175). A disk confined within the plasma treatment area was spot inoculated with a 2 μl suspension ($\sim 8 \times 10^9$ CFU/ml) of *S. mutans* and dried for 10 min. After 3 min plasma treatment at a distance of ~ 3 mm from the end of the plasma jet, each disk was vortexed in 1 ml of phosphate buffered saline and serial dilutions were spread on brain heart infusion (BHI) agar plates. The plates were incubated at 37 °C for 24 h to determine bacterial colony counts. Inactivation was evaluated by determining the ratio of colony counts after treatment (N) to control ($N_0 \approx 8 \times 10^9$ CFU/ml). All data presented are mean ±standard error of measurement of three independent experiments. As shown in the left axis of Fig. 4, the bacterial inactivation rate increased with H_2O_2 vapor up to 0.6% but decreased at higher concentrations. Inactivation efficacy was highly dependent on the •OH concentration (determined by optical emission spectroscopy) as plotted on the right axis.

For direct treatment of dental germs in the oral cavity, a plasma jet must be small and obviously nonthermal. In this study, we achieved a 5-$\log_{10}$ reduction in *S. mutans* using a 1 mm diameter rf plasma jet operating below 43 °C. While ozone, often generated by atmospheric pressure plasmas, is highly effective in killing bacteria, its toxicity presents a disadvantage for use in the clinical setting. By adding H_2O_2 vapor to the plasma feedstock gas, we reduce ozone formation yet still inactivate bacteria through short-lived •OH.

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