Plasma Processing for Biomedical Applications Focus Topic
Room 101A - Session PB+BI+PS-MoA

Plasma Processing of Biomaterials

Moderators: Denis Dowling, University College Dublin, Deborah O'Connell, University of York, UK

2:00pm **PB+BI+PS-MoA2 Atmospheric Plasma Deposition of Antimicrobial Nano-Coatings on Biomedical Textiles**, *A. Nikiforov, I. Kuchakova, T. Coenye, C. Leys,* Ghent University, Belgium*; N. Hojnik, M. Modic,* ***Uroš Cvelbar,*** Jozef Stefan Institute, Slovenia

In this work, the antimicrobial non-woven fabrics were prepared with the use of atmospheric pressure plasma deposition.Atmospheric pressure DC jet operating in N_2 at current density of 6 mA/cm^2 and voltage of 15 kV is used as a source of non-thermal plasma for engineering of the antibacterial nano-composites on surface of polymeric polyethylene terephthalate (PET) meshes. Nano-particles of Ag, Cu and ZnO are tested as antimicrobial agents through incorporation in to the structure of the plasma deposited composite film. The deposition process is carried out in three steps process. The fabric is first pretreated by depositing a first layer (250 nm - 500 nm) of organosilicon thin film using an atmospheric pressure plasma system, then nano-particles are incorporated by a dipping-dry, and finally the nano-particles are covered by a second organosilicon layer of 10-50 nm thickness. Top layer in the composite coating of “sandwich-like structure” with variable thickness is used for precise control of metal ions release and so to tune antimicrobial efficiency of the material. The deposition process and surface chemistry of the coatings are studied by emission spectroscopy, and surface analysis techniques: XPS, AFM and SEM. The antimicrobial activity of the treated fabrics is also tested against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It is revealed that thickness of top (barrier) layer plays a key role in release of metal ions and negligible small antibacterial activity is observed if barrier thickness exceeds 50 nm. Tests with *S. aureus* show that the highest 98% bacterial reduction is achieved with Cu NPs whereas Ag NPs are much less effective and can provide only 79% reduction. In contrast, the fabric antibacterial efficiency against of *Pseudomonas aeruginosa* is very low for both Cu and ZnO nanoparticles in spite of the load and only Ag NPs are proved to be effective (2 orders reduction) against of *P. Aeruginosa*. The results clearly indicate that plasma of atmospheric pressure can be used as effective tool for immobilization of nano-particles in composite coatings. Control of antibacterial activity can be achieved through variation of deposition parameters and a type of incorporate nanoparticles. The approach might present a new route to preparation of effective antimicrobial materials against of certain class of bacteria.

This work is partially supported by the M.Era-Net project “PlasmaTex”.

2:20pm **PB+BI+PS-MoA3 Plasma Polymers for Biomedical Applications**, ***Farzaneh Arefi-Khonsari,*** l'université Pierre et Marie Curie, France*; A. Baitukha, J. Pulpytel, A. Valinataj Omran,* Sorbonne Universités, UPMC, France **INVITED**

In this talk, different nonequilibrium atmospheric pressure plasmas used for biomedical applications such as planar DBD, single and double barrier DBD plasma jets, and transported discharges in tubes will be discussed. Indeed in the case of the latter, deposition and surface treatment, by means of a He cold transported discharge in tubes as long as 200 cm and tube inner diameters ranging from 1 to 20 mm, can present a great potential for surface modification of polymers used as biomaterials. We have, as well as several research groups, succeeded to retain the precursor moieties to obtain PEG like polymers which present interesting antifouling properties by using planar DBD and jets. However for particular plasma applications such as making a Drug Delivery System (DDS) based on several polymer or copolymer layers, encapsulating the drug, it is more reasonable to use a low pressure plasma which can give rise to dense crosslinked barrier films. The latter are less flexible and develop microcracks due to swelling and curvature of the host biocompatible and biodegradable substrate. In order to obtain good cohesive coatings with excellent barrier and mechanical properties, it is very important to deposit layers presenting a vertical chemical gradient, where stress is gradually distributed over the rigid and flexible zones of the DDS, which is more easily deposited in low pressure plasmas. Our recent results in copolymerizing amphiphilic precursors for the use of cell adhesive or nonadhesive surfaces will be presented. Such copolymers can be also used as biodegradable multi-layer copolymers for drug delivery applications. Human ovarian carcinoma cell lines (NIH:OVCAR–3) were used for *in vitro* measurements of cell interactions with the surface of fabricated DDS. Proposed model of DDS on collagen films prevents migration, adhesion and growth of cancer cells on its surface, and by tuning the thickness of the dense barrier films, encapsulating the drug, it is possible to control the drug release kinetics and to improve the therapeutic effect. *In vivo* experiments were carried out by injecting OVCAR3 cells in mice lymph nodes to develop a tumor, followed by implantation of the DDS membranes to evaluate the feasibility of the proposed model.

3:00pm **PB+BI+PS-MoA5 Plasma Coating Using Biologics: Degradation or Polymerisation?**, ***Liam O'Neill,*** *J. O'Donoghue,* TheraDep, Ireland **INVITED**

The interaction of plasma with biomolecules is generally viewed as being a simple degradation reaction in which the plasma denatures any biologic material it encounters. Using a combination of heat, UV, free radicals, electrons and ions from the plasma, it is possible to cut, oxidise, burn and even ablate biological materials and this has established plasma sterilisation as a trusted technique in science, medicine and engineering.

However, recent research in our labs has shown that it is possible to minimise these effects and to instead use the plasma to cross-link biologic materials with retention of the biological properties of the precursor materials. Using low levels of applied plasma power, it is possible to produce low energy helium and argon plasma discharges. When biomolecules are nebulised into such a low temperature plasma, the materials are activated without losing their chemical structure. This activation can then effectively cross-link or coagulate the biomolecule without significant degradation. In addition, the plasma can activate substrates and effectively bind the biomolecules to the substrate as a thin nano-scale coating.

The result is a one-step process capable of modifying the surface of medical devices, research and diagnostic lab ware, implants and even living tissue. Tailored biological surfaces can be grown in situ over large areas using established equipment systems. The mechanisms used to control such reactions and to move the plasma from degradation to cross-linking modes are now being established and will be discussed. Examples of protein and polysaccharide coatings produced to date will also be presented.

4:00pm **PB+BI+PS-MoA8 Low and Atmospheric Pressure Plasma Polymerization for Immunosensing and Tissue Engineering**, ***Lenka Zajickova,*** *A. Manakhov, E. Makhneva, J. Medalova, D. Necas,* Masaryk University, Czech Republic*; L. Strbkova,* Brno University of Technology, Czech Republic*; A. Obrusnik, M. Landova,* Masaryk University, Czech Republic **INVITED**

Plasma polymerization provides a large playground for the preparation of surfaces suitable for immobilization of biomolecules and colonization by cells because chemical, structural and functional properties of plasma polymerized thin films can be tuned accordingly. The key decision for the particular application is the selection of functional chemical group that the final plasma polymer should contain. This contribution is going to discuss deposition of plasma polymers containing amine and carboxyl groups, functional groups that are typically used in biochemical applications and that are proposed to influence positively the attachment and proliferation of cells at surfaces. Amine-rich films were deposited in the low pressure pulsed radio frequency discharge using vapors of cyclopropylamine mixed with argon. The films contained primary and secondary amines and a small amount of oxygen. The structure of the films, reflected in their stability in water, could be tuned by the plasma conditions. The relationship between the amount of amine groups and the water stability was not straitforward because the films with similar amount of primary amine groups but different cross-linking could be prepared. The plasma polymers containing anhydride groups that hydrolyzed fastly at air into carboxyl groups were deposited in kHz-frequency dielectric barrier discharge at atmospheric pressure from the mixture of maleic anhydride and acetylene. The variation of the flow rate ratio was used to optimize the stability of films together with the amount of functional groups. Amine and carboxyl plasma polymers proved to be useful for the preparation of immunosensors based either on the principle of quartz crystal microbalance or surface plasmon resonance because in both these methods it is necessary to prepare a stable and reactive film on the gold surface. The amine films were also tested for the cultivation of human dermal fibroblasts and mouse myoblasts. It was identified that the water stability of the films is very important for succesfull experiments

4:40pm **PB+BI+PS-MoA10 Low-Temperature Plasma Processing of Polymeric Materials for Biomedical Applications**, ***Michelle Mann***, *M.R. Maynard, E.R. Fisher,* Colorado State University

Polymeric biomaterials are widely used in medical applications such as wound healing, drug release, and blood dialysis. For example, Tygon® and similar thermoplastics are chosen for these applications because of excellent mechanical strength and flexibility but often suffer from bacterial attachment and proliferation that ultimately leads to infection and fouling of the biomedical device. Biocidal agents can be incorporated into the polymer to actively eradicate bacteria, but it is difficult to ensure that biocidal action is localized at the material-biological interface. As a result, changing the surface properties of the polymer ensures a second mechanism by which to discourage bacterial attachment and growth. Plasmas are frequently used to alter the surfaces of biomaterials, most often by surface modification or deposition of a film to discourage bacterial attachment, while retaining the bulk properties critical to device performance. Specifically, H_2O (v) plasma treatment can enhance the compatibility of biomaterials by increasing hydrophilicity and altering surface chemistry; here, we demonstrate the use of this treatment method specifically for antibacterial materials. First, we have used H_2O (v) plasmas to tune the release of an antibacterial agent (NO) from drug-releasing polymers. Composition of treated drug-releasing polymers measured via X-ray photoelectron spectroscopy demonstrates a 100% increase in oxygen content and an associated increase in wettability, as observed via water contact angle goniometry. Compared to the untreated polymer, H_2O (v) plasma treated polymers had a delayed, but equally dramatic 8-log reduction in growth of both gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*. Second, in a related study, we utilized plasma-enhanced chemical vapor deposition to deposit a film of 1,8-cineole, an antibacterial constituent of tea tree oil. Bacterial attachment and biofilm formation assays reveal significantly reduced growth of both bacterial strains on plasma polymerized cineole films. H_2O (v) plasma treatment of these materials will also be discussed. Furthermore, optical emission spectroscopy allows correlation of gas phase excited state species in our plasmas under various plasma conditions to the resulting 1,8-cineole film surface properties, thereby allowing for fine-tuning of film surface properties for deposition onto biomedically-relevant polymer structures such as 3D polycaprolactone scaffolds. Collectively, our studies of plasma processing of antibacterial materials demonstrate this technique is a valuable tool in the production of next generation biomedical devices.

5:00pm **PB+BI+PS-MoA11 Plasma-based Functionalization of Polystyrene Surfaces of Cell Culture Plates**, ***Kazuma Nishiyama***, *T. Ito, S. Sugimoto, K. Gotoh, M. Isobe,* Osaka University, Japan*; M. Okamoto,* Osaka University Hospital, Japan*; A. Myoui,* Osaka University Hospital, Japanl*; H. Yoshikawa, S. Hamaguchi,* Osaka University, Japan

Polystyrene is one of the most widely used cell-culture plate materials. Amino and/or carboxyl coated cell culture plates are commercially available and such surface functionalizations are known to contribute effectively to the control of growth and differentiation of various stem cells. Plasma-enhanced chemical vapor deposition (PECVD) or plasma ion implantation may be used to functionalize polystyrene surfaces of cell culture dishes. The goal of this research is to understand how such surface functionalizations are affected by plasma conditions. In this study, we have used molecular dynamics (MD) simulation to understand how incident ions and free radicals affect the formation of amines and carboxyl groups. The simulation is based on interatomic reactive potential functions developed in-house based on quantum mechanical calculations. Results of MD simulations under the conditions similar to PE-CVD by ammonia (NH3), cyclopropylamine (CPA), or N2/CH3OH plasmas or ion implantation by NH3, N2/H2, or N2/CH3OH plasmas suggest that, with energetic ion bombardment, functional groups such as primary amines are less likely to form and nitridation of the surface tends to occur. Some simulation results have been compared with experimental data obtained from parallel-plate discharges with an inverter power supply at a relatively high gas pressure of 250 - 2,500 Pa and found to be in good quantitative agreement.

Plasma Processing for Biomedical Applications Focus Topic
Room 101A - Session PB+BI+PS-TuM

Plasma Processing of Biological/Biomimetic Surfaces

Moderators: Uroš Cvelbar, Jozef Stefan Institute, Slovenia, Satoshi Hamaguchi, Osaka University, Japan

8:00am **PB+BI+PS-TuM1 Investigation of Discharge Propagation on Cell and Plasmid Suspension in Plasma Gene Transfection**, ***Yugo Kido***, Pearl Kogyo Co., Ltd., Japan; *H. Motomura, Y. Ikeda,* Ehime University, Japan; *S. Satoh,* Y's Corp., Japan; *M. Jinno,* Ehime University, Japan

The authors have been developing a plasma gene transfection technique and averaged transfection efficiency up to 20% and cell survivability up to 90% are achieved for more than 20 kinds of cells. A typical procedure of this method is as follows. Target cells are cultured on a 96 well plate and gene suspension is added. The plate is placed between a high voltage electrode made of copper capillary with the diameter of 70 µm and a copper plate grounded electrode. By exposing the suspension to a microplasma generated at the tip of the capillary electrode, the cells are transfected by the genes. In this method, both chemically reactive species (chemical factors) and discharge current (electrical factors) are indispensable to the transfection process and their synergistic effect has been experimentally verified. Moreover, the transfection occurs on the whole area of each well although only the central area is exposed to the microplasma. In this study, to clarify how the discharge current contributes the transfection process with the synergistic effect with the chemical factor, discharge propagation phenomenon on the cell and plasmid suspension is investigated.

As a target, COS-7 cells are cultured on a 35 mm dish and 24 µg of plasmid pCX-EGFP suspended in 120 µL of TE/PBS buffer is added. The applied voltage between the electrodes is 20 kHz sinusoidal waveform and the amplitude is set at 10 kV peak to peak. The gap length between the capillary electrode and the suspension is set at 1 to 5 mm. The discharge propagation is observed with an ICCD camera equipped with a UV lens.

When only TE/PBS buffer solution exists in the dish, the discharges reach the buffer solution surface and then they propagate radially up to 15-20 mm of diameter. On the other hand, when the cells are cultured on the dish, remarkable radial propagation of the discharge is not observed and the discharge irradiation area is limited within the diameter of about 1 mm, which is narrow compared with the area in which the transfection occurs. Therefore, as a contribution of the electrical factors, not only the direct effect of the discharge current, charge on the plasmids, conduction current in the solution etc. should be analyzed. As a first step of the chemical factor investigation, similar observation is performed by the ICCD camera with an interference filter to observe the emission of OH radicals. The results of the discharge propagation paths study by means of equivalent circuit simulation and comparative analysis between the discharge propagation and the transfection will be shown at the symposium.

8:20am **PB+BI+PS-TuM2 Spectroscopic Study of Permeability of Stratum Corneum by Plasma Treatment for Transdermal Drug Delivery**, ***Jaroslav Kristof***, *N. Tran, M. Blajan, K. Shimizu,* Shizuoka University, Japan

Application of drugs by needles presents risk of infections and causes pain. On the other side, oral application of drugs can be toxic for human body because drug has to be transported through alimentary tract and higher amount of active agent is required. Transdermal delivery could be ideal painless and effective way but barrier function of skin has to be reduced for improving permeability of drugs. Research of last years proves that plasma can interact with skin and cause decreasing barrier function of skin [1-3].

We used plasma jet and microplasma discharge for investigation of barrier function of stratum corneum – horny layer of of Yucatan micropig skin. Helium or argon was used as working gas. These rare gases were later enriched by liquids like water or ethanol through the bubbling system to achieve higher amount of active particles like OH.

Physical changes of the pig skin were observed by microscope. As the human body is not non-conductive, we can expect different results when conductivity of layer under skin is changed. We compared effect of plasma on conductive and non-conductive material. Placement of skin on conductive material caused burned spots on skin by plasma jet [3]. While it was isolated, no damage was observed with plasma jet irradiation. In case of treatment of skin by microplasma, physical damage was hardly observed.

Changes in stratum corneum layer were observed by Attenuated Total Reflectance – Fourier Transform InfraRed (ATR-FTIR) spectroscopy. ATR-FTIR spectrum offer information about water, lipid bilayer and proteins in stratum corneum. Permeability of skin for drugs correlates with shift of asymmetric stretch of CH_2 band to higher wavenumbers. This information describes behavior of lipid bilayer. Information about reaction of proteins on plasma treatment content Amide I and Amide II bands. Reaction of stratum corneum layer of pig skin depended on used discharge type and gas. Effectivity of plasma sources and used gases or gas mixtures for transdermal drug delivery was analysed.

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8:40am **PB+BI+PS-TuM3 Cell Attachment to Microwave Plasma-oxidized Titanium and Titanium Alloy Substrates**, ***Denis Dowling***, University College Dublin, Ireland; *M. Naciri,* University Mohamed V of Rabat, Morocco; *M. Al-Rubeai, A. Breen,* University College Dublin, Ireland **INVITED**

Titanium and its alloys have been widely investigated for use in orthopedic and dental implant devices, particularly for osteointegration and bio-compatibility. This paper evaluates the influence of titanium surface oxidation using a microwave plasma treatment technique on cell attachment. Commercially pure titanium (CpTi) and titanium alloy (Ti6Al4V) discs were treated in an oxygen atmosphere for 5 min-utes at 850 °C using a microwave (2.45 GHz) plasma system, operating at 2 kPa. After the 5-minute treatment, the thickness of the oxidized layer was 2.3 µm on the CpTi discs and 4.7 µm on the Ti6Al4V discs, with growth rates of 0.5 and 1 $\mu m.min^{-1}$ respectively. Reduced plasma oxidation rates were observed on a high surface area beaded surface (Porocoat). In contrast to the plasma treatments, the use of air furnace oxidation only achieved an oxide layer thickness for the CpTi of 1 µm, when treated at the same temperature. Optical profilometry measurements were performed to determine the surface roughness; XRD, EDX, and SEM examinations were also car-ried out to determine the properties of the oxide layers and their morphologies. Cell attachment to the treated discs was also assessed after exposure times of 25 and 100 minutes. A 40% increase in MG63 osteoblast cell attachment on the Ti6Al4V discs was observed, when compared with that on the CpTi discs. Alkaline phosphatase (ALP) specific activity of MG63 cells grown on control and plasma oxidised surfaces were compared after 21 days. A statistically significant difference between Ti6Al4V and CpTi oxidised surfaces ($P<0.05$), when compared to that obtained for the control surface that had not been plasma treated. The acicular morphology of the oxidised Ti6Al4V surface was found to have the most significant influence on enhancing cell attachment, combined with higher oxide layer roughness and thickness.

9:20am **PB+BI+PS-TuM5 The Role of Electrical and Chemical Factors in the Molecular/Gene Transfection by Micro-Plasma Irradiation**, ***Masafumi Jinno***, *Y. Ikeda, H. Motomura,* Ehime University, Japan; *Y. Kido,* Pearl Kogyo Co. Ltd., Japan; *S. Satoh,* Y's Crop., Japan **INVITED**

The plasma gene transfection is expected as a safe and useful method of gene transfection. However, this method had a problem of a difficulty in keeping both high transfection efficiency and less cell damage simultaneously. The authors have evaluated four different plasma sources, such as arc discharge, plasma jet, DBD (dielectric barrier discharge) and microplasma, in terms of the transfection efficiency and the cell viability. High transfection efficiency is achieved by the styles of arc discharge and microplasma in which the electric current flows via the cells. Our experimental results suggests that an electric current may play an important role in plasma gene transfection, and that total volume of the gas flow must be small or zero and the area in which the cells are directly irradiated by plasma must be small in order to achieve higher cell viability. Among the various types of plasmas, which the authors have tried, the microplasma satisfies these conditions and brings both the high transfection efficiency and the high cell viability simultaneously.

We evaluated the contribution weight of three groups of the effects and processes inducing gene transfection, i.e. electrical, chemical and biochemical ones through three experiments. The laser produced plasma (LPP) was employed to estimate the contribution of the chemical factors. The liposomes were fabricated and employed to evaluate the effects of plasma irradiation on membrane under the condition without biochemical

reaction. The clathrin-dependent endocytosis, one of the biochemical processes was suppressed. It also turned out the clathrin-dependent endocytosis is the process of the transfection against the 60% in all the transfected cells. The endocytosis and electrical poration are dominant in plasma gene transfection, and neither permeation through ion channels nor chemical poration is dominant processes.

By scavenging the H_2O_2 generated by plasma irradiation using catalase, the transfection efficiency decreased to 40% of that of without catalase. On the other hand, when the H_2O_2 solution is dropped in the cell suspension without plasma irradiation, the transfection is not observed. These results suggest that the synergistic effect of H_2O_2 with electrical factors or with other reactive species generated by plasma irradiation is important. Consequently it becomes clear that chemical factors, radicals such as H_2O_2 and reactive oxygen/nitrogen species, do not work by itself alone, and that the electrical factors (electrical current, charge and field) are essential to plasma gene transfection.

11:00am **PB+BI+PS-TuM10 Control of Plant Growth by RONS Produced Using Nonthermal Atmospheric Air Plasma**, ***Kazunori Koga,*** Kyushu University, Japan; *T. Sarinont,* Kyushu University; *M. Shiratani,* Kyushu University, Japan

Nonthermal atmospheric plasmas have been widely used for biomedical applications because of their non-equilibrium feature and synergy effects [1-3]. The non-equilibrium feature allows us to introduce reactive oxygen and nitrogen species (RONS) to biomaterials with a significantly wide dose range compared with the conventional irradiation methods such as X-ray and γ-ray [4]. For an agricultural application, we succeeded in reducing harvest period and enhancing crop yield by plasma irradiation to plant seeds [5]. We found irradiation of RONS with an appropriate dose to seeds brings about growth enhancement in all growth stages of plants. To understand the growth enhancement mechanism, here we have studied dependence of irradiation dose of RONS produced by plasma to seeds on growth of *Arabidopsis thaliana L.* Experiments were carried out using a scalable DBD device [2, 3, 5]. The device consisted of 20 electrodes of a stainless rod of 1 mm in outer diameter and 60 mm in length covered with a ceramic tube of 2 mm in outer diameter. The discharge voltage and current were 9.2 kV and 0.2 A. 20 seeds of Arabidopsis thaliana L. were set 3 mm below the electrodes. The RONS dose was controlled by the irradiation time. After plasma irradiation, they were grown on soil tab in incubators. To evaluate plant growth, the stem length was measured as a function of cultivation days. The stem length was normalized by the stem length of the plants without plasma irradiation. To evaluate statics of the measured values, we used a two-tailed ANOVA statistically significance different at $\alpha = 0.05$ ($p < 0.05$). The normalized stem length increases to 1.3 for 3 min irradiation, then decreases to zero for 10 min irradiation. The results indicate the plant growth is activated by plasma irradiation less than 3 min and inactivated by plasma irradiation of 5-10 min. Above 10 min irradiation, no seeds were germinated. We have succeeded in growth control of plants from death to activation with irradiation dose of RONS produced by plasma. The mechanism will be discussed in the presentation.

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11:20am **PB+BI+PS-TuM11 Generation of Reactive Species in Medium Irradiated Laser-Induced-Plasmas**, ***Yukihiro Kurokawa,*** *N. Kurake, K. Takeda, K. Ishikawa, H. Hashizume, H. Tanaka, H. Kondo, M. Sekine, M. Hori,* Nagoya University, Japan

The non-equilibrium atmospheric pressure plasma (NEAPP) was irradiated to the cell culture medium as liquid. The antitumor effect, showing the selective killing effect for cancer cells without killing normal human cells, was reported [1,2]. This effect are considered to be caused by large amounts of reactive nitrogen and oxygen species (RONS) generated by the plasma. However, chemical reactions during transport of plasma in ambient to the liquid surface is complicated; therefore we have applied the laser-induced plasma.

Previously, we reported that the high ratio of NO_2^-/ H_2O_2, even in low H_2O_2 contained in the plasma activated medium [3]. However, relations of reactive species concentrations with antitumor effects have not been fully elucidated. Here, we focus on the concentrations of reactive species generated in culture media by the laser-induced plasma.

A Nd:YAG laser and harmonic generators (Quanta Ray Pro 230, Spectra Physics) provided the pulsed-laser light with a wavelength of 266 nm, a frequency of 30 Hz, a power at sample surface of 25 mW. The light was focused on the gas-liquid interface of ultrapure water or Dulbecco's Modified eagle Medium (DMEM; cat. no. 5796; Sigma) by using plano-convex lens, made of synthetic quartz. 2 mL of the liquid was typically irradiated for 5 min. This is called as LPAM. Just after irradiation, H_2O_2 and NO_2^- concentrations were measured by using absorption that was measured by ultraviolet-visible near infrared spectrometer (V-650, JASCO). Moreover, HeLa cells were incubated in the LPAM and cell survival was measured after 24 h incubation. For analysis of killing mechanism, activated caspase3/7 as apoptosis marker (CellEvent Caspase-3/7) was measured after fluorescent staining by a fluorescent microscope.

The LPAM generated effectively H_2O_2 causing by photo-dissociation of water, hydroxyl radicals (・OH) works a precursor of H_2O_2 with the reaction of ・OH + ・OH →H_2O_2. Survival of HeLa cells in the LPAM was dependent on dilution of the LPAM and standard DMEM. We prepared the diluted LPAM for a half of killing of HeLa cells. After the cultivation for 24 h in the diluted LPAM, the caspase-3/7 activity of dead cells as apoptosis death was observed clearly. Notably, the cell-death was almost inhibited by catalase.

We will discuss on the generation mechanism of active species and the mechanism of antitumor effect of the LPAM with comparison of the PAM.

This work was partly supported by MEXT KAKENHI on Innovative Areas Grant no. 24108002.

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11:40am **PB+BI+PS-TuM12 Electric Fields in kHz-driven Plasma Jets**, *ET. Slikboer, Y.N. Nguyen,* Eindhoven University of Technology, The Netherlands; *O.Y.N. Guaitella,* Ecole Polytechnique, Palaiseau, France; *G. Sretenović,* University of Belgrade; *A. Obrusnik,* Masaryk University, Brno; ***Ana Sobota,*** Eindhoven University of Technology, The Netherlands **INVITED**

Non-thermal atmospheric pressure plasma jets have been developed for use on thermosensitive targets at atmospheric pressure, for example polymers or for biomedical applications. Diagnostics on these plasma sources is challenging because of their transient nature, often associated jitter and very small volume. Electric fields, fundamental property essential for the understanding of the discharge, are not well known. In this talk two methods of electric field measurements will be shown applied to a He kHz-driven jet, one based on spectroscopy and one on polarimetry and the obtained results will be discussed.

Biomaterial Interfaces
Room Hall D - Session BI+PB-TuP

Biomaterial Interfaces Poster Session (preceded by Oral Flash Presentations)

BI+PB-TuP2 Quantitative Sensing of Pancreatic Enzymes using Gelatin, ***George Banis***, University of Maryland, College Park; *L. Beardslee,* Walter Reed National Military Medical Center; *R. Ghodssi,* University of Maryland, College Park

We present an investigation of gelatin film response to an array of pancreas-specific enzymes using a Quartz Crystal Microbalance (QCM) system. In fluids secreted from the pancreas into the upper small intestine, highly concentrated enzymes (α-amylase, trypsin, and lipase) are mixed to complete digestion of partially broken-down materials entering from the stomach. Sensors, such as that illustrated in Fig 1, are utilizing biomaterials such as gelatin, as it can be used to enter the body due to its biocompatibility and tailorability both chemically and structurally. Gelatin is known to be highly sensitive to degradation by trypsin, one of the aforementioned enzymatic pancreas biomarkers, thereby offering potential to indirectly monitor trypsin levels [1]. The composition of pancreatic fluid is a biomarker in testing exocrine function of the pancreas, a process often involving invasive procedures toward quantifying losses in enzyme levels [2]. However, its interactions with other interfering enzymes such as lipase or α-amylase have not been studied. While these enzymes are believed to cleave specific to bonds not found in gelatin, it is critical to be able to determine how these non-specific enzymes impact the signals produced when gelatin interacts with specific enzymes, i.e. trypsin, when they are each in the media.

In this work, we utilize a QCM system in sensing the mass change of gelatin films deposited onto standard crystals with gold electrodes. Films are subjected to a constant flow rate of buffer before introducing pancreatic enzymes in the setup illustrated in Fig 2. After system stabilization under buffer flow, material loss is quantified from the surface of the crystal. For trypsin, as expected, we observe degradation in a concentration-dependent manner, shown in Fig. 3. With either lipase or α-amylase, however, we observe no change, as illustrated in the top of Fig 4. After rinsing with buffer, we reintroduce trypsin in combination with each enzyme to determine if the presence of nonspecific enzymes affected the sensitivity of the gelatin to proteolytic activity, shown in the bottom of Fig 4. Digestion rates are found to decrease by -----83% and 77% with exposure to lipase or α-amylase, respectively, indicating a decrease in gelatin sensitivity to trypsin in the presence of these enzymes. The next phase in this work will be to combine all three enzymes to further model pancreatic juices. This work emphasizes the necessity in characterizing gelatin's response to other enzymes in understanding its sensitivity and specificity in the digestive environment, leading the avenue for devices designed to monitor gastrointestinal health.

BI+PB-TuP3 Evaluation of Printed Cell Viability, Proliferation, and Insulin Production on Various Alginate-Gelatin Hydrogels, ***Luis Solis***, *J. Rincon, A. Varela-Ramirez, R. Aguilera, T. Boland,* University of Texas at El Paso

Over the past couple of decades, encapsulation of islets or beta cells has emerged as the new modality for the treatment of Type 1 Diabetes Mellitus (T1DM). A major setback in bioengineering encapsulated cells however, is the formation of fibrosis from immunologic defenses rendering the cells ineffective. This study proposes the use of an inkjet bioprinter to allow arrangement of βTC-6 mouse pancreatic beta cells and improve vascular ingrowth among alginate hydrogels. In addition, different concentrations of gelatin were tested in order to determine printable alginate-gelatin ratios for optimal vascular ingrowth, proliferation, viability, and insulin production of cells. Cell proliferation cultures were monitored daily for a total duration of 14 days. Cell viability and glucose stimulated insulin production were assessed at day 14. In-vitro alginate-gelatin hydrogels promoted proliferation of spherical insulinoma clusters and increased insulin secretion as compared to the monolayer of cells without hydrogels. These findings demonstrate that the alginate-gelatin hydrogels support the proliferation, viability, and insulin production of βTC-6 cells. These results will also allow to formulate improved bioinks for automated cell encapsulation applications.

BI+PB-TuP4 Synchrotron Radiation Studies of the Bonding and X-Ray Induced Reactions of Bacteriorhodopsin Adsorbed on Gold, ***Richard Rosenberg***, Argonne National Laboratory; *D. Mishra, R. Naaman,* Weizmann Institute of Science, Israel

Bacteriorhodopsin (bR) is the integral protein of the purple membrane of *Halobacterium salinarum* and is the most studied proton pump. It is a chiral system composed of seven parallel, upright-oriented alpha-helices. Recent photoemission and electrochemical studies have shown that it can act as a natural electron spin filter as a result of the chiral-induced spin selectivity effect.[1] Previous structural studies using hard x-ray synchrotron radiation (SR) have shown that such radiation can significantly impact the structural integrity of bR,[2] while earlier work has demonstrated that X-ray-induced, low energy secondary electrons can play a major role in surface chemical reactions of adsorbed biological molecules.[3] In the present study we use SR x-ray absorption and photoelectron spectroscopy (XPS) to characterize the initial state of the adsorbed bR. Time-dependent changes in the core-level XPS spectra are utilized to follow the dynamics of the X-ray/secondary electron induced reaction. The results will be discussed in terms of previous studies of x-ray induced reactions in bR and other biological molecules.

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BI+PB-TuP5 Investigations on Peptide Incorporation and Peptide Yields in ME-SIMS, ***Martin Körsgen***, *A. Pelster, M. Heeger, B.J. Tyler, K. Dreisewerd, H.F. Arlinghaus,* Universität Münster, Germany

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a powerful technique for the nanoanalysis of biological samples, but improvements in sensitivity are needed in order to detect large biomolecules, such as peptides, on the individual cell level at physiological concentrations. An increase in the detection efficiency for larger molecules and reduced fragmentation rates could be obtained by a) the use of cluster ion beams such as Au_n^+ , Bi_n^+ , C_{60}^+ , or even large Ar_n^+ clusters in order to maximize the energy deposited close to the surface or b) by modifying the surface by organic matrices in the so-called matrix-enhanced SIMS (ME-SIMS). This approach is based on embedding analyte molecules in low weight organic matrices, like common MALDI matrices, prior to ion bombardment

We used dual beam ToF-SIMS to image the incorporation of three peptides with different hydrophobicities, bradykinin, substance P, and vasopressin, into two classical MALDI matrices, 2,5-dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnamic acid (HCCA) prepared with dried droplet sample preparation method. For depth profiling, an Ar cluster ion beam was used to gradually sputter through the matrix crystals without causing significant degradation of matrix or biomolecules. A pulsed Bi_3^+ ion cluster beam was used to image the lateral analyte distribution in the center of the sputter crater. Using this dual beam technique, the 3D distribution of the analytes and spatial segregation effects within the matrix crystals were imaged with sub-µm resolution.

Combining cluster ion beams and ME-SIMS we were able to investigate the molecular yield of two peptides (bradykinin and melittin) under various primary ions and preparation methods. Large argon clusters in the mass range between 1000 and 2500 atoms per cluster and several bismuth primary ions were used to determine molecular yields. Preparation utilized spin coating of pure peptide solutions and spray coating of matrix-peptide mixtures on silicon wafers. With the data obtained we were able to describe the molecular yield of the analyzed peptides. For bismuth primary ions the yield obtained by the use of cluster primary ions is nearly constant in the case of ME-SIMS, whereas for the neat sample an increase of the molecular yield is observable. In contrast to the molecular yield decrease with larger argon clusters for neat samples, an increase of the molecular yield is observable for larger argon clusters in the case of ME-SIMS.

BI+PB-TuP6 Developments of Non-Stick Surfaces for Medical Devices: Beneficial Effects of Thin Film Metallic Glass Coating, *G.H. Jiang, C.C. Yu, C.L. Li, Y. Tanatsugu,* ***Jinn P. Chu,*** National Taiwan University of Science and Technology, Taiwan, Republic of China*; M.J. Chen, S.H. Chang,* Mackay Memorial Hospital Tamsui Campus, Taiwan, Republic of China

This presentation reports on the use of Zr-based ($Zr_{53}Cu_{33}Al_9Ta_5$) thin film metallic glass (TFMG) for the coating of various medical devices and compares the results with those obtained using conventional titanium nitride and pure titanium coatings . TFMG was selected as the coating material for its unique properties such as good biocompatibility and antibacterial property due to its amorphous atomic structure, revealing a great potential for biomedical applications. The TFMG coating was shown to reduce insertion forces and retraction forces by up to over seventy percent when tested using polyurethane rubber block. The benefits of TFMG-coated needles were also seen when tested using pig muscle tissues. Based on the nano-scratch test, the TFMG coatings achieved a low coefficient of friction (COF), about one order of magnitude lower than those of bare surface and other coatings. Furthermore, the adhesions of cancer cells and platelets to coatings are also examined. TFMG coating is shown to appreciably minimize the attachment of cancer cells and platelets by more than eighty percent in relative to those of Ti coating and bare surface. The low COF and non-stick coated surfaces by TFMG can be attributed to the absence of grain boundaries in the TFMG coating, smooth surface and low surface free energy.

BI+PB-TuP7 Polyurethane Degradation by Wild Type and Hydrolase Deficient *Pseudomonas protegens* Pf-5 Unsaturated Biofilms, ***Daniel Barlow,*** US Naval Research Laboratory*; LJ. Nadeau, C.S. Hung,* Air Force Research Laboratory*; J.C. Biffinger,* US Naval Research Laboratory*; A.L. Crouch,* Air Force Research Laboratory*; J.N. Russell,* US Naval Research Laboratory*; W.J. Crookes-Goodson,* Air Force Research Laboratory

Two hydrolases secreted by *Pseudomonas protegens* Pf-5 bacteria, PueA and PueB, have been demonstrated to be active towards polyester - polyurethane (PU) hydrolysis. In this work, the impact of these enzymes towards PU degradation was directly compared at biofilm / PU interfaces through deletion of PueA and PueB genes. Unsaturated biofilm assays were used where biofilm growth took place on solid, hydrated PU discs in air. PU degradation was analyzed using confocal Raman microscopy of intact samples. Additionally, cross-sectional analysis of microtomed sections was done using FTIR microscopy and combined atomic force microscopy – infrared spectroscopy (AFM-IR). Results showed varying degrees of biofilm related permeation and polymer degradation within the ~300 um thick discs. Degradation took place through a pitting process involving preferential loss of the ester component. Wild type and PueB knockout mutants showed the highest levels of hydrolysis, measured by loss of carbonyl intensity in vibrational spectra, while the PueA and double knockout mutants showed lower hydrolysis levels. The apparent higher level of PueA activity is consistent with higher enzymatic activity for the hydrophobic lipase substrate, p-nitrophenyl palmitate. Relationships between biofilm morphology and PU degradation were also observed for the wild type and mutant biofilms.

BI+PB-TuP8 Laser Irradiation of Mg Alloys: Reduced Kinetics and Enhanced Biocompatibility, *M.A. Melia,* ***David Florian,*** *W. Steuer, J.R. Scully, J.M. Fitz-Gerald,* University of Virginia

Until recently, biodegradable implants have exclusively been used in non-load bearing applications such as stents and sutures. Mg-Al-Zn alloys like AZ31B are currently being considered as biodegradable materials because of their similar mechanical properties to that of bone. In addition, the corrosion product resulting from Mg alloys in body fluid contains Mg and Ca-phosphates (hydroxyapatite) and has been shown to stimulate bone regeneration. The degradation of Mg alloys is also considered non-toxic when corroding in the human body. However, the structural integrity is poor due to rapid corrosion caused by microstructural heterogeneities in the form of electrochemically noble secondary phases leading to micro-galvanic couples and preferential dissolution of the α-Mg matrix in physiological media. Laser surface processing of the Mg-Al-Zn alloy, AZ31B, reduced the corrosion rate in simulated body fluid (SBF) experiments by minimizing the impact of secondary phases.

Experiments utilized a pulsed excimer laser (λ = 248 nm and FWHM = 25 ns) in combination with a novel surface modification chamber. Samples of AZ31B in the as-received and laser processed condition were submerged in a 27 mM HCO_3^- Tris ($(HOCH_2)_3CNH_2$) variant of SBF. The corrosion resistance was investigated through optical microscopy, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), gravimetric mass loss, and polarization measurements.

No major corrosion product variations were observed for the as-received / laser processed specimens by SEM and EDS, both showing a similar amount of Ca and P. The laser processed alloy exhibited a reduction in anodic kinetics compared to the as-received material, suggesting the corrosion product is more compact and passivating. Furthermore, the laser processed surface exhibited a 50% reduction in mass loss after 24 hours immersion in SBF in comparison to the as-received samples. Optical micrographs of samples immersed in SBF reveal a reduction in the H_2 evolution rate of the laser processed versus as-received material. In addition, the laser treated specimens exhibited a significant increase in wettability with a 10^o contact angle compared to the 45^o angle of the as-received materials. The increased wettability of the laser processed samples may decrease the time required for osseointegration through allowing cells to more readily bind to the surface of an implant.

BI+PB-TuP9 Plasma-assisted Fabrication of Silver/Bacterial Cellulose/Chitosan Functional Nano-composites and Their Properties, ***Shuquan Chang,*** *A.R. Shetty, S.L. Arias Suarez, J.P. Allain,* University of Illinois at Urbana-Champaign

Bacterial cellulose and chitosan are renewable natural polymers and have many favorable properties such as biocompatibility, biodegradability and low toxicity. So, they have been extensively used in drug delivery systems, gene therapy, tissue engineering, and biosensor applications. Silver nanoparticles have attracted much attention for their unusual chemical and physical properties and have been widely applied in sensors, antibacterial and photocatalytic areas. The synthesis of nanomaterials with different chemical composition, size distribution, and controlled monodispersity has become an important research area in nanotechnology. Many kinds of methods such as vapor deposition, solvent-thermal, sol-gel, electrochemistry and microwave have been developed to fabricate nanomaterials. So far, plasma technology has become an important approach to prepare and reinforce materials and surfaces. This work seeks to fabricate nanoparticles/natural polymer functional composites via an atmospheric pressure plasma method. Comparing many traditional methods, atmospheric pressure plasma jet can induce chemical reactions in mild conditions, which can guarantee the purity of system and will not destroy the structure of natural polymers.

In this work, silver/bacterial cellulose/chitosan functional composites are fabricated via an atmospheric pressure plasma method. Plasma can produce many active radicals including reduction species and oxygen species, which can trigger chemical reaction. Ag+ in the reaction system can attach to the surface of bacterial cellulose and chitosan via bonding. By controlling the reaction condition, Ag+ can be reduced to Ag(0) and form Ag nanoparticles under plasma treatment. The existence of bacterial cellulose and chitosan can limit the growth and prevent the aggregation of particles, which is very critical to form nanostructure. SEM, XRD, XPS, FT-IR are employed to examine the morphology and structures of as prepared nano-composites. The biocompatibility and antibacterial properties are also studied. All results reveal that Ag nanoparticles are successfully formed and well dispersed in bacterial cellulose/chitosan. The as prepared silver/bacterial cellulose/chitosan nano-composites have excellent biocompatibility and antibacterial abilities, which can be used in biomedical areas. This convenient synthesis strategy based on atmospheric pressure plasma could be extended to fabricate other nanoparticles/bacterial cellulose/chitosan composites.

BI+PB-TuP11 A Non-toxic, Super-Hydrophilic Anti-Fog Coating for Lenses used in Closed Body Cavity Surgery: VitreOx TM– In Vivo Animal Clinical Trials, ***Nicole Herbots,*** SiO2 NanoTech LLC*; C.F. Watson,* SiO2 NanoTech LLC/Arizona State University Physics Dpt*; EJ. Culbertson,* University of California at Los Angeles*; PR. Thilmany, IPO. Martins,* SiO2 NanoTech LLC

Laparoscopes, arthroscopes, and laryngoscopes lenses are hydrophobic and fog during closed body surgery, due to bodily fluids and differences between body and operating room temperatures [1,2]. Surgeons must repeatedly remove, clean, and reinsert scopes obscured by fog. Hencesurgery duration, infection risks, and scarring from air exposure increase. Methods to address fogging introduce other complications [3]. Alcohol-based coatings scar tissue and quickly evaporate, heated lenses require reheating every 5 to 20 minutes. A non-toxic, super-hydrophilic, anti-fog coating that is pH neutral (7.2-7.4), long-lasting has been developed VitreOx™ . [4] VitreOx™ can be used wet or dry, without alcohol, heat, or fluid evacuation. When applied in liquid form, it easily espouses a lens's surface and edges, and dries within seconds to form a permanently

super-hydrophilic surface on silica and most polymer surfaces . VitreOx™ avoids current shortfalls by foregoing frequent reapplications.

VitreOx™'s anti - fog properties can be explained by nucleation and growth theory, and the three mechanisms for condensation: 1) 3-D droplets, resulting in fogging; 2) 2-D sheets resulting in a flat transparent film . ; or 3) mixed, resulting in optical distortion. On hydrophobic surfaces (e.g. optical lenses), condensation occurs with fogging via spherical 3-D droplets (Volmer-Weber model). 3 - D droplets scatter light in all directions through refraction yielding opaque or translucent films, or fog. VitreOx™ applied to hydrophobic lenses renders them super-hydrophilic. Similar to the 2-D Frank Van-der-Merwe growth mode, ***condensation with uniform wetting*** yields transparent 2-D films that don't distort light.

In-vitro and *in-vivo* animal studies of VitreOx™ were conducted to measure performance and duration of anti-fog effectiveness and bio-compatibility. *In-vitro* testing spanned from 3 to 72 hours over a 3-year range. Side-by-side *in-vivo* gastro-endoscopies were conducted using a lens coated with VitreOx™ and a Covidien Clearify ™ warmer with anti-fog, on Yucatan™ swine for 90 minutes . The VitreOx™ coating lasted without fogging nor reapplication, while Covidien Clearify™ only l ast ed at for 38 minutes without fogging, and required retreatment and reapplication . No adverse reaction was observed on swine in surgery, and in the 18 months that follows.

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