Biomaterial Interfaces
Room 101A - Session BI+AS+SA-ThM

Synthesis and Processing of Biomaterials/Biologically Inspired Materials

Moderators: Daniel Barlow, US Naval Research Laboratory, Lara Gamble, University of Washington

8:00am **BI+AS+SA-ThM1 Response of PC 12 Cells to Mesoporous Substrates with and without DC Bias**, *F. Sabri,* University of Memphis*; **Kyle Lynch**,* Universtiy of Memphis*; O. Skalli,* University of Memphis

The interaction of nerve cells with nanostructured surfaces and substrates is of great importance to the field of tissue engineering and artificial substrates developed for biomedical applications. It has been established that cells respond to different polymer surface characteristics such as roughness, surface free energy, topography, chemistry, charge, and other properties including electrical conductivity. It has also been recognized that the nanotopography can affect and influence cell morphology, cell alignment, cell signaling and extension of neurites. Here, we discuss the influence of the mesoporous structure of crosslinked silica aerogels on the adhesion, proliferation, and neurite extension of PC 12 cells, in the presence and absence of applied DC bias. The behavior of cultured PC 12cells on the aerogel substrates is compared to the behavior of cells cultured on cell culture plastic (control) and the affect of applied DC bias of different magnitudes is carefully investigated. The neurite extensions clearly show a preferred growth direction and the rate of growth of extensions is also influenced by the varying conditions.

8:20am **BI+AS+SA-ThM2 Collagen Functionalized with ALD-TiO_2: A Novel Biomaterial for Bone Grafting**, ***ArghyaKamal Bishal***, *C. Sukotjo, C.G. Takoudis,* University of Illinois at Chicago

In medicine, the use of implants is growing rapidly. Some patients may not have enough bone to support such implants.[1, 2] Therefore, those patients are required to have augmentation, a procedure to increase the height or width of inserted bone-like supporting materials, prior to implantation.[1] Collagen resorbable membrane is used as a bone grafting material which acts as supporting material and facilitates new bone formation.[3] Sometimes, titanium reinforced collagen membrane is used for improved stability.[2]

Collagen is an important biomaterial which is used in several biomedical applications. It has a triple helix structure made of polypeptide chains.[3, 4] Hydrogen bonds play an important role in keeping together these peptide chains. Glycine, proline are the most abundant amino acids found in its structure. Collagen has also the ability to be reorganized and crosslinked and thus turn into flexible fibrils with higher tensile strength.[3]There are four main types of collagen: type I, II, III and V. Among them mostly type I and little amount of type V construct the bone structure by forming a composite with hydroxyapatite (HA) crystals.[4]

Titania (TiO_2) itself is biocompatible.[5] Additionally, it has the ability to attract Calcium and Phosphate in a liquid environment.[6] Therefore TiO_2 coated collagen may be used as an excellent bone grafting material to nucleate Ca and P and thus reconstructing a stable bone structure. In this work, we present ALD of TiO_2 on collagen membrane in a custom-made ALD reactor. The deposition was performed at room temperature. Tetrakis(dimethylamido)titanium (TDMAT) and ozone were used as metal precursor and oxidizer, respectively. Samples were characterized for their surface morphology, composition and mechanical properties. Energy dispersive spectroscopy confirmed the presence of Ti on coated collagen and electron microscopy showed an increase in fiber diameter after deposition by more than a factor of 2:

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8:40am **BI+AS+SA-ThM3 Nanostructure Formation on Biomaterials by Directed Irradiation Synthesis (DIS) for Tissue Regeneration and Maximize Corrosion Resistance**, ***Jean Paul Allain***, *A.R. Shetty,* University of Illinois at Urbana-Champaign*; S. Arias, A. Barnwell,* University of Illinois at Urbana Champaign*; F. Echeverria, L.F. Berrio,* University of Antioquia, Colombia

An important aspect of tissue engineering is to create a favorable extracellular microenvironment, mainly the extracellular matrix (ECM) which can guide cell differentiation and tissue regeneration. The ECM consists of a number of cues that can be guided by surface topography and matrix stiffness [1]. Recent studies [2,3] have demonstrated that depending on the type of surface structuring and patterning, cell adhesion can be controlled with potential applications in smart cell culture systems and biosensors. Many of the desired biomaterial properties that require a combination of metal alloy and soft material interfaces cannot be processed with conventional bottom-up techniques. Directed irradiation synthesis (DIS) address this limitation by introducing a synthesis process that is scalable to high-volume manufacturing by virtue of its intrinsic large-area simultaneous exposure of materials surfaces and interfaces.

In this study, we have employed directed irradiation synthesis to induce nanostructure formation on two commonly used biomaterials: 1) Ti_6Al_4V and 2) magnesium (Mg). The goal is to examine the role of surface nanostructuring on the stimulation of cells and tissues in order to provide important cues for tissue regeneration as well as guarantee a good corrosion resistance and to minimize bacteria adhesion. Detailed characterization, establishing processing conditions and correlating them to surface and biomaterial properties have been successfully performed on nanostructured medical grade Ti_6Al_4V and Mg. These irradiated surfaces were biologically evaluated by using human aortic smooth muscle cells (HASMCs) for cytotoxicity and cell/surface adhesion and interactions. This analysis allowed us to determine connections with processing, structure, surface energy, and biointerface properties. Biological response of these new surfaces has also lead us, for the first time, to establish correlations between nanostructuring by DIS and cell stimulation, as well as to show the real potential of these new surfaces to favorably stimulate cells and tissues different than bone. The corrosion behavior of these biomaterials in a phosphate buffered simulated body fluid (SBF) has also been investigated for bone implant application.

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9:00am **BI+AS+SA-ThM4 Controlled Peptide Surfaces of Various Ratios that Guide Neural Stem Cell Differentiation**, ***HalaShakib Dhowre***, *M. Zelzer, H. Sahaf, C. Towlson, N.A. Russell,* University of Nottingham, UK

Cell instructive biointerfaces represent an essential aspect for the advancement of regenerative medicine. Currently, a major issue in biointerface design is the limited ability to mimic the complex interactions of the natural processes in the extracellular matrix (ECM) with artificially designed surfaces and interfaces [1]. While biomaterial surfaces have been shown to be able to elicit specific cell responses (e.g. adhesion, proliferation, differentiation), precise control akin to that of natural cellular environments is still lacking[2].

AIM:

The present work aims to address this challenge by designing new synthetic peptide surfaces with well controlled composition and functionality able to impact control over the differentiation of neuronal stem cells with the ultimate goal to understand and control how neuronal networks function.

METHODS:

Compositionally well defined surface concentrations of two short laminin peptide sequences, Arg-Gly-Asp (RGD) and Ile-Lys-Val-Ala-Val (IKVAV) were prepared of various ratios via the “grafting from” stepwise approach and the surface modification was confirmed with surface analysis techniques to

indicate successful peptide functionalisation. The neural stem and progenitor cells (NSPC) were set up from embryonic rat hippocampi (E18). Immunocytochemistry (ICC) observed cell viability and differentiation to specific NSPC lineages for Nestin, βIII-Tubulin and GFAP.

RESULTS:

Surface characterising techniques (WCA, AFM and ToF-SIMS) verified the successful amino acid build-up to peptides on the surfaces, allowing modification of the surfaces with RGD and IKVAV. Enhanced NSPC adhesion, proliferation and differentiation were observed on the peptide surfaces. ICC demonstrated Nestin expression decrease after the removal of the growth factors (EGF and FGF) and an increase in the expression of βIII-Tubulin and GFAP; thus illustrating cells differentiating from stem cells to neurons or astrocytes due to peptide surface influence.

CONCLUSION:

Well defined peptide surfaces were designed successfully, the various ratios of RGD and IKVAV surfaces demonstrated cell adhesion, proliferation and i desirable effects in controlling different populations of stem cell fate. These surfaces may advance new insight in understanding how surface properties affect the regulation of physiological relevance in directing neural cell differentiation, which will be essential to understand how neural networks function.

References

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9:20am **BI+AS+SA-ThM5 Biofunctional Hydrogels for Tissue Repair***, **Andres Garcia,*** Georgia Institute of Technology **INVITED**

Hydrogels, highly hydrated cross-linked polymer networks, have emerged as powerful synthetic analogs of extracellular matrices for basic cell studies as well as promising biomaterials for regenerative medicine applications. A critical advantage of these synthetic matrices over natural networks is that bioactive functionalities, such as cell adhesive sequences and growth factors, can be incorporated in precise densities while the substrate mechanical properties are independently controlled. We have engineered poly(ethylene glycol) [PEG]-maleimide hydrogels to study epithelial morphogenesis and identified independent contributions of biophysical and biochemical properties of these materials to this developmental process. In another application, we have developed synthetic hydrogels that support improved pancreatic islet engraftment, vascularization and function in diabetic models. These studies establish these biofunctional hydrogels as promising platforms for basic science studies and biomaterial carriers for cell delivery, engraftment and enhanced tissue repair.

11:00am **BI+AS+SA-ThM10 Nanoscale Domain Formation Induced by Partial Polymerization Creates Planar Supported Lipid Bilayers that are Fluid and Stable***, **N.Malithi Fonseka**, B. Liang, K.S. Orosz, C.A. Aspinwall, S.S. Saavedra,* University of Arizona

Planar supported lipid bilayers (PSLBs) are widely explored bilayer platforms for receptor-based biosensors. PSLBs composed of fluid lipids lack the stability necessary for many technological applications due to the relatively weak non-covalent interactions between lipid molecules. Lipid polymerization enhances bilayer stability, but may greatly reduces lipid mobility and membrane fluidity. In an effort to enhance bilayer stability while maintaining fluidity, we have prepared and characterized PSLBs composed of mixtures of the polymerizable lipid bis-Sorbyl phosphatidylcholine (bis-SorbPC), and the fluid lipid diphytanoyl phosphatidylcholine (DPhPC) to form mixed PSLBs. We measured lateral diffusion coefficients (*D*) as a function of the bis-SorbPC/DPhPC molar ratio using fluorescence recovery after photobleaching (FRAP). In pure DPhPC PSLBs, D = 0.66 μm^2/sec. In equimolar poly(bis-SorbPC)/DPhPC, D = 0.36 μm^2/sec, whereas when the ratio is greater than 0.7, D decreased to 0.13 μm^2/sec. These data show that considerable fluidity is retained even when the poly(bis-SorbPC) fraction is substantial, which suggests that these bilayers are phase segregated, composed of polymerized and fluid domains. However domains were not observed with fluorescence microscopy techniques. The sub-µm morphology of these PSLBs was therefore investigated using atomic force microscopy (AFM). Nano-scale phase segregation of the two lipids was observed. DPhPC forms a continuous lipid matrix that is 0.2-0.4 nm thicker than the island-like poly(bis-SorbPC) domains. This height difference agrees with bilayer thicknesses measured for pure DPhPC and poly(bis-SorbPC) PSLBs. Furthermore, it was observed that the size of the poly(bis-SorbPC) domains increased with the percentage of poly(bis-SorbPC) in the PSLB. In summary, mixed lipid bilayers composed of poly(bis-SorbPC) and DPhPC form nano-structured membranes with retained lipid diffusivity, and thus they have considerable potential for creating membrane-based biosensors in which receptor activity depends on bilayer fluidity.

11:20am **BI+AS+SA-ThM11 Stabilization of Lipid Films by Hyaluronic Acid and Polymeric Substitutes in a Joint Model System***, **Felicitas Schwoerer,*** Universität Heidelberg, Germany*; M. Trapp, R. Steitz,* Helmholtz-Zentrum Berlin für Materialien und Energie GmbH*; R. Dahint,* Universität Heidelberg

In the United States there are 27 million people suffering from osteoarthritis. The disease is primarily caused by the degeneration of cartilage, which covers the bone ends of the joints and is in turn decorated with a phospholipid (PL) layer. The bone ends are separated by the synovial fluid containing the polysaccharide hyaluronic acid (HA) as a main component. It is generally assumed that both HA and PLs reduce friction and protect the cartilage. Based on the observation that HA concentration is reduced in diseased joints, a new cure called viscosupplementation has been developed, where HA or mixtures of HA and PLs are injected into the joints. However, until now the positive effect of such therapy is under debate.

To elucidate the importance of HA and PLs for joint lubrication and protection on a molecular level we investigate their interaction using a simplified model system for natural joints. A silicon wafer (representing the bone end) is covered with PL oligobilayers and incubated in an aqueous solution containing HA or polymeric substitutes (representing the synovial fluid). To mimic the forces in joint movement, we expose the model surfaces to a home-built shear apparatus facilitating *in situ* measurements at a rotational speed between 0 rpm and 6000 rpm. Measurements were performed at *BioRef* (Helmholtz-Zentrum Berlin), a time-of-flight neutron reflectometer with integrated infrared spectroscopy.

Upon contact with both HA and poly(allylamine hydrochloride) (PAH) solutions a tremendous swelling of the lipid film occurs. Film thickness increases by a factor of about four compared to pure D_2O exposure due to a drastic increase in the thickness of the interstitial water layers located between adjacent lipid bilayers. This effect is most likely due to the adsorption of charged polymers at the lipid headgroups leading to electrostatic repulsion. Despite their high film thickness and water content, the polymer-exposed lipid films exhibit approximately ten times higher shear stability than the respective systems incubated in pure water. With increasing rotational speed the lipid films contain substantially enhanced water fractions, which we attribute to increasing lateral fragmentation. Present investigations aim at the question whether HA and PAH are incorporated into the lipid tail region and bridge adjacent bilayers as this might explain the observed higher stability.

11:40am **BI+AS+SA-ThM12 New Substrates and Patterning Methods for Supported Lipid Bilayers***, **Sally McArthur,*** *L. Askew,* Swinburne University of Technology, Australia **INVITED**

The cell membrane encases and protects cellular components and plays an important role in transport, signalling and disease. Studying membrane behaviour is a challenging task due to the complexity and scale on which these processes occur. Supported lipid bilayers (SLBs) have provided researchers with stable and reproducible platforms to recreate cell membrane environments. The planar structure of the model means a variety of patterning techniques can be employed to recreate membrane architecture on both a micro and nanoscale. In particular, pre-patterned substrates are of great interest as they eliminate complications associated with preserving membrane integrity during patterning. Plasma polymers provide a versatile method of creating thin films with a variety of different surface chemistries. In this work we explore the behaviour of plasma coatings in aqueous conditions and the use of plasma films for creating patterned SLBs using vesicle collapse. The results demonstrate that variations in plasma polymer chemistry can be used to control lipid bilayer formation and the locations of different lipid species. Characterisation of film behaviour and bilayer formation was conducted using a variety of techniques including ellipsometry, quartz crystal microbalance with dissipation (QCM-D), confocal microscopy, atomic force microscopy (AFM) and Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS).

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