Plasma Processing for Biomedical Applications Focus Topic
Room: 12 - Session PB+BI+PS-TuM

Plasma Medicine
Moderator: Katharina Stapelmann, Ruhr-University Bochum, Germany

8:00am **PB+BI+PS-TuM1 Spatial Distribution of Biological Effects Induced by Plasma Reactive Species**, ***Sylwia Ptasinska***, University of Notre Dame **INVITED**

Several *in vitro* and *in vivo* studies have been conducted in a variety of cancer cell lines that demonstrate the efficacy of cold plasmas in causing cell death since the advent of this new research area in the plasma physics community in 2010. Due to the complexity of both the plasma and biological systems, many questions must be answered to sharply improve our understanding of the physical, chemical, and biological processes underlying their interactions. However, since cold plasmas produce a cocktail of reactive oxygen species (ROS) and reactive nitrogen species (RNS), these species are believed to be key agents that can induce a number of biological effects, including impairment of cell substructures and even cell death. Moreover, cancer cells have proven to be more susceptible to damage by these reactive species than normal cells subjected to plasma exposure. The outcome of cell responses to plasma treatment has inspired the potential application of plasma as an effective and safe tool for novel cancer therapy. Our research focuses on investigations of nucleus DNA damage in cancer cells and bacterial inactivation caused by exposure to plasma reactive species. Initially, to detect ROS and RNS that reached the targeted biological systems we used semi-quantitative test strips, while to investigate biological effects in cells we used digital imaging or immunofluorescence microscopy. Recently, to obtain the high-resolved spatial distribution of DNA strand breaks we developed a workflow with algorithms for image analysis using CellProfiler and MATLAB, including background correction, cell segmentation, feature extraction, cell classification, and data visualization. This method well preserves the essential spatial information about cell distribution, which is critical because of the localized nature of the plasma jet treatment. By applying both supervised and unsupervised machine learning techniques to the images, we were also able to classify the cells according to different cell cycle phases, and thus obtain spatial information regarding plasma jet effects on cell cycle progression.

8:40am **PB+BI+PS-TuM3 Mechanisms of Cell Death in Prostate Epithelial Cells after Treatment with Low Temperature Plasma**, *J. Packer, A.M. Hirst, F.M. Frame,* ***Deborah O'Connell***, *N.J. Maitland*, University of York, UK

Low-temperature plasma (LTP) treatment of cancer cells have been explored for a variety of malignancies. These plasmas, operated at atmospheric pressure and close to room temperature, are efficient sources of reactive oxygen and nitrogen species (RONS), electric fields and photons, and can induce a variety of biological responses. There is an increasing clinical move towards focal therapy for more conservative management of prostate cancer, with reduced levels of common side effects such as incontinence and impotence compared with radical treatments, and promising outcomes. Low-temperature plasmas may offer such potential.

A dielectric barrier discharge jet, created within a glass tube surrounded by two electrodes (~ 6 kV applied sinusoidal voltage), with a helium plus 0.3% oxygen gas flow is used for these investigations. We have employed both purified tumour cells freshly extracted from prostate cancer patients, and matching, non-tumour cells from a distant region of the same prostate. Freshly isolated primary tumour cells acts as a near patient model, which has recently confirmed differences in pharmacological susceptibility as compared with 30 year old established cell lines.

Treatment of primary prostate epithelial cells with LTP resulted in sigificant cell death in both normal and cancer cells; and no significant selectivity observed, as commonly reported. In addition, most cells appeared to die via a necrotic mechanism, rather than apoptosis, maybe as a result of the mitochondrial toxicities of the LTP-activated reactive oxygen species (ROS). However, some autophagy was also detected, which has been shown to act as a salvage pathway for sub-lethally damaged cells.

To determine which of the multiple plasma activated bio-reactive species are responsible for the cytotoxicity, we have explored immediate and longer-term effects on gene expression, with a particular focus on oxidative responses, in multiple patient samples. Comparative studies in the established cell lines indicated a delayed and different response, highlighting that cell lines don't always reflect the response of primary cells. Expression of 84 genes (mRNA by RT^2 arrays from Qiagen) was assessed at multiple time points, after a 3 minute LTP treatment, and candidate genes/response pathways were identified. Immunofluorescence and western blotting were used to verify changes in protein expression. The response varied according to the clinical grade of the tumour (including a remarkable downregulation of 18 factors only seen in the highest grade tumours). All epithelial cells showed a stimulation of transcription factor-driven anti-oxidative response, as a potential resistance mechanism.

9:00am **PB+BI+PS-TuM4 Selective Antitumor Effect of the Plasma-Activated Medium Produced by Atmospheric Pressure Plasma with High Plasma Density**, ***Yohei Takahashi***, *Y. Taki*, Nikon Corporation, Japan, *K. Takeda*, Meijo University, Japan, *H. Hashizume, H. Tanaka, M. Hori*, Nagoya University, Japan

Recently, atmospheric pressure plasma has been widely developed for the applications on various fields, such as synthesis approaches, surface modification, sterilization, etc. Especially, cancer therapy using atmospheric pressure plasma is one of the most attractive applications. The culture medium irradiated with the atmospheric pressure plasma was called Plasma-Activated Medium (PAM), which exhibited the selective apoptotic cell death of cancer cells. In this study, we have demonstrated the antitumor effect of medium induced by irradiation of atmospheric pressure plasma with high plasma density and compared the cell survival between cancer and normal cells, which showed that the selective apoptotic cell death was achieved. Additionally, the basic diagnostics of the plasma and the analysis of the PAM were performed and the relation with the antitumor effects was discussed. The emission peak of OH radical ($A^2\Sigma$-$X^2\Pi$) was observed in the atmospheric pressure plasma. This transition is the intense systems emitted by low temperature plasmas containing even a small amount of H_2O. The selective apoptotic cell death effect by treatment with PAM produced by atmospheric pressure plasma irradiation was confirmed. The survival of cancer cell after incubation in PAM was greatly lower than that of normal cell was. The productions of H_2O_2 and NO_2^- by irradiation of high density plasma were detected by the colorimetric assay. The synergistic effect of H_2O_2 and NO_2^- in PAM is considered to affect the proliferation of cancer cells.

9:20am **PB+BI+PS-TuM5 Multiplex Coherent Anti-Stokes Raman Scattering (CARS) Observations of HeLa Cells Cultured in Non-equilibrium Atmospheric Pressure-Plasma-Activated Medium (PAM)**, ***Kenji Ishikawa***, *R. Furuta,* Nagoya University, Japan, *K. Takeda, T. Ohta, M. Ito,* Meijo University, Japan, *H. Hashizume, H. Tanaka, H. Kondo, M. Sekine, M. Hori*, Nagoya University, Japan

Non-equilibrium atmospheric-pressure plasma (NEAPP) affects cancer cells not only directly[1] but also indirectly through exposure of cells to medium irradiated beforehand with NEAPP (i.e., plasma-activated medium [PAM]).[2] Recent studies have revealed that NEAPP irradiation generates reactive oxygen and nitrogen species (RONS) in the gas phase and relatively long-lived RONS such as hydrogen peroxide, nitrites and nitrates in the aqueous phase.[3] To further elucidate a cell-death mechanism in more detail, the present study focused on the direct analysis of PAM-induced intracellular molecules such as lipids, acylglycerol, triglyceride, adiposome in HeLa cells as cervical cancer cells. Lipid droplets (LDs) are dynamic organelles with complex and interesting biological functions that go beyond mere energy storage and are important in lipid homeostasis and metabolism. To evaluate LDs, coherent anti-Stokes Raman scattering (CARS) microscopy was used. The observation-results by multiplex coherent anti-Stokes Raman scattering (CARS) microscopy elucidated the mechanism underlying the apoptosis of HeLa cells in cultivating in PAM, leading to be simultaneously occurred the exhaustion of LDs in the cells in contrast to the accumulation, while the activation of caspase-3/7 was induced, though accumulation in lipid droplets (LDs) and lipid metabolism in the normal apoptosis of HeLa cells with activation of caspase-3/7 was previously reported.

Acknowledgement: This study was supported in part by the JSPS-KAKENHI (No. 24108002).

1 S. Iseki et al., Appl. Phys. Lett. **100**, 113702 (2012); 2 H. Tanaka et al., Plasma Med. **2**, 207 (2012); 3 N. Kurake et al., Arch. Biochem. Biophys. **605**, 102 (2016).

9:40am **PB+BI+PS-TuM6 Plasma Medicine - From Bench to Bedside**, ***Kai Masur***, *T. von Woedtke, K.D. Weltmann*, Leibniz Institute for Plasma Research and Technology, Germany

During the last decade it became possible to stimulate eukaryotic cells by applying non-thermal plasma. The same plasmas can be used to kill micororganisms - both in vitro and in vivo. However, there is the need to understand the processes of how electrical fields, ROS /RNS and UV

generation influence the cellular activities in order to find the balance between stimulating or killing biological matter. Therefore, much effort had been done by in order to control the plasma components and finally modulate biological activities. It was shown before that argon plasma treatment leads in a time dependent manner to an activation of cell proliferation in human skin samples. Furthermore, it is known that non-thermal plasma is able to diminish bacterial load of cultured microorganisms *in vitro* independent of the strain. Even more, plasma reduces the amount of antibiotic resistant bacteria in the same manner as their non-resistant strains.

In 2013, new developed plasma sources were certified as medical products and since than those devises are in clinical application. Here we report on our findings on plasma treated chronic wounds and the efficacy of non-thermal plasma. There is a very promising rate of healed and improved wounds, which demonstrate that plasma indeed can help patients with chronic wounds. However, there are some discrepancies between *in vitro* findings and results from patient treatment. The bacterial reduction is lower than in *in vitro* studies, but skin regeneration seems not to be dependent on complete bacterial removal. On the other hand, patient treatment reveals new facts about the positive effects of plasma treatment of persisting wounds. Here we summarize the positive results of plasma mediated stimulation of patients with chronic wounds.

11:00am **PB+BI+PS-TuM10 Plasma Medicine, RONS, Tissue and Cell Models**, ***Rob Short***, University of Lancaster, UK, *E. Szili*, University of South Australia, Australia **INVITED**

Electrically-generated cold plasma gas discharges are being intensively researched for novel applications in medicine and biology. Significant attention is being given to the reactive oxygen and nitrogen species (RONS), initially generated upon plasma-air interactions that are delivered to biological systems. The effects of plasma exposure are observed deep within tissue, to millimetre depths and within cells. However, very little is known about the exact nature of the initial plasma-tissue interactions, including RONS speciation and delivery depth, or how plasma RONS intervene in biological processes. In this presentation I will focus on current research using tissue and cell models to learn more about the plasma delivery and transport of RONS into tissue and cells. I will argue this research is vital to establishing an underpinning knowledge that is needed to realise the full potential of plasma in medicine and biology.

11:40am **PB+BI+PS-TuM12 Non-thermal Plasmas in Biomedical Applications– Beyond the Long Lived Species**, ***Kristian Wende***, *J. Volzke*, INP Greifswald, Germany, *J-W. Lackmann*, Ruhr University Bochum, Germany, *H. Jablonowski*, *S. Bekeschus*, INP Greifswald, Germany, *K. Stapelmann*, Ruhr-University Bochum, Germany, *S. Hasse*, INP Greifswald, Germany, *P.J. Bruggeman*, University of Minnesota, *K.D. Weltmann*, INP Greifswald, Germany

Non-thermal plasmas have reached evidence level 2 regarding acceleration of wound healing and in certain aspects of cancer treatment, with a growing community of physicians successfully using it (plasma medicine). Key players in such biomedical applications are reactive oxygen or nitrogen species (ROS/RNS), which are deposited in either tissue (in vivo) or liquid (in vitro) and subsequently influence cellular redox signaling. A huge variety of plasma sources for potential application has been developed and comparing these sources in respect of safety and efficacy remains challenging but desirable.

One aspect can be the identification and quantification of the sources ROS/RNS deposition in liquids. However, due to the short lifetime of many ROS/RNS and limited specificity of available probes their detection is demanding. To meet this challenge, we applied a variety of analytical techniques including high-resolution mass spectrometry of small molecules (cysteine, tyrosine), ion chromatography (RNS detection), electron paramagnetic resonance spectroscopy (O, O_3, $^1O_2$, O_2^-, OH), and colorimetric assays to infer on dominant active species. Two argon plasma jets (MHz jet kinpen, RF jet) and a helium based RF jet (COST jet) were investigated. In addition, cell biology experiments allowed a first estimation of the biological impact of plasma treated small molecules.

A large number of covalent modifications have been detected and in part identified. The majority of changes to the chemical structure of cysteine was found in the vicinity of the thiol group, while in tyrosine the aromatic ring was targeted. The resulting products also occur in physiological situations in vivo, allowing to conclude that the covalent modification of small organic molecules is part of the mechanism of direct plasma-cell interaction. Predominantly short-lived oxygen species were found to be of relevance regarding the chemical and biological impact of plasma, challenging the popular concept of remote treatment (e.g. plasma treated buffers).

12:00pm **PB+BI+PS-TuM13 Effects of Oxygen or Water in Plasma Jet Environment and Feed Gas on DNA Damage**, ***Ek Adhikari***, *V. Samara*, *S. Ptasinska*, University of Notre Dame

Atmospheric pressure plasma jet (APPJ) sources have been explored for applications in industry and medicine. Since environmental conditions such as room temperature and humidity fluctuate, two identical APPJ sources operating at various places and time might perform differently. An APPJ operating in a controlled environment may be able to overcome that issue. Moreover, the interaction of plasma components (e.g., ions, electrons, UV light) with the air in the atmosphere generates the reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the plasma jet [1]. These reactive species can be controlled by adjusting a fraction of oxygen and water vapor in the plasma jet environment and the feed gas. To create a controlled environment for a plasma source, a helium APPJ source was operated in a cylindrical glass chamber with an ambient pressure and filled with pure nitrogen gas along with a fraction of oxygen and water vapor. This APPJ source was used to induce damage in aqueous DNA. The fraction of different types of damaged DNA such as single strand breaks (SSBs) and double strand breaks (DSBs), which were induced due to plasma irradiation, and undamaged DNA were quantified by using agarose gel electrophoresis. We observed that a moderate amount of oxygen and water vapor in the environment, as well as in the feed gas, increases the level of DNA damage.

1. K. Arjunan, V. Sharma, and S. Ptasinska, Int. J. Mol. Sci. **16**, 2971 (2015).

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