Surface Modification of Materials by Plasmas for Medical Purposes Focus Topic
Room: 315 - Session SM+AS+BI+PS-ThM

Plasma Processing of Antimicrobial Materials and Devices

Moderator: Heather Canavan, University of New Mexico, Morgan Hawker, Colorado State University

8:00am **SM+AS+BI+PS-ThM1 Plasma Polymers: Dogma, Characteristaion and Challenges**, ***Sally McArthur***, Swinburne University of Technology, Australia **INVITED**

Plasma polymers, the dogma tells us are densly cross-linked, pinhole free films that adhere to virtually any dry surface. But when you are working at low power and trying to retain specific functional groups within your films, is this still true? How does environment (pH, salt concentration) effect film behaviour and what do respnses to change in environment tell us about the nature of these films? This talk will explore methods for studying the physicochemical behaviours of plasma polymer films and discuss how these films can be manipulated address specific biomaterials challenges.

8:40am **SM+AS+BI+PS-ThM3 The Role of Plasma Surface Modification in Antimicrobial Thin Films and Strategies**, ***Renate Foerch***, FhG-ICT-IMM, Germany **INVITED**

"Delivery on demand" has become a key issue in the development of solutions for bacterial infection and the evolution of resistance. Antimicrobial bioactive coatings may be thin layers, scaffolds or hybrid materials with chemically immobilized or physically embedded antimicrobial substances that act while tethered to a surface or that are released either passively or upon a stimulus. Examples include burst release systems of an antimicrobial from plasma polymerised thin films that have fed into a recent efforts aiming to develop, characterize and evaluate nanocomposite coatings consisting of thin films, nanoparticles and nanocarrier systems. The nanocomposite coatings are formulated to respond to specific changes in the surrounding environment. The work to be described is part of a European-Australian effort to investigate new strategies to combat microbial infection; it draws expertise from plasma assisted technologies and wet chemical post plasma attachment of responsive nanocontainers carrying an antimicrobial to treat bacterial infection.

9:20am **SM+AS+BI+PS-ThM5 Plasma Modification of Drug-Eluting Materials for Localized Action at Medical Device Interfaces**, *J. Joslin, A. Pegalajar-Jurado, M.J. Hawker, E.R. Fisher,* ***Melissa Reynolds***, Colorado State University **INVITED**

To direct protein and cellular behavior at the surface of synthetic materials, both localized chemical signaling and control over surface properties are required. To achieve requisite drug delivery dosages, hydrophobic polymers are often employed that slowly elute a therapeutic agent from the bulk material into systemic circulation. However, the surface free energy of the hydrophobic material can lead to deposition of undesired proteins and activation of the clotting. To overcome these challenges, advanced material platforms are needed to achieve localized therapeutic action and customizable surface properties. Herein, we present the development of H_2O(v) plasma-treated PLGA-nitric oxide (NO) releasing materials. NO is a well-established anti-platelet and anti-microbial agent, and the NO release rate can be controlled by the hydrophobic nature of the bulk material where it was incorporated. Plasma treatment conditions were optimized to maintaining the NO release function while rendering the surface hydrophilicity. Despite the plasma conditions employed, the material retained 80-90% of the *S*-nitrosothiol content, while the NO release profiles were unaltered compared to the control. The change in the surface wettability was confirmed by water contact angle measurements. Extensive surface (XPS) and bulk (ATR FT-IR) chemical characterization demonstrated that the changes in wettability was due to the implantation of O-containing surface functional groups such as carbonyl and hydroxyl groups. In addition, optical profilometry analysis confirmed no statistically significant changes in the surface roughness compared to the control. Furthermore, the materials show minimal hydrophobic recovery after several days stored at -20°C. By combining both chemical signaling and surface treatments into one material, we expect to reduce activation of clotting cascade and enhance the biocompatibility of the materials.

11:00am **SM+AS+BI+PS-ThM10 Plasma Treated Substrates Reduce Protein Adsorption**, ***Marvin Mecwan***, *J. Stein, W. Ciridon,* University of Washington, *X. Dong,* Eli Lilly and Company, *B. Ratner*, University of Washington

Proteins irreversibly adsorb onto surface, causing losses from solution, denaturation, as well as aggregation. Hence, there have been recent efforts in the pharmaceutical industry to addressing the manufacture, packaging and delivery of protein-based pharmaceuticals. We propose the use of radio-frequency (RF) plasma deposition to create coatings on substrates relevant to the pharmaceutical industry—glass, stainless steel and cyclic olefin polymer (COP). The monomers of choice were acrylic acid (AA) and tetraglyme (TG) (hydrophilic), and perfluoropropylene (C3F6) and perfluoromethyl vinyl ether (C3F6O) (hydrophobic). All monomers were successfully plasma coated on all substrates, and did not delaminate as was determined from survey and detailed ESCA scans. Furthermore, no peaks associated with the substrates were seen in the scans, which indicate that the plasma coating are at least 100Å thick. Protein adsorption studies were carried out using 0.1mg/mL solution of I-125 tagged bovine IgG by adsorbing the tagged protein on the plasma treated substrates for an hour. All hydrophilic monomer plasma treated substrates had lesser protein adsorbed on their surfaces (< 2ng/cm^2) as compared to hydrophobic plasma treated substrates (10-14 ng/cm^2). This is in comparison to untreated controls that had 200-300 ng/cm^2 protein adsorbed on the surface. Furthermore, following ISO 10993-5 guidelines, by performing cytotoxic studies using NIH-3T3 fibroblasts all plasma treated substrates were determined to be non-cytotoxic. Hence, these results indicate that radio-frequency plasma treatment could lead to a new generation of surfaces that will be particularly effective for protein manufacture, storage and delivery. Future studies will be aimed at determining plasma coating thickness, protein aggregation assessment as well as studying the bonding strength of the proteins to the plasma treated surfaces.

11:20am **SM+AS+BI+PS-ThM11 Modification of Porous Materials by Low Temperature Plasma Treatment to Achieve Low-Fouling Membranes**, ***Adoracion Pegalajar-Jurado***, *B.D. Tompkins, E.R. Fisher*, Colorado State University

Artificial porous polymeric membranes are used in many applications including water filtration systems and devices to treat blood for a broad variety of therapeutic purposes. In water filtration systems, membranes are used to remove colloidal particles and organic molecules from the watercourse and, in medical treatments, they function primarily to eliminate toxins from the blood before it is returned to the patient's body. Although these are very different applications, both are affected by membrane fouling from proteins, toxins, bacteria, and cells, which significantly decrease flow through the porous material. Surface modification techniques that retain the desired bulk properties are the ideal method for obtaining low-fouling membranes, thus extending their life-time in applications where they are exposed to fouling conditions. Here, we will present the properties of polysulfone ultrafiltration membranes subjected to H_2O plasma and their performance when exposed to proteins and bacteria. Plasma treated membranes showed enhanced hydrodynamic characteristics (i.e. increase in water flux) as a result of their high hydrophilicity. Notably, hydrophilic characteristics were retained for more than six months, ensuring top-shelf stability of the surface treatment. In terms of protein fouling performance, treated membranes show less bovine serum albumin adsorption than untreated membranes and cleaning of treated fouled membranes yields 70-90% flux recovery depending on plasma treatment time. This surface modification provides a mechanism for extending the life-time of the membranes.

11:40am **SM+AS+BI+PS-ThM12 Immobilized Laminin Concentration Gradients on Electrospun Fiber Scaffolds for Controlled Neurite Outgrowth**, ***Nicole Zander***, US Army Research Laboratory, *T. Beebe Jr.*, University of Delaware

Neuronal process growth is guided by extrinsic environmental cues such as extracellular matrix proteins (ECM). Recent reports have described that the growth cone extension is superior across gradients of the ECM protein laminin compared to growth across uniformly distributed laminin. In this work, we have prepared gradients of laminin on aligned electrospun nanofibers for use as substrates for neuronal growth. The substrates therefore presented both topographical and chemical guidance cues. Step gradients were prepared by the controlled robotic immersion of plasma-treated polycaprolactone fibers reacted with N-hydroxysuccinimide into the protein solution. The gradients were analyzed using x-ray photoelectron spectroscopy and confocal laser scanning microscopy. Gradients with a dynamic range of protein concentrations were successfully generated and

neurite outgrowth was evaluated using neuron-like PC12 cells. After 10 days of culture, PC12 neurite lengths varied from 32.7 ± 14.2 μm to 76.3 ± 9.1 μm across the protein concentration gradient. Neurite lengths at the highest concentration end of the gradient were significantly longer than neurite lengths observed for cells cultured on samples with uniform protein coverage. Gradients were prepared both in the fiber direction and transverse to the fiber direction. Neurites preferentially aligned with the fiber direction in both cases indicating that fiber alignment has a more dominant role in controlling neurite orientation, compared to the chemical gradient.

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Plasma Processing of Biomemetic Materials
Moderator: Sally McArthur, Swinburne University of Technology, Adoracion Pegalajar-Jurado, Colorado State University

2:20pm **SM+AS+BI+PS-ThA1 The Chemistry of Plasma Modified 3D Biomaterials**, ***Eloisa Sardella***, CNR-IMIP, Italy **INVITED**

Plasma processing has become a most powerful and versatile tool for surface functionalization of porous materials in biomedical field.

Non equilibrium plasmas have many advantages over wet chemistry approaches: they are highly eco-friendly, have high potentialities in developing surfaces with peculiar characteristics, are capable to be part of in-line material processing and most importantly, can be applied to any material. Consequently, it has opened many new opportunities for investigation of surface modification in various fields like tissue and organ regeneration and biosensing. In this talk, we shall give a brief review on the recent developments of plasma processing of porous materials. We shall describe our experience on non-equilibrium plasmas to modify materials of biomedical interest like: scaffolds for tissue engineering and 3D carbon nanotubes carpets for bio-sensing. This research is aimed to gain new insights on the potentialities of plasma processing of biomedical materials. This work is encouraged by a deep characterization of material's surface and investigation of the material/ bio-environment interface.

3:00pm **SM+AS+BI+PS-ThA3 Advantages of Plasma Polymerized Surfaces for Cell Sheet Engineering over Other Deposition Techniques**, ***Heather Canavan***, *M.A. Cooperstein*, University of New Mexico, *B. Bluestein*, University of Washington, *J.A. Reed*, University of New Mexico **INVITED**

Poly(N-isopropyl acrylamide) (pNIPAM) undergoes a conformation change in a physiologically relevant temperature range: it is relatively hydrophobic above its lower critical solution temperature (LCST, ~32oC), and mammalian cells are easily cultured on pNIPAM-grafted surfaces. When the temperature is lowered below the LCST, the polymer's chains rapidly hydrate, and cells detach as intact sheets capable of being used to engineer tissues ("cell sheet engineering"). This behavior has led to a great deal of interest from the bioengineering community, resulting in a variety of film deposition methods, substrate storage techniques, and cell release methods. Unfortunately, this has also resulted in widely varying responses (e.g., % of cells released, biocompatibility and stability of surfaces, etc.) from the resulting cell sheets. In this work, we present a comprehensive comparison of the surface chemistry, biocompatibility, and effect on reversible cell adhesion that results from pNIPAM substrates fabricated using the most common polymerization (free radical and plasma polymerization) and deposition (spin coating and plasma polymerization) techniques. The relative biocompatibility of different mammalian cells (e.g., endothelial, epithelial, smooth muscle, and fibroblasts) was evaluated using appropriate cytotoxicity tests (MTS, Live/Dead, plating efficiency). The pNIPAM-coated surfaces were evaluated for their thermoresponse and surface chemistry using X-ray photoelectron spectroscopy and goniometry. We find that plasma polymerized NIPAM substrates (ppNIPAM) are more stable under a variety of storage conditions prior to their use. Furthermore, when used for cell culture, ppNIPAM films exhibit no cytotoxicity toward any of the cell types tested and yield excellent cell detachment (~85%), which is an important consideration for their ultimate use in engineered tissues.

4:00pm **SM+AS+BI+PS-ThA6 Biofunctionalization of Surfaces by Energetic Ion Implantation: Fundamentals and Recent Progress on Applications**, ***Marcela Bilek***, *A. Kondyurin, E. Kosobrodova, G. Yeo*, University of Sydney, Australia, *S. Wise*, Heart Research Institute, Australia, *N.J. Nosworthy, C.G. dos Remedios, A.S. Weiss, D.R. McKenzie*, University of Sydney, Australia **INVITED**

Despite major research efforts in the field of biomaterials, rejection, severe immune responses, scar tissue and poor integration continue to seriously limit the performance of today's implantable biomedical devices. Implantable biomaterials that interact with their host via an interfacial layer of active biomolecules to direct a desired cellular response to the implant would represent a major leap forward. Another, perhaps equally revolutionary, development that is on the biomedical horizon is the introduction of cost-effective microarrays for fast, highly multiplexed screening for biomarkers on cell membranes and in a variety of analyte solutions.

Both of these advances will rely on the availability of methods to strongly attach biomolecules to surfaces whilst retaining their biological activity. Radicals embedded in nanoscale carbon rich surface layers by energetic ion bombardment can covalently immobilize bioactive proteins [*Proc. Nat. Acad. Sci* **108**(35) pp.14405-14410 (2011)] onto the surfaces of a wide range of materials, including polymers, metals, semiconductors and ceramics . This new approach delivers the strength and stability of covalent coupling without the need for chemical linker molecules and multi-step wet chemistry. Immobilization occurs in a single step directly from solution and the hydrophilic nature of the surface ensures that the bioactive 3D shapes of the protein molecules are minimally disturbed.

This presentation will describe recently developed approaches that use energetic ions extracted from plasma to facilitate simple, one-step covalent surface immobilization of bioactive molecules. A kinetic theory model of the biomolecule immobilization process via reactions with long-lived, mobile, surface-embedded radicals and supporting experimental data will be presented. Progress on applications of this technology to create antibody microarrays for highly multiplexed, simple analysis of cell surface markers and to engineer bioactive surfaces for implantable biomedical devices will be reviewed.

4:40pm **SM+AS+BI+PS-ThA8 Three-Dimensional Biopolymeric Scaffold Surface Modification Using Plasma Enhanced Chemical Vapor Deposition: The Effect of Functionality and Wettability on Cell and Bacterial Attachment**, ***Morgan Hawker***, *A. Pegalajar-Jurado, E.R. Fisher*, Colorado State University

Three-dimensional (3D) bioresorbable polymeric materials, such as porous scaffolds made of poly(ε-caprolactone) (PCL), have desirable bulk properties for tissue engineering, wound healing, and controlled-release drug delivery applications. However, the surface properties (e.g., chemical functionality and wettability) are often undesirable for certain biomedical applications. Therefore, the ability to fabricate 3D materials with ideal bulk properties and customizable surface properties is a critical aspect of biomaterial development. Here, we demonstrate the deposition of conformal films throughout the 3D porous scaffold network using plasma enhanced chemical vapor deposition (PECVD). Resulting film properties can be tailored by using different precursor species. Octofluoropropane (C_3F_8) and hexafluoropropylene oxide (HFPO) precursors were chosen as model hydrophobic film PECVD systems, whereas a copolymerization system consisting of allylamine/allyl alcohol (allylNH/allylOH) precursors was chosen as a model hydrophilic, nitrogen containing PECVD system. To ensure the efficiency and reproducibility of the treatments, both the exterior and interior of the plasma treated scaffolds were characterized using contact angle goniometry, X-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (SEM) to assess changes in wettability, chemical functionality, and scaffold architecture in comparison to untreated scaffolds. C_3F_8 and HFPO PECVD on scaffolds resulted in fluorocarbon films on the exterior of the scaffold, and the extent of deposition throughout the scaffold's 3D structure was controlled by treatment time. The nitrogen content of the allylNH/allylOH films was tailored by changing the feed gas composition of the copolymerized films. After surface modifications, modified PCL scaffold surface interactions with cells and bacteria were assessed to confirm the relevance of these coatings for the biomedical field. We also explored the effect of different plasma treatments on cell adhesion/proliferation using both human dermal fibroblasts and endothelial cells, bacterial attachment, and biofilm formation using *Escherichia coli*.

5:00pm **SM+AS+BI+PS-ThA9 Plasma Polymerized Bandages for Wound Healing**, ***Jason Whittle***, *L.E. Smith, T.L. Fernandez*, University of South Australia

Wound healing is a multi-billion dollar drain on healthcare systems around the work. This is particularly true in developed countries as they deal with aging populations and conditions such as vascular disease and diabetes. More than 30% of the costs associated with treating diabetes can be attributed to management of chronic wounds. Dressings for the clinical management of wounds are constantly evolving to provide antimicrobial environments and optimal gas exchange, pH and hydration to facilitate wound healing. Ideally, the next generation of wound dressings will also provide a favourable surface for cell attachment, proliferation and migration to further promote the healing process. A number of approaches have been developed for healing chronic wounds, many of which involve culturing of explanted cells, or donor cells, and returning them to the wound site. In this paper, we have used plasma polymerisation to develop surfaces which influence the migration rate of primary cells (keratinocytes, fibroblasts and endothelial cells). A pro-migratory surface will enable cell transport into the

wound bed. Earlier workers have concentrated on cell attachment as a key measurement of clinical potential, but we have observed that cell mobility exhibits a preference for different surface chemistry to attachment, and this preference depends on cell type. We show how plasma polymerization can be used to produce surfaces with controllable chemistry, and explore the effect of changing surface chemistry on the migration rate of primary fibroblasts and keratinocytes in vitro. We also investigate the effect of these surfaces on wound closure rate using an in-vitro wounding model based on an engineered skin composite. We also explore the application of plasma polymerized pro-migratory surfaces to electrospun scaffolds for use with deeper wounds.

Authors Index

Bold page numbers indicate the presenter