Biological, Organic, and Soft Materials Focus Topic
Room: 201 - Session BO+NS+BI+NC-ThA

Biological and Molecular Applications of Nanostructures

Moderator: P. Kingshott, The University of Aarhus, Denmark

2:00pm **BO+NS+BI+NC-ThA1 Fabrication of Nanoscale Bioarrays for the Study of Cytoskeletal Protein Binding Interactions Using Nano-Imprint Lithography**, ***M. Schvartzman***, *M. Palma, J. Abramson, J. Sable, J. Hone, M.P. Sheetz, S.J. Wind*, Columbia University

Recent advances in solid-state nanofabrication technology now make it possible to fabricate structures in the size regime of biomolecules, i.e., ~ tens of nanometers and below. We are developing a system that mimics biological spatial order by using nanofabricated structures which are organized into hierarchical arrays in which structural parameters, such as spacing and orientation, are systematically varied, and which provide multiple protein binding sites with nanometer-scale separations. The aim of the work is to study the dependence of large cytoskeletal protein binding on the geometrical arrangement of extracellular matrix (ECM) proteins and integrins. Nanoscale patterns are formed in arrays containing metal dots 5 - 10 nm in diameter, which are functionalized with linker molecules that specifically interact with individual protein binding sites. These dots can be arranged individually, in pairs, or in more complex patterns based on the structure of the molecules under investigation. In particular, we are interested in understanding of the importance of the spacing between integrin cytoplasmic tails on the binding of other proteins, such as talin, that are involved in the building of focal adhesion (FA) complexes by which the actin cytoskeleton attaches to the ECM. The nano-arrays fabrication process uses thermal nanoimprint lithography and pattern transfer by Au/Pd deposition and lift-off. For the lift-off process for such small features and relatively thin resist layer, an angle evaporated metal hard mask is deposited after the NIL step, followed by resist descum. A post-lift-off annealing step at 400 – 500 oC results in further reduction of feature size and a high degree of uniformity. Spheroidal dots are formed with diameters ~5 - 10 nm. The pattern is functionalized with fibronectin RGD motif through a biotin-avidin-biotin linkage. Total-Internal-Reflectance Fluorescence (TIRF) is used for the monitoring of the bio-functionalization with fluorescene labeled molecules. In-vitro study of cells spreading on the patterned and bio-functionalized surfaces is performed on the patterns with different geometries. This presentation will describe the fabrication arrays of ultra-small metal features using NIL technology, functionalization and implementation of these arrays in the study of the fundamentals of cell behavior, representing a new example of the enormous impact of nanofabrication on the life sciences.

2:20pm **BO+NS+BI+NC-ThA2 Universal Method for Forming Various Metal Particles as Multiplexed Labels for Electron Microscopy in the Backscattering Mode**, ***H. Takei***, *H. Kim, K. Yasuda*, Kanagawa Academy of Science and Technology, Japan

Scanning electron microscopy is a powerful technique in terms of resolution, three dimensional rendition of the object and use of ease. Life science is one among many fields for which SEM is an indispensable tool, and it continues to find new uses. One way to enhance its utility in the field of life science is to multiplex labeling as carried out routinely with fluorescence microscopy; labels with different emission spectra. For this purpose, one approach would be to use particles made from different metals because different metals backscatter electrons differently in accordance to the atomic weight. In the backscattering mode, particles made from different metals can be readily distinguished by the brightness of the image so that labeling each type of the particle with distinct biomolecules such as an antibody or DNA would allow one to observe distributions of distinct molecular species simultaneously, as with multiplexed fluorescence labeling, albeit at much higher resolutions. What is needed is thus a method to produce various metal particles at will. For this purpose, we will demonstrate a universal method consisting of formion of a dense monolayer of monodisperse Latex spheres with a self-assembling technique and then evaporating a metal that can be readily evaporated or sputtered. With the above method, the sphere adsorption is accomplished through mild physisorption so that application of mild sonication to surface bound metal particles in the presence of a liquid such as water leads readily to re-dispersal of Latex sphere coated with the metal. We show that resulting particles made from different metals such as gold, silver, nickel or aluminum can be readily distinguished. The sphere size used ranges typically from 50 to 100 nm with the deposition thickness anywhere between 5 to 20 nm. Beside its flexibility with respect to the type of metal that can be used, another advantage of this method lies with a fact that particles can be surface-modified while still adsorbed on a surface. This is particularly advantageous from the perspective of rinsing after each surface treatment protocol. It should be mentioned that because particles are covered only on one side, two types of surfaces, metal and exposed polystyrene, can be selectively used for surface modifications; if necessary two sides can be modified with two distinct species at the same time to give higher functionalities.

2:40pm **BO+NS+BI+NC-ThA3 Nanoscopic Presentation of Peptides at Cell Interfaces**, *J.P. Spatz*, ***V. Hirschfeld-Warneken***, MPI for Metals Research and U. of Heidelberg, Germany **INVITED**

Engineering of cellular environments has become a valuable tool for guiding cellular activity such as differentiation, spreading, motility, proliferation or apoptosis which altogether regulates tissue development in a complex manner. The adhesion of cells to its environment is involved in nearly every cellular decision in vivo and in vitro. Its detailed understanding and defined control also opens new strategies for medical technologies with respect to, e.g., stem cell regulation, tissue scaffolds, cell selection due to their disease state, artificial blood vessels, or immunology. Our approach to engineer cellular environments is based on self-organizing spatial positioning of single signaling molecules attached to inorganic or polymeric supports, which offers the highest spatial resolution with respect to the position of single signaling molecules. This approach allows tuning cellular material with respect to its most relevant properties, i.e., viscoelasticity, peptide composition, nanotopography and spatial nanopatterning of signaling molecule. Such materials are defined as "nano-digital materials" since they enable the counting of individual signaling molecules, separated by a biologically inert background. Within these materials, the regulation of cellular responses is based on a biologically inert background which does not trigger any cell activation, which is then patterned with specific signaling molecules such as peptide ligands in well defined nanoscopic geometries. This approach is very powerful, since it enables the testing of cellular responses to individual, specific signaling molecules and their spatial ordering. Detailed consideration is also given to the fact that protein clusters such as those found at focal adhesion sites represent, to a large extent, hierarchically-organized cooperativity among various proteins. Moreover, "nano-digital supports" such as those described herein are clearly capable of involvement in such dynamic cellular processes as protein ordering at the cell's periphery which in turn leads to programming cell responses.

3:20pm **BO+NS+BI+NC-ThA5 Bioadhesive/Bioresistant Nanopatterns: Fabrication, Characterization and Investigation of Their Effect on Enhancing Biomolecule Affinity Reactions**, ***I. Mannelli***, *A. Valsesia, P. Lisboa, P. Colpo, F. Rossi*, European Commission Joint Research Centre, Italy

Fabrication and characterisation of nanopatterned surfaces are topics of many past and present research studies. Recently many techniques have been investigated for the fabrication of nanopatterned surfaces. Among them colloidal lithography combined with plasma polymer deposition and/or self-assembly has shown to be a flexible technology for producing large area nanostructured surfaces. At the same time investigations have increasingly focused on the behavior of biomolecules (i.e. protein, nucleic acid) when interacting with surfaces and/or particles with nanometre dimensions. Although many studies have been done on the surface/biomolecule interaction mechanisms and the conformation changes that molecules undergo after the interaction with macroscopic surfaces it is much less well understood how the biomolecules interact with surfaces that have been previously functionalised with nanofeatures. In particular little is known about the interactions which occur with features with dimensions comparable with those of the biomolecules themselves and how, after the interaction, the native molecule conformation changes. We have optimized a procedure for fabricating nanopatterned surfaces at the sub500 nm scale in which a hexagonal close packed array of bioadhesive gold nanoareas are embedded in an anti-fouling matrix (PEO-like polymer). The surfaces fabricated in this way were characterized by AFM analysis and their interaction with amino functionalised Au nanoparticles were investigated. The AFM images show the crystalline arrangement of nanopattern array and the localization of the H_2N-Au nanoparticles in the bioadhesive areas. A SPR Imaging system was used to perform kinetics studies on the adsorption and interaction behaviour of biomolecules on these surfaces. At the same time, the detection performance of these surfaces when employed as a transduction platform for studying biomolecule interactions has been investigated. To do this, a recognition biomolecule was immobilized on the surface and the affinity reaction with a specific target molecule was

monitored in real time by means of the SPRi system. The investigated surfaces showed an enhancement of the affinity reaction efficiency with respect to the non structured surfaces. The results obtained show that nanostructuring the surfaces makes it possible to improve the binding site accessibility of the immobilized biological probes without significantly modifying the native biomolecule conformation.

4:00pm **BO+NS+BI+NC-ThA7 Control and Separation of Proteins in a Nanofluidic FET Device, using pH Gradient and Valence Charge**, ***Y.-J. Oh***, University of New Mexico, *D.R. Bottenus, C.F. Ivory,* Washington State University, *S.M. Han*, University of New Mexico

We have fabricated Si multiple internal reflection infrared waveguides embedded with a parallel array of nanofluidic channels (100 nm W ×500 nm D) and studied field-effect-transistor (FET) flow control and separation of proteins, using scanning laser confocal fluorescence microscopy (SL-CFM) and multiple internal reflection Fourier transform infrared spectroscopy (MIR-FTIRS). For fluidic FET, a DC potential is applied to a highly doped gate area in the mid-section of nanochannels, in addition to a longitudinal electric field along the nanochannels. The gate potential controls the surface charge on SiO_2 channel walls and therefore their ζ-potential. Depending on the polarity and magnitude, the gate potential can accelerate, decelerate, or reverse the flow of proteins. In addition, our MIR-FTIR analysis demonstrates that fluorescein dye molecules, used here as a pH indicator, are hydrogenated and dehydrogenated in response to the gate bias and subsequent pH shift. Using fluorescein, we have thus measured a pH shift caused by the surface charge modulation and longitudinal electrical field. We observe that this pH shift is further influenced by water electrolysis occurring at the electrodes that drive the electroosmotic flow as well as at the gate where a leakage current unavoidably flows through a thermal SiO_2 layer. Using this pH manipulation and generating a pH gradient along the nanochannels, we have conducted isoelectric focusing and separation of proteins with different isoelectric points (Ip). In this presentation, we will further discuss protein separations, using transverse electromigration based on their different valence charges in relation to the surface charge on channel walls.

4:20pm **BO+NS+BI+NC-ThA8 High Throughput Device for Surface Modification Studies**, ***S. Saxer***, *S. Tosatti, S. Zuercher,* ETH Zurich, Switzerland, *K. Gademann,* EPF Lausanne, Switzerland, *M. Textor*, ETH Zurich, Switzerland

Surface coatings are often used to control the degree of the interaction between the material and the surrounding environment. Paints and lacquers are the most common and widespread coatings. Recent developments in the process of micro- and nanostructures lead to the demand of thinner coatings, which maintain the aspect ratio and preserve the fine structures. Such conditions can be fulfilled by the use of self-assembled monolayers.[1] Due to the large spectrum of applications are nowadays high-throughput approaches required to screen through a large number of parameters, ranging from the substrate up to the different assembly conditions (temperature, solvent, pH). We designed a surface modification screening platform (SuMo-device), which allows the parallel execution of different surface–modification experiments while allowing a single measure for the adlayers characterization, thus enhancing the efficiency of the experiment. The seventy wells (working area/well: 7mm^2; Volume/well: 20µmL) provided by the SuMo-device, were verified with solutions having different concentrations of the grafted copolymer; poly (L-lysine)-grafted-poly ethylene glycol, which is known to adsorb electrostatic to different metal oxide substrates and to render the surface resistant against non-specific protein adsorption (non-fouling), and thus resulting in adlayers with different coverage and thickness.[2] A secondary adsorption of fluorescein isothiocyanate labeled fibrinogen (FITC-fbg) enables to test the non-fouling behavior and therefore the quality of the polymer layer, by the measurement of the fluorescence with a microarray scanner.[3] A standard evaluation procedure was introduced to enable a quantification of the fluorescent response. The Limit of Detection (LOD) was calculated from the standard curve and requires a minimum FITC-fbg concentration of 0.002mg/mL (incubation: 1 h at 25°C). The fluorescence data were compared and found to correlate with layer thickness (ellipsometry) and with the in situ mass adsorption curve obtained by optical biosensor devices. Thus, we conclude that our approach offers a faster and more efficient way to screen between different possible coatings strategies, similarly to drug discovery processes.

[1] F. Schreiber, Prog. Surf. Sci. 65, 151 (2000)
[2] G.L. Kenausis et al., J. Phys. Chem. B 104, 3298 (2000)
[3] S. Pasche et al., Langmuir 19, 9216 (2003).

4:40pm **BO+NS+BI+NC-ThA9 Nanopatterned Functional Surfaces by Electron Beam Lithography of Plasma Assisted Chemical Vapour Deposited Polymers**, *F. Bretagnol, A. Valsesia,* ***G. Ceccone***, *D. Gilliland, P. Colpo, F. Rossi*, European Commission, Joint Research Centre Italy

We presents a method for the fabrication of well defined chemically active nano-patterned surfaces. Electron-beam lithography is applied on plasma-deposited polymers in order to create sub-micron carboxylic functional areas over a non-bio-adhesive matrix. Characterization of the patterned surface demonstrates that this technique can be used to fabricate chemically active features with lateral size down to 200 nm. Moreover, experiments with a model protein (bovine serum albumin) on the patterned surfaces show preferential adhesion on the active region indicating the ability of this method for the design of biosensing platforms.

5:00pm **BO+NS+BI+NC-ThA10 Application of Plasma Polymerised Microchannel Surfaces for IEF**, ***M. Salim***, *B. O'Sullivan, S.Y. Ow, P.C. Wright, S.L. McArthur*, The University of Sheffield, UK

The emerging proteomics field has triggered the development of many bioanalytical tools and technologies. Since biological samples are usually present in small quantities and volume, there is a high priority for the analytical tools to be able to process these minute samples. The use of microfluidics for this purpose has thus emerged. Plasma polymerisation has been shown to be an attractive method to coat microchannel surfaces. Here, we apply the plasma surface modified microchannels for on-chip IEF protein separation (separation based on the isoelectric points of the proteins). A common problem associated with the use of non-coated glass or fused silica substrates in IEF is the high electroosmotic flow (EOF) and high extent of protein adsorption. Therefore, coated microchannel surfaces are usually introduced to reduce the EOF and reduce protein adsorption, and improve the resolution of the separation. Lower EOF can also permit IEF separation in short channel lengths. In this study, we investigate the applicability of plasma polymerised tetraglyme surface for IEF-facilitated protein separation at a 2.2 cm channel length, since it exhibits slower EOF compared to non-coated microchannel surfaces, and is low-fouling. Comparisons were also made to non-coated microchannels and other types of plasma polymers, i.e. plasma polymerised acrylic acid and plasma polymerised allylamine coated surfaces. IEF performed on the low-fouling plasma polymerised tetraglyme microchannel surface was shown to exhibit better separation compared to non-coated microchannel, as observed from the clearer formation of focused protein bands. Also, due to the presence of protein and carrier ampholytes adsorption in both plasma polymerised acrylic acid and allylamine coated microchannels, these surfaces may not be suitable for IEF protein separation over a wide range of pH, with the separation resolutions decreasing after repeating experimental runs.

5:20pm **BO+NS+BI+NC-ThA11 Plasma Deposited Polyethylene Oxide as a Platform for Proteins and Stem Cells Microarrays**, *L. Ceriotti,* JRC, Ispra, Italy, *L. Buzanska,* JRC, Ispra, Italy and Polish Academy of Science, *H. Rauscher, I. Mannelli,* ***L. Sirghi***, *D. Gilliland, M. Hasiwa, F. Bretagnol, A. Ruiz, S. Bremer, S. Coecke,* JRC, Ispra, Italy, *P. Colpo,* Joint Research Center, IHCP, Italy, *F. Rossi*, JRC, Ispra, Italy

In this work we fabricated and characterized microarrays of proteins of the extra cellular matrix (ECM) for stem cells adhesion studies. Plasma deposited poly(ethylene) oxide (PEO-like) film coated glass slides has been chosen for its dual properties, being protein and cell repellent in wet conditions and protein adhesive in dried conditions. The microarrays were created by direct microspotting of the proteins on the PEO films with optimized printing buffer by using a non-contact printing technology. The stability and the quality of the spots of fibronectin used as model protein were assessed by Time of Flight- Secondary Ion Mass Spectrometry (ToF-SIMS) and ellipsometry was used to determine the amount of protein immobilized on each spot after rinsing of the substrate with water. It was found that when fibronectin is spotted at a concentration higher than 84 µmg/ml, the protein forms a monolayer with a density of 112 ± 4 ng/cm^2 with a low surface coverage but quite regular spatial distribution as confirmed by Atomic Force Microscopy (AFM) measurements. The active conformation of the spotted fibronectin as a function of the spotted concentration was verified by performing an immunoassay with antibodies specific for the fibronectin RGD sequence by Surface Plasmon Resonance (SPR) imaging. Human Umbilical Cord Blood Neural Stem Cells (HUCB-NSCs) were cultured on different ECM protein arrays (fibronectin, laminin, collagen I, collagen III and collagen V) showing a protein type and concentration dependent adhesion and growth on the micro-spots. No cells were found in-between the spots thanks to the anti adhesive properties of the PEO-like film. The cell nuclei were stained for cell counting and preliminary specific cell staining was performed to evaluate the differentiation stage of HUCB-NSCs on fibronectin spots. The array platform developed in this study provides a promising approach to investigate in a high throughput manner how insoluble factors patterned on the surface influence stem cell adhesion and development.

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