Plasma Science and Technology Division
Room 104C - Session PS+PB-TuM

Plasma Medicine

Moderator: Deborah O'Connell, University of York, UK

8:00am **PS+PB-TuM1 Lessons from Tesla for Plasma Medicine**, ***David Graves,*** University of California at Berkeley

It can be argued that plasma medicine originated with Nikolai Tesla in the late 19th century when he showed that one could pass large quantities of high frequency currents through a human body with no apparent damage. [1, 2] Tesla interpreted the significant sensations he experienced following exposure to these currents as being potentially valuable therapeutically. Tesla's work inspired much more extensive investigations over a period of several decades by numerous other researchers, on both the physics and biomedical effects of these currents. Researchers such as Arsene d'Arsonval and Paul Oudin in France and Frederick Strong in the United States, among others, were important pioneers. These early pioneers had a surprisingly modern view of some aspects of the therapeutic mechanisms of high frequency currents that clearly overlap with recent results. The perspective of this community was that the most important physiological effects are associated with the high frequency currents rather than the gas phase plasma. Some early work, such as the analgesic effects of dielectric barrier air plasma on tissue, is not well known today. [3] The range of afflictions that early practitioners treated successfully is remarkable. This body of work, in some cases almost 130 years old, has important lessons for current investigations into plasma medicine. Observations from Tesla and other early practitioners suggests that high frequency currents are potentially important and plasma medicine researchers should probably pay more attention to them.

[1] F.F. Strong, High Frequency Currents, Herman Company, New York, 1908.

[2] S.H. Monell, High Frequency Electric Currents in Medicine and Dentistry, WR Jenkins, New York, 1910.

[3] P. Oudin, Application therapeutique locale des courants de haute frequence en gynecologie, Archives d'électricité médicale, 287, 10 juin, 1910.

8:20am **PS+PB-TuM2 Characterization of a Helium Atmospheric Pressure Plasma Jet by Measuring the Total Yield of Reactive Species in Real Time**, ***Ek Adhikari,*** *V. Samara, S. Ptasinska,* University of Notre Dame

Recently, we performed in-situ measurements of reactive oxygen species of a helium atmospheric pressure plasma jet (APPJ) using an acidified ferrous sulfate solution (Fricke) as a sample. The total yield of reactive species reached to or originated in the solution corresponds to the amount of the yield of Fe^{3+} from reactions that transform Fe^{2+} into Fe^{3+} during plasma irradiation. The measurements indicated that the number of reactive species formed in the plasma jet is proportional to the applied pulse voltage and repetition frequency. However, there is a decrease in the yield of Fe^{3+} per pulse for an increase in the frequency. For higher frequencies, there is not enough time to complete all reactions before the next pulse arrives to the sample. Whereas, for lower frequencies, this yield is higher due to the relatively longer time period necessary for reactions to complete. Further, the flow rate of feed gas and treatment distance, which is the distance between the sample and glass capillary, have a minor effect on the formation of reactive species, but the yield of Fe^{3+} gradually decreases for a treatment distance longer than 20 mm. Moreover, we calculated the yield of Fe^{3+} in a very short time (equivalent to time period used in the experiment), and compared with the experimental results. The yield of Fe^{3+} formed within 15 s of plasma irradiation was also compared with the fractions of plasma induced DNA damage level under similar experimental conditions.

8:40am **PS+PB-TuM3 Dry Etching of Patterned Medical Grade Titanium Alloys**, ***Eitan Barlaz,*** *J. Mettler, D.N. Ruzic,* University of Illinois at Urbana-Champaign

We report on the development of a plasma etch process for Ti-6Al-7Nb, an alloy of titanium common for performance biomedical implants due to its excellent mechanical properties and corrosion resistance. The process uses the same chlorine and oxygen chemistry common to etches of pure titanium, with added ion bombardment to remove reaction products with low volatility and to ensure minimal texturing of the surface. The process is capable of etch rates in excess of 50 nm/min in 50 mTorr of Argon and 20 mTorr of CCl_4 using 50 W of RF power and a negative sample bias of > 500 Volts. Due to the need to produce irregularly shaped geometries, relative etch rates are reported for a variety of features including through holes and posts on representative parts. Plasma diagnostics including Langmuir and radical were applied to the process to compare the efficacy of both inductively coupled and surface wave plasma sources over large part sizes.

9:00am **PS+PB-TuM4 Electron Temperature And Plasma Density Of Ar Plasma In Atmospheric Pressure Micro-DBD**, ***Pradoong Suanpoot,*** *J. Sornsakdanuphap,* Maejo University Phrae Campus, Thailand; *B. Ghimire, Y.J. Hong,* Plasma Bioscience Research Center, Republic of Korea; *G. Cho,* Charged Particle Beam and Plasma Laboratory, Republic of Korea; *E.H. Choi,* Plasma Bioscience Research Center, Republic of Korea

A model based on plasma propagation velocity has been recently developed to estimate the electron temperature (T_e) of atmospheric pressure μ-DBD plasma. In this work, we have extended this model to calculate T_e for plasma generated with Ar gas. Plasma has been generated by input discharge voltage of 2.7 kV at driving frequency of ≈ 45 kHz. A high-speed single-frame intensified charged coupled device (ICCD) has been used to observe the space and time-resolved discharge images and estimate the value of plasma propagation velocity (u_g). The value of u_g for Ar plasma has been obtained in the range of $6.2\acute{}10^3$ m/s. The electron temperature has been calculated for this plasma. The average electron temperature has been found to be about 1.18 eV and the average plasma density has been found to be about 3.62×10^{14} cm^{-3} for Ar plasma. Our results obtained with modified convective-wave packet model can be a new contribution to plasma medicine.

Keywords: Atmospheric-pressure μ-DBD plasma, Ar plasma, plasma propagation speed, electron temperature, plasma density

9:20am **PS+PB-TuM5 Plasma Immunotherapy of Cancers**, ***Vandana Miller,*** *A. Lin, P. Ranieri,* Drexel University; *A. Snook,* Thomas Jefferson University; *A. Fridman,* Drexel University **INVITED**

Non-thermal plasmas are currently being developed as an alternative therapy for cancer. Local application of plasma to tumors *in vivo* has led to reduced tumor size and increased life expectancy of treated animals.[1] The body's immune system plays a vital role in the control of cancer.[3] In fact, cancer immunotherapy, the control of cancer by employing components of the patient's own immune system, is emerging as an appealing strategy.[3] New approaches being explored include increasing the immunogenicity of tumor cells by inducing immunogenic cancer cell death (ICD).[4] ICD of cancerous cells has been demonstrated with certain chemotherapeutic drugs and through physical methods such as X-ray therapy and UVC.[5, 6] Cells undergoing ICD express damage associated molecular patterns (DAMPs) which assist immune responses that may mediate systemic elimination of cancer.[4] We have demonstrated that non-thermal plasma is a good candidate for cancer therapy via immunomodulation by:

direct effects on immune cells[7, 8] and

indirect effects of cancer cell ICD.[8]

The role of plasma augmentation on the immune system, based on our *in vitro* and *in vivo* studies, will be discussed as a potential modality for clinical application in cancers. *In vivo* studies using Balb/c mice inoculated with subcutaneous CT26 colorectal cancer cells, treated with nspDBD plasma showed DAMP signal expression and recruitment of immune cells in the local tumor environment. Furthermore, there was development of a systemic, tumor-specific immune response. This demonstrates that plasma elicits ICD locally, in the treatment area, which leads to beneficial host immune responses both locally and systemically. The clinical potential of plasma cancer immunotherapy will be discussed and the challenges to address will be identified for further development of this technology. Results from a small clinical trial will also be presented.

References

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[5] Kalbasi, A., et al., *The Journal of clinical investigation*, **123**, 2013.

[6]Zitvogel, L., et al., *Nature Reviews Immunology*, **8**, 2008.

[7] Kaushik, N.K., et al., *Journal of Physics D: Applied Physics*, **49**, 2016.

[8] Lin, A., et al., *Plasma Processes and Polymers*, **12**, 2015.

11:00am **PS+PB-TuM10 Hydroxyl Radical Footprinting with Plasma-Induced Modification of Biomolecules (PLIMB): A Novel Tool for Protein Structural Analysis**, ***Faraz Choudhury**, D.I. Benjamin, B.B. Minkoff, J. Blatz, M.R. Sussman, J.L. Shohet,* University of Wisconsin-Madison

The protein-therapeutic industry, typified by anti-cancer proteins like Herceptin, has accelerated the need to be able to analyze the 3-dimensional structure of proteins in solution. Mass spectrometry is widely used for this. This has generated a need for new types of probes that covalently label protein solvent-accessible sites. Two common ones rely upon the creation of and covalent modification with highly reactive hydroxyl (OH) radicals. The first, fast photochemical oxidation of proteins (FPOP), generates hydroxyls from H_2O_2 via laser photolysis, and the second, synchrotron X-ray beam exposure, produces OH radicals via direct radiolysis of water. These techniques suffer from issues such as: a reaction times that are potentially too long to correctly measure protein structure or the necessity to add chemicals. Furthermore, both are cumbersome & expensive, either by accessing a synchrotron or building an instrument to perform FPOP. As a result of a collaboration at the interface of plasma physics and biochemistry, we developed a technique that generates µsecond bursts of OH radicals, using a surface-barrier discharge, for labeling proteins at solvent-accessible amino acid side chains. We call this Plasma-Induced Modification of Biomolecules (PLIMB). PLIMB does not require chemical additives, circumvents the issues associated with reaction timescale, and ultimately costs far less. Using a model protein in solution, cytochrome C, the protein was modified in a dose-dependent fashion in only a lightly buffered water-based solution, demonstrating that the system can generate OH radicals capable of labeling proteins without additional reagents. In addition, only discrete peptides within the protein are modified. Perturbing protein structure via digestion prior to plasma exposure significantly increases the observed covalent modification, suggesting that conformational structure is maintained during exposure. Experiments with myoglobin, a second protein, also revealed distinct regions of modification despite examining in depth the entirety of the protein's sequence. Mapping the oxidized peptides to myoglobin's crystal structure reveals that all of these peptides fall within the same face of the protein, suggesting the preservation of a higher-order structure under the solution conditions described. These experiments suggest that PLIMB provides a means of efficaciously generating microsecond bursts of OH radicals while providing a low cost and readily accessible means of probing the conformation of proteins in solution using mass spectrometry. We envision PLIMB being useful in a wide range of biological, medical and pharmaceutical fields.

11:20am **PS+PB-TuM11 Biological Effects of Plasma-Irradiated Organic Molecules in Plasma- Treated Liquids**, ***Kenji Ishikawa**, Y. Hosoi, D. Kanno, Y. Kurokawa, H. Tanaka, M. Mizuno, F. Kikkawa, M. Hori,* Nagoya University, Japan

Selective killing of cancer cells incubated in non-equilibrium atmospheric pressure plasma (NEAPP)-activated medium (PAM) has been reported.[1] This antitumor effect revealed by involvement of reactive oxygen and nitrogen species (RONS) in PAM.[2] The effect was also found in plasma-activated lactate in Ringer's solution (Lactec), so called as PAL.[3] We found that the cancer cells incubated in the PAL received lesser oxidative stress than that of the PAM.[3] A cause of intracellular oxidation with respect to the RONS reactions has been studied by nuclear magnetic resonance (NMR) analysis of organic substances in the Lactec solution.

From the NMR measurements, reactive organic acids, that is, plasma activated lactate (LA) involved pyruvic acid (PA) and acetic acid (AA)-like components in the PAL were detected. The plasma activated organic acids act potentially as antitumor agents other than RONS.

Furthermore, NEAPPs irradiated to fullerenol. The plasma irradiated fullerenol demonstrated a cytotoxic effect on cells. The PF was modified by the plasma irradiation, arising carbonyl groups, ether bonds, and intercalated nitrate anion. Endocytosis of the PF induced to apoptotic cell death and generated intracellular RONS on cells cultured in the PF-added cell culture medium.

1 mL of fullerenol-added water (1.6 mM) was irradiated by the NEAPP (Ar 2 slm) for 3 min. Precipitates of the PF were collected by drying water at 70°C for 1 hr. The collected PF dissolved again into 50 µM of cell culture media (DMEM). 5×10^3 of HeLa cells were cultured for 24 hr in the PF-added DMEM and the same amount (50 µM) of fullerenol-added DMEM, respectively. Cell viability was evaluated by the MTS assay. Caspase activation and fullerenol permeation of the cell membrane were observed by fluorescent microscopy.

Although the viability of the fullerenol-added DMEM remained at a constant of 110 %, the HeLa cell viability decreased to 70 %, when the cells were incubated in the PF-added DMEM. The cells showed caspase 3/7 activation. The PF activate the caspase cascade pathway to induce apoptosis and permeation of the fullerenol into the cells. Therefore, the fullerenol properties were modified by the plasma-irradiation to enhance the cytotoxicity of PF.

Acknowledgments This study was supported by KAKENHI 24108002.

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[2] N. Kurake et al., Arch. Biochem. Biophys. **605**, 102 (2016).

[3] H. Tanaka et al., Sci. Rep. **6**, 36282 (2016).

11:40am **PS+PB-TuM12 OH-Radical Generation in an Atmospheric-Pressure Plasma Discharge for use in Three-Dimensional Protein Structural Analysis**, ***Joshua Blatz**, B.B. Minkoff, F.A. Choudhury, D.I. Benjamin, J.L. Shohet, M.R. Sussman,* University of Wisconsin-Madison

Atmospheric-pressure plasma discharges are well-known to generate OH radicals when interacting with water. However, the use of radicals created in this way for protein footprinting is virtually non-existent. Here, we describe a novel technique which utilizes a dielectric-barrier plasma discharge to oxidize proteins in solution. These oxidation events are quantified using mass spectrometry and allows us to determine the exterior domain and solvent accessibility of a protein.

To generate the plasma a high-voltage A.C. signal is applied to a needle electrode in close proximity to the surface of the liquid sample. This causes the gas to breakdown and form the plasma. These plasma discharges have been measured to be on the order of microseconds which prevents the protein from cleaving. During plasma exposure the sample is cooled to maintain temperature and prevent denaturation.

Protein bovine serum albumin was exposed to plasma. Labeling of the exterior domain was found in a manner consistent with other protein structural analysis techniques. Additionally, initial measurements have been collected which indicate that by changing various electrical, physical, and chemical parameters the technique may still be optimized. This could lead to greater OH-radical generation, reduced sample heating, and reduced pH change.

In addition to the consistent initial results and optimization potential there are many advantages it holds over competing techniques. It can be built inexpensively and on a space-limited benchtop. There are no necessary chemical additives which may interfere with the results and there seems to be no limit to the size of the protein which can be treated. All the samples are treated in liquid solution so they are free to move as they would *in vivo*.

12:00pm **PS+PB-TuM13 Plasma-Surface Interaction at Atmospheric Pressure: From Mechanisms with Model Polymers to Applications for Sterilization**, ***Pingshan Luan***[1], *G.S. Oehrlein,* University of Maryland, College Park

Cold atmospheric plasma (CAP) produces many types of chemically reactive species and is capable of modifying materials at atmospheric pressure. Studying plasma-surface interaction (PSI) at such pressure has been challenging due to the small mean-free-path (< 100 nm) which prohibits the method of using independently controlled beams of ions/neutrals. In the past few years, we developed an alternative approach of studying PSI at atmospheric pressure using well-controlled source-ambient-sample systems and comprehensive characterization techniques. First, we characterized and compared a few types of CAP sources such as atmospheric pressure plasma jet (APPJ) and surface micro-discharge (SMD). We found that the dominant reactive species generated by different CAP sources can be dramatically different. By tuning source operating parameters, we were able to manipulate the dominant reactants generated by these sources. Second, by controlling the gaseous environment wherein PSI took place, we could suppress certain unwanted interactions of plasma species with the ambient and regulate the delivery of reactive species to material surfaces. Lastly, we used polymers with representative functional groups to study the effect of reactive species on certain surface moieties. Due to the multi-phase nature of PSI, we integrated many characterization techniques in our study, including that of

[1] **Coburn & Winters Student Award Finalist**

plasma/gas phases such as optical emission spectroscopy (OES), Fourier transform infrared spectroscopy (FTIR) and UV absorption, and that of material surfaces such as X-ray photoelectron spectroscopy (XPS), attenuated total reflection (ATR) FTIR and Ellipsometry. To our knowledge, the perpendicular electric field enhanced ATR-FTIR was used for the first time to study plasma processed polymer films less than 10 nm-thick. Combined with XPS, these techniques provide rich chemical information of both surface and subsurface modifications. By correlating plasma/gas phase with surface/subsurface measurements, we showed the dominant effect of a few types of reactive species such as O, OH and N_2O_5 on materials. We also provided evidence showing the competition between etching and surface modification during plasma treatment. Besides, we extended our investigation to studying the CAP-induced bacterial membrane damage, which might help understand the sterilization mechanism of CAP. We gratefully acknowledge funding from National Science Foundation (PHY-1415353) and US Department of Energy (DE-SC0001939). We thank Andrew J. Knoll, Elliot A. J. Bartis, V. S. S. K. Kondeti, Peter J. Bruggeman, Andrea Gilbert, Rohan Tikekar and David B. Graves for collaborations.

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