

Effect of cold atmospheric pressure plasma needle on DNA

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Abstract: Non-thermal argon plasma needle at atmospheric pressure was constructed. The experimental setup was based on a simple and low cost electric components that generate a sufficiently high electric field at the electrodes to ionize various gases which flow at atmospheric pressure. A high AC power supply was used with 1.1Kv and 19.57KHz. Non-thermal atmospheric pressure plasma has much potential for medical applications. The atmospheric pressure plasma needle contains almost equal amounts of positive and negative charges. In this research we focused on the possible biomedical application of the atmospheric pressure plasma needle, where we used plasma ignited in Argon (Ar) gas flow to cause strand breaks in DNA. However, the mechanism by which plasma destroys DNA is not yet understood. In this study we investigated the effects of exposure time and distance between the plasma source and the DNA sample. In this study we found that plasma does indeed alter DNA. We came to this conclusion through qualitative interpretations using a standard gel electrophoresis technique on DNA samples treated with the atmospheric pressure plasma needle.

Keyword: Plasma treatment, Low temperature plasma, Plasma needle, Plasma medicine.

I. Introduction

Non-thermal atmospheric pressure plasma is composed of UV light, radicals, positive and negative charges traveling in a flow of gas in a plasma needle. One reason for why the plasma needle is advantageous is because even though the electrons and other species which are generated might be hot due to their high kinetic energy, the overall gas is at room temperature. This property of the plasma needle can be used to treat living tissue. Non-thermal atmospheric pressure plasmas can be used for biomedical applications such as in dentistry in which it is used for teeth bleaching[1]. and in material processing such as cleaning of surfaces[2]. In the case of micro plasmas, they have been found to be able to “inactivate microorganisms and destroy biomolecules,” such as DNA and proteins. Hence, plasma has gained the function of a “bactericidal” agent and “decontamination technology to address contamination of surgical instruments.” [3]. Plasma can generate a wide array of species and therefore the manner by which plasma attacks DNA can occur through various different reaction pathways. Plasma can generate “excited atoms and molecules, charged particles, electrons, and UV light,” all of which can have different effects on the DNA molecule [4]. electrophoresis can serve as a way to find if plasma can break chemical bonds in the plasmid DNA.

II. Experimental work

2.1. The atmospheric pressure plasma needle:

Plasma needle consists of a hollow stainless steel pipe of 100mm long with inner diameter 1mm and outer diameter 2.7mm inserted inside a Teflon pipe as shown in figure (1). The stainless steel connected to the high voltage power supply. As put between Teflon pipe and stainless steel pipe filled with Teflon tape. Under certain conditions an argon plasma needle can be extracted from the downstream tube end since there is no discharge inside the plastic tube. The plasma needle obtained by this method is cold enough to be put in direct contact with human skin without electric shock and can be used for medical treatment and decontamination. All configuration the high voltage power supply generates high voltage of sinusoidal shape of 1.1kV peak to peak and frequency of 19.57kHz.

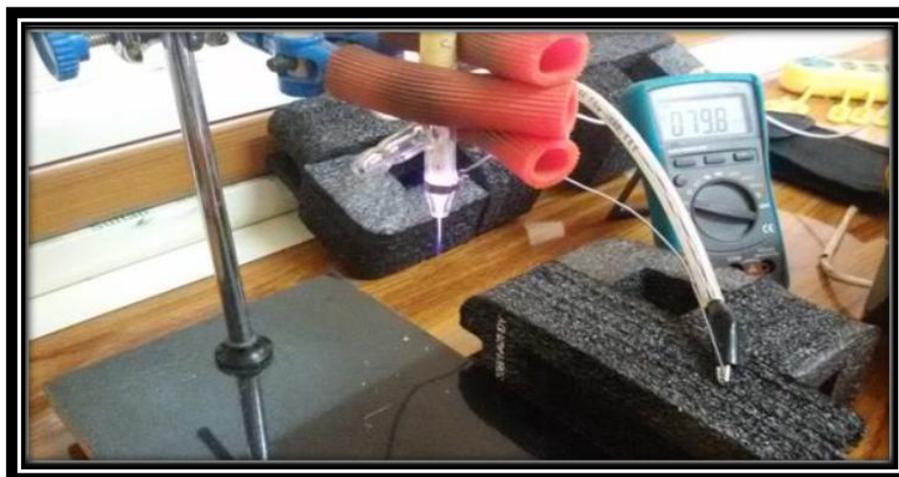


Figure 1: photograph of the plasma needle system.

2.2. Plasma needle system:

Plasma system include four main parts:

1. High voltage AC power supply.
2. Plasma needle.
3. Argon gas.
4. Flow meter.

Figure (2) shows the schematic diagram of plasma needle system. Which consist of high voltage source, plasma torch, Argon gas and gas flow meter.

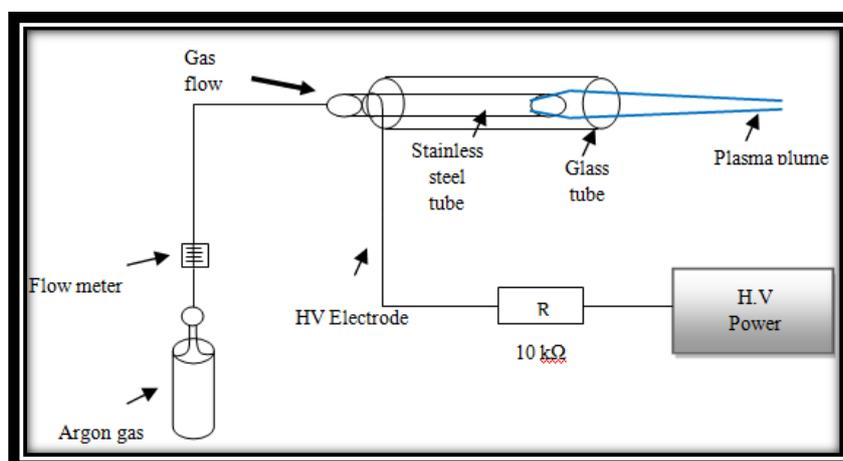


Figure 2: The plasma needle system.

III. Biomedical applications (DNA treatment):

The Non- thermal plasma needle shown in fig (1), which is constructed and designed locally was used to treat DNA samples for different exposure time and distances.

3.1 Sample preparation:

2.2.1. Salting out method (Pospiech and Neumann 1995):

1. Inoculate single Escherichia coli bacterial cell in 50 ml brain heart infusion broth and incubate it in shaker incubator for overnight 37 °C.
2. Harvesting the bacterial growth for 15 min. at 6000 rpm in cooling centrifuge at 4°C, pour off supernatant.
3. Suspend the pellet in 5ml SET buffer, mix well by vortex, spin for 15 min. At 6000rpm by using cool centrifuge pour off supernatant, suspend the pellet in 1.6 ml SET buffer.
4. Add 100 µl lysozyme solution, incubate (30-60) min. At 37 °C using water bath.
5. Add 600 µl of 10% SDS, mix by inversion for 5 min, incubate 1hr. In water bath at 55°C.
6. Add 2ml from 5M NaCl, mix thoroughly by inversion.
7. Add 5ml chloroform, mix by inversion for 30 min.

8. Spin for 20 min. at 6000 rpm by using cool centrifuge, three layers are separated first one is aqueous phase contain DNA, interphase layer contain protein and the lower one organic phase contain the organic solvent chloroform.
9. Transfer the upper layer to another tube, add twice volume from ethanol, and mix by inversion, after 3 min spool DNA on to pasture pipette.
10. Dissolve the DNA in (50-100) μ l TE buffer .

3.1.2 Preparation of 1.0% Agarose Gel :

It was prepared as follows:

1. Agarose (1g) was added to 100 ml of TBE 1X buffer, placed in a microwave for 1.5 min until the agarose granules were completely melted and the solution looks clear.
2. Ethidium bromide (2 μ l) was added to the agarose solution after cooled down to 50-60 $^{\circ}$ C.
3. The solution was then poured into a tray, the comb was inserted and the agarose gel was allowed to solidify for about 30 min.
4. The electrophoresis apparatus was assembled and plugged in a direct current, agarose gel tray was placed into a tank, filled with 1X TBE buffer sufficient to cover the entire gel.
5. The comb was removed carefully, and then each well was loaded with total DNA.

3.1.3 DNA Visualization :

1. Each well was loaded with 5 μ l of loading dye mixed with 7 μ l of total DNA.
2. The electrical power was turned on after closing the tank lid then adjusted at 80 volt for 1 hr.
3. The migration of DNA was from cathode (-) to anode (+) poles.
4. Agarose gel tray was placed in gel documentation system for visualization of DNA bands under ultraviolet light (350 nm) and photographed (Couto et al., 2013) [5].

3.2 Sample treatment by plasma needle:

DNA samples were treated with plasma needle at argon gas flow rate of (1) L/min. , two tests has been made to show the effect of plasma on DNA samples the first experiment was done to determine the effect of different time durations of treatment on wet samples placed 6cm from the plasma tube. The second experiment was done to determine the effect of different exposure distances of treatment on wet samples placed at time 20 second.

IV. Result and Discussion

Our first experiment has been done to investigate the effect of plasma on wet DNA samples placed at distance 6cm from the tip of the plasma for different exposure time (10,20,30,40,50 second), Since the atmospheric plasma needle contains multiple types of species, such as radicals, electrons, photons, and charged particles, direct plasma radiation on a target, e.g., a cancer cell, can cause ionization or excitation at the molecular level. In cells, these processes may initiate an event cascade that could lead to biological changes in DNA[6]. figure(3) displays a representative gel image for the DNA samples treated by the plasma at fixed distance.

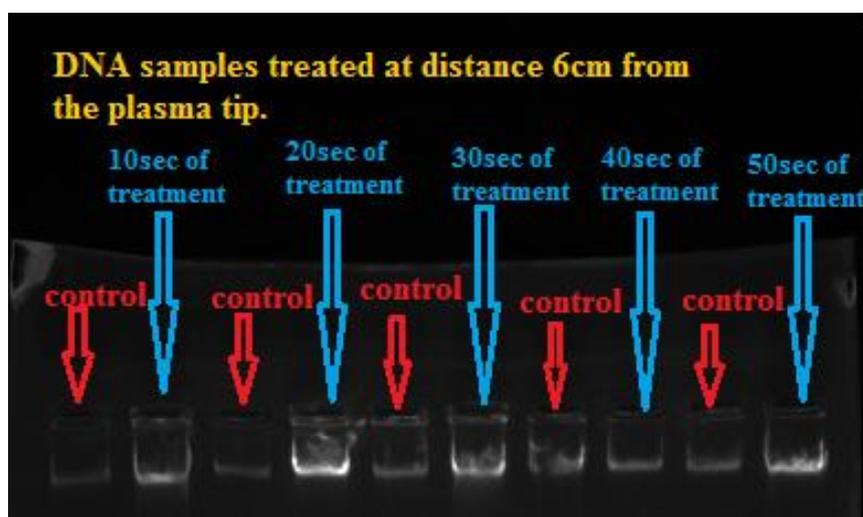


Fig .(3): the image shows the distribution of bands, a brighter band indicates a larger amount of DNA conformers.

From the image above one can conclude that as the time of exposure increase the effect of plasma on DNA is increased but it do not change much at the time 10,40 sec.

The second experiment has been done to investigate the effect of plasma on wet DNA samples placed at different distances (11,12,13,14,15cm) and fixed exposure time 20second.

Figure (4) displays a representative gel image for the DNA samples treated by the plasma at fixed time.

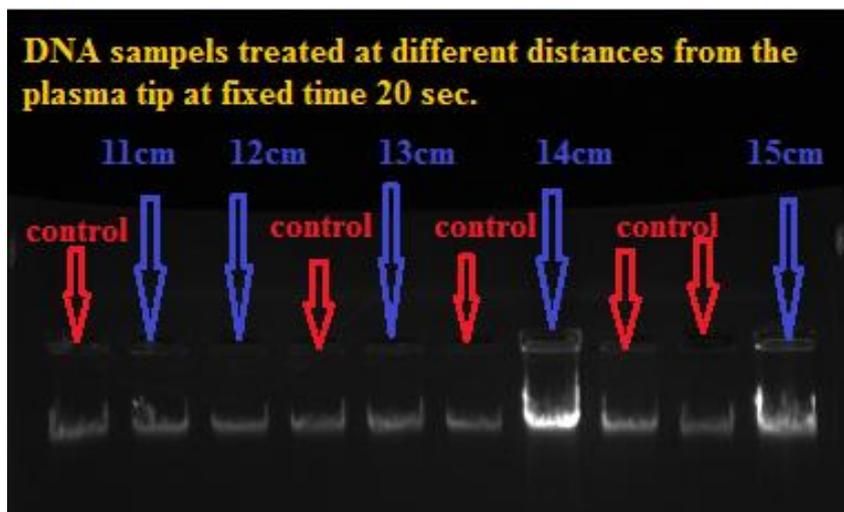


Fig .(4) the image shows the distribution of bands, a brighter band indicates a larger amount of DNA conformers.

From the image above one can conclude that as the distance increased between the plasma tip and the DNA sample the effect of plasma on DNA sample increased (DNA destruction).

The interactions at the plasma liquid interface are complex with a large variety of relevant species and a broad range of densities and particle fluxes, Reactive oxygen species and reactive nitrogen species are particularly known for their aggressive influence on biomolecules, Atomic oxygen can be expected to be one of the more important species for DNA damage [7].

V. Conclusions

Strand breaks in aqueous DNA samples were induced by Argon atmospheric pressure plasma jet. The contribution to DNA damage of variations in both distance from the plasma tube and exposure time has been discussed with respect to the electrical parameter settings of the plasma source. The damage level was shown to rise when the sample was close to the needle tip region. A high effect on DNA sample was shown when increasing the exposure time of plasma treatment. which supports the effectiveness of plasma for inducing DNA scissions. A low level of DNA damage was observed mainly under certain exposure distances (11,12,13)cm. Possible fast chemical reactions and diffusion processes between reactive radicals in the jet and the liquid environment may be largely responsible for the generation of the strand breaks. Thus, the detailed determination of the species types generated in liquid during plasma irradiation will be essential to further explore the mechanisms of DNA damage.

References

- [1] Lee, Hyun Woo et al. (2009) Tooth bleaching with nonthermal atmospheric pressure plasma. JOE (587- 591).
- [2] Belkind, A. and Gershman S. (2008) Plasma Cleaning of Surfaces. Vacuum Technology and Coating.
- [3] Felipe Iza, et al. (2008). Microplasmas: Sources, Particle Kinetics, and Biomedical Applications. Plasma Processes and Polymers, 322- 344.
- [4] Sylwia Ptasinska, et al. (2010) DNA strand scission induced by a non-thermal atmospheric pressue plasma jet. Physical Chemistry Chemical Physics, 12, (7779- 7781).
- [5] Couto, M. C.; Sudre, A. P.; Lima, M. F. and Bromfim, T. C. (2013). Comparison of techniques for DNA extraction and agarose gel staining of DNA fragments using samples of Cryptosporidium. Vet. Med., 58(10): 535-542.
- [6] Xu Han, W.A. Cantrell, E.E. Escobar, S. Ptasinska,(2014), Plasmid DNA damage induced by helium atmospheric pressure plasma jet. Eur.J.D;DOI:101140/epjd/e2014-40753-y.
- [7] D. O'Connell, L.J. Cox, W.B. Hyland, S.J. McMahon, S. Rreuter, W.G. Graham, T. Gans, and F.J.Currell, (2011), Cold atmospheric pressure plasma jet interactions with plasmid DNA, Applied Physics letters98, 043701;doi:10.10631/1.3521502.