Biomaterial Interfaces
Room: 202 - Session BI+NC-ThM

Engineering Biointerfaces
Moderator: S. Zauscher, Duke University

8:00am **BI+NC-ThM1 Engineering Membrane Physical Properties and Dynamics using Structured Interfaces**, ***A.N. Parikh***, *B. Sanii, A.M. Smith, M. Howland, A.M.A.M. Brozell*, University of California, Davis **INVITED**

Interfacial organization of lipids and amphiphiles into a discrete number of molecular layers provides,arguably, one of the most pristine experimental realizations of elf-organized, two-dimensional systems. it provides an experimental test-bed for the study of a rich variety of interface-dominated processes, including surface melting, low-dimensional phase transitions, surface dynamics, and phase coexistence and separation. This talk will present recent experimental evidence from our laboratories which highlight the importance of substrate structure (e.g., topography, charge, and surface energies) in engineering the physical properties, namely curvature, morphology, and lateral dynamics, in supported lipid bilayers. Applications of such engineered surfaces in examining the dependence of membrane phase separation and phase transition on bilayer curvature and morphology will be discussed.

8:40am **BI+NC-ThM3 Fluidic and Air-Stable Supported Lipid Bilayer and Cell-Mimicking Microarrays**, ***X.-Y. Zhu***, University of Minnesota

As drug delivery, therapy, and medical imaging are becoming increasingly cell-specific, there is a critical need for high fidelity and high-throughput screening methods for cell surface interactions. Cell membrane-mimicking surfaces, i.e., supported lipid bilayers (SLBs), are currently not sufficiently robust to meet this need. Here we describe a method of forming fluidic and air-stable SLBs through tethered and dispersed cholesterol groups incorporated into the bottom leaflet. Achieving air-stability allows us to easily fabricate SLB microarrays from direct robotic spotting of vesicle solutions. We demonstrate their application as cell membrane-mimicking microarrays by reconstituting peripheral as well as integral membrane components that can be recognized by their respective targets. These demonstrations establish the viability of the fluidic and air-stable SLB platform for generating content microarrays in high throughput studies, e.g., the screening of drugs and nanomedicine targeting cell surface receptors.

9:00am **BI+NC-ThM4 Supported Lipid Membranes as Biomimetic Model Systems**, ***S. Svedhem***, *A. Kunze, H. Ekstrand*, Chalmers University of Technology, Sweden, *P. Sjövall*, SP Technical Research Institute of Sweden, *R. Frost, M. Edvardsson, B. Kasemo*, Chalmers University of Technology, Sweden

Engineering of surface-supported lipid membrane model systems is currently a very active field of research. The present contribution will focus on two recent examples from our group in this area; (i) Lipid exchange between liposomes and supported lipid membranes of opposite charge, and (ii) The action of lipases on supported lipid membrane structures. These examples cover different kinds of supported lipid structures; both (planar) supported lipid bilayers and (intact) supported liposomes, as well as different kinds of biomolecular interactions associated with them. Key experimental techniques used to follow processes at these interfaces are the quartz crystal microbalance with dissipation monitoring (QCM-D), optical reflectometry, surface plasmon resonance (SPR), fluorescence microscopy, atomic force microscopy (AFM) and time-of-flight secondary ion mass spectrometry (TOF-SIMS). Our first examples deals with lipid exchange/transfer between lipid membranes, which is important for many biological functions, but which has also the potential for in situ engineering of supported membranes. To learn more about how the dynamics of such processes can be studied, we have investigated the interaction of positively and negatively charged lipid vesicles with supported lipid bilayers (SLBs) of opposite charge. In particular, it was possible to follow the different steps during such modification processes both by QCM-D and TOF-SIMS, the latter allowing direct estimation of the fraction of different lipids in the membrane. These results have also implications for studies of how nanoparticles interact with membranes. The second example covers how lipases (PLA2 and PLD) act on membranes, and in particular how lag phases for such interactions can be monitored by QCM-D. Depending on the type of lipase under study, either dissolution or membrane morphology changes were observed. In conclusion, the combination of surface-supported lipid membranes and surface-sensitive analytical techniques allows for detailed studies of processes of relevance for biological membranes. In particular, the molecular composition can be controlled, and morphological changes of the membrane structure can be induced and visualized

9:20am **BI+NC-ThM5 Nanopatterning Proteins Over Large Areas for Biological Applications**, *J. Malmström, H. Agheli, P. Kingshott*, ***D. Sutherland***, University of Aarhus, Denmark

The recent decade has seen a rapid expansion in the ability to create and study nanometer scale objects and these new methods are being applied to the study of biological systems. The immobilisation of bioactive molecules has long been a goal in biomaterials and tissue engineering research, for use as stimulatory cues or model systems to study biointeractions. The advent of soft lithographic routes and efficient approaches to minimise non-specific protein interactions for example through immobilised polyethylene oxide coatings has lead to microscale pattens of proteins were routinely demonstrated and applied as model systems to study biological systems. While patterns at the micrometer scale of considerable interest and application, the size of and lengthscale at which proteins and other macromolecules are structured in vivo is in most cases at the nanoscale. Patterning biomolecules at the nanometer scale gives a significant potential for studying how biological systems function at the macromolecular length scale or to mimic the structure of biological interfaces with macromolecular resolution. A key requisite for the study of cellular biosystems is the ability to robustly generate large areas of patterns. In this work colloidal lithographic routes utilising electrostatic self assembly to generate dispersed monolayers of colloidal particles as masks for pattern generation have been used to generate nanostructured interfaces. Substrates with nanopatterned surface chemistry have been used as templates for generation of nanopatterns of proteins. Hydrophobically modified gold nanopatches in a silicon oxide background have been used to open up arrays of 100nm nanometer diameter regions within a protein rejecting background (based on PLL-g-PEG) and used to demonstrate nanopatterning of a number of protein systems (Laminin, Osteopontin and Ferritin). Nanostructured interfaces have also been fabricated on QCM-D sensors and used to study insitu protein and antibody binding at nanoscale patches while AFM microscopy of dried samples was used to quantify protein and antibody binding utilising height histograms. A combination of QCM-D, AFM and SPR derived data was used to establish the thickness and density of the adsorbed laminin layers at both nanoscale patches and homogeneous surfaces.

9:40am **BI+NC-ThM6 Fabrication and Testing of Electrospun Novel Biodegradable Polyurethane Scaffolds**, ***N. Brown***, *C. Zhang, T. Boland*, Clemson University

Synthesis and fabrication of biomaterials that can temporarily mimic the native tissue is a lofty aim in Tissue Engineering. It is also paramount in Regenerative Medicine material research. Such a biomaterial could be formed into scaffolds and be temporary replacements of tissues or for other internal biomaterial corporal needs. Our work here is on the use of a novel biodegradable polyurethane (BPU) that was electrospun and fabricated into tubes. Once fabricated, smooth muscle cells (RASMC) were ink-jet printed onto the same scaffolds and tested for degrees of cell alignment BPU are biopolymers that are designed to mimic the elasticity and memory of native tissue. These biopolymers can be designed to fit the application. This BPU was synthesized from methylene di-p-phenyl-diisocyanate (MDI), polycaprolactone diol (PCL-diol) and N, N-bis (2-hydorxyethyl)-2-aminoethane-sulfonic acid (BES), serving as a hard segment, soft segment and chain extender respectively. The BPU was then electrospun into nanofibers and formed small diameter (4 mm) blood vessels. The blood vessels were electrospun at various extrusion rates to determine optimum pore size and fiber diameters. This was accomplished by SEM imaging. The mechanical testing included tensile and burst pressure testing to determine if the scaffold could withstand extreme physiological conditions. Burst pressure testing results were from 1600-2900 mm Hg. Fiber diameters were in the 700-1000 nm range. Pore sizes were in the 50-90 um range. Mechanical testing results indicated a elongation of 620+/-120% with memory. The mechanical testing indicated that these scaffolds could withstand extreme mechanical physiological conditions well exceeding what they would experience in vivo. The imaging indicated fiber formation that could mimic an extracellular matrix or act as an internal physical barrier. Lastly, ink-jet printing was used as a cell placement method to control the location of cells on material. Cell printing was used to determine if RASMC cell alignment was possible and to what degree patterns could be printed to conjure alignment on the fibrous scaffolds. Histological results of the RASMC patterns on the electrospun scaffolds will be presented.

10:40am **BI+NC-ThM9 Electrically Insulating Pore-Suspending Membranes on Highly Ordered Porous Alumina**, ***C. Steinem***, University of Goettingen, Germany **INVITED**

In the last years, we have developed a membrane system that combines the merits of freestanding and solid supported bilayers. These membranes suspend the pores of a highly ordered porous material such as porous alumina (nano-BLMs) or porous silicon (micro-BLMs). In this talk, I will discuss the electrical properties and stability of these membranes as a function of lipid composition and under flow conditions. We were able to demonstrate that a buffer solution exchange can be readily achieved by placing the membranes in a flow system. The membranes turned out to be stable as evaluated by the changes in membrane resistance obtained from impedance analysis. The membrane resistances are sufficiently high to analyze ion channel activity on the single channel level. In particular, we have demonstrated that connexins can be inserted into nano-BLMs exhibiting full functionality.

11:20am **BI+NC-ThM11 Supported Lipid Bilayers on Nanoporous Substrates for Multi-technique Membrane Sensing**, ***K. Kumar***, *S. Kaufmann, A.M. Tabari, M. Textor, E. Reimhult*, ETH Zürich, Switzerland

Supported lipid bilayers (SLBs) formed from the rupture of liposomes have the advantage over other planar membrane architectures in that they can be formed completely bereft of organic solvents, enabling the further incorporation of sensitive ion channels or membrane proteins.[1] Porous structures allow the use of fusogenic surfaces which enhance the formation of SLBs, while accommodating the incorporation of larger membrane proteins. By integrating these porous structures with suitable gravimetric or optical sensor surfaces that could double up as electrodes, it would be possible to conduct electrochemical measurements in tandem with, e.g., affinity measurements. For optical sensing techniques such as waveguide spectroscopy, if the pores are small enough, it would also be possible to discriminate between processes occurring on the surface and within the pores.[2] We have developed a process where it is possible to etch high aspect ratio pores into silicon nitride and silicon oxide with a tunable diameter between 50 nm and 150 nm using particle lithography for patterning etch masks.[3] Sensor substrates for coupled plasmon waveguide resonance (CPWR) measurements, quartz crystals for quartz crystal microbalance with dissipation (QCM-D) measurements and glass slides for microscopy techniques were fabricated. The influence of nanopore density and size on the kinetics of formation of SLBs by liposome fusion was investigated by QCM-D and the structure of the lipid bilayer in the pore area was investigated by atomic force microscopy, confocal fluorescence microscopy and nanoscopy.[4] QCM-D, microscopy and nanoscopy measurements suggest the formation of fully covering SLBs by liposome fusion on such substrates. Atomic force microscope (AFM) images and force distance measurements on individual SLBs over pores seem to indicate that the formed SLB also spans the nanopores, but are strongly deformed by the tip interaction. These results set the stage for the next phase of experiments, where electrochemical measurements can be made in situ on the waveguide or quartz crystal after the confirmed formation of a pore-spanning SLB.

[1] Reimhult, E. and Kumar, K. TIBTECH, 2008. 26(2): p. 82-89.
[2] Lau, K.H.A., et al. J Phys Chem B, 2004. 108(30): p. 10812-10818.
[3] Reimhult, E., et al. Nanotechnology, 2007. 18(27): p. 7.
[4] Donnert, G., et al. PNAS, 2006. 103(31): p. 11440-11445.

11:40am **BI+NC-ThM12 Patterning of Plasma Polymers for Bioarray**, ***G. Mishra***, *S.L. McArthur*, University of Sheffield, UK

The high-density, multi-anaylate chips required for genomic and proteomic research can be successfully produced using a precise surface patterning methodology that allows controlled positioning of chemically distinct active areas. A major challenge with current bio-sensing devices which requires addressing is the need for surface chemistry that allows immobilised biomolecules of diverse types to retain their biological activity. Plasma polymerisation presents a versatile approach to surface modification of these devices. The range of monomers available for plasma polymerisation makes this manufacturing approach even more suitable for use in systems where multiple coatings with specific properties are required for a single device. The control offered by this surface modification technique and the ability to spatially define reactive regions to reduce non-specific background adsorption is integral to this project. This study highlights the efficacy of photolithographic plasma polymer patterning and provides a rare insight into issues associated with achievable chemical specificity and spatial resolution. A multi-technique investigation (XPS, ToF-SIMS, AFM, fluorescence microscopy) of surface chemistry and its biological response forms the focus of the study. Using ToF-SIMS data and multivariate analysis, we highlight the intricacies of pulsed plasma polymerised surface chemistry and propose a unique approach to optimising these parameters in order to maximise functional group retention. ToF-SIMS data has also been used to provide new insight into the mechanism of pulsed plasma polymerisation.

Authors Index

Bold page numbers indicate the presenter