CAMT Seminar

“On the Effect of Plasma (Media) Treatment on Cell Division”

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“The importance of understanding cold atmospheric plasma interactions with tissue fluid and tissue”

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Date: March 6, 2017 (Mon) 14:00-16:00
Location: Main Conference Room (1st floor), Bldg. A12
Center for Atomic and Molecular Technologies (CAMT)
(A12 棟 1 階会議室)

Abstracts are attached blow.

(Host: Satoshi Hamaguchi Ext：7913)

On the effect of plasma (media) treatment on cell division

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In this paper we will describe our efforts to adapt an established protocol (the CBMN assay) for assessing changes in "normal" cell division process as a result of plasma treatment of cell culture media, prior to the addition of cells.

1. Introduction

There is a significant and growing body of literature appearing concerning the use of atmospheric pressure plasma jets as an emerging treatment strategy for a range of diseases including non-healing wounds and cancers[1]; these are now being supported by clinical data [2,3]. The mechanism of action is thought to involve the longer-lived reactive oxygen and nitrogen species (RONS) generated by the plasma, but the effects from plasma radiation and electric fields have also been recognized.

In contrast to the rapidly expanding interest in applications, there is comparatively little research being conducted on the effects of plasma jet treatment, direct or indirect, at the chromosomal level. And, particularly in the context of potential plasma effects on the normal cell division processes. In this study we use the established cytokinesis block micronucleus (CBMN) assay [4] to assess the effect of plasma jet treatment at the chromosomal level in cell division. We investigate the effects of plasma action indirectly through the treatment of cell culture media which is subsequently applied to WIL2-NS human lymphoblastoid cells. This approach removes any complicating effects from radiation and electric fields.

2. Results

We find that increasing the concentration of plasma treated media applied to the WIL2-NS cells resulted in significant elevated levels of cell death and there was a significant increase in occurrence of chromosomal damage, measured through raised levels of micronuclei (MN), nuclear plasmic bridges (NPB) and nuclear buds (NB). These are shown in Fig. 1. The occurrence of these MNs, NPBs, and NBs were rigorously scored (the experimenter was blinded to the exact sample they were scoring). The results to be presented will highlight the significant changes in normal cell division (in WIL2-NS cells) that have been indirectly exposed to plasma. The results also validate the suitability of CBMN assay to examine the plasma jet induced damage of genetic material and provides a first insight into understanding the mechanism involved in the fatal effect of plasma on all cells.

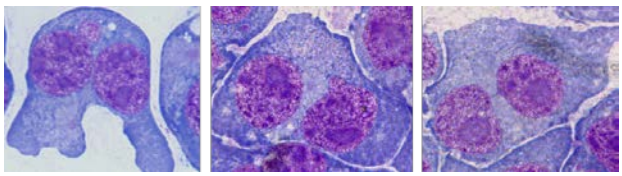

Fig 1: LHS, binucleated cell with MN, Centre, Bud and RHS, NPB

Acknowledgments

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References

[1] Von Woedtke, T., H.R. Metelmann, and K.D. Weltmann, *Clinical plasma medicine: state and perspectives of in vivo application of cold atmospheric plasma.* Contributions to Plasma Physics, 2014. **54**(2): p. 104-117.

[2] Keidar, M., et al., *Cold plasma selectivity and the possibility of a paradigm shift in cancer therapy.* British journal of cancer, 2011. **105**(9): p. 1295-1301.

[3] Isbary, G., et al., *Successful and safe use of 2 min cold atmospheric argon plasma in chronic wounds: results of a randomized controlled trial.* British Journal of Dermatology, 2012. **167**(2): p. 404-410.

[4] Fenech, M., *Cytokinesis-block micronucleus cytome assay.* Nature protocols, 2007. **2**(5): p. 1084-1104.

The importance of understanding cold atmospheric plasma interactions with tissue fluid and tissue

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There is significant optimism that cold atmospheric plasma could play a role in the treatment of diseases and infection, particularly those that are refractory and potentially life-threatening such as non-healing chronic wounds and cancers. The medical benefits from plasma are assigned to reactive oxygen and nitrogen species (RONS) that are generated by plasma upon interaction with air and liquids. However, we still do not have a sufficient understanding of (1) what RONS are delivered by plasma, (2) the rate RONS are delivered, (3) how deep RONS are delivered into tissue and (4) how RONS interact with cellular membranes. This knowledge is essential in order to obtain a mechanistic understanding of plasma in biology and medicine. In this talk, I will discuss simple biological mimics of 3D tissue or cells membranes, which are utilized to gain new insight into the plasma generation and transport of RONS and molecular oxygen into tissue fluid, tissue and cells. Surprisingly, we discovered that plasma can directly transport RONS and molecular oxygen deep within tissue to millimeter depths and across cellular membranes without physically damaging the tissue or cell membrane. In addition, I will discuss how the combined dynamic changes in the concentrations of RONS and molecular oxygen in the biological fluid can significantly impact cell viability during and after the plasma treatment. Finally, I will discuss how the above assays can support the future development of plasma sources to deliver metered doses of RONS and molecular oxygen within tissue for treatment of diseases such as chronic wounds and cancers.

References

[1] How to assess the plasma delivery of RONS into tissue fluid and tissue, J-S OH, EJ Szili, N Gaur, S-H Hong, H Furuta, H Kurita, A Mizuno, A Hatta, RD Short, Journal of Physics D: Applied Physics **49:** 304005 (2016).

[2] How plasma induced oxidation, oxygenation, and de-oxygenation influences viability of skin cells, J-S Oh, X Strudwick, RD Short, K Ogawa, A Hatta, H Furuta, N Gaur, S-H Hong, A J Cowin, H Fukuhara, K Inoue, M Ito, C Charles, RW Boswell, JW Bradley, DB Graves, EJ Szili, Applied Physics Letters **109:** 203701 (2016).