## Effects of Atmospheric Pressure Plasma on Cellular Components: An Insight into Bacterial Destruction Mechanisms

<u>Mahmoud Y. Alkawareek</u><sup>1</sup>, Sean P. Gorman<sup>1</sup>, William G. Graham<sup>2</sup>, Deborah O'Connell<sup>3</sup>, and Brendan F. Gilmore<sup>1</sup>

<sup>1</sup> School of Pharmacy, Queen's University of Belfast, Belfast, BT9 7BL, UK <sup>2</sup> Centre for Plasma Physics, Queen's University of Belfast, Belfast, BT7 1NN, UK <sup>3</sup> York Plasma Institute, Department of Physics, University of York, York, YO10 5DD, UK E-mail: malkawareek01@qub.ac.uk

Atmospheric pressure non-thermal plasmas have proven to be effective in the eradication of bacteria in both planktonic and biofilm modes of growth [1]. These observations make this a promising approach for surface decontamination of medical devices and even viable tissues. However, the exact mechanisms of bacterial cell destruction mediated by cold plasmas are not fully understood. Cell killing mediated by the plasma is thought to be a complex and heterogeneous process, which involves a sequence of reactions [2]. In the present study, interactions of atmospheric pressure non-thermal plasma with different bacterial cell components are being explored in order to identify cell components most vulnerable to plasma exposure. Coupling this with the knowledge of plasma chemistry will help in identifying the specific reactions leading to cell death.

In this study, the efficacy of a 20 kHz atmospheric pressure dielectric-barrier type plasma jet [3], operating in helium and oxygen, was evaluated against a set of clinically significant bacterial strains in both of their planktonic and biofilm forms. Optical diagnostics for relevant reactive oxygen species have also been applied for direct correlations; absolute densities of metastable singlet delta molecular oxygen and ozone, relevant reactive oxygen species have been measured [4]. All planktonic bacteria were inactivated within four minutes of plasma exposure. Although 48-hour old bacterial biofilms, of the same strains, were more resistant to the plasma treatment, all biofilms were still completely eradicated within ten minutes.

Isolated bacterial plasmid DNA has also been exposed to the plasma and analysed using gel electrophoresis. Changes in plasmid structural conformation were quantitatively assessed and the rates of single and double strand breaks were calculated. Catalytic activity of certain bacterial enzymes was also evaluated after plasma exposure using fluorogenic assay, which allows the determination of the maximum retained enzyme activity after each plasma exposure. Peroxidation of lipid content of bacterial cells was also studied to evaluate the effect of plasma exposure on the phospholipid bilayer in the cell membrane. Furthermore, cell wall integrity and change in membrane permeability were assessed by measuring the leakage of Adenosine-5'-triphosphate (ATP), while electron microscopy studies are underway to examine the morphological changes to the cell surface following plasma exposure.

## References

[1] Alkawareek MY, Algwari QT, Gorman SP, Graham WG, O'Connell D & Gilmore BF, *FEMS Immunol. Med. Microbiol.* (2012), 'Accepted Article', **doi:** 10.1111/j.1574-695X.2012.00942.x.

[2] Gaunt LF, Beggs CB & Georghiou GE, IEEE Trans. Plasma Sci. (2006), 34: 1257-1269.

[3] Algwari QT & O'Connell D, Appl. Phys. Lett. (2011), 99, 121501

[4] Sousa JS, Niemi K, Cox LJ, Algwari QT, Gans T & O'Connell D, J. Appl. Phys. (2011), 109, 123302