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Feasibility of Leukemia Cancer Treatment (K562) by Atmospheric Pressure Plasma Jet

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***Abstract*—**A new and novel approach in medicine is the use of cold plasma for various applications such as sterilization blood coagulation and cancer cell treatment. In this paper a pin-to-hole plasma jet suitable for biological applications is investigated and characterized and the possibility and feasibility of cancer cell treatment is evaluated. The characterization includes power consumption via Lissajous method, thermal behavior of plasma using Infra-red camera as a novel method, Optical Emission Spectroscopy (OES) to determine the species that are generated. Treatment of leukemia cancer cells is also implemented and MTT assay is used to evaluate viability.

***Keywords*—**Atmospheric Pressure Plasma Jet (APPJ), Plasma Medicine, Cancer cell treatment, leukemia.

I. Introduction

NON-EQUILIBRIUM plasma is known because of the difference between its electron temperature and gas temperature. Such kind of plasma has an electron temperature in order of few electron volts to roughly 20 electron volts while the actual gas temperature stays in order of room (low) temperature.

Non-Equilibrium discharges in atmospheric pressure attracted lots of attentions, providing this kind of plasma with a vast area of applications such as polymer deposition [1], light sources [2], biological applications [3], microelectronics [4] and so on [5].

In this Paper, implementation and characterization of an Atmospheric Pressure Plasma Jet (APPJ) has been made to do a feasibility study of leukemia cancer cell treatment (K562) in an in-vitro situation.

The plasma jet structure has been made by using a dielectric material as a barrier between two conductor which will reduce the passing current and prevents electrical discharge so there won't be any electrical danger for user and specimen and also will help keeping the temperature as low as possible which is essential for cells treatments .in general these devices has a low power consumption [6].

On the other hand discharge will produce oxygen and nitrogen species which can affect living cells, Due to the mentioned reactive species, plasma can have effects such as sterilization [7], cancer cell treatment [8], blood coagulation [9], wound healing [10], and teeth treatment [11].

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II. Experimental Setup

In the plasma jet device a copper tube used as the first electrode and a copper ring used as the second electrode. Dielectric barrier was made of a carbonic Teflon (PTFE). Modeling and manufacturing drawings has been done by Autodesk inventor software. A high voltage, high frequency power supply with a maximum power capacity of 200Watts was used to generate the mentioned plasma. Helium Gas has been chosen as the carrier gas because of low breakdown voltage and also providing homogenous uniform plasma as well.

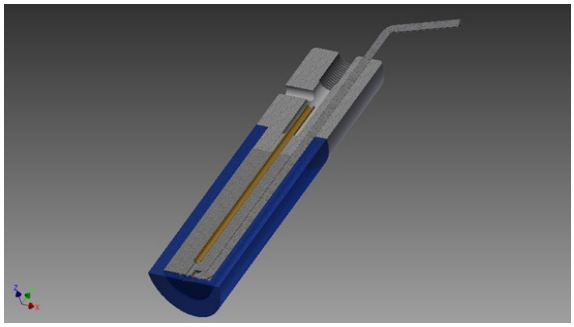


Fig. 1 Atmospheric Pressure Plasma Device

A mass flow meter (APEX, AX-MC-5SLPM-D) (He) was used to control the carrier gas flow. Varies voltages and different gas flows with a constant frequency of 20 kHz, cold plasma has been produced and different parameters were inspected.

III. Result and Discussion

Plasma temperature is one the most important parameters in medical and biological applications Due to the sensitivity of living cells and their inner organs like proteins. Plasma temperature must stay below a certain amount of degree. This way plasma will not have any thermal effects such as ablation and coagulation. To determine the plasma temperature an infra-red camera was implemented to observe and save the plasma temperature behavior. In this experiment two variables were examined, temperature of different distance from nozzle and effect of working voltage on temperature has been measured.

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In this setup IR camera was fixed toward the center of the plasma jet. Photos were taken every 20 seconds and recorded. This operation was repeated for varies length in 1.5 and 2cm and for each distance three voltages of 3, 3.5, and 4kV has been inspected, as shown in Fig. 2.

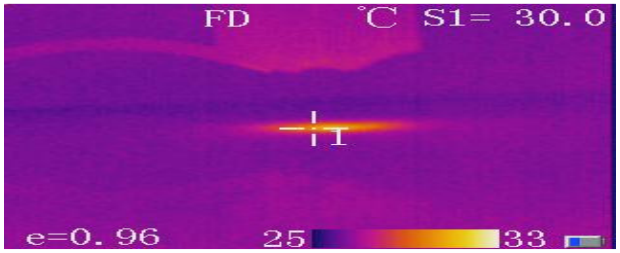


Fig. 2 Infra-Red image of APPJ

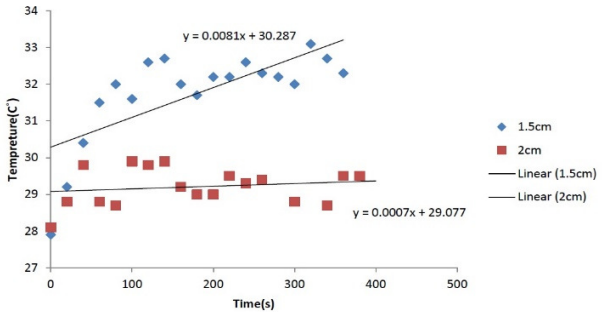


Fig. 3 Temperature measurement in different distance from nozzle

By considering the drawings in Fig. 3 increasing the distance from nozzle not only concludes to a decrease in the temperature but also the slope well

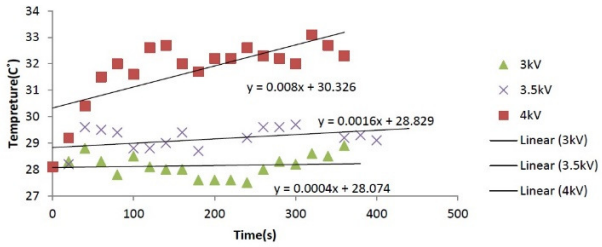


Fig. 4 Temperature measurement in different voltages

In fixed distance, increasing the given voltage to the carrier gas will lead to an increase in ionizing rate which will lead to a higher temperature.

To calculate power consumption of APPJ device, lissajous method was used. In the experimental setup a capacitor was connected to the device in serial, so the current passes through the device will also pass through the capacitor and hence the capacitor will be charged. Through the measurement of the charge and the current, the lissajous curve was drawn. The area of the curve multiplied by the working frequency and the capacitor value will provide the power consumption of the APPJ device. The Reported power of the device is in order of 1 watt.

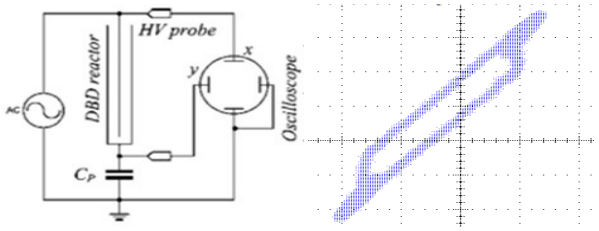


Fig. 5 Lissajous setup and curve

Cold atmospheric plasma is mostly known for the ability to generate and produce a sufficient amount of active radicals. As it is known lasers mostly have thermal effects have the ability to generate these radicals with high energy photons but compared to plasma they may induce very high concentrations of these radicals which will affect normal cells in respect. To observe the existence of these radicals, Optical Emission Spectroscopy (OES) was used. Through the analysis and inspection of these spectrums and relevant peaks different radicals was observed and determined [12].

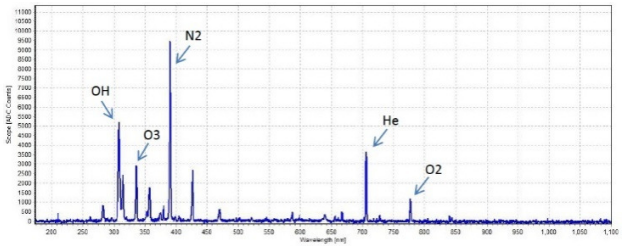


Fig. 6 Optical Emission Spectroscopy

The OES has been taken at 3.5kV, in 2cm distance from the nozzle. Different radicals were determined on the spectrum due to their wavelength. Oxygen reactive species and nitrogen reactive species had the highest concentration in the sample. These reactive species have the ability to oxidase and peroxidase which can lead to blood coagulation, wound healing and apoptosis of cancerous cells, so the existence of these products is one of the most important issues.

Different parameters could influence the rate of reactive species such as voltage and the distance from the nozzle

Fig. 7 shows that by increasing the applied voltage, generation of these radicals' increases respectively. This happens because of higher discharge energy which leads to a higher ionization rate.

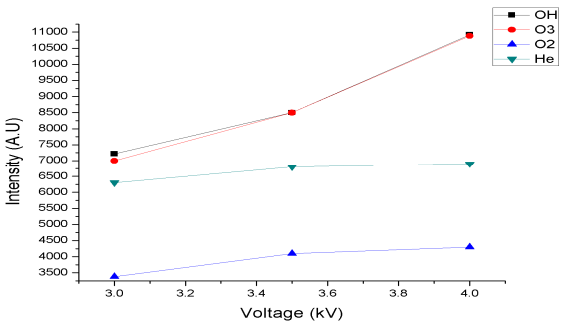


Fig. 7 Species Vs Voltage

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Due to the recombination of reactive species their concentration would vary with distance. Spectrums were taken in three different distances and compared in Fig. 8. By increasing the distance from nozzle it is obvious that these active radical will recombine and form stable.

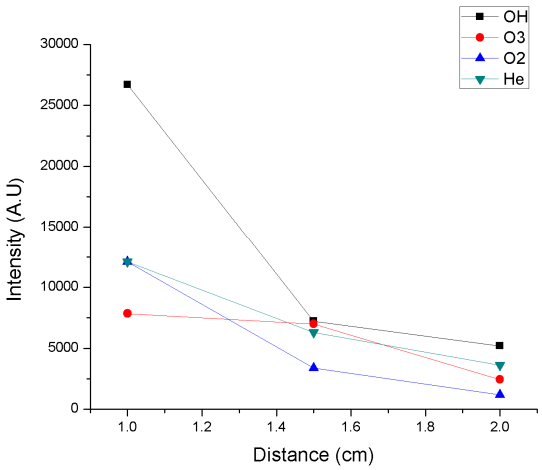
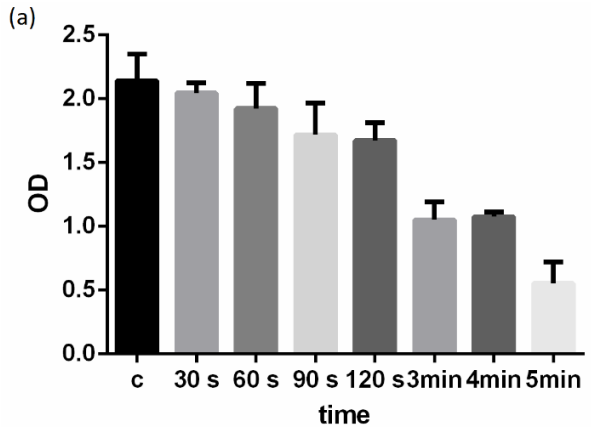


Fig. 8 Species Vs Distance from nozzle

The human chronic myelogenous leukemia K562 cell line was bought from Iranian Biological Resource Center (IBRC) and cultured at 37 C with 5% CO2. The culture medium was Dulbecco`s modified eagle medium (DMEM; Gibco, Invitrogen, CA, USA) with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin/streptomycin (Sigma). For the cytotoxicity assay, first, $5*10^4$ K562 cells were seeded on 96-well plates for 24h. Three plates were devoted in order to analysis. In intervals of 0, 24 and 48, the wells were analyzed in triplicate and ultimately the cytotoxicity was evaluated by MTT assay.



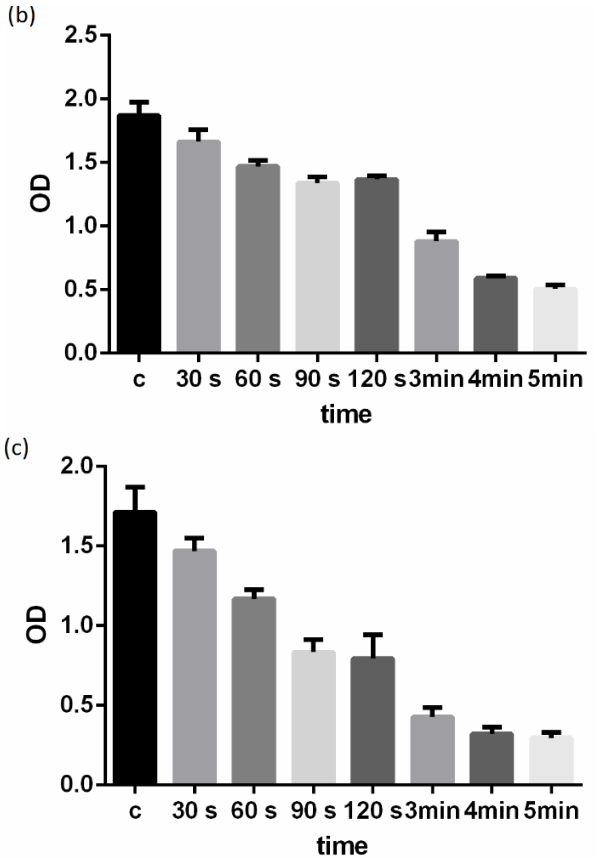


Fig. 9 The effect of Different plasma exposure time (a) Cell viability at 0 h after plasma exposure, (b) Cell viability at 24 h after plasma exposure, (c) Cell viability at 48 h after plasma exposure

As it can be seen in Fig. 9 plasma has more effect through time and it could be due to chain reactions that plasma may have triaged in the process. Each cell flux has a unique control group of its own so if there is any unwanted extrinsic factor it would affect all the samples equally and the only parameter separating these samples would be the time of their exposure to plasma treatment. Each group of samples was compared with control groups in the relevant time and the most effective was for 5 minute treatment in 48hours past plasma treatment.

IV. Conclusion

In this study an Atmospheric Pressure Plasma Jet device was designed and manufactured in order to generate col plasma for medicinal and biological purposes. The generated plasma was fully studied and characterized including power consumption via Lissajous method, thermal behavior of plasma using Infra-red camera, Optical Emission Spectroscopy (OES) to determine the species and gas flow rate.by finding the best operating conditions, leukemia cells that are generated. The effect of flow and the type of working gas is also inspected to obtain the best condition for our plasma device after that leukemia cancer cells were treated in various exposure times and their viabilities were examined in different post exposure times.it was seen that plasma treatment was more effective after time and the best result was after 5

minutes of treatment for 48 h after plasma exposure, although plasma was effective on killing cancerous cells it is possible that it may have had side effects on normal cells as well and more experiments should be done to identify and observe the possible side effects.

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