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Atmospheric Pressure Single Electrode Argon Plasma Jet for Biomedical Applications

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***Abstract*-Atmospheric pressure single electrode argon /oxygen plasma jet has been generated between a high-voltage electrode and the surrounding room air for treating thermally sensitive materials is reported. The discharge has been characterized by electrical and optical emission spectroscopic technique. The microplasma jet device operated by an electrical power less than 10 W exhibited a long plasma jet of about 8.0 cm with temperature near 300 K, not causing any harm to human skin. Optical emission spectra measured in the range of 200–900 nm showed that various reactive species such as O, OH, N^{2+}, Ar^{+} etc., are present in the plasma plume. Optical emission spectrometry has been used for the determination of electron temperature (T_e) and electron density (n_e) of the produced plasma. The analyses provided that electron density and electron temperature vary with plasma jet length. This plasma jet has been applied for inactivation of prokaryotic cells (Escherichia coli, Staphylococcus aureus) and eukaryotic cells (Candida albicans, Saccharomyces cerevisae) to obtain >4 $\log_{10}$ reduction in E. coli and < 2,000 cells reduction in eukaryotic microalgae C. vulgaris, operating at a voltage of 3.5 kV, frequency of 27 kHz and gas flow rate of 2 L/min at jet. We also investigated the effect of plasma jet in pH and temperature of cell culture medium to demonstrate that plasma species are solely responsible for inactivating living cells.**

Keywords: **Bacterial Inactivation, DBD Plasma Jet, Line Broadening, Spectroscopic Diagnostic,**

I. INTRODUCTION

At atmospheric pressure dielectric barrier discharge (DBD), the discharge gaps is very small due to the relatively high breakdown voltage of working gases, which limit the size of materials to be directly treated. If indirect treatment (remote exposure) is used, some short lifetime active species, such as oxygen atom O, OH, N^{2+}, Ar^{+} etc. charge particles, may already disappear before reaching the object to be treated, which makes the efficiency of treatment much lower[1].

To address these concerns, Various types of atmospheric pressure plasma sources have been designed for a wide biomedical and industrial applications range, like plasma needle [2], floating-electrode DBD [3,4], microhollow cathode discharge air plasma jet [5] or different types of plasma jets [6-8]. Atmospheric pressure plasma jets have been established as suitable sources of low-temperature and non-equilibrium atmospheric pressure plasmas. The plasma jet devices generate plasma plumes in open space rather than in confined discharge gaps only. Thus, they can be used for direct treatment and there is no limitation on the size of the object to be treated [9].

The number of applications of plasma technology in many fields including microelectronics, metallurgy, polymer engineering, and biomedical engineering, is growing rapidly. Biomedical applications generally require that the gas temperature of the plasma plume is close or equal to room temperature. In medicine direct plasma treatment for sterilization, deactivating pathogens, blood clotting, wound healing, cancer treatment, etc. is more effective than any other method. Thus, plasma and plasma modified materials play an important role in our daily live, making it more convenient and healthier. [10-15]

The present work focused on the generation of single electrode atmospheric pressure argon plasma jet for biomedical applications. APPJ has been characterized by optical and electrical methods. This plasma jet has been applied for inactivation of prokaryotic cells (Escherichia coli, Staphylococcus aureus) and eukaryotic cells (Candida albicans, Saccharomyces cerevisae, and Chlorella vulgaris). We also investigated the effect of plasma jet in pH and temperature of cell culture medium to demonstrate that plasma species are solely responsible for inactivating living cells

II. EXPERIMENTALS

Plasma jets described in this paper are generated in a glass capillary tube with an inner diameter of 3.0 mm and an outer diameter of 5.0 mm.

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High-purity argon is used as the working gas; and the flow rate is controlled by a volume flow meter. The flow rate of the argon gas was restricted below 15 l/mim so that the flow velocity would not exceed the limit for a laminar argon flow. A sinusoidal voltage at 27 kHz is applied for the excitation and sustaining of the discharges. The schematic diagram of experimental setup is shown in Fig.1. The electrodes, 1.0 cm wide, are made of aluminum foil wrapping the glass capillary tube, and the gap between the inner edges of the aluminum strips is 15.0 cm. The ground electrode is on the upstream side; the active electrode is on the downstream side and 5.0mm away from the tube orifice. In Fig.1 (b and c) the grounded electrode is removed. This is then a single electrode configuration. As there is no another physical electrode, the plasma jet thus generated was terminated through a virtual ground plane, i.e., a closed circuit of the discharge is only completed with the remote ground. Linear array optical spectrometer is used to study the optical emission from the plasma jet. The resistor R in the circuit shown in Fig.1 is utilized for the measurement of discharge current. The voltage and current waveforms are recorded using digital oscilloscope.

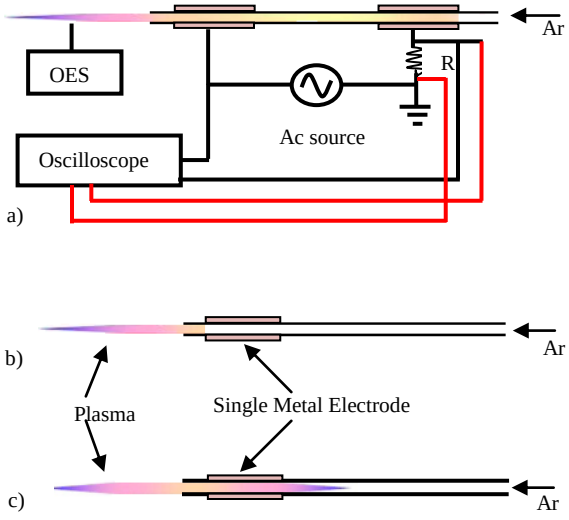

Fig.1: Schematic diagram of experimental setup for generation of atmospheric pressure argon plasma jet. a) plasma jet with double electrode b)plasma jet downstream with single electrode c)plasma jet downstream and upstream with single electrode

A. Electrical Characterization

The current and voltage are recorded digitally by using Tektronix TDS oscilloscope.

The data obtained can be transferred to a personal computer for further analyses. The estimation of electron density can be done by the power balance method, in which the total energy lost by the electron in the plasma is balanced by the input power. [16]

$$n_e = \frac{P_{ab}}{2Aev_bE_{lost}} \text{----------} (1)$$

Where $P=VI$ is the input power during the discharge, A is the surface area of the electrode, e is the charge on the electron, v_b is the Bohm velocity. Electron density in the discharge was found to be 2.1×10^{16} cm^{-3}. It was also observed that the density varied with applied voltage.

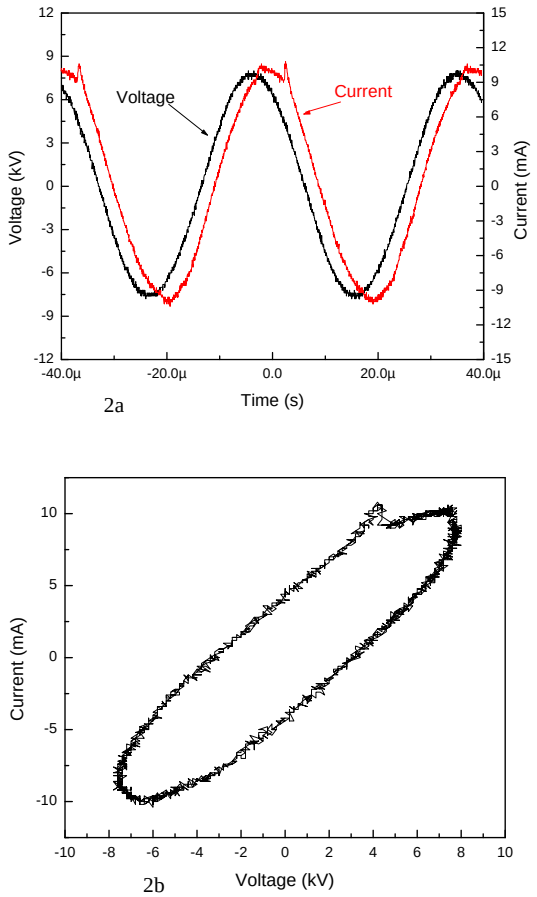

Fig.2: Current voltage signal of APPJ and Lissajous figure

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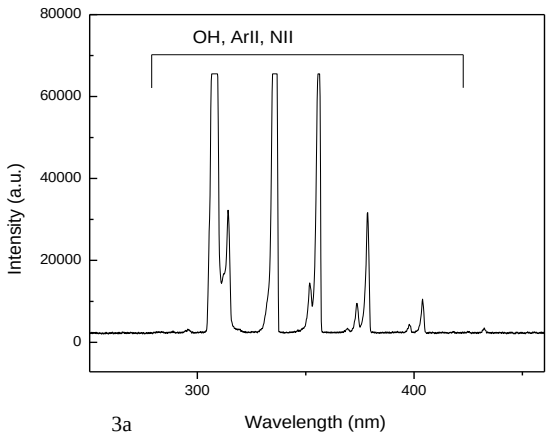
The electric energy dissipated in the reactor in each voltage cycle is calculated by measuring the area of the Lissajous figure, and the average discharge power can be obtained by multiplying the energy per voltage cycle and the frequency of the ac power.

B. Optical Characterizations

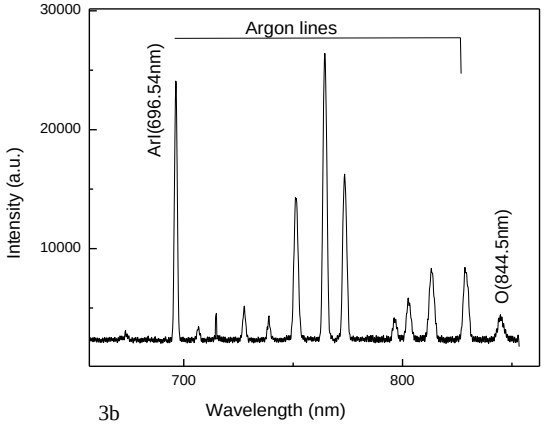
The Spectra of discharge in the range 190nm- 900nm were recorded by the optical emission spectrometer with focal length 140mm and typical resolution 100μm core fiber (Linear array spectrometer VS 140). The recorded spectra were analyzed to determine the electron temperature and electron density. A commonly employed convenient method of temperature determination is the two-line emission ratio methods [17].

$$KT_e = \frac{E_2 - E_1}{Log\left[\frac{I_1}{I_2}\frac{\lambda_1}{\lambda_2}\frac{g_2}{g_1}\frac{A_2}{A_1}\right]} \text{----------(2)}$$

In equation (2), Indices 1 and 2 refer to the first and second spectral lines, *I* is the measured intensity of selected spectral line, *k* is the Boltzmann constant, *E* is the excited state energy, *g* is the statistical weight, and *A* is the transition probability.



3a



3b

Fig.3: Optical emission spectra of atmospheric pressure plasma jet at jet length of 3.5cm

The Boltzmann plot method is valid if the discharge plasma under study is in complete local thermodynamic equilibrium (LTE). But in the plasma jet LTE is not valid. Therefore, this method may not be used for the exact determination of T_e and n_e. It can only provide us estimated values of these plasma parameters under varying working condition of discharge plasma in the APPJ and found that the electron temperature at plasma jet length of 3.5cm was 0.35eV.

IV. Application Of Atmospheric Pressure Plasma Jet

A. Plasma Treatment

One ml of cell suspension ($O.D._{600}$ = 0.132) was loaded on 12 well flat bottom tissue culture plates (Corning Inc.). Cells were treated with APAPJ for four different time lengths (60, 120, 180 and 240 s). APAPJ was operated at high voltage of 3.5 kV, high frequency of 27 kHz and argon gas flow rate of 2 L/min. The distance between APAPJ nozzle and suspension surface was adjusted at 3.5 cm. Movement of jet was not required as the argon gas flow facilitates the vortexing of cell suspension during treatment

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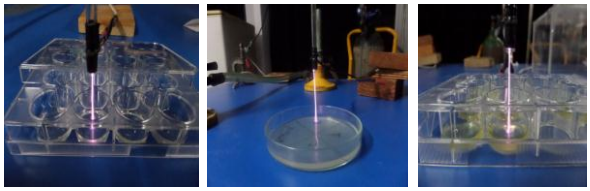
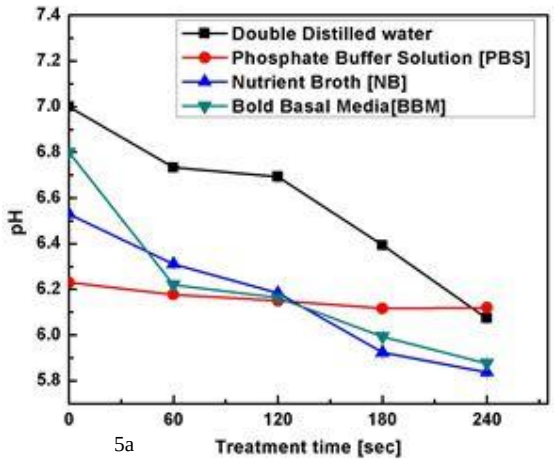


Fig.4: Photograph of biomedical application of plasma

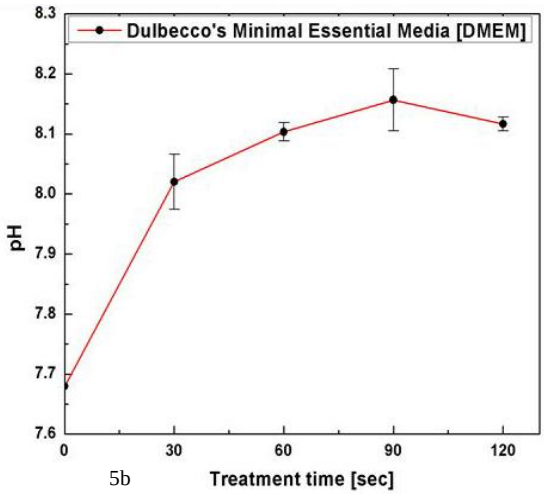
B. Ph and Temperature Measurement

Temperature and pH of distilled water, Dulbecco's Minimal Essential Media (DMEM), Phosphate Buffer Solution (PBS), Nutrient Broth (NB) and Bold Basal Media (BBM) before and after plasma treatment was measured using IR thermometer and calibrated pH meter. There was no significant change in pH and temperature of the medium used in preparation of sample of cell suspension. The results of treatment of media are shown in Fig.5. In the experiment, there was greater than 1 log change of pH in distilled water, no change in highly buffered salt solution PBS and less than 1 log decrease in pH of NB media and BBM but High nutrient media like DMEM shows slight increase in pH. The pH difference was less than 1 log.

There was no significant change of temperature in media but in many experiments, there was decrease in temperature due to cooling effect of gas flow which is shown in Fig.6.



5a



5b

Fig.5: A study of variation pH of different media with treatment time

C. CFU Analysis

Colony Forming Unit abbreviated as CFU is the analysis done to investigate the no. of dead cells and no. of viable cells (live) in microbiology. Treated and untreated samples were diluted by 10 to 10^4 times. 100 µl of diluted sample was spread on Plate Count Agar (PCA) using sterile bent glass rod. Then, the samples were incubated for 24 hours at 37^o C. After incubation viable colony of microbes were count using Quebec colony counter. By spread plate technique, viable cell colony was counted using Quebec Colony Counter. Greater than 4 log reductions were observed in E. coli, Staphylococcus, Candida albicans and Saccharomyces cerevisae. In eukaryotic microalgae greater than 2,000 cells were killed by jet at a maximum treatment time of 240 seconds, measured using UV-VIS spectrophotometer taking into account the absorbance of the cell suspension before and after treatment.

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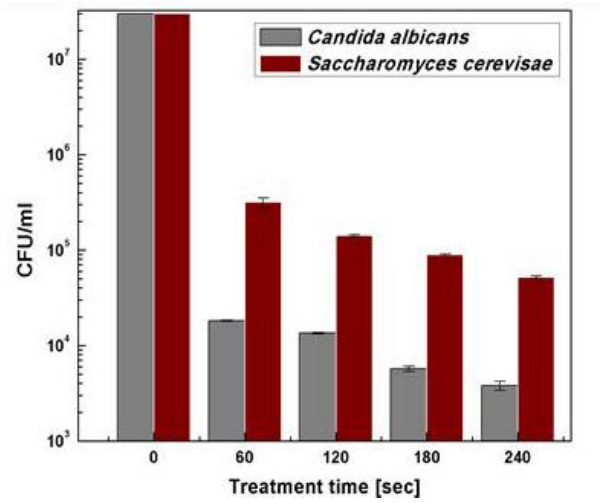

Fig.7: APAPJ treatment on eukaryotic yeast cells at distance of 3.5cm from nozzle in 12well plate

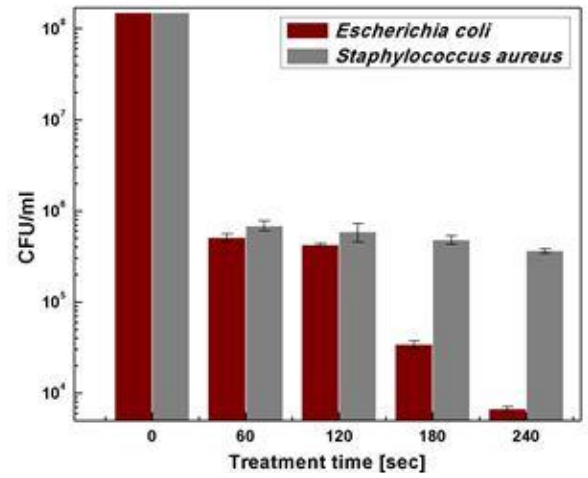

Fig.8: APAPJ treatment on prokaryotic bacteria cells at distance of 3.5cm from nozzle in 12 well plates

V. CONCLUSION

In this study, we have generated the APAPJ by using the low frequency operation of 27 kHz and electrical power of 12W with Ar gas flow. Also we have investigated electron temperature and electron density along the plasma jet length by electrical and optical methods for the gas flow of 2l/min. Electron temperature and electron density along the plasma jet length indicates that the plasma generated in our laboratory is non-thermal or cold plasma and suitable for biomedical application.

The pH of distilled water, Phosphate Buffer Solution (PBS), Nutrient Broth (NB) and Bold Basal Media (BBM) slightly decreases but pH of Dulbecco's Minimal Essential Media (DMEM) slightly increased after plasma treatment. Fig.7 and Fig.8 indicates that Plasma jet is responsible for the killing prokaryotic and eukaryotic cells. The inactivation killing of both prokaryotic and eukaryotic cells, increased as the treatment time increased with argon as carrier gases.

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