Bacterial inactivation using atmospheric pressure single pin electrode microplasma jet with a ground ring

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Bacterial inactivation experiment was performed using atmospheric pressure microplasma jets driven by radio-frequency wave of 13.56 MHz and by low frequency wave of several kilohertz. With addition of a ground ring electrode, the discharge current, the optical emission intensities from reactive radicals, and the sterilization efficiency were enhanced significantly. When oxygen gas was added to helium at the flow rate of 5 SCCM, the sterilization efficiency was enhanced. From the survival curve of *Escherichia coli*, the primary role in the inactivation was played by reactive species with minor aid from heat, UV photons, charged particles, and electric fields. © *2009 American Institute of Physics*. [DOI: 10.1063/1.3114407]

Atmospheric pressure glow discharges and their applications in biomedical treatments have become hot issues of current low-temperature plasma research.[1–3] They have been operated at an excitation frequency either in the several tens of kilohertz ac range (or pulsed mode) or in the radio-frequency (rf) range.[4–8] Atmospheric pressure glow discharges with different designs have been shown to inactivate many different microorganisms including the bacteria,[9–11] fungus,[12] bacterial spores,[13–15] plant cells,[9] cancer cells,[2] proteinaceous matters,[16,17] and even genetic DNA.[8] Atmospheric pressure microplasma jets are among the most plausible candidates for that purpose. Therapeutic effects of plasma treatment are caused by chemical reactions with atomic oxygen and nitrogen, hydroxyl radical, nitrogen oxide. Addition of a small amount of impurity gas to pure noble gas (here helium) modifies the charged particle balance and discharge impedance, thus altering the composition of reactive species in the plasma.[18,19] The effect of mixing oxygen gas to helium on the composition of reactive species and on the biomedical treatment is one of the concerns of this study.

A key issue for atmospheric-pressure microplasma jets is to expand their stability range to a moderate discharge current with improved plasma reactivity. A possible way to achieve this is to place a ground ring electrode at the surface of the outlet glass tube. In contrast to the single pin electrode jet, spatially confined gas ionization occurs in the interelectrode region. The rapid oscillation of the excitation voltage imparts a radial directed momentum on electrons, making it difficult for electrons to be transported axially toward the downstream region.[20] Thus the plume length is reduced, but the plume radius becomes thick. In this paper, specially designed microplasma jets driven by a several tens of kilohertz ac and 13.56 MHz rf voltages are reported. The effect of a ground ring on the electrical characteristics and optical emission spectra of the plasma jets and their interaction with biomaterials was explored.

Figure 1(a) shows the jet source driven by low frequency (LF) of several tens of kilohertz ac voltage. At the center of the glass tube is a copper wire with a diameter of 0.9 mm and a pencil-shaped tapered end. The wire was concentric with a *T*-shaped cylindrical glass tube, which has an inside diameter of 6 mm and an outside diameter of 8 mm. The wire shaft was covered with a polyethylene insulator tube, leaving a length of 4 mm of the wire exposed to gas. The tip-to-nozzle distance was 3 mm. The power source (FTLab HPSI200) of several tens of kilohertz was applied to the copper wire. Figure 1(b) shows the jet driven by 13.56 MHz rf voltage. The tungsten pin wire (0.3 mm diameter) with sharpened tip was inserted coaxially in a Perspex tube (7 mm inner diameter, 10 mm outer diameter) and it protruded from the stainless steel holder by 1.3 cm. The tip-to-nozzle distance for the rf jet was 1 mm. The glass tube was filled with helium gas (99.999%) delivered at a flow rate in the range of 1–2 l/min, controlled by a flow meter (Kofloc RK1600R). The electrical properties were measured by using a high voltage probe (Tektronix P5100) and a current probe (Pearson 3972). The current probe monitors the oscillating current in the circuit. The results were recorded on a real time digital oscilloscope (LeCroy WS44Xs).

Figure 2(a) shows the wave forms of the applied voltage and total current for different discharge modes operating at 50 kHz. The gas flow rate for the LF jet was kept constant at 2 l/min. When a copper ring (width 3 mm, thickness 0.4 mm,

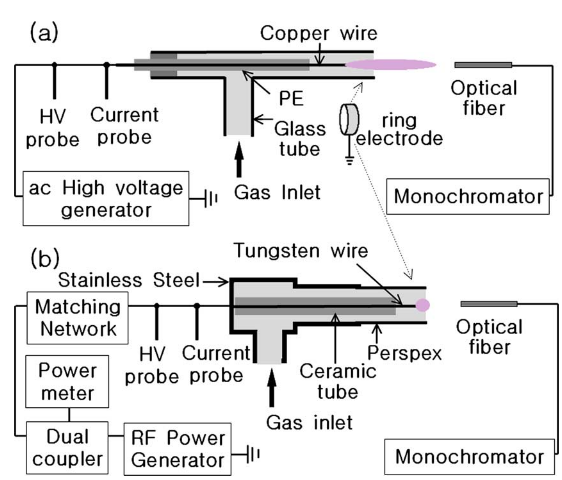

FIG. 1. (Color online) Schematic of the experimental setup. (a) LF microplasma jet and (b) rf microplasma jet with diagnostics systems.

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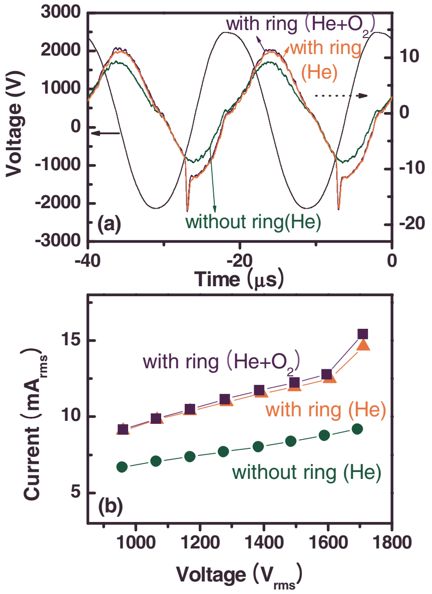

FIG. 2. (Color online) (a) Waveforms of the total current and the applied voltage for different discharge conditions. (b) Current vs applied voltage for three operating conditions.

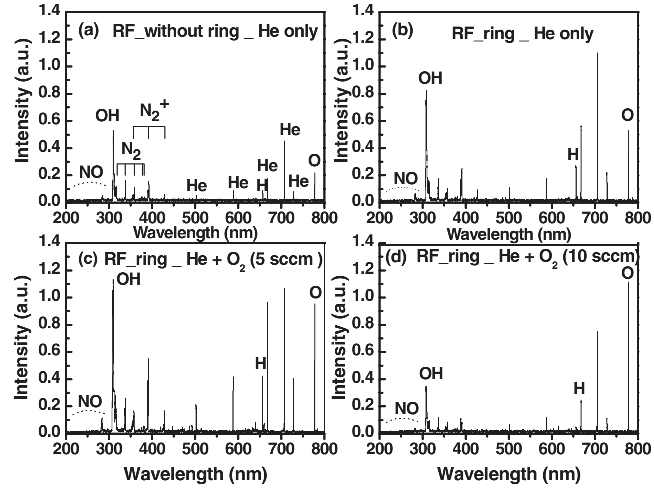

FIG. 3. Emission spectrum from 200 to 800 nm observed in rf plasma jet (6 W) with different conditions: (a) He only without a ring electrode, (b) He only, (c) He with the 5 SCCM of oxygen gas, and (d) He with the 10 SCCM of oxygen gas. The (b)–(d) are with a ground ring.

diameter 8.2 mm (LF) and 10.2 mm (rf)) serves as a ground electrode, the total current is observed to increase. Surface charge accumulated on the dielectric layer beneath the ring during one half period favors the discharge breakdown in the next half. This typically leads to the current spikes of much shorter duration than the ac excitation period. With addition of oxygen gas, the total current was observed to increase slightly. However, when the oxygen flow rate increased to a specific value, the plasma extinguished. This resulted from an excessive oxygen attachment, which caused the plasma to become unstable, reducing the electron density in plasma at a high oxygen flow.[13] The total current versus the applied voltage for three different operating conditions (He only without a ring electrode, and He/O_2 with, and He/O_2 without the ring) are presented in Fig. 2(b). The applied voltage is in the range of 950–1700 V_{rms} and the measured total current without a ring electrode is 6.7–9.2 mA_{rms}. The plasma with a ground ring electrode has larger total current amplitude up to 15.5 mA_{rms}. The difference in the current amplitude between the He only case and the He/O_2 case is observed to be small, but the difference is expected to become large as the applied voltage is increased. The gas temperature was measured by using a fiber optic temperature sensor (FISO UM14&FOTL-L). The gas flow rate for the RF jet was kept constant at 2 l/min. The continuous increase in the gas temperature from 300 to 309 K was seen as the rf power increased from 5 to 10 W. With a ground ring electrode, a continuous increase from 308 to 352 K was seen as the rf power increased from 5 to 10 W. On the other hand, the gas temperature of the LF plasma was as low as the room temperature (294 K). Even with a ring, the gas temperature did not deviate much from the room temperature, varying from 300 to 306 K as the applied voltage increased from 900 to 1600 V_{rms}.

Optical spectra were recorded for emission along the axis of the plasma jet (detected at the distance of 2 cm from the nozzle) in the range from 200 to 800 nm. The emitted light was focused by means of optical fiber into entrance slit of 0.75 m monochromator (SPEX 1702), equipped with a grating of 1200 grooves/mm and slit width of 100 μm. Figure 3 shows the emission spectra observed in the rf plasma jet. Four different spectra are compared. They are (a) the He only case without a ring, (b) the He only case with a ring, (c) the He/O_2 (5 SCCM) (SCCM denotes cubic centimeter per minutes at STP) with a ring, and (d) the He/O_2 (10 SCCM) with a ring. Compared with the LF jet spectra (not shown) the rf jet spectra have strong lines of He, OH, and O.[2,4,6] It should be noted that there appear the emission lines from nitrogen oxide (NO) (at 282 nm) and the H_a line (at 656 nm). With addition of a ring electrode, the spectra have higher intensity in helium atomic lines indicating larger plasma density and/or electron temperature. With the ring electrode, the intensities of O, OH, H, and NO peaks were enhanced at the O_2 flow rate of 5 SCCM. As the oxygen flow rate was increased to 10 SCCM (which is equivalent to 1 vol % in helium), the intensities from all the emission line except oxygen (at 777 nm) diminished, which indicated that electrons dissipated more of their energy through collisions with oxygen molecules rather than helium. Since the critical radicals to cell death are O, NO, and OH radicals, the richness of these species may make the rf plasma jet utilizing a proper ratio of He/O_2 mixture suitable for biomedical applications.[12,13]

The bacterial inactivation experiment was performed. The *Escherichia coli* (K-12 DH10B) suspension samples of 1 μl was deposited on the cover glass and directly exposed to the plasma. The distances of 2.5 mm (the rf jet), and 1.5 cm (the LF jet) were chosen to place the samples from the tip of the pin electrode for exposure. The nozzle-to-sample distances were chosen for convenience in treatment. The gas flow rate was kept constant (1 l/min for the rf jet, 2 l/min for the LF jet). The treated samples were then placed in a 10 ml of sterile distilled water and were thoroughly detached from the cover glass. The mixtures were serially diluted from 10^{-2} to 10^{-4}. Afterwards, the diluted solution were spread onto Petri dishes with the culture media (LB agar, Miller) and incubated at 37 °C for 20 h. After the incubation, the resulting colony forming units were counted. For each experimental condition, five samples were prepared, three submitted to the plasma flowing afterglow, and one stored in the laboratory atmosphere for the same dura-

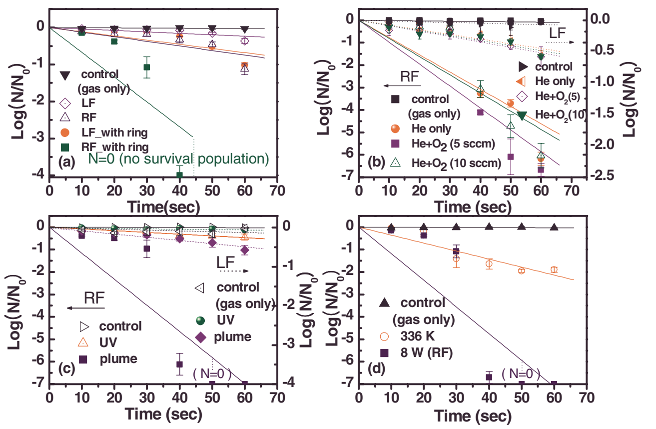

FIG. 4. (Color online) (a) Survival curves of *E. coli* for different discharge conditions: the LF jets in 1400 V with and without a ring electrode (distance 1.5 cm), the rf jets in 8 W with and without a ring electrode (distance 5 mm). (b) The effect of added oxygen gas on the sterilization efficiency for the LF jet (with ring, 1200 V, 1.5 cm) and the rf jet (with ring, 6 W, 2 mm), respectively. The solid lines are for the rf jets and the dotted lines are for the LF jets. (c) The effects of UV radiation on the survival of *E. coli* for the LF jet (with ring, 1200 V, 1.5 cm) and the rf jet (with ring, 8 W, 2 mm). The circle symbols correspond to the results for the LF jets. The UV only cases are compared with the control and the plasma treatments. (d) The effect of hot plate temperature on the survival of *E. coli* (circle symbols) and the comparison with the control and the plasma treatments.

tion, as a control. Another control experiment was performed with the same gas flow, except with the plasma turned off. For the treated sample with the gas flow only, the same result was obtained as that for the sample control.

Figure 4(a) shows the survival curve of *E. coli* for different discharge conditions. Two cases of LF (50 kHz) jets (with and without a ring, applied voltage 1400 V, distance 1.5 cm) and two cases of rf jets (with and without a ring, input power 8 W, 5 mm distance) are considered. The vertical axis is the log of the number of viable *E. coli* remaining (N) to the control number (N_0). The solid and dotted lines represent fitting curves using the least-squares linear regression. The decimal reduction time (D value) for the rf plasma jet with a ring is 36 s (8 W, 5 mm), and D value for the jet without the ring is 58 s (8 W, 5 mm). Almost all *E. coli* were killed in about 40 s at 5 mm distance for the rf jet with a ring. The D value for the LF plasma jet with the ring is 55 s (1400 V, 1.5 cm). The experimental data show that the sterilization efficiency of the rf plasma jet is generally better than that of LF plasma jet. The sterilization efficiency was proportional to the input power (or the applied voltage). However, the current results indicate that the LF plasma jet with a little higher voltage can achieve a level of sterilization efficiency comparable to that of the rf jet.

Figure 4(b) shows that the addition of a small amount of O_2 gas to pure helium results in an efficient sterilization. Higher sterilization efficiency was achieved in both the rf (solid lines) and LF jets (dotted lines) with the O_2 flow rate of 5 SCCM. In many cases, oxygen radicals were considered as one of the important contributor to sterilization. As was observed in Fig. 3(d), the oxygen content was high in the O_2 flow rate of 10 SCCM, while the contents of OH, H, and NO were lower that those in the O_2 flow rate of 5 SCCM. The survival curve demonstrates that the sterilization strongly depends on all active radicals rather than oxygen radical only.

Figure 4(c) illustrates the effect of UV radiation. When UV pass filter (sapphire glass) was set above the sample, the number of survivor decreased only slightly for the rf jet (triangle symbols) and remained almost unchanged for the LF jet (circle symbols). From the results, it is concluded that UV photon is not a significant sterilizing factor in the case of low current (low power) plasma jets. To explore the heat effect of sterilization, the substrate was heated to 336 K (corresponding to the gas temperatures for the rf jet with a ring at 8 W) using a hot plate (WiseStir MSH-20D), and the gas was flown. As can be seen in Fig. 4(d), the populations of *E. coli* heated on the hot plate were decreased until 35 s, and then the survival population was saturated. However, the plasma treatment resulted in a drastic reduction at 40 s. The inactivation efficiency of the plasma rf jets is significantly greater than that of the hot gas only case as the exposure time is increased above 35 s. Therefore, the primary role in the inactivation is expected to be played by reactive species including oxygen, OH, H, and NO with minor aid from heat, UV photons, charged particles, and electric fields.[15]

In conclusion, the addition of a ring electrode enhanced the bacterial inactivation. The primary role in the inactivation was played by reactive oxygen species such as O, NO, and OH species. The rf plasma jets utilizing a proper ratio of He/O_2 suitable for the biomedical applications.

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