**Biomaterial Interfaces**
**Room: 23 - Session BI+SS+NS-WeM**

## Bio/Nano Interfaces with Applications in Biomedicine and Energy

**Moderator:** G.J. Leggett, University of Sheffield, UK

8:00am **BI+SS+NS-WeM1 Combining Colloidal Lithography and Photolithography to Create Dual Length-Scale Topographical Features to Study Stem Cell Behavior**, ***D.T. Bennetsen***, *D.C.E. Kraft, R. Ogaki, M. Foss*, Aarhus University, Denmark

It is well known that topographical features influence cellular response. A novel combination of colloidal- and photolithography has been developed to create a dual length scale topographical platform. The presented approach permits rapid parallel fabrication of micro/nanoscale patterns. The aim is to study the response of primary human dental pulp stem cells (hDPSC) to such topographies in a systematic way.

Colloidal lithography is performed using the "lift-off" method, which is applicable to surfaces with a non-flat surface. This enables the combination of using photolithography pre-made wafers as substrates, resulting in a complex topographical structure, spanning two length scales (Figure 1). Topographical patterns are created using the colloidal mask with either evaporation or sputtering via physical vapor deposition (PVD). The principle combination of materials investigated is tantalum covered with tantalum features. These dual scale substrates are exposed to hDPSC and proliferation, attachment and differentiation are examined. Differentiation is examined using osteogenic markers and MyoD1 expression.

Initial cell proliferation data indicates that variations in the colloidal pattern heights do not seem to elicit a statistical significant response (Figure 2). A set of experiments to clarify the effect of the colloidal pattern on the proliferation and cell cycle of the hDPSC is thus currently being performed. Furthermore, the effect of the dual scale topographical substrates on proliferation, differentiation and cell cycle is also being explored.

Concurrently we are investigating the combined effects of topographical/chemical patterns on cellular response. This can be achieved by depositing different materials site-specifically, followed by a material-specific self-assembly route. E.g. silanes and thiols with specific chemical moieties on oxides and gold, respectively. Characterization is performed using atomic force microscopy (AFM), scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS).

Our fabrication approach enables the opportunity to increase the complexity of artificial 2D platforms thus by gaining a better understanding of cellular behavior for a range of biomedical and biotechnological applications.

8:20am **BI+SS+NS-WeM2 Genetically Modified *Tobacco Mosaic Virus* (TMV)-based Electrochemical Detection of 2, 4, 6-trinitrotoluene (TNT)**, ***F. Zang***, *H. Ben-Yoav, X. Fan, A. Brown, J. Culver, R. Ghodssi*, University of Maryland

Detection of chemical hazards and explosive compounds has received growing attention for applications in environmental monitoring, food science, and national security. Explosives, such as TNT, show low vapor pressure, molecular mass, and volume, which makes the detection of these molecules challenging for most mass and refractive index based sensors. Thanks to the redox reaction of nitro groups in TNT molecules, electrochemical methods may be used for detection of low concentrations of TNT in aqueous environments. Electrochemical sensors are suited for on-site explosive detection due to high sensitivity, low volume and convenient integration with miniaturized devices. However, to distinguish TNT from other electrochemically active compounds in complex environments, high selectivity is a more critical factor for development of TNT sensors.

The TMV has a high aspect ratio, rod-like nanostructure that can be genetically modified to express tailored chemical receptors. In this work, a 12-amino acid (WHWQRPLMPVSI) sequence peptide with multivalent recognition properties of TNT was expressed on the coat protein of TMV (TMV-p) which was utilized to develop a sensitive and selective electrochemical sensing mechanism for TNT detection. Selective binding of TMV-p with TNT molecules will decrease the free TNT concentration in solution, reducing the number of nitro groups available for redox reactions.

In preliminary studies, background signals generated from electrolytes were characterized and the signal-to-noise ratio was optimized by long term scans of square wave voltammetry. Three concentration dependent current peaks from the reduction of nitro groups in TNT were observed at the potentials of -0.53V, -0.72V and -0.86V vs. Ag/AgCl reference electrode, respectively, which agreed with the results in literatures. The initial results showed a stable and reliable electrochemical response by the TMV-p sensing system. By comparing the reduction currents in the mixtures of TMV-p and unmodified TMV with TNT solutions, we will demonstrate that TMV-p preserves the peptide binding affinity to TNT molecules while increasing the binding site density.

The approach described in this study is a sensitive and selective label-free method to detect TNT based on the binding of target molecules with peptide modified TMV. In addition to the highly selective peptide binding with analytes and a high binding site density, the genetically modified TMV is also capable of self-assembly, coating the active surfaces of a wide range of transducers. This work can potentially be implemented in the development of miniature sensors for selective TNT detection in complex environments.

8:40am **BI+SS+NS-WeM3 Nanoparticles in Biology: Engineering the Interface for Sensing and Delivery**, ***V. Rotello***, University of Massachusetts **INVITED**

A key issue in the use of nanomaterials is controlling how they interact with themselves and with the outer world. Our research program focuses on the tailoring of nanoparticles of surfaces for a variety of applications, coupling the atomic-level control provided by organic synthesis with the fundamental principles of supramolecular chemistry. Using these engineered nanoparticles, we are developing particles for biological applications, in particular delivery and sensing. This talk will focus on the interfacing of nanoparticles with biosystems, and will discuss our use of nanoparticles for delivery applications including our *in vitro* studies of small molecule, nucleic acid, and protein delivery. This presentation will also feature the use of nanoparticles for diagnostic applications, including the use of array-based sensing paradigms for the sensing and identification of proteins, bacteria and cell type and state.

9:20am **BI+SS+NS-WeM5 Hydrophobic Forces, Electrostatic Steering, and Acid–Base Bridging between Atomically Smooth Self-Assembled Monolayers and End-Functionalized PEGolated Lipid Bilayers**, ***M. Valtiner***, Max-Planck-Institut fur Eisenforschung, Germany, *S.H. Donaldson, M.A. Gebbie, J.N. Israelachvili*, University of California, Santa Barbara

A molecular-level understanding of interaction forces and dynamics between *asymmetric* apposing surfaces plays a key-role in utilizing molecular structures for functional surfaces in biological and materials applications. To quantify interaction forces and binding dynamics between apposing surfaces in terms of their molecular architecture we developed a novel surface-forces-apparatus experiment, using self-assembled monolayers (SAMs) on *atomically-smooth* gold. Varying the SAM head-group allowed to quantitatively identify and control which interaction forces dominated between the SAM surfaces and surfaces coated with short-chain, end-functionalized polyethylene-glycol (PEG) polymers extending from lipid-bilayers [1].

Three different SAM-terminations were studied: (a) carboxylic-acid, (b) alcohol, and (c) methyl head-group terminations. These functionalities allowed for the quantification of (a) specific acid-base bindings, (b) steric effects of PEG chains, and (c) adhesion of hydrophobic segments of the polymer-backbone, all as function of the solution pH. The pH-dependent acid-base binding appears to be a *specific, charge-mediated hydrogen bond* between oppositely-charged carboxylic-acid and amine functionalities, above the acid-p$K_A$ and below the amine-p$K_A$. The long-range electrostatic "steering" of acid-base pairs leads to high binding probability even at distances close-to-full-extension of the PEG tethers, a result which has potentially important implications for protein-folding, enzymatic catalysis and biomaterial development.

[1] M. Valtiner et al., *JACS*, **2012**, 1746

9:40am **BI+SS+NS-WeM6 Viral Encapsulation in Lecithin Liposomes to Enhance the Therapeutic Effect of Oncolytic Viral Therapy**, ***N. Mendez***, *V. Herrera, A.C. Kummel*, University of California San Diego

Oncolytic viruses have emerged as a novel platform for cancer therapeutics due to their tumor-selective replication in cancer cells. In particular, the oncolytic virus TAV-255 has shown viral replication attenuation in normal cells while retaining cytolytic activity in tumor cells by taking advantage of defects in the p53-tumor suppressor pathway. Extensive testing of oncolytic viruses has shown a limited therapeutic effect due to rapid clearance by the reticuloendothelial (RE) system and antibody neutralization. With the aim to overcome an immune response and to enhance localized delivery, an oncolytic virus-liposomal encapsulation method has been designed to increase tumor uptake and the therapeutic efficacy of oncolytic viruses in

cancer cells. An inexpensive, non-toxic liposome has been prepared by self-assembly of Lecithin phospholipid bilayers around the Adenovirus capsid. Cholesterol and DSPE-PEG were incorporated into the lipid formulation to improve retention and stability. The developed method has shown that non-targeted encapsulated viral particles retain their ability to transfect cancer cells. In addition, surface functionalization of the liposomes may be applied to specifically target cancer cells and to compensate for decreased infectivity due to viral encapsulation.

10:40am **BI+SS+NS-WeM9 Engineering Bio-Interfaces using Electric Field-Induced Nanolithography**, ***S. Zauscher***, *R.J. Ferris, B. Yellen*, Duke University

Field-Induced Nanolithography (FINL) offers a convenient tool to create physically or chemically distinct patterns for bio-interfacial sensing applications. For pattern transfer, FINL merely requires a conductively coated SPM tip or stamp, connected to a conductive substrate via a voltage source. The patterning electrode is placed in contact with the target surface and a bias voltage is applied. Few sub-diffraction limit surface patterning techniques offer FINL's versatility to function in both a serial and parallel fashion. Recently we demonstrated the use of FINL to pattern a range of polymer brushes: poly(acrylic acid) (PAA), poly(N-isopropylacrylamide) (PNIPAAm), poly(sulfobetaine methacrylate) (PSBMA), and poly(oligo(ethylene glycol) methyl methacrylate) (POEGMA). Our results show that FINL of non-fouling polymer brushes provides a novel patterning technique that results in the localized topographical and chemical modification of the polymer brush surface only. The resulting chemical modification allowed selective addressing of the brush surface with aldehyde reactive coupling chemistries. Our approach thus shows significant promise for fabricating large-scale sensing devices, as patterning can be accomplished in a step-and-repeat fashion. Using FINL, we also demonstrated patterning of surface charges onto ferroelectric thin films (FETFs). FETFs are materials that are able to maintain a bi-stable polarization state, and that once polarized, maintain a high surface charge density. Using FINL, it is possible to locally align unit-cell dipole moments within the film to produce nano-scale polarization patterns. Although to date the use of FETFs is isolated to semiconductor and memory applications, we demonstrate that FETFs have great potential for biological and interfacial sensing applications. We show that FETF surface charge patterns can be used to control the lateral extent of electric double layer formation in dilute electrolyte solutions, with clear implications for field assisted particle deposition and programmed self assembly.

11:00am **BI+SS+NS-WeM10 Supramolecular Bioassemblies at Solid-Liquid Interfaces: Binding Control through Redox-Driven Multivalent Host-Guest Interactions**, ***G.V. Dubacheva***, CIC biomaGUNE, Spain, *L. Guerente, D. Boturyn,* Joseph Fourier University, France, *R. Auzély,* CERMAV, France, *R.P. Richter,* CIC biomaGUNE, Spain; Joseph Fourier University, France; Max Planck Institute for Intelligent Systems, Germany, *P. Labbé*, Joseph Fourier University, France

The design of kinetically stable bioassemblies while keeping binding control is of high current interest for bioanalytical and biomedical sciences. The development of tunable biointerfaces is also a key issue in nanobiotechnology as they can be used for modeling cell surface-associated biological processes. In this context, supramolecular host-guest chemistry is particularly attractive as it allows controllable molecular recognition and structural modification at specific areas of a nanoassembly, i.e. purpose-designed molecules can be confined in time and space in a highly controlled manner.

Cyclodextrin (CD) is well-known to form host-guest complexes with hydrophobic molecules while being soluble at physiological conditions. Taking advantage of redox-driven β-CD-ferrocene (Fc) multivalent interactions, we designed stimuli-responsive biomaterials composed of linear polymers, their multilayer assemblies and biomolecules. For this aim, we developed a new method to create β-CD self-assembled monolayers (SAMs) allowing precise varying β-CD surface density.1 We showed that Fc-functionalized polymers can be reversibly attached to such β-CD SAMs.1 We also showed a possibility to build up multilayer host-guest polymer assemblies on β-CD surfaces.2 In addition, we applied these β-CD SAMs for the reversible attachment of biomolecules using orthogonal Fc/β-CD- and specific bio-interactions under biological conditions.3 Finally, combined with guest-modified polysaccharide hyaluronan, the β-CD surfaces were explored as a model system to understand multivalent interactions at the cell-hyaluronan matrix interface associated to a variety of cellular functions and biological processes.

Physico-chemical properties of supramolecular assemblies were characterized by QCM-D, ellipsometry, cyclic voltammetry and contact angle goniometry. The redox-driven binding of polymers and biomolecules to β-CD surfaces was assessed by *in situ* combining electrochemistry/QCM-D and SPR ellipsometry/microfluidic systems. The developed tunable biointerfaces can be applied to investigate other topics in soft condensed matter physics, molecular physics and biophysics.

1Dubacheva et al., *Langmuir*, **2010**, 26:13976

2Dubacheva et al., *Soft Matter*, **2010**, 6:3747

3Dubacheva et al., *Chem Commun*, **2011**, 47:3565

11:20am **BI+SS+NS-WeM11 High-resolution *In Situ* Electrochemical STM Imaging of Phospholipid Model Cell Membrane**, ***H. Shimizu***, *S. Matsunaga,* University of Tokyo, Japan, *T. Yamada, T. Kobayashi,* RIKEN, Japan, *M. Kawai*, University of Tokyo, Japan

We obtained molecular-scale images of phospholipid layers spread on a modified Au(111) immersed in a buffer solution, by means of *in situ* electrochemical scanning tunneling microscopy (EC-STM). Real cell membranes consist of a bilayer of phospholipids which continually gather and interact. There are various kinds of phospholipids in the real cell membranes. To understand the action of these molecules, a dynamic molecular-scale method of observation is necessary. Lipkowski [1] first visualized static monolayers of phospholipid on Au(111) by *in situ* EC-STM. Matsunaga *et al.* [2] revealed dynamic, microscopic motion of phospholipid monolayer on alkanethiol-modified Au(111) immersed in a buffer solution. We intended to compose a bilayer of phospholipid on a hydrophilic substrate in order to mimic the real cell membrane more truly. We used a hydrophilically modified Au(111), anticipating that the first lipid monolayer with the hydrophilic head group down to the surface, and the second lipid monolayer with the hydrophobic alkyl chains down, all spontaneously in aqueous buffer solution.

For this purpose, we used 3-mercaptopropionic acid (MPA) self-assembled monolayer (SAM) on Au(111), in which the COOH groups are expected to be exposed out of the surface. We first observed a ($\sqrt{3}$ x $\sqrt{3}$) type adlattice of MPA SAM by STM. Then the sample was immersed in 50 mM phosphate buffer containing minimal lipid particles of 200 μM 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) with or without 50 μM cholesterol.

We uniquely observed a 2-dimensional adlattice with a parallelogram unit cell of 1.0 nm x 1.9 nm. Along the short segment, blight spots are aligned. The adlattice did not change with or without cholesterol, indicating that it was composed exclusively of pure POPC. The interval of 1.9 nm is apparently shorter than the full length of POPC molecule (≈2.5 nm). To interpret the adlattice structure, we considered a model structure composed of tilted POPC, with the head group attached to the MPA SAM. This model involves a strong affinity between the hydrophilic groups.

Although this frozen adlattice does not completely match our target structure of mobile lipid bilayer, we consider we could partly utilize the hydrophobicity/hydrophilicity of the phospholipid molecules to compose a uni-directional membrane. We will further develop this kind of methods by choosing the proper modifier on Au(111), aiming the bilayer structure. By this we expect to go closer to the nanometer-scale reality of cell membranes containing functional proteins.

[1] J. Lipkowski, *Phys. Chem. Chem. Phys.* **12**, 13874 (2010).

[2] S. Matsunaga *et al.*, *Electrochem. Commun.* **9**, 645 (2007).

11:40am **BI+SS+NS-WeM12 Characterization of Polymer/Drug Films as Model for Drug Eluting Coronary Stent Coating Layers**, ***V. Ciarnelli***, *M.R. Alexander, M.C. Davies, C.J. Roberts*, University of Nottingham, UK

This work describes the characterization of a polymeric based drug eluting stent coating, used in coronary stenting to prevent restenosis [1]. The work examines thin films as models for drug eluting stent coatings. Complementary surface analysis techniques are used to investigate the drug polymer distribution on the surface and throughout the depth of the model films.

The first goal of this project is to establish the feasibility of certain surface analysis techniques in the characterisation of a drug eluting stent coating layer. Secondly, this study will act as a standard reference to determine the ideal operating conditions for characterizing the more complex stent device.

Thin film models were produced varying the substrate materials (silicon or glass), preparation procedures (spin casting or spray coating) and drying methods (oven or warm air). The different drug to polymer ratios used were: 1:3, 1:1 and 3:1 (w:w).

Complementary surface analysis techniques such as atomic force microscopy (AFM), time of flight secondary ion mass spectrometry (ToF-SIMS) and x-ray photoelectron spectroscopy (XPS) were employed for the characterization of the films. Depth profiling has also been performed using XPS and ToF-SIMS.

AFM imaging of the oven dried spun cast films shows domains of drug, characterized by a circular organization with features of 100 - 250 nm in

diameter. These domains are not observed in other samples and appear to be related to phase separation during the drying step.

Surface characterization using XPS shows enrichment of the drug at the surface for all the model films with the exception of the spray coated films at the 1:3 drug-to-polymer weight ratio.

Depth profiling using both ToF-SIMS and XPS confirms that the drug is enriched at the surface, posing significant implications for drug loaded polymer delivery systems.

Complementary surface analysis techniques have proven extremely successful in characterizing the model films. Suitable techniques and their operative conditions have now been established for the characterization of a stent device.

[1] I. Iakovou et al., Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. JAMA,. **293** (2005): p. 2126-2130.

# Authors Index

## Bold page numbers indicate the presenter


**— A —**

Alexander, M.R.: BI+SS+NS-WeM12, 2
Auzély, R.: BI+SS+NS-WeM10, 2

**— B —**

Bennetsen, D.T.: BI+SS+NS-WeM1, **1**
Ben-Yoav, H.: BI+SS+NS-WeM2, 1
Boturyn, D.: BI+SS+NS-WeM10, 2
Brown, A.: BI+SS+NS-WeM2, 1

**— C —**

Ciarnelli, V.: BI+SS+NS-WeM12, **2**
Culver, J.: BI+SS+NS-WeM2, 1

**— D —**

Davies, M.C.: BI+SS+NS-WeM12, 2
Donaldson, S.H.: BI+SS+NS-WeM5, 1
Dubacheva, G.V.: BI+SS+NS-WeM10, **2**

**— F —**

Fan, X.: BI+SS+NS-WeM2, 1
Ferris, R.J.: BI+SS+NS-WeM9, 2
Foss, M.: BI+SS+NS-WeM1, 1

**— G —**

Gebbie, M.A.: BI+SS+NS-WeM5, 1
Ghodssi, R.: BI+SS+NS-WeM2, 1
Guerente, L.: BI+SS+NS-WeM10, 2

**— H —**

Herrera, V.: BI+SS+NS-WeM6, 1

**— I —**

Israelachvili, J.N.: BI+SS+NS-WeM5, 1

**— K —**

Kawai, M.: BI+SS+NS-WeM11, 2
Kobayashi, T.: BI+SS+NS-WeM11, 2
Kraft, D.C.E.: BI+SS+NS-WeM1, 1
Kummel, A.C.: BI+SS+NS-WeM6, 1

**— L —**

Labbé, P.: BI+SS+NS-WeM10, 2

**— M —**

Matsunaga, S.: BI+SS+NS-WeM11, 2
Mendez, N.: BI+SS+NS-WeM6, **1**

**— O —**

Ogaki, R.: BI+SS+NS-WeM1, 1

**— R —**

Richter, R.P.: BI+SS+NS-WeM10, 2
Roberts, C.J.: BI+SS+NS-WeM12, 2
Rotello, V.: BI+SS+NS-WeM3, **1**

**— S —**

Shimizu, H.: BI+SS+NS-WeM11, **2**

**— V —**

Valtiner, M.: BI+SS+NS-WeM5, **1**

**— Y —**

Yamada, T.: BI+SS+NS-WeM11, 2
Yellen, B.: BI+SS+NS-WeM9, 2

**— Z —**

Zang, F.: BI+SS+NS-WeM2, **1**
Zauscher, S.: BI+SS+NS-WeM9, **2**