Article

Journal of
Nanoscience and Nanotechnology
Vol. 17, 8446–8449, 2017
www.aspbs.com/jnn

Feasibility Studies on Inactivation of *Staphylococcus aureus* with a High Voltage DCDC Atmospheric Plasma Source

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Inactivation of pathogenic bacteria, *Staphylococcus aureus* by a high voltage DCDC atmospheric pressure air plasma (APAP) generated by a AC adapter of smart devices such as mobiles and tablets has been performed. 4 kV DC is developed from 5VDC-2A by a high voltage (HV) DCDC converter to be utilize for generation of an arc discharge. This is applied to a coaxial pin with a nozzle configuration, which leads to generate a small APAP. Fourier transform infrared (FT-IR) analysis shows little generation of ozone (O_3) due to conversion of O_3 in high temperature arc discharge region. Current and voltage characteristics, and optical diagnostics are to be detailed along with inactivation test of bacteria. This non-jet air plasma weakly inactivates *Staphylococcus aureus*.

Keywords: Inactivation of Bacteria, Atmospheric Pressure Air Plasma, Smart Device Power Supply, Portable Plasma Source.

1. INTRODUCTION

Atmospheric pressure air plasmas (APAP) have been variously applied in material processing and medical applications, such as surface modification and etching of polymer films in micromechanics, sterilizing medical tools, inactivating microbes, and treating cancer cells and healing wounds in bio-medical fields.[1–5] In order to generate high density plasmas ($10^{13}\sim10^{16}$ #/cm^3) in various atmospheric pressure plasma sources such as Corona discharge and Dielectric Barrier Discharge (DBD), one should utilize high power inverters, such as RF (kHz∼MHz) and pulsed DC),[6–8] whose volume is generally large ($\sim24\times48\times13$ cm^3), weight is heavy (∼7 kg), and cost is high (∼3 kUSD in 2017 currency), comparing a smart device power supplies including DCDC transformer (volume $\sim6\times4\times2$ cm^3, weight ∼0.1 kg, and cost ∼0.3 kUSD).[9]

Recently, several portable atmospheric plasma sources for bio-medical applications have been reported. Choi et al., reported a portable microwave-excited atmospheric pressure plasma jet operating at 900 MHz, and showed blood coagulation with 20 s treatment time with the jet.[10] Won et al., developed a compact air microwave plasma jet operating at 1.29 GHz, and showed no ozone (O_3) generation in the high plasma gas temperature in the jet.[11] However, their devices are expensive and have complicated circuits. Jiang et al., developed a portable atmospheric pressure plasma sources operating at AC kHz and nano-second pulsed DC, and showed inactivation of bacteria (*E. Coli* and *Enterococus faecalis*).[12–14] However, their sources are not free from ozone.

In this work, a simple high voltage DC atmospheric pressure air plasma (APAP) source generating little ozone powered by a ubiquitous smart device power supply (5 VDC 2 A) is developed, which can be portably used in bio-medical fields with a portable lithium ion battery.

Molecules from the source and discharge are characterized by voltage–current probe, ultra-violet (UV) light absorption spectroscopy, and Fourier transform infrared (FT-IR) analysis. Inactivation of *Staphylococcus aureus* by using developed the source is investigated.

2. EXPERIMENTAL DETAILS

2.1. Plasma Source

Figures 1(a) and (b) show photographic views of plasma source to be operated in HVDC with ground electrode.

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 1533-4880/2017/17/8446/004 doi:10.1166/jnn.2017.15157

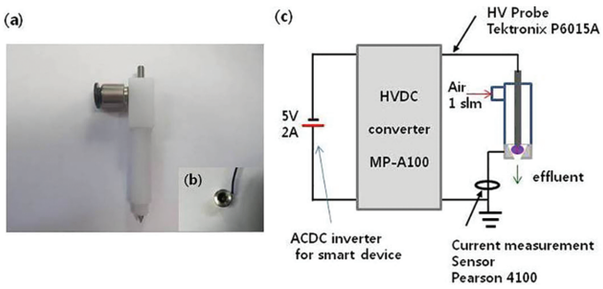

Figure 1. (a) Designed HV DC plasma source with a coaxial pin to nozzle configuration.[6] (b) Ground nozzle electrode. (c) A schematic view of experimental set-up for measuring voltage and current.

The main body (insulator) was made of polyacetal $(–O–CH_2–)_n$ resin, and the coaxial pin electrode and ground electrode are made of SUS304. Figure 1(c) is a schematic view of experimental setup. 4 kV DC is generated by using HVDC converter (MP-A100, FT-LAB) from a ACDC inverter (5 V DC 2 A) insulated hybrid structure for smart devices.[15] Tektronix P6015A high voltage probe and Pearson 4100 are used for measuring voltage and current during discharge, respectively. Compressed air at 1 slm is injected into the source, then jetted outside through the nozzle with chemicals including reactive species generated between the pin electrode and ground nozzle.[6]

With our simple DC source, no air jet from nozzle is obtained, because of

(1) using air gas;
(2) DC power;
(3) low secondary electron emission from electrodes (SUS304);
(4) oxidization of electrode surface (not shown);
(5) relatively low voltage (1.5 kV) comparing to that of Walsh's report (15 kV), which leads to no emission of charged particles from the nozzle.[6]

Figure 2 shows a measured voltage–current waveform during discharge. No displacement currents are observed due to DC operation.[11] Discharge currents are measured

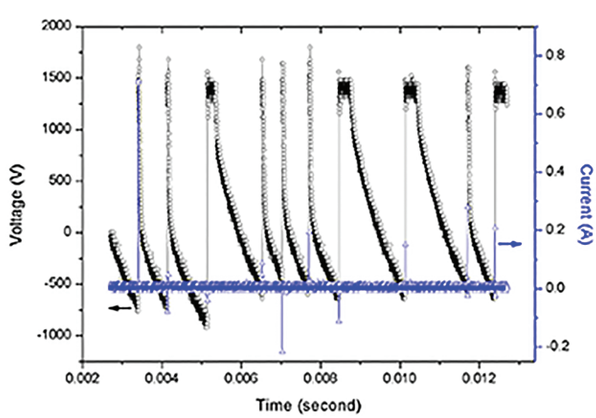

Figure 2. Voltage–current waveform. Black line with open circle is for the voltage and the blue with open triangle is for the current.

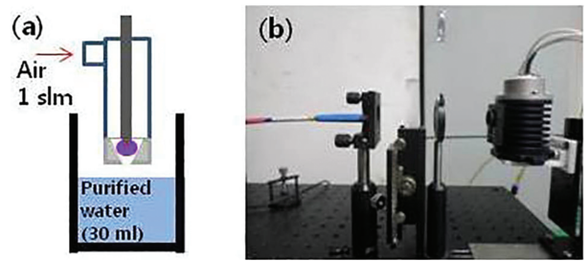

Figure 3. (a) A schematic view of making plasma activated water from the source. (b) A figure of experimental setup of UV-LAS.

during 0.01 s indicating about 1 kHz discharge frequency. Discharge duration of one peak current is about 20 ns to be estimated from the figure, indicating filamentary discharge mode in air with HVDC.[6] During each of discharges, drastic voltage drop from 4 kV to 1.5 kV is measured due to accumulated charges between oxidized electrodes.

2.2. Measurement of Chemicals

Ultra-Violet Light Absorption Spectroscopy (UV-LAS) is one of simple diagnostics techniques for detecting long-lived species in liquid. Purified water with amount of 30 ml in a glass beaker was activated by the effluent from the source for 3 min as shown in Figure 3(a), then 5 ml plasma activated water was put into a high precision cell made of quartz SUPRASIL 300 of which light path is 10 mm. For ultra violet light of which range is from 185 nm to 400 nm, 30 W Deuterium lamp (Hamamatsu) is employed. Absorption spectra were detected by USB-4000 ($\Delta = 1.5$ nm Oceans optics). Calculating absorbance of being measured spectra is well-described in Baek et al.'s work.[16] Detail identifying long living species from the sources were performed by using Fourier Transform Infrared Ray (FT-IR) (Vertex 80, BRUKER). The length of urethane ($NH_2COOC_2H_5$) tube between the gas cell of FT-IR and the source were 100 mm.

2.3. Inactivation of *Staphylococcus aureus*

For checking inactivation effect of pathogenic bacteria of the developed system, *Staphylococcus aureus* (gram+) was employed. The diluted culture was uniformly spread over 12 commercialized agar plates in plastic Petri dishes, especially designed for culture of the *Staphylococcus aureus*. Then, every 3 agar plates were irradiated by the effluent from the source with different irradiation time, 1 min, 3 min, 5 min. The distance between the nozzle and the agar plates was 10 mm. Then, 12 agar plates including 3 plates of controls had been incubated for 48 hours at 37 °C.

3. RESULTS AND DISCUSSION

Figure 4 shows measured UV absorbance spectra. The absorbance, indicating the densities of all long-lived (H_2O_2, NO_2^-, NO_3^-, and O_3) reactive species in plasma

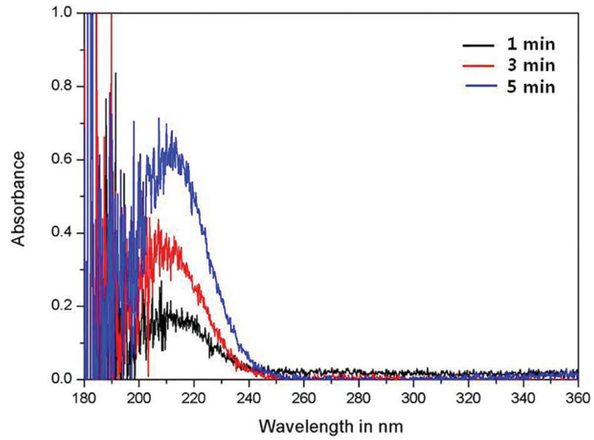

Figure 4. Absorbance spectra observed by using UV-LAS.

activated water (PAW), certainly increases with irradiation time. In despite of low power DC discharge, calculated absorbance in this work is 3 times higher than other reports.[16]

Figure 5 shows a measured FT-IR waveform CO_2, H_2O, NO_2, N_2O, were observed. The CO_2 and H_2O seemed to be generated by chemical interactions between reactive oxygen species, such as O (short-living radical) and urethane ($NH_2COOC_2H_5$) tube. NO_2 and N_2O are metabolic produce of NO having various positive effects such as signal transduction and stimulation of angiogenesis.[3–5] Although O_3 is known as having strong sterilization effect to microbes and wound healing, O_3 was very weakly detected because ozone is converted into O_2 at high temperature.[6, 10, 17, 18] CO_2 can decrease pH, generate carbonic acid (HCO_3^-) in liquid, and induce increasing O_2 partial pressure in blood (Bohr effect), which could lead to the treatment of chronic wound as reported by Brandi et al.[19]

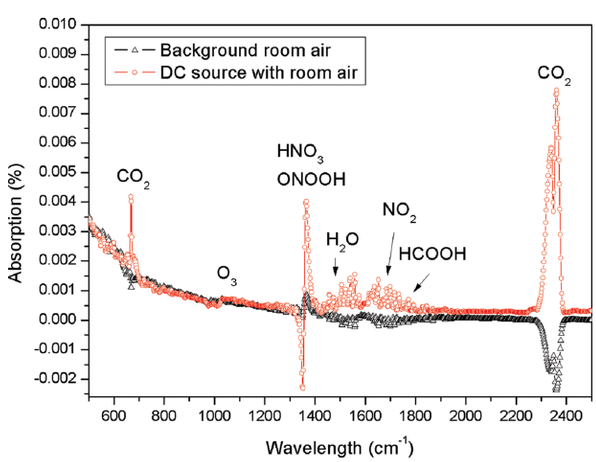

Figure 5. FT-IR waveform. Black line with open triangle is FT-IR waveform of room air. Red line with open circle is FT-IR waveform of the effluent from the source. Minus (−) absorption of CO_2 in black line is due to calibration of CO_2 included in room air prior to measurement.

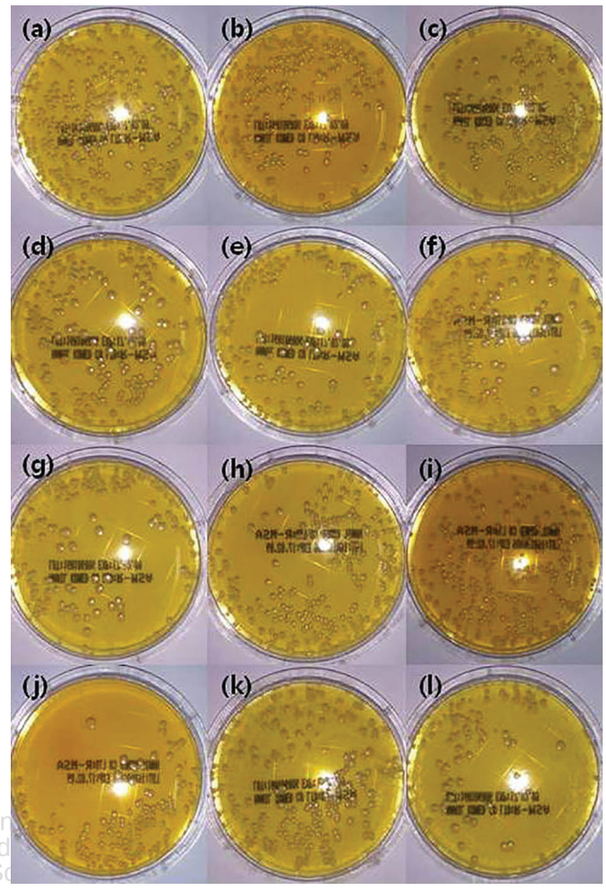

Figure 6. Inactivation of *Staphylococcus aureus* with developed DC devices. (a–c) refer control (no expose), (d–f), (g–i), and (j–l) show cultured colonies during 48 hours after 1 min, 3 min, and 5 min expose, respectively. Red dot lines indicate no colony region.

Figure 6 shows cultured colonies with different experiment conditions. Comparing of Kim's work, inactivation of *Staphylococcus aureus* by using film-type surface dielectric barrier discharge at 25 kHz AC, the *Staphylococcus*

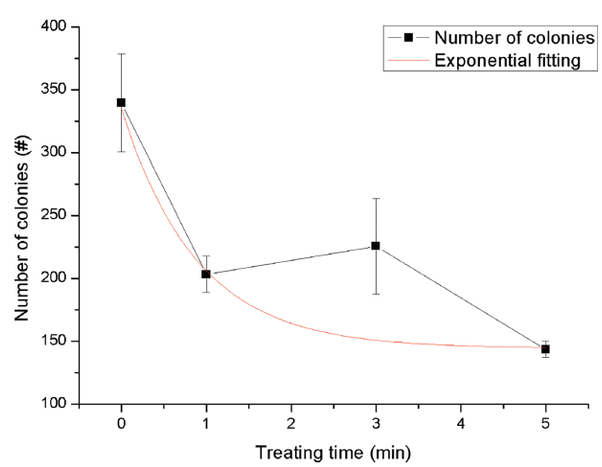

Figure 7. Counted colony numbers on 12 agar plates. The number of colony decrease with increasing irradiation time.

aureus with the developed DC device is not perfectly inactivated.[20] Areas circled by dotted line in Figure 6 indicate no cultured colony due to influence of air flow including reactive species. *Staphylococcus aureus* was weakly inactivated due to O, ROS (reactive nitrogen species), a very small amount of O_3, and pH decease by reactive nitrogen species and CO_2 from the source shown in Figure 5.[21]

Figure 7 shows the cultured colonies (C_N), which decreases with increasing exposed time with exponential decay time (T) of 0.88592 minutes according to the fitting of $C_N = 144.46973 + 190.82704 \exp[-t/0.88592]$, although a little longer treated time would be better for more reliable data analysis.

4. CONCLUSION

In this work, a simple and compact high voltage DC atmospheric pressure air plasma (APAP) source is developed by a smart device power supply with upgrading to 4 kV. Long lived chemicals such as CO_2, NO_2 from the source, and weak inactivation of *Straphylococcus aureus* have been observed. Our conclusion are as the following:

(1) Discharge frequency is about 1 kHz in the DC source;

(2) Various chemicals (O_3, N_2O, NO_2) generating in discharge are observed. Especially, H_2O and CO_2 generation is observed to be formed due to polymer tube like urethane near to source nozzle, which lead to the effects such as sterilization (O and O_3), pH decrease (NO_2 and CO_2),[21] and various medical effect (NO converted from NO_2^- in low pH);[22]

(3) *Staphylococcus aureus* was weakly inactivated;

(4) A small concentration of O_3 is detected.

Developed simple DC source could be portably applied to bio-medical fields such as inactivation of bacteria and pH decrease of tissue, where low ozone concentration is required.

Acknowledgment: Technical supports of NFRI (National Fusion Research Institute), Gunsan, Korea are acknowledged in using their equipments. This research had been supported by the Brain Korea 21 Program, and by the National R&D Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2015M1A7A1A01002784).

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Received: 9 November 2016. Accepted: 22 May 2017.