Frontiers in Inkjet Technology Topical Conference
Room: Tesuque - Session IJ+BI+MN-MoM

Frontiers in Inkjet Technology
Moderator: T. Boland, University of Texas at El Paso

8:20am **IJ+BI+MN-MoM1 Drop Impact on Liquid, Solid and Porous Surfaces**, ***A.L. Yarin***, University of Illinois at Chicago **INVITED**

The talk covers drop impacts on thin liquid layers, dry impermeable surfaces, and porous surfaces with nano-scale texture. Splashing and corona formation and propagation on liquid layers are discussed first. Then, some additional kindred, albeit non-splashing, phenomena like drop spreading and deposition, receding (recoil), jetting, fingering and rebound on liquid and dry impermeable solid surfaces are covered. A number of practical applications of drop impacts are mentioned and relevant experimental, theoretical and computational aspects are considered.

After that, a novel method of enhancement of drop and spray cooling for microelectronic, optical and radiological elements and server rooms, which require extremely high heat fluxes, is discussed. The key idea of the method is to cover the heat transfer surfaces with electrospun nonwoven polymer nanofiber mats. The experiments reveal that drop impacts on nanotextured surfaces of nanofiber mats produce spreading similar to that on the impermeable dry surfaces. However, at the end of the spreading stage the contact line is pinned and drop receding is prevented. All the mats appear to be dynamically permeable for water drops. The enhanced efficiency of drop cooling in the presence of nanofiber mats observed experimentally results from a complete elimination of drop receding and bouncing characteristic of the current spray cooling technology. Therefore, the drops evaporate completely, and the large cooling potential associated with the latent heat of water evaporation is more fully exploited. This is paradoxical: the best cooling can be provided by a "furry overcoat"! The results on drop impact on porous surfaces are also relevant for drop impacts on paper and nonwovens in the context of ink-jet-printed microelectronics.

9:00am **IJ+BI+MN-MoM3 Upper and Lower Bounds for the Stability of Inkjet Printed Lines**, ***B. Derby***, *J. Stringer*, University of Manchester, UK

Many applications for inkjet printing require the ability to print continuous linear features. Inkjet printing achieves this through the overlap and coalescence of a series of liquid drops on a planar substrate, which then transform to a solid through phase change or solvent loss. In order to produce regular parallel sided printed lines, the intermediate fluid thread must retain morphhological stability prior to solidification. Drying fluid drops often show considerable hysteresis between the advancing and receding contact angles. This behaviour is shown to impose upper and lower bounds for the width of a line formed by the overlap of printed drops. The lower bound for line width is determined by the minimum separation distance for spreading drops to spread, overlap and coalesce. However, for liquids with zero receding contact angles (as is the case for many evaporating solvents) there is a further limit for parallel sided lines [1]. The upper bound for line width is determined by a dynamic fluid instability that occurs through competing fluid flows between the spreading and coalescence processes [2]. This dynamic instability is a function of both drop spacing and the rate of droplet deposition. By considering both the upper and lower bound limits we can construct a map in a parameter space defined by drop size, drop spacing, drop/substrate contact angle and linear printing velocity that shows the conditions under which stable linear features can be printed.

9:20am **IJ+BI+MN-MoM4 Particle Deposition and Assembly of Inkjet-Printed Colloidal Drops in Line and Pattern Printing**, *A. Joshi, V. Chhasatia,* ***Y. Sun***, Drexel University

Precise control of process parameters during inkjet printing is essential to enable uniform, accurate and repeatable deposition of functional materials. In this work, we present a combined in-situ observation and computational study to examine particle deposition and assembly during evaporation of inkjet-printed colloidal drops. Unlike previous computational models that use tracer particles and known velocity fields inside the drop, our computational model uses a multi-phase lattice Boltzmann method (LBM) that directly simulates the flow of the liquid drop, surrounding vapor phase and the motion of the liquid-vapor interface. The motion of suspended particles within the liquid phase is directly coupled to the fluid flow and also influences the velocity field in the liquid. Evaporation is accomplished by reducing the vapor pressure above the drop and different evaporation modes including evaporation with contact line pinning and self-similar evaporation with a constant contact angle are examined. A novel visualization technique is developed wherein aqueous suspensions of fluorescent particles are jetted onto transparent surfaces and the evaporation dynamics are observed in real-time using a fluorescence microscope. The effects of drop spacing, jetting frequency, substrate wettability, particle size and volume fraction, and environmental conditions (temperature and humidity) on the final deposition morphology are presented for line and pattern printing of functional materials on substrates.

9:40am **IJ+BI+MN-MoM5 Inkjet Printing of Flexible Hybrid Solar Cells based on P3HT and ZnO**, ***G. Carryon***, *J.B. Baxter, Y. Sun*, Drexel University

Inkjet printing of organic solar cells offers an inexpensive alternative to conventional solar cell fabrication methods. Despite the attractiveness of organic solar cells, they have demonstrated some degradation problems and have yet to achieve the efficiencies necessary to make them economically viable. In contrast to their organic counterparts, inorganic semiconductors have demonstrated advantages in their high dielectric constant which facilitates carrier generation processes, high carrier mobility, and thermal morphological stability. In recent years, improvements in device performance have been seen in the development of organic-inorganic hybrid materials (e.g., ZnO nanoparticle-polymer composites or CdSe quantum dot-polymer composites) as the photoactive layer. To date, most studies on hybrid solar cell fabrication have focused on using lab-scale spin-coating methods to deposit ZnO nanoparticle-polymer materials. In this paper, we present our results in using an industrial piezoelectric-driven printing device for inkjet printing of ZnO nanoparticles/nanorods-polymer [e.g., poly(3-hexylthiophene (P3HT)] ink materials for large-scale processing of hybrid solar cells. The deposition morphology and properties of printed photoactive layer are examined as a function of the solvent properties (e.g., wettability and vapor pressure), particle size, volume fraction, and polydispersity, as well as the aspect ratio of nanorod. The effects of jetting parameters (e.g., wave form and jetting frequency) and printing patterns on film thickness and uniformity are also discussed in detail. Finally, the feasibility of printing P3HT onto aligned ZnO nanorod arrays for novel heterogonous nanostructures for reduced exciton diffusion lengths is explored.

10:00am **IJ+BI+MN-MoM6 Anomalies in Applications of Inkjet Printing in Microfluidic Device Fabrication**, ***W.E. Dieterle***, *C.P. McNary*, California University of Pennsylvania

Utilization of inkjet-generated masks for UV photosensitive materials as a cost-effective method for the generation of microfluidic devices requires resolution of certain anomalies related to combinations of various color components. These anomalies are demonstrated for UV exposures with a high-pressure mercury vapor source and possible solutions are discussed, including implications for inkjet manufacture designs targeted to similar applications.

10:40am **IJ+BI+MN-MoM8 Fabrication of Miniature Drug Screening Platform Utilizing Low Cost Bioprinting Technology**, ***J. Rodriguez***, *T. Xu*, University of Texas at el Paso

In the pharmaceutical industry, new chemicals and substances are being tested to find appropriate compounds for treating a specific disease. The demand for screening large compound collections against and increasing number of therapeutic targets has stimulated technology development in the areas of assay automation and miniaturization. Current methods for evaluating the reactions of cells use a relatively large volume in the range from microliters to milliliters; since reliability has to be met, it exists the need to have several assays to confirm the biochemical reactions, which ultimately cause the usage of large amounts of volume for each substance. Unfortunately, some of these new compounds are rather hard to obtain, which causes an expensive researched and limited material availability; therefore, increasing the time development for future cures. We have developed a new and low-cost deposition method to fabricate miniature drug screening platform that can realistically and inexpensively evaluate biochemical reactions up to 4 substances per trial in a picoliter-scale volume.

This study focues on the development of the controls for a deposition method (inkjet printing technology) which will simultaneously place therapeutic drugs and cells onto target sites to fabricate cell/drug chips for drug screening application. Using a modified HP 5360 CD printer, droplets of GFP expressing Escherichia coli have been deposited in an agar coated coverslip chip as small reliable volume of 180 picoliters per each colony dot, along with this bacteria it has been patterned different antibiotics in

such a way that we evaluated the growth of the bacteria under antibiotics presence. The viability and function of the printed cells were evaluated by the live/dead and plasmid gene transfection experiments resulting in 98% viability and maintaining DNA function. Moreover, it has been recorded as high throughput process printing 250,000 droplets/second. Due to the reduction of volume, this method will increase the effectiveness of the resources utilized for emerging drug screening processes. The results show promising usage of resources for future drug screening through new biochemicals.

11:00am **IJ+BI+MN-MoM9 Inkjet Printing of Oxygen Releasing Materials for Improved Cell Survival and Growth**, ***A. Arteaga***, *T. Xu*, University of Texas at el Paso

Introduction: A major barrier in tissue engineering is the impossibility of providing adequate oxygen to all cells within the engineered tissue before a full vascularization is achieved. To overcome this limitation, a variety of oxygen-producing particles have been developed for improving tissue survival. However, most of these particles are used in random mixtures with scaffolding materials, which usually leads to an uneven distribution of oxygen in bioengineered tissues. An ideal oxygen supply requires a precise spatial-temporal control of the oxygen-producing particles in scaffolds. Unfortunately, current oxygen delivering scaffold techniques are unable to perform as described and to precisely incorporate oxygen particles into the scaffolds. Cell inkjet printing is a novel tissue fabrication approach, in which a special inkjet printer can be programmed to deposit cells and/or biomaterials of various types and sizes in a very precise pattern. In this study we have applied the inkjet printing technology to allocate oxygen releasing materials to their designed positions for optimal cell viability and growth.

Methods: The controlled oxygen-releasing platform is fabricated by printing different patterns ("Black", "White", "Grey", and "Dots" to represent different densities of the oxygen particles on the substrates) of encapsulated calcium peroxide (CPO) particles that were analyzed against C2C12 mouse myoblast cell line for cell viability. CPO has been found to release its oxygen over an extended timeframe. The effects of controlled oxygen-releasing particles on cell viability were analyzed using the cell morphology study, live/dead assay, and the MTS assay.

Results: These analyses showed the concentration of the oxygen-releasing particles in "Black" was toxic to the cells based on the decreasing trend in cell viability. The "White" did not have oxygen-releasing particles, which correlates to the decrease in cell viability over time due to oxygen deprivation. Both "Grey" and "Dots" showed a similar trend in absorbency, in which the absorbency was low at 24 hours, there was an increase in absorbency at 48 hours, and then an abrupt decrease at 72 hours. Both these results suggest that the amount of oxygen released was beneficial to the cells within the first 48 hours, yet may not have been sufficient to sustain cell viability after that time span. The cells treated in the printed "Dots" showed to have the most compatible treatment for an overall increase in cell viability.

Conclusion: The amount of oxygen released can be controlled to optimize the cell by bioprinting different densities of the oxygen releasing materials onto a substrate.

11:20am **IJ+BI+MN-MoM10 Understanding Volume Ejection of Complex Fluids through Pressure Measurements**, ***G.E. Mårtensson***, *W. Holm*, Micronic Mydata AB, Sweden

In conventional ink jetting applications, a pressure difference is used to ensure the continuous and prompt filling of the jetting chamber between jetting actuations. The delivery of precise fluid volumes utilizing inkjet-like drop-on-demand jetting technology is primarily controlled by the piezo voltage that actuates the jetting chamber (Gerhauser et al. 1983, SID 83 Digest). The jetting of large volumes, in excess of 1 nL, of complex viscous fluids is complicated by the difficulty of filling the ejection chamber quickly after the previous droplet ejection.

A novel jetting mechanism for highly viscous complex fluids, that utilizes a viscous micropump to control the amount of fluid that is ejected by a piezo actuated mechanism, has been developed and implemented by the authors. In this paper , we report results of volume, exit velocity, and pressure measurements. The piezo voltage, V_p, and the angular speed, N, of the viscous pump has been varied. A chosen V_p translates directly to displaced volume and N to flow rate.

The ejected volume has been measured by weighing a large number of droplets and via 3D profilometry methods. It has been shown in the experimental jetting setup that the volume of a jetted deposit is only affected to a minor degree, of the order of 5% of the target volume, by the chosen piezo voltage, V_p. Thus the ejected volume is almost independent of the displaced volume within the experimental range and determined by the flow rate, which in turn is controlled by N.

The exit velocity of the jet has been measured using high speed double exposure imaging. It has been shown that the speed of the ejected droplet has a nearly linear response to V_p (at least for all but the smallest N). For a given V_p, the exit velocity increase with increasing N. Thus, it seems that a larger displaced volume results in a higher exit velocity, but the ejected amount is unaffected.

In order to probe these seemingly counterintuitive results, the pressure in the ejection chamber, as well as in the viscous micropump, was measured over a large number of ejection cycles. The volume of the resulting depositions were correlated with the chamber and pump pressures. Additional measurements were performed to correlate the speed of the resulting shot with the chamber and pump pressures. Simple models are proposed to correlate the above mentioned quantities.

11:40am **IJ+BI+MN-MoM11 Determination of Effective Jet Radius from Measurements of the Perturbation Growth Rate in Thermally Stimulated Continuous Microjets**, ***J.M. Grace***, *G. Farruggia, E.P. Furlani, Z.J. Gao, K.C. Ng*, Eastman Kodak Company

Drop formation in continuous inkjet devices is based upon the Rayleigh-Plateau instability – a phenomenon in which surface tension drives the break-up of a column of fluid into droplets. In thermally stimulated continuous inkjet devices, heat pulses applied to the jet at the nozzle couple to the instability to stimulate drop formation. The level of stimulation depends upon the size of the effective perturbation and its growth rate along the jet. While the growth rate depends upon characteristics of the jet itself (fluid properties and jet diameter), the effective perturbation depends upon coupling between the source of stimulation and the jet, as well as the fluid properties. The coupling efficiency can be inferred from measurements of the perturbation growth rate and the jet diameter. For liquid microjets with diameters of 10 micrometers or less, direct determination of the jet diameter by optical microscopy is extremely challenging. Although the lateral dimensions of the microjets may be difficult to measure precisely, the break-up length can be determined with relatively good precision. Measurements of break-up length as a function of input power provide a means to determine the perturbation growth rate. From the experimentally determined growth rate as a function of stimulation frequency, the diameter of the microjet can be determined by fitting to a model for jet break-up. The experimentally determined growth rate and jet diameter provide a basis for comparing the effective coupling for different designs of jetting modules. Measurements of jet break-up and methods for determining the effective jet diameter will be presented and discussed.

Marine Biofouling Topical Conference
Room: Navajo - Session MB+BI-MoM

Understanding Marine Biofouling
Moderator: S. Zauscher, Duke University

8:40am **MB+BI-MoM2 Colloidal Theories of Bacterial Attachment as Applied to Marine Bacteria: A Necessary Revision?**, ***L.K. Ista***, University of New Mexico, *G.P. Lopez*, Duke University

The majority of our knowledge of bacterial attachment and subsequent biofilm formation has been gleaned from studies on human pathogens and commensal bacteria with specialized attachment mechanisms and attachment substrata. In contrast many marine microorganisms have a variety of substratum choices, with the added challenge of new types of introduced substrata (boats, piers, pilings) as possible biofilm supports. Maintaining the genetic information needed to produce specific attachment mechanisms for each possible substratum would be maladaptive; it is very likely that marine bacteria exploit their colloid-like size and rely on colloidal interactions to drive attachment. Thus, colloidal models of bacterial attachment are of particular interest to understanding marine microbial attachment. Current models of bacterial attachment are useful for describing some bacterial attachment, but cannot predict attachment behavior in most cases. In this work we examine 3 basic assumptions of the preeminent model used for bacterial attachment, the Lewis Acid Base (LAB) model proposed by van Oss. We used gold-alkanethilate self-assembled monolayers (SAMs) and three marine bacteria to test these assumptions. The first assumption is that apolar interactions include both London dispersion (induced dipole/induced dipole) interactions and those

based on fixed dipoles. Using apolar contact angle liquids of either purely London dispersion or London dispersion and dipole/induced dipole interactions we calculated the apolar component of the surface tensions of SAMs and bacteria and observed differences in the estimation of the apolar surface tension and, thus, the total surface tension on polar surfaces. The second assumption is that the Lewis acid and base components of H_2O surface tension are equal, which frequently leads to overestimation of Lewis basicity. We calculated surface tensions of bacteria and SAM surfaces with both LAB values and those based on solvatometric hydrogen bonding calculations and observed that the latter gave more reasonable estimation of the free energy of attachment. The third assumption is that interactions can be correlated with average surface energy for the cell. Both our observations and those in the literature have led us to believe this is untrue. We present scanning electron microscopy data that demonstrate that different parts of bacterial cells are in contact with the surface of different SAMs and that SAMs on nanoparticles can identify specific regions of heterogeneity on bacterial cell surfaces. Based on our results we propose modifications to the LAB model that may make it more able to model and predict marine bacterial attachment.

9:00am **MB+BI-MoM3 Reversible Adhesion in Barnacle Cyprids: the Weak Link in Surface Colonisation?**, *N. Aldred,* Newcastle Univ., UK, *I.Y. Phang,* MESA+ Institute for Nanotech. and Dutch Polymer Inst., Netherlands, *T. Ekblad, O. Andersson, B. Liedberg,* Linköping Univ., Sweden, *G.J. Vancso,* MESA+ Institute for Nanotech. and Dutch Polymer Inst., Netherlands, ***A.S. Clare***, Newcastle Univ., UK **INVITED**

Reversible or temporary adhesion allows barnacle cyprids to explore surfaces before they commit to permanent settlement. As such this scarcely studied system is an obvious point of attack for fouling control. The remarkable paired antennules of the cyprid bear attachment discs; hairy adhesive structures that enable the cyprid to walk over surfaces in a stilt-like fashion while resisting detachment by hydrodynamic forces. A proteinaceous secretion at the surface of the disc has been postulated to function as a 'temporary adhesive'. Here evidence is presented that suggests the antennular secretion is related to the adult settlement pheromone – the settlement-inducing protein complex – and that it functions as a reversible adhesive, but in a hybrid wet/dry adhesive system somewhat akin to insect reversible adhesion. While a complete characterisation of the antennular secretion remains an aim, the application of surface analytical techniques (atomic force microscopy and imaging surface plasmon resonance) and direct measures of cyprid behaviour, go some way to providing a mechanistic understanding of why cyprids settle at low rates on certain surfaces, which can be applied to future developments in antifouling technology.

9:40am **MB+BI-MoM5 Chemical Insights on How Shellfish Stick**, *J.D. White, C.R. Matos-Perez, J.R. Burkett, T.W. McCarthy,* ***J.W. Wilker***, Purdue University **INVITED**

Since the very first mariners traversed ocean waters, hulls have become encrusted with fouling organisms such as barnacles, oysters, tube worms, and algae. Antifouling coatings can prevent the resulting loss of vessel performance, but at a cost. Current coatings release toxins into the water, thereby killing the foulers as well as other species. Rather that destroying marine life, stopping bioadhesion processes may provide a benign means of antifouling. Consequently we have been seeking detailed knowledge of how shellfish attach themselves to surfaces. The resulting insights can be used to develop mechanism-based antifouling coatings for inhibiting the production of bioadhesives. Our characterization efforts have focused on the intractable glues and cements of mussels, barnacles, and oysters. A fruitful approach has been to work simultaneously with synthetic peptide models, extracted adhesive proteins, and material produced by the animals. Perspectives from each class of experiments can be complimentary and used to build pictures of how the animals generate their adhesives. Themes in marine bioadhesion are beginning to emerge as well as evidence for unique aspects within each system. Cross-linking of proteins plays a prominent role in curing the glues. Inorganic reactions and related oxidative chemistry also contribute to formation of the materials. Here we will present a summary of our latest findings on how shellfish stick.

10:40am **MB+BI-MoM8 Investigation of Early Marine Biofouling Events on Model Organic and Polymeric Surfaces**, ***G.P. Lopez***, Duke University **INVITED**

Marine biofouling -the accumulation of unwanted biomass on solid structures- is of major concern to maritime pursuits. Biofouling can not only decrease performance of deployed marine equipment, such as ships or oil rigs, but can also result in the transport of invasive species between ports of call. The problem of biofouling is at first a problem of microbial interaction with the water-solid interface; bacteria and diatoms themselves can form detrimental biofilms and can further enable the settlement of macrofoulers. This talk will present studies that seek to shed light on underlying chemical factors that lead to the initial attachment of metabolically homogenous populations of model marine bacterial populations to well-defined organic and polymeric surfaces. It will also present studies of the use of stimuli-responsive surfaces to allow release of attached marine biofilms.

11:20am **MB+BI-MoM10 The Promise of Fouling Deterrence as a Natural Marine Antifouling Strategy**, ***A.S. Mount***, Clemson University **INVITED**

Marine biofouling is the unwanted accumulation of bacteria, algae, plants and marine animals on submerged structures including ships. Unfortunately, man's attempts to develop effective antifouling coatings have had deleterious effects on marine life and a less toxic deterrent to cuprous oxide based paints are needed. Larval marine invertebrates have highly developed sensory organs which investigate surfaces prior to settlement, attachment and metamorphosis. We investigated this tactile chemical sense as a potential natural antifouling strategy by covalently linking the neuroendocrine hormone noradrenaline (NA) to poly(hydroxyethylmethacrylate) and to poly(methacrylic acid) polymer surfaces. NA was selected since it is well established that the soluble form it inhibits larval settlement in mollusks, barnacles, bryozoans and annelid tube worms, all of which are major macrofoulers. The NA conjugate polymer surfaces induced oyster cellular apoptosis when compared to negative controls and also deter the settlement of barnacle and oyster larvae. Fouling deterrence is promising strategy in that only treated surfaces would deter biofouling thus eliminating the need to release of any toxic substances into the oceans.

Marine Biofouling Topical Conference
Room: Navajo - Session MB+BI+AS-MoA

Preventing & Characterizing Marine Biofouling
Moderator: G.P. Lopez, Duke University

2:00pm **MB+BI+AS-MoA1 Zwitterionic Polymers for Non-Fouling Coatings**, ***G. Tew***, University of Massachusetts Amherst **INVITED**
Biofouling remains a challenging problem for various fields ranging from biomedical applications and marine coatings technology, to water purification, transport, and storage systems. To date, the most widely employed protein repellent materials are poly(ethylene glycol) (PEG) or oligo(ethylene glycol) (OEG) based. Even though PEG shows excellent nonfouling character, it has low stability in the presence of oxygen and transition metal ions found in most biochemical solutions, which pushed the field to search for more robust non-fouling materials. Having hygroscopic nature similar to PEG as well as a biomimetic character, arising from their structural similarity to the head groups of lipids comprising cell membranes, zwitterions such as 2-methacryloyloxyethyl phosphorylcholine (MPC) and more recently carboxy/sulfobetaines have also been investigated as protein resistant materials. These materials concentrate only on hydrophilic modification of the substrates. However, the real biological environment is populated by different species, which have different attachment mechanisms; some prefer to adhere more on hydrophilic surfaces whereas others prefer more hydrophobic substrates. The solution to this problem has been investigated by engineering surfaces that reconstruct depending on the environment they are being exposed to, which has been found to be relatively easy to obtain with amphiphilic materials. However, these approaches are either still not sufficient to inhibit bioadhesion by themselves or they suffer from complex or labor intensive coatings preparation conditions. In this work, we are introducing a new polymeric system which carries dual functionality at the repeat unit level, a zwitterionic functionality coupled with an alkyl moiety that can be varied to adjust the amphiphilicity of the overall system. The alkyl group is varied to include PEG based, hydrocarbon, and fluorinated chains. Using these ring-opening metathesis polymerization (ROMP) based zwitterionic polymers as the foundation for non-fouling coatings, we are trying to understand what role the overall hydrophilicity/amphiphilicity of the materials play in fouling prevention.

2:40pm **MB+BI+AS-MoA3 Resistance of Saccharide-Terminated Alkylthiol Self-Assembled Monolayers to Protein Adsorption and Marine Biofouling**, ***T. Ederth***, *T. Fyrner, T. Ekblad, M. Hederos, H.-H. Lee, A. Mangone, P. Konradsson, C.-X. Du,* Linköping University, Sweden, *M.E. Pettitt, M.E. Callow, J.A. Callow,* University of Birmingham, UK, *S.L. Conlan, A.S. Clare,* University of Newcastle, UK, *F. D'Souza, G.T. Donnelly, A. Bruin, P.R. Willemsen,* TNO Science and Industry, The Netherlands, *B.G. Liedberg*, Linköping University, Sweden
The protein resistance of galactoside-terminated alkanethiol self-assembled monolayers (SAMs) can be tuned by partial methylation of the terminating saccharides, and has a non-trivial dependence on the degree of methylation [1], and for other mono- and oligosaccharide-terminated SAMs, protein resistance may vary considerably with small changes in sugar structure. We have used such mono- and oligosaccharide-terminated SAMs in a series of assays using marine fouling organisms as biological model systems, representing common micro- and macrofoulers. We investigate to what extent protein resistance properties are related to effective prevention of fouling by the marine model organisms, and discuss the results in terms of physicochemical properties of the SAMs.

[1] Hederos, M.; Konradsson, P.; Liedberg, B., *Langmuir* **2005,** 21(7), 2971-2980. DOI: 10.1021/la047203b

3:00pm **MB+BI+AS-MoA4 Influence of the Characteristics of a Mineral Coating on its Ability to Resist to the Biofouling**, ***T.H. Tran***, Ecole Nationale Supérieure des Mines de Saint Etienne, France
Mortars are building material with a high primary bioreceptivity and thus, they are easily colonized by different microorganisms... But green algae and cyanobacteria are the main which affect the aesthetics of the facade. Besides the aesthetical problem, an economical problem exists because of the expensive restoration of facade.

This work aimed to study the influence of the intrinsic parameters of a Portland cementitious mortar (roughness, porosity and surface alkalinity) on the algae development in laboratory and also in situ experiments. The degree of fouling was evaluated by means of colorimetric measurements and image analysis.

The roughness played an important role in algae establishment: the higher the roughness, the easier the algae adhesion. The carbonation, reducing surface alkalinity, shortened remarkably the latency time of the fouling onset.

From experimental results, a model was built to predict the fouling of mortar. This model was based on processes such as "germination" - growth. Each rate law was determined separately by image analysis.

3:40pm **MB+BI+AS-MoA6 Influence of Physicochemical Surface Properties on the Settlement of Biofouling Microorganisms**, ***A. Rosenhahn***, Karlsruhe Institute of Technology, Germany **INVITED**
When manmade surfaces are immersed into the ocean, biofouling rapidly occurs. To support the outphase of toxic coating formulations from the market we derive design rules for environmental benign alternatives. Therefore we study the interaction of biofouling organisms such as zoospores of the green seaweed Ulva linza with well defined surfaces and disentangle the influence of wetting, hydration, morphology, and charge. The obtained results are discussed in the context of time depending formation of conditioning layers. Especially because of its motility, the settlement step of Ulva is highly selective and crucial in their life cycle. A detailed investigation of the relevant phases of approach, exploration and eventually settlement is desired but challenging due to the quick, three dimensional swimming motions of spores. Digital in-line holography is suited for this application as time lapse holograms recorded with a single detector provide the 3D position of microorganisms with high accuracy and at a high frame rate. From such 4D tracking data, the sensitive response of spores and their interaction with surfaces has been studied. Statistical analysis of the motion pattern occurrence, velocity distributions and turning motions on surfaces with different chemical termination can be correlated with the accumulated biomass. By this we obtain quantitative access to the interaction between single spores and surfaces.

4:20pm **MB+BI+AS-MoA8 Interfacial Spectroscopy: *In situ* Approaches to Understand Sticky Contacts**, ***K.J. Wahl***, *D.E. Barlow, R.K. Everett, C.M. Spillmann,* Naval Research Laboratory, *G.H. Dickinson, B. Orihuela, D. Rittschof*, Duke University Marine Laboratory **INVITED**
Proteinaceous secretions are widely recognized to be significant contributors to marine biofouling. The resulting interfacial films can be physisorbed or chemisorbed, and have varying degrees of permanency – they may be highly polymerized and cross-linked, or simply sticky enough to allow surface exploration. Conventional approaches to examining interfacial films derived from bioadhesive junctions is forensic in nature – foulant removal (separating the surfaces) followed by *ex situ* examination of the adhesive composition and surface morphology. While "what" the adhesive is may be gleaned from *ex situ* approaches, "how" the adhesive is applied and cures cannot. These time dependent changes can't be examined "after the fact" and instead require real-time measures of interfacial interactions.

At NRL, we have made significant progress in developing *in situ* methods to demonstrate the chemical, mechanical and rheological processes in interfaces. We are now applying and extending these approaches to examine underwater adhesion in marine organisms, specifically the little striped barnacle, *Balanus amphitrite*. We are developing *in situ* and *in vivo* spectroscopic approaches to determine how protein structure and chemistry influence marine foulant adhesion. We are particularly interested in determining the structure and chemistry of the cement, the biochemical processes influencing polymerization, cross-linking, and water displacement, as well as the physiochemical nature of the adhesion. Our *in situ* approaches include performing temporally- and spatially-resolved microscopy and spectroscopy through adhesive interfaces transparent at UV, visible, IR, and x-ray wavelengths. I will describe how we have used these tools to develop new understanding of the properties and development of the adhesive interface of barnacles.

5:00pm **MB+BI+AS-MoA10 Solid State Circular Dichroism of Insoluble Bioadhesive Films: Determining Protein Secondary Structure by Concentration Independent Analysis**, ***D.E. Barlow***, *J.L. Kulp, K.J. Wahl*, U.S. Naval Research Laboratory
Far-UV circular dichroism (CD) is a valuable method for estimating protein structure components. Analysis of protein CD spectra typically requires

deconvolution to resolve overlapping bands and standard methods require that the concentration and pathlength of the sample are accurately known. While this is usually not an issue for the solution state, it is sometimes desirable or a necessity to analyze samples as solid films, complicating deconvolution. Barnacle cement is one example of a proteinaceous bioadhesive that is insoluble by standard biochemical methods and of inconsistent thickness in the native state. To analyze such samples by CD, we have applied g-factor analysis,[1] where the CD spectra are normalized by absorption spectra. This has been demonstrated as a valid, concentration independent deconvolution method, but so far has not been widely used. We will present protein secondary structure estimation results of barnacle cement films as determined by g-factor analysis and show how these results compare with those obtained by infrared spectroscopy. Potential issues and further applicability of solid state CD for bioadhesion studies will be discussed.

[1] McPhie, P. *Anal. Biochem.* **2001**, *293*, 109.

5:20pm **MB+BI+AS-MoA11 Dissipative Microbalance (QCM-D) Studies of Interfacial Processes at the Nanoscale***,* ***M.A. Poggi***, Biolin Scientific

Currently there are many technologies that can study the bulk properties of nanoparticles in solution (such as light scattering) as well as experimental methods that allow one to visualize particles (microscopy or fluorescence). However, there are few technologies that can provide real-time in-situ information regarding how nanoparticles interact with other molecules or materials. Recently we have been using the quartz crystal microbalance with dissipation monitoring technology (QCM-D) to quantify the interaction of particles with surfaces and other materials (biological and organic). We will first present recently published results that address the effect of stagnant and dynamic motion of chemically modified nanoparticles on their adsorption onto silica surfaces. We were able to follow the real-time assembly (in liquid) of these chemically-modified particles. By simultaneously quantifying the changes in surface mass and viscoelasticity during the adsorption process, we were subsequently able to model the adsorption characteristics of these nanoparticles. We will also discuss recent advances that have been made in regards to using QCM-D to follow the assembly of biological nanoparticles (such as cells, viruses and lipids) and polyelectrolytes and touch upon recent electrochemical work that we have been using to study electroactive processes at interfaces.

Biomaterial Interfaces
Room: Taos - Session BI-TuM

Cells on Surfaces
Moderator: M.R. Alexander, University of Nottingham, UK

8:00am **BI-TuM1 Co-Culturing of Osteocytes and Neurons on a Unique Micropatterned Surface: Bone Pain in Cancer**, *M.E. Boggs, W. Thompson,* University of Delaware*, M.C. Farach-Carson,* Rice University*, R.L. Duncan,* ***T.P. Beebe***, University of Delaware

Bone pain is a debilitating problem for patients with bone cancer. Bone pain is the result of communication between the neural and skeletal systems of the body, and yet the mechanisms of communication or signal transduction between cells and tissues comprising these bodily systems is poorly understood. We have developed and are now using a unique micropatterned substrate as a platform consisting of two different proteins to investigate the mechanisms of cell-cell communication between co-cultured osteocyte-like cells and neurons. We have found that an optimal matrix for neurons is laminin type-1, while an optimal matrix for osteocytes is the perlecan domain-IV peptide. The use of micropatterned lanes 40-micron-wide by 1-cm long consisting of alternating laminin type-1 and perlecan domain-IV peptide creates a unique environment in which several osteocyte-neuron meeting events can be observed and probed under controlled conditions. This presentation will build upon our prior work with neuronal cell culture substrates, starting from how they are made, characterized, and then used in experiments to study cell-cell interactions for different cell types.

8:20am **BI-TuM2 Patterned Growth of Human Neural Stem Cells on Surfaces Prepared by Microcontact Printing**, *A. Ruiz,* University Milan, Italy*, L.M. Buzanska, M. Zychowicz,* Polish Academy of Sciences, Poland*, P. Colpo,* ***F. Rossi***, Joint Research Centre, Italy

This work relates to a method of patterning human neural stem cells as a suitable platform for performing different studies of cell proliferation, migration, and differentiation. The patterning of cells has been achieved by using microcontact printing (MCP) to create micropatterns of Poly-L-Lysine (PLL) and Fibronectin (FN), on substrates coated with cell repellent poly ethylene glycol (PEG) deposited by plasma. This substrate is particularly interesting since it is anti adhesive in liquid, but protein adhesive in the dry state. More details of the preparation method are described in [1]. Briefly, microstructured polydimethilsiloxane stamps have been fabricated by casting silicon masters produced by photolithography. The PDMS stamps have been inked with a FN or PLL solution, then dried with a nitrogen stream and put in conformal contact with the PEG substrate. By this method we are able to create fouling (PLL, FN) / antifouling (PEG) contrast on the surfaces where the cells are incubated and by modification of the spot size and distance, the influence of the cell environment on stem cells maintenance and fate studied.

Human Umbilical Cord Blood - Neural Stem Cells [2] were grown on the platforms with PLL or fibronectin pattern. After incubation for 4 days on PLL patterns consisting of 105µm squares spaced 300 µm, the cells are predominantly localized within the square. Such behavior is conditioned by agents added to the incubating medium. After being exposed to dBcAMP, the cells extend neuronal projections outside the squares, but cell bodies are patterned within the active domain. Immunocytochemistry was applied to trace neuronal lineage specific markers and their redistribution on the pattern domain upon influence of differentiating agents. It is found that the maintenance and fate of stem cells can be controlled by a combination of the protein type layer deposited by MCP and culture medium composition: presence of serum, neuromorphogenes and growth factors. Proper arrangement of soluble factors and bioactive surface domains allowed to work out conditions for developmental stage-specific immobilization of neural stem cells to the surface.

1. A. Ruiz, L. Buzanska, D. Gilliland, T. Sobanski, L. Ceriotti, S. Coecke, P. Colpo, F. Rossi. *Micro-stamped surfaces for the patterned growth of neural stem cells.* Biomaterials 29 (2008) 4766-4774.

2. Buzanska, L., Jurga, M., Stachowiak, E.K., Stachowiak, M.K., Domanska-Janik, K. Stem cells Dev., 15, 391–406, 2006.

9:00am **BI-TuM4 Biomolecule Microarrays in Neural Stem Cell Assay Development**, ***D. Mehn***, *J. Nowak,* Joint Research Centre, Italy*, L.M. Buzanska,* Polish Academy of Sciences, Poland*, A. Ruiz, H. Rauscher, G.R. Marchesini, P. Colpo, F. Rossi*, Joint Research Centre, Italy

Human umbilical cord blood originated neural stem cells (HUCB-NSC) are promising candidates for developing human cell based *in vitro* neurotoxicity assays. The origin of the cells makes possible to avoid species specificity extrapolation problems and avoid ethical issues compared to the embryonic stem cell research. Besides the easy cell line like culturing procedure of these cells they were shown to be able to differentiate to neuronal, asctrocytic and oligodendroglial phenotypes. This feature makes them also excellent subjects for developmental neurotoxicity studies.

The extracellular matrix compounds (ECM) used for anchoring the cells on culture dish or device surfaces may induce various ECM-dependent stem cell developmental responses mediated by integrin receptors. Investigation of stem cell - extracellular matrix interactions on biomolecule microarrays provides fundamental knowledge on neural differentiation as well as key inputs for stem cell based assay development.

The most powerful tools for microarray fabrication are microspotting and microcontact printing, offering flexibility regarding composition, concentration, spotted volumes or printed pattern geometry. Both methods are proven to be gentle enough to handle biomolecules for cell interaction studies.

In this work, the piezoelectric spotting and microcontact printing technologies were tested for rapid fabrication of biomolecule arrays on cell repellent surfaces.

Cell anchoring molecules, like extracellular matrix proteins (fibronectin and vitronectin) and poly-L-lysine (a polycationic polypeptide) were printed on anti-adhesive poly(ethylene) oxide-like films deposited by plasma-enhanced chemical vapor deposition. The microspotted and printed patterns were characterized by ellipsometry and microscopic techniques. The morphology of seeded stem cells and expression of certain differentiation marker proteins (GFAP, beta-tubulin III) were visualized by immunostaining and fluorescent microscopy, including also characterization after treatment with various concentrations of a known neurotoxic compound (MeHgCl). Toxicity results obtained on adhesive molecule spots were compared with results of a conventional neutral red uptake assay performed in coated 96 well plates.

Effect of the nature of biomolecules on cell behavior (including cell adhesion, morphology and survival) were investigated and are discussed focusing on generation long term cell-pattern stability, as well as stem cell differentiation into particular cell types.

9:20am **BI-TuM5 Imaging Conformational States of Fibronectin on Patterned Poly(dimethylsiloxane) Surfaces**, ***J. Dechene***, *A. Leclair, P.R. Norton*, University of Western Ontario, Canada

The ability to both understand and direct cellular adhesion is vital in a variety of fields including bioanalysis, medical diagnostics and implant materials design. When a cell approaches a material, its ability to adhere and proliferate will depend greatly on the surface properties of the underlying material. One such material, poly(dimethylsiloxane) (PDMS), has many ideal bulk characteristics but its surface is hydrophobic and does not inherently promote cell growth. To remedy this, many surface treatments of PDMS, particularly plasma treatments, have been used to increase the adhesion strength and bioactivity of PDMS towards cells.

We have recently reported a novel method of patterning the bioactivity of PDMS [1]. We have further exploited the patterning by spatially-selective modification with various biologically active functionalities that permit cell patterning. A different, photolithographic technique has also been developed that exploits the hydrophobic recovery of PDM S, and while we were successful at patterning hydrophobic and hydrophilic areas using this method, cell growth was unfortunately not limited to only the hydrophilic regions on the photolithographically produced patterns.

To understand why the photolithographic method was unsuccessful, we looked both at the ability of a cell to proliferate on the different surfaces, as well as the adsorption of serum proteins, the presence and conformation of which directly affect the adherence of a cell to the surface. To better understand the cell selectivity or lack thereof, fibronectin was adsorbed on the patterned surfaces. A monoclonal antibody HFN7.1 was used to identify exposed integrin binding sites. The antibody was immunofluorescently labeled using a secondary FITC conjugated antibody. The difference in relative availability of binding sites was visualized using confocal microscopy and compared to the relative adsorption of fibronectin, and cell proliferation on the patterned surfaces. The availability of binding sites was

shown to correlate with cell attachment on the stencil-masked patterned surfaces. However an increased availability of binding sites was seen on the hydrophobic recovered regions of the photolithographic patterned surfaces. We plan to directly measure the adhesion forces of chosen cells on the areas of "good" and "bad" conformation.

[1] N. Patrito, C. McCague, P. R. Norton, N. O. Petersen, *Langmuir* **2007**; 23, 2, 715 - 719

9:40am **BI-TuM6 Measuring Contractile Cell Forces on Rigid Substrates**, ***B. Müller***, University of Basel, Switzerland, *J. Köser*, University of Applied Sciences, Switzerland

Mechanical properties of substrates have been shown to be crucial factors for cell behavior, which includes the differentiation of stem cells or the malignant transformation into cancerogeneous cells (Engler et al. (2006) *Cell* **126**, 677 and Cross et al. (2007) *Nature Nanotechnol* **2**, 780). There have been published several techniques to measure contractile forces of cells, exerted onto the underlying substrate, since 1980. These approaches, however, only rely on compliant substrates and not on rigid ones as well known from load-bearing implants and culture dishes.

We present an approach to quantify the contractile forces of an ensemble of cells growing on rigid substrates based on nanomechanical cantilever sensors. In particular, we measured the relaxation of micro-cantilevers as the result of trypsin-release of about 100 fibroblasts. The optically measured change in cantilever bending, detected by means of the Cantisens Research system (Concentris GmbH, Basel), together with the number of fibroblasts counted was converted to the contractile cell force using the STONEY formula.

For the measurement of the contractile cell forces the selected cells were cultured on silicon cantilever arrays. The arrays consist of 8 micro-cantilevers each 500 µm long, 100 µm wide and 1 µm thin that enables us to detect stresses as small as 0.01 mN/m. Following adhesion and contractile force generation over night, the cantilever arrays were introduced into the Cantisens Research system to monitor the cantilever relaxations upon trypsin-mediated release of the cells from the substrate.

When rat2 fibroblasts are seeded on the silicon cantilevers they adhere and develop the morphology indistinguishable from that on standard culture dishes. Upon trypsin-induced release of the cells from the silicon substrate bending, the free end of the cantilever changes its position according to the following function $D = 0.5D_0\ (1\text{-}\tanh(t/\tau))$, where D_0 corresponds to the deflection amplitude and τ the time constant of the cell release. While τ is apparatus and process specific, D_0 directly relates to the contractile forces of an ensemble of cells. The contractile force of an individual rat2 fibroblast on silicon corresponds to (17±7) µN. This value is reasonable, but high compared to the contractile forces of fibroblasts exerted on compliant substrates, a behavior expected from the studies on differently stiff compliant substrates. The contractile cell forces are strongly dependent on the state of the cell that explains the rather large error bar.

The method will support the fundamental understanding of cell-materials interactions with implications for cell-based biosensing and implant design.

10:40am **BI-TuM9 Biological Cell Detachment from Poly(*N*-Isopropyl Acrylamide) and its Applications**, ***H.E. Canavan***, University of New Mexico **INVITED**

Over the past two decades, poly(*N*-isopropyl acrylamide) (pNIPAM) has become widely used for bioengineering applications. In particular, pNIPAM substrates have been used for the non-destructive release of biological cells and proteins. In this work, we review the applications for which pNIPAM substrates have been used to release biological cells, including for the study of the extracellular matrix (ECM), for cell sheet engineering and tissue transplantation, the formation of tumor-like spheroids, the study of bioadhesion and bioadsorption, and the manipulation or deformation of individual cells. The work reviewed includes that of our own group, as well as from those performing research in the field world-wide.

11:20am **BI-TuM11 Rare Earth Nanoparticles - Biocompatibility Studies: Interaction with Human Neutrophil Granulocytes**, *N. Abrikossova,* ***C. Skoglund***, *M. Ahren, L. Selegård,* Linköping University, Sweden, *T. Bengtsson,* Örebro University, Sweden, *K. Uvdal*, Linköping University, Sweden

Rare-earth metal nanoparticles are among the most promising candidates to be used as probes for visualization and targeted drug delivery. Compared to the ion-based gadolinium containing complexes used clinically as contrast agents today, gadolinium oxide (Gd_2O_3) nanoparticles show a considerably improved relaxivity and thus enable an increased resolution and an increased contrast enhancement. However, surface modification of these nanoparticles is essential in order to improve the biocompatibility and diminish any potential toxic effects. In the present study we have evaluated the impact of Gd_2O_3 nanoparticles (as synthesized, dialyzed, and functionalized with polyethyleneglycol, PEG) on the production of reactive oxygen species (ROS) from human neutrophils.

Gd_2O_3 nanoparticles were synthesized (via the polyol route), functionalized with PEG and characterized as previously described in Ahren *et al.*[1]. Neutrophil granulocytes were isolated from heparinized whole blood using density gradient centrifugation. Generation of ROS by neutrophils upon addition of IgG-opsonized yeast, in presence and/or absence of as synthesized, dialyzed and functionalized Gd_2O_3 nanoparticles was studied with luminol-dependent chemiluminescence. In addition, the morphology of neutrophils after interaction with Gd_2O_3 nanoparticles was evaluated by fluorescence microscopy.

The ROS production from neutrophils challenged with IgG-opsonized yeast after exposure to as synthesized Gd_2O_3 nanoparticles was significantly decreased compared to control without nanoparticles. This indicates that the as synthesized nanoparticles are not well suited to be directly used in a living system without further modification. However, after dialysis and functionalization with PEG, no inhibitory effects were observed, possibly indicating that the high concentration of diethylene glycol (DEG) present in the as synthesized nanoparticle preparation is responsible for the inhibitory effects. Indeed, we in the present study also show that even a low concentration of DEG (0.3%) inhibits neutrophil ROS production. Our results indicate that dialyzed and PEG-functionalized Gd_2O_3 particles may be suitable in an in vivo situation as they do not impair the neutrophils capacity to produce ROS in response to a pray.

[1] Ahren M, Selegård L, Klasson A, Söderlund F, Abrikossova N, Skoglund C, Bengtsson T, Engström M, Käll P.O, and Uvdal K, *Synthesis and characterization of PEGylated Gd2O3 nanoparticles for MRI contrast enhancement*. Langmuir 2010, 26 (8) 5753-5762

11:40am **BI-TuM12 Synchrotron Radiation X-Ray Fluorescence Mapping of Cobalt Ferrite Nanoparticles in BALB 3T3 Fibroblast Cells**, ***G. Ceccone***, *P. Marmorato, J. Ponti, F. Rossi,* EC-JRC-IHCP, Italy, *B. Kaulich, A. Gianoncelli, M. Kiskinova,* Elettra Sincrotrone Trieste, Italy, *M. Salomé,* ESRF Grenoble France, *R. Ortega, G. Deves, A. Carmona,* University of Bordeaux, France, *L. Pascolo*, Elettra Sincrotrone Trieste, Italy

Recent efforts in development of multifunctional nanoscale materials, and in particular nanoparticles (NPs) for use as drugs delivery, targeted therapeutic and imaging agent have made significant progress [1, 2]. Magnetic NPs and in particular cobalt ferrites (CoFe2O4), offer some attractive possibilities in biomedicine as drug delivery carriers, hyperthermia treatments in cancer therapy and magnetic resonance imaging (MRI) contrast enhancement [3, 4].. However, along with developing their vast implementation there is a growing concern about the health hazards related to the possible toxic effects of the NPs. The lacking information about the NPs impact on environment and on human health as well as data on risk assessment requires reliable methodology for control and prediction. Among the promising detection methods is the synchrotron radiation x-ray fluorescence (SRXRF) that has already demonstrated its potential in bio-medical research exploring e.g. neurodegenerative disorders [5, 6]. Extending the application of SRXRF using soft X-rays [7] has also provided access to light elements natural constituents of the living matter. In this work we report SRXRF investigation of the distribution and chemistry of CoFe2O4nanoparticles on balb3T3 mouse fibroblast cells exposed to NPs concentrations ranging between 40 and 1000 uM for 24h. The SRXRF maps and micro-spot spectra indicate that, for concentrations below 500 uM, the NPs are localized in the perinuclear region, whilst at higher concentration they penetrate also in the nuclei, where the Fe/Co ratio indicates that the cells are not able to counteract the toxic effect of NPs chemical components (i.e. cobalt). A co-localization of P, Ca and Fe at high concentration has also been observed indicating intracellular sequestration mechanisms as a response or in an attempt to reduce the nanoparticles toxic effects. Preliminary PIXE (Proton Induced Ion Emission) measurements support the XRF results indicating that at 250uM the Fe and Co are localized around the nucleus whilst Ca, P, S, K, Zn and Na are uniformly distributed in the cell.

References

[1] T. Paunesku at al., Nanoletters, 7(3), (2007), 596.

[2] L.A. Nagahara, M. Ferrari, T. Groodzinski, MRS bulletin, 34(6), (2009), 406.

[3] D.H. Kim et al, J. Mag.Mag. Mat. 320(2008), 2390

[4] J.A. Ritter, et al., J. Mag. Mag. Mat., 288, (2005), 403

[5] A. Ide-Ektessabi, Application of Synchrotron Radiation: Microbeams in cell Microbiology and Medicine, Springer, (2007)

[6] R. Ortega et al., Nucl. Inst. Met. B210 (2003), 325

[7] R. Alberti et al., X-ray Spectrometry 38 (2009), 205-209

Biomaterial Interfaces
Room: Taos - Session BI1-TuA

Bacteria on Surfaces
Moderator: L.J. Gamble, University of Washington

2:00pm **BI1-TuA1 High Throughput Methodologies for the Discovery of Materials Resistant to Biofilm Formation**, *A.L. Hook, J. Yang, C.-Y. Chang,* University of Nottingham, UK, *D.G. Anderson, R. Langer,* Massachusetts Institute of Technology, *S. Atkinson, P. Williams, M.C. Davies,* ***M.R. Alexander***, University of Nottingham, UK

Biofilm formation leads to a 1000 times increase in antibiotic tolerance compared with planktonic bacteria and is associated with 80% of hospital acquired infections, resulting in $3.0 billion in excess health-care costs each year in the U.S alone. Thus, new materials for biomedical devices that prevent biofilm formation would offer enormous benefits to the health industry and patient welfare. Polymer microarrays are emerging as a key enabling technology for the discovery of new biomaterials.[1] This platform enables a large combinatorial space to be rapidly screened by a biological assay to identify new materials that fulfil a given performance criterion.[2] Furthermore, utilising a high throughput surface characterisation approach the surface chemical and physical properties of each material can be understood and related to the biological performance in order to understand the material-biological interaction.[3] A method for forming polymer microarrays has been developed using contact printing to deposit nanolitre volumes of premixed acrylate monomer and initiator to defined locations on a poly(HEMA) coated glass slide with UV photo-initiation.[4] We have developed a high throughput bacterial attachment assay based on GFP transfected pathogens that is compatible with the polymer microarray format. In our high throughput strategy we initially produced an array containing hundreds of unique materials that was designed to maximise the combinatorial space explored. From this array 'hit' monomer compositions were identified that were used to design a second generation array that explored systematic variations in material compositions in order to focus onto the optimal material composition. This has been utilised to identify new materials that resist the formation of bacteria and show promise for implementation to various biomedical devices such as urinary tract catheters that are susceptible to bacterial colonisation.

[1] A. L. Hook, D. Anderson, R. Langer, P. Williams, M. C. Davies, and M. R. Alexander, *Biomaterials* **2010**, *31*(2), 187.

[2] Y. Mei, S. Gerecht, M. Taylor, A. J. Urquhart, S. R. Bogatyrev, S. W. Cho, M. C. Davies, M. R. Alexander, R. S. Langer, D. G. Anderson, *Adv. Mater.* **2009**, *21*(27), 2781.

[3] A. J. Urquhart, D. G. Anderson, M. Taylor, M. R. Alexander, R. Langer, M. C. Davies, *Adv.Mater.* **2007**, *19*(18), 2486.

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2:20pm **BI1-TuA2 Surface Self-Assembled PEG Gel Particles to Control Bacteria-Biomaterial Interactions**, ***Y. Wu***, *Q. Wang, M. Libera*, Stevens Institute of Technology

The fact that desirable tissue cells and undesirable bacteria compete for the surface colonization of an implanted biomaterial is now well recognized. When bacteria win this competition, the resulting infection can lead to device failure with substantial consequences to both the patient and the health-care system. We are developing poly(ethylene glycol) [PEG] -based gel particles with which to modify surfaces and differentially control surface interactions with both tissue cells and bacteria. We are particularly interested in modulating the surface cell adhesiveness at micro/nano length scales with the goal of reducing staphylococcal adhesion while still enabling the adhesion, spreading, and proliferation of desirable tissue cells. In short to preserve healing while reducing the probability of infection. We have synthesized anionically charged PEG-acrylic acid (AA) copolymer hydrogel particles by inverse emulsion polymerization and used a bottom-up electrostatic self-assembly approach to modify otherwise cell-adhesive surfaces with cell-repulsive gel particles. Zeta potential measurements confirm that the gel particles are negatively charged because of the acid groups. SEM imaging and dynamic light scattering show that the particle diameters range from ~10's to ~ 100's of nm. We have electrostatically deposited them on both polylysine-modified silicon wafers and titanium metal coupons. By varying the concentration of gel particles in solution and the deposition time, we can control the area density of particles deposited on the substrate surface to levels of ~ 0.1 – 2 particles/sq micron. Immunofluorescence imaging shows that, relative to unmodified Si and PLL primed Si, PEG-modified Si has substantially lower colonization by *S. epidermidis* after innoculation and 4 hrs of culture. Confocal imaging of PEG-modified surfaces after 4 days of osteoblast culture show good osteoblast spreading and proliferation. SEM images indicate that the osteoblasts grow over the cell-repulsive particles while adhering to the remaining adhesive surface. Such surfaces may be useful in reducing the susceptibility of biomedical devices to biomaterials-associated infection.

2:40pm **BI1-TuA3 Some Strategies and Results for Antibacterial Coatings**, ***H.J. Griesser***, *K. Vasilev, H. Ys, C.P. Ndi, S.S. Griesser, S. Al-Bataineh, S. Semple*, University of South Australia **INVITED**

Bacterial attachment and subsequent biofilm formation might be reduced by application of a thin coating that deters bacterial colonisation. For biomedical devices a coating should also allow good attachment of human tissue to facilitate wound healing, or for catheters and contact lenses be lubricious and not bio-adhesive. Requirements differ for antibacterial coatings for different implants and devices; accordingly we have used different approaches for the fabrication of several antibacterial coatings. For long-lasting effect, we prefer the approach of covalently immobilising antibacterial molecules; we have also investigated the alternative approach of release of silver ions. This presentation will review advantages and disadvantages of various approaches, and discuss open questions.

Our strategies are based on plasma polymer thin film coatings, because this approach can be transferred to coat many polymeric, metallic and ceramic materials. Plasma polymers with chemically reactive surface groups enable covalent immobilisation of antibacterial compounds onto their surface. Alternatively, we load plasma polymer coatings with silver nanoparticles, from which Ag^+ ions can outdiffuse. Organic antibacterial compounds investigated were furanones, novobiocin, and serrulatanes, the latter are novel substituted diterpenes extracted from Australian plants used in traditional medicine. The chemical composition of coatings was assessed by XPS and ToF-SIMS to ensure that the intended coatings were achieved. Samples were tested for bacterial attachment and for biofilm formation, as well as for mouse 3T3 fibroblast cell attachment.

Surface-immobilised furanones, Novobiocin, and serrulatanes reduced bacterial attachment by up to 99.8%. While large biofilm communities formed on control surfaces within 48 hrs, these coatings prevented biofilm formation. Plasma polymer coatings loaded with Ag nanoparticles also were effective; Ag^+ delivery can be adjusted via the properties and thickness of the plasma polymer film and the silver loading. Testing of coatings with m3T3 fibroblast cell cultures showed, however, that in many cases there were adverse effects. Silver in particular affected 3T3 cells. With organic antibiotics, the surface density appears important and an optimum must be found between deleterious cell effects and antibacterial effectiveness.

Important questions remain: do surface-immobilised antibiotics act as in solution, as quorum sensing inhibitors (furanones) or gyrase inhibitors (Novobiocin)? Do in vitro and in vivo tests correlate? How to mitigate adverse effects on mammalian cells? Why is there contradictory literature especially on Ag?

Biomaterial Interfaces
Room: Taos A - Session BI2+AS-TuA

Combining Techniques for Biointerface Characterization
Moderator: L.J. Gamble, University of Washington

4:00pm **BI2+AS-TuA7 Spatial and Depth Characterisation of Immobilised Biomolecules on Surfaces**, ***G. Mishra***, *A.J. Roberts,* Kratos Analytical Ltd., UK, *D.J. Surman,* Kratos Analytical Ltd., *S.L. McArthur*, Swinburne University of Technology, Australia

Analysis of biomolecules on surfaces is essential to various applications of biosensors and biomolecule engineering. Matrix-assisted laser desorption/ionisation (MALDI) is now a well established technique for mass spectrometry of biomolecules. Different matrix-analyte preparation protocols have been shown to influence the desorption or ablation process resulting in either high or low metastable fragmentation. It has been speculated that following laser ablation the velocities of the analyte and matrix can be regarded as a valuable and meaningful characteristic of the MALDI process. However, the interaction and distribution of the analyte with respect to the matrix is poorly understood. Here we study the distribution of a selection of biomolecules as a function of matrix material using high resolution imaging x-ray photoelectron spectroscopy (XPS). We feel that understanding the depth distribution of biomolecules in the matrix

remains factor which would enable us to draw conclusions on the incorporation of the analyte in the matrix. Thus in this study we also investigate the depth distribution using organic depth profiling techniques.

4:20pm **BI2+AS-TuA8 Measuring the Orientation of Electrostatically Immobilized Proteins by Time-of-Flight Secondary Ion Mass Spectrometry and Sum Frequency Generation: From a Model Protein G B1 System to Cytochrome**, ***J.E. Baio***, *T.M. Weidner, L. Baugh, P.S. Stayton, L.J. Gamble, D.G. Castner*, University of Washington

The ability to orient proteins on surfaces to control exposure of their biologically active sites will benefit a wide range of applications including protein microarrays and biomaterials that present ligands to bind cell receptors. As methods to orient proteins are developed, techniques are required to provide an accurate picture of their orientation. Since no single technique provides a high-resolution image of surface-bound proteins, combinations of surface analytical techniques are required. In this study, we have developed a model system based on the electrostatic immobilization of a small rigid protein (Protein G B1 domain, 6kDa) to further develop the capabilities of time-of-flight secondary ion mass spectrometry (ToF-SIMS) and sum frequency generation (SFG) spectroscopy as tools to probe the orientation of surface immobilized proteins. A Protein G mutant (D4) exhibiting net positive and negative charges at either end (for pH 6-8) was produced by neutralizing four negatively charged residues closest to the end of the protein (Asp to Asn or Glu to Gln mutations). These mutants were then immobilized onto NH_3^+ and COO^- terminated self assembled monolayers (SAMs) to induce opposite end-on orientations. ToF-SIMS data from the D4 variant on both NH_3^+ and COO^- SAMs showed intensity differences from secondary ions originating from asymmetric amino acids (Asn:70, 87, and 98m/z; Met:62m/z; Tyr:107 and 136m/z at the N-terminus. Leu:86m/z at the C-terminus). For a more quantitative examination of orientation, we developed a ratio comparing the sum of the intensities of ions stemming from residues at either end of the protein. The 50% increase in this ratio, observed between the NH_3^+ and COO^- SAMs, indicated opposite orientations of the D4 variant on the two different surfaces. In addition, SFG spectral peaks characteristic of ordered α-helix ($1645cm^{-1}$) and β-sheet (1624 and $1675cm^{-1}$) elements were observed, with a phase that indicated a predominantly upright orientation for the α-helix, consistent with an end-on protein orientation. We then moved from this model system and extended this analysis to examine the change in orientation of horse heart Cytochrome c on both NH_3^+ and COO^- SAMs. The positively charged region at one end of Cytochrome c binds to the COO^- substrate while the NH_3^+ surface elicits the opposite binding orientation. Again, within the SFG spectra, ordering of the protein α-helices were confirmed by the feature at $1645cm^{-1}$ and the change in orientation, induced by the two different substrates, is confirmed by intensity differences within ToF-SIMS spectra between ions stemming from asymmetric amino acids (Glu:84 and 102m/z; Asp:72 and 88m/z).

4:40pm **BI2+AS-TuA9 NanoBio Imaging for Cardiovascular Researches**, ***D.W. Moon***, *T.G. Lee, J.Y. Lee, W. Jegal, S.W. Kim*, KRISS, Republic of Korea **INVITED**

NanoScience has been developed to meet the demands on atomic scale characterization and manipulation of materials and devices from semiconductor industries based on the scaling down law. KRISS has been trying to extend the application scope of nanoscience and technology from microelectronics to biomedical areas. Biochemical imaging of cells and tissues is a basic infra-technology in various bio-medical applications. Instead of conventional labeling methodology for bio-molecular imaging with fluorescent dyes, label-free biochemical imaging methodologies for single cells and tissues such as coherent anti-stokes Raman scattering (CARS), secondary ion mass spectrometry (SIMS), and surface plasmon resonance imaging ellipsometer (SPRIE) has been developed and integrated for new biomedical applications, especially for cardiovascular researches.

Preliminary results of nanobio imaging for cardiovascular researches will be reported on the following issues 1) Three-dimensional visualization of atherosclerotic tissue and prompt on-site analysis of chemical profiles by multiplex CARS with intracellular lipids at the single-cell level as well as crystallized cholesterol in necrotic cores. (1) 2) Histological Imaging based on SIMS analysis of myocardial infarction tissues. (2) 3) cell adhesion dynamics of human carotid smooth muscle cells and human umbilical endothelial cells on fibronectin thin films with SPRIE.

Finally, the present status and future challenges of nano-bio technology based on laser, mass spectrometry, and nanoprobe for biochemical imaging of single cells and tissues at KRISS will be discussed for practical applications in bio, medical, and pharmaceutical researches.

(1) "Multiplex coherent anti-Stokes Raman spectroscopy images intact atheromatous lesions and concomitantly identifies distinct chemical profiles of atherosclerotic lipids", Se-Hwa Kim, Eun-Soo Lee, JaeYong Lee, EunSeong Lee, Bok-Soo Lee, JeongEuy Park ,and DaeWon Moon, Circulation Research, in press (2010)

(2) "ToF-SIMS Analysis of Myocardial Infarcted Tissue", J.-W. Park, M.-J. Cha, H. K. Shon, S.-H. Kim, T. G. Lee, D. W. Moon, and K.-C. Hwang, Surface and Interface Analysis, in press (2010)

5:20pm **BI2+AS-TuA11 Determining Antibody Orientation using ToF-SIMS and Fluorescence Imaging of Affinity-generated Patterns**, ***M. Dubey***, Los Alamos National Laboratory, *F. Liu, H. Takahashi, D.W. Grainger,* University of Utah, *D.G. Castner*, University of Washington

This study assesses the capability of high-resolution surface analytical tools to distinguish immobilized antibody orientations on patterned surfaces designed for antibody affinity capture. High-fidelity, side-by-side co-patterning of protein A (antibody Fc domain affinity reagent) and fluorescein (antibody Fab domain hapten) was achieved photo-lithographically on commercial amine-reactive hydrogel polymer surfaces. This was verified from fluorescence imaging using fluorescently labeled protein A and intrinsic fluorescence from fluorescein. Subsequently, dye-labeled murine anti-fluorescein antibody (4-4-20), and antibody Fab and Fc fragments were immobilized from solution onto respective protein A- and fluorescein- co-patterned or control surfaces using antibody-ligand affinity interactions. Fluorescence assays support specific immobilization to fluorescein hapten- and protein A-patterned regions through antigen-antibody recognition and natural protein A-Fc domain interactions, respectively. Affinity-based antibody immobilization on the two different co-patterned surfaces generated side-by-side full antibody "heads-up" and "tails-up" oriented surface patterns. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) analysis, sensitive to chemical information from the top 2-3 nm of the surface, provided ion-specific images of these antibody patterned regions, imaging and distinguishing characteristic ions from amino acids enriched in Fab domains for antibodies oriented in "heads-up" regions, and ions from amino acids enriched in Fc domains for antibodies oriented in "tails-up" regions. Principal component analysis (PCA) improved the distinct ToF-SIMS amino acid compositional and ion-specific surface mapping sensitivity for each "heads-up" versus "tails-up" patterned region. Characteristic Fab and Fc fragment immobilized patterns served as controls. This provides first demonstration of pattern-specific, antibody orientation-dependent surface maps based on antibody domain- and structure- specific compositional differences by ToF-SIMS analysis. Since antibody immobilization and orientation are critical to many technologies, orientation characterization using ToF-SIMS could be very useful and convenient for immobilization quality control and understanding methods for improving the performance of antibody-based surface capture assays.

5:40pm **BI2+AS-TuA12 Molecular Depth Profiling and 3D Imaging of Biological Samples by ToF-SIMS: From Model Amino Acid Films to Real Biological Cells**, ***J.P. Brison***, *N. Wehbe,* University of Namur, Belgium, *D.G. Castner,* University of Washington, *L. Houssiau*, University of Namur, Belgium

Time-of-Flight Secondary Ion Mass Spectrometry is now routinely used to obtain molecular information about the surface of complex biological samples and biomaterials. The recent advent of cluster ion beams such as Bi_3^+ and C_{60}^+ provides enhanced sensitivity for high mass fragments, which in turn allows 2D imaging of specific biomolecules with improved sensitivity and sub-micrometer lateral resolution. Molecular depth profiling of biological samples with low chemical damage and high depth resolution (*i.e.*, < 10 nm) has also been shown to be possible by etching with cluster projectiles such as C_{60} and with low energy cesium ions. Since ToF-SIMS also allows the detection of all elements without the need of specific markers, the technique shows great potential for molecular 3D imaging of single cells and could become an inevitable complementary tool to MALDI and fluorescence microscopy for biomedical research in the near future.

However, only few examples of full 3D images of biological cells have been reported in the literature at this time. This limitation probably comes from the facts that preparing the cells for chemical analysis under UHV environment is tedious, and that our understanding of energetic primary ions/biological matter interactions is limited. Fundamental studies of these aspects are indeed difficult during 3D imaging because the cells are complex, heterogeneous, non-flat samples with relatively unknown molecular composition.

In this work, fundamental biological matter/projectile interactions were studied by depth profiling model amino acid films under different ToF-SIMS conditions. The influence of the experimental parameters on the quality of the depth profiles was investigated by measuring the sputter rates, the depth resolutions and the intensities of the molecular ion signals with respect to the chemistry of the target (*e.g.*, arginine vs phenylalanine), the nature of the primary ion species (*e.g.*, C_{60}^+ vs Cs^+) and the bombardment conditions (*e.g.*, fluence and energy). Then the complexity of the model

samples was increased by mixing several amino acids and by creating multilayer films. This approach was another step toward the analysis of real biological samples. Again, ToF-SIMS fundamentals were investigated by measuring, *e.g.*, the interface widths between the different organic layers and the signal decay due to the increasing etching fluence. Finally, optimal 3D images of single HeLa cells were acquired and were discussed based on the data obtained for the model amino acid films. The effect of the sample preparation on the quality of the images was also investigated.

Biomaterial Interfaces
Room: Taos - Session BI1-WeM

Biomolecules at Interfaces
Moderator: P. Kingshott, Aarhus University, Denmark

8:00am **BI1-WeM1 Lipid Membrane Interface Mediated Protein Misfolding and Aggregation**, ***E.Y. Chi***, University of New Mexico, *J. Majewski,* Los Alamos National Laboratory, *E. Mandelkow,* Max Planck Unit for Structural Molecular Biology, Germany, *K.Y. Lee*, University of Chicago

The misfolding and aggregation of the amyloid-beta (Ab) peptide and tau protein into fibrillar deposits are linked to the pathogenesis of Alzheimer's disease (AD). However, the molecular basis of the early events during the aggregation process and the nature of the structural fluctuations that triggers the misfolding and association of Ab and tau remain poorly understood. The lipid membrane interface has been implicated to mediate the fibrillogenesis of both proteins. Using model lipid membranes, we studied the nature and mode of lipid-protein interactions and characterized the effect of these interactions on the conformation and assembly of Ab and tau.

Both Ab and tau exhibit strong interactions with membranes composed of charged lipids, but interact weakly with zwitterionic lipids. To elucidate the molecular-scale structural details of Ab-membrane association, we used complementary X-ray and neutron scattering techniques (grazing-incidence X-ray diffraction, X-ray reflectivity, and neutron reflectivity) to investigate *in situ* the association of Ab with lipid monolayers at the air/water interface composed of either the negatively charged lipid DPPG, the zwitterionic lipid DPPC, or the cationic lipid DPTAP at the air/water interface. We found that the anionic lipid DPPG uniquely induced crystalline ordering of Ab at the membrane surface that closely mimicked the beta-sheet structures in fibrils, revealing an intriguing templated ordering effect of DPPG on Ab. Furthermore, incubating Ab with lipid vesicles containing the anionic lipid POPG induced the formation of amyloid fibrils, confirming that the templated ordering of Ab at the membrane surface seeded fibril formation. By measuring the interaction between different tau constructs (hTau40, K18 and K32) with membranes composed of different lipids, our data showed that tau's C-terminus, the microtubule binding domain, is responsible for its association with the lipid membrane. Moreover, hyperphophorylation which is an early and critical event in the pathogenesis of AD, as mimicked by a tau mutant, did not prevent tau from binding to lipid membrane.

Our study provides a detailed molecular-scale characterization of the early structural fluctuation and assembly events that may trigger the misfolding and aggregation of Ab *in vivo.* Our study suggests that the "soft", intrinsically unfolded nature of both Ab and tau can give rise to rich dynamic behaviors at interfaces, such as the lipid membrane interface. Our data implicate that the adsorption of Ab and tau to anionic lipids in the cell membrane may serve as an in vivo mechanism of templated aggregation and drive the pathogenesis of AD.

8:20am **BI1-WeM2 Effect of Metal Ions on Lipid Bilayer Formation on Semiconductor Surfaces**, ***R. Jain***, *A.J. Muscat*, University of Arizona

Lipid bilayers have applications in drug delivery, bio-sensing for clinical diagnosis, and device fabrication. Just as in a living cell where a lipid bilayer separates aqueous compartments from their surroundings, a lipid membrane supported on a surface can function as a mask that allows selective mass transport via intermembrane proteins. Lipid bilayers have been used primarily to support proton channel proteins in sensors, but there are many other types of intermembrane proteins with different functions. With an aim to extend the use of biomolecules in device fabrication, the effect of heavy metal ions on bilayer formation was investigated using atomic force microscopy (AFM) and x-ray photoelectron spectroscopy (XPS). 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) lipid molecules were used to form supported bilayers by the vesicle fusion method on hydroxylated Si, As_2O_3-terminated GaAs, and Al_2O_3 surfaces. Alumina was deposited on a p-Si(100) surface by atomic layer deposition (ALD) at 170°C using trimethylaluminum (TMA) and water as precursors. The bilayer was formed on an AFM stage for 6 hr and the stage was heated to 41.5±5°C, which is 17.5°C above the DMPC phase transition temperature. The height of lipid membranes measured with AFM after digging a hole was 4.9±0.5 nm on a hydroxylated Si surface and 4.0±0.6 nm on an alumina surface, which correspond to the thickness of a bilayer. The mechanical strength, uniformity, and integrity of membranes were measured after flowing copper sulfate solutions (100-3000 ppm) through a liquid flow cell over the formed bilayer on the Al_2O_3 surface. Al_2O_3 was chosen because Cu^{2+} ions are reduced to Cu^0 on alumina, but not on SiO_2. It was found by XPS that the copper permeates through the lipid bilayer and deposits on the alumina surface as Cu^0. Force distance measurements were made to understand copper permeation. The adhesion force of copper on Al_2O_3 was 2-3 times higher than that of lipid molecules, leading to breaking of the bilayer and deposition of copper. AFM confirmed the breakage and the bilayer thickness after copper exposure was 1.4±0.2 nm. This study shows that metal ions with a higher adhesion force than lipid molecules on an insulator surface disrupt bilayer formation, placing limitations on how bilayers can be used in device fabrication. These results also suggest an additional mechanism for the antibacterial properties of copper.

8:40am **BI1-WeM3 Characterizing Carbohydrate-Modified Surfaces: Advancing the Glycomics Paradigm**, ***D.M. Ratner***, University of Washington **INVITED**

Carbohydrates and glycoconjugates are involved in a myriad of biological processes, including fertility, cancer, the immune response, and host-pathogen interactions. The carbohydrate microarray (or glycoarray) has emerged as one of the most promising technologies capable of revealing the complex roles played by carbohydrates in biology and medicine. While the glycoarray has had a significant impact on the field of glycomics (the study of carbohydrates in biology), little is known about the function of surface chemistry on array performance. In addition, existing glycoarray technologies are non-standardized, utilize disparate chemistries, and are only partially optimized to interrogate low affinity interactions or discriminate between mono- and multivalent binding. To help realize the glycoarray's full potential in glycomics research, new label-free and high-throughput diagnostic tools are needed to screen glycan-dependent interactions and expand our fundamental understanding of glycobiology. The reengineered glycoarray must also include a quantitative picture of glycan surface chemistry to advance our ability to match array results with the biological question or hypothesis being tested.

This study details the development a panel of carbohydrate-functionalized ultrasensitive label-free biosensors based on surface plasmon resonance imaging (SPRi), novel silicon photonic devices, and microelectrode microarrays. To define the role of biosensor surface chemistries, we describe the application of advanced surface analytical techniques to exhaustively characterize the biointerface of carbohydrate-modified surfaces for biosensor applications. X-Ray Photoelectron Spectroscopy (XPS), Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), and Scanning Probe Microscopy (SPM) are used to examine the chemistries of glycoarray surfaces to establish the relationship between biosensor performance and glycan surface density and molecular conformation—highlighting the contributions of the surface analysis and biointerfaces communities in advancing the nascent field of glycomics.

9:20am **BI1-WeM5 *In situ* Characterization of DNA Films**, ***C. Howell***, *P. Koelsch*, University of Heidelberg and Karlsruhe Institute of Technology, Germany

Films of thymine, adenine and cytosine single-stranded DNA (homo-oligonucleotides) immobilized on gold were characterized in liquid under various conditions using broadband sum-frequency-generation (SFG) spectroscopy. Spectra of the three film types under these *in situ* conditions in the C-H stretching region were significantly different than those obtained in air and appeared to show unique contributions based on the nucleobase composition of the film. This could be the result of base-specific hydration differences for these films. Data in air showed no identifiable unique base contributions in this region; however, significant differences in peak intensities among the three film types were evident in the spectra. These differences appeared to correlate with the degree of order of the films, suggesting that SFG spectroscopy can be useful for detecting overall order in these types of systems. Hybridization behavior of these systems was also studied, with results showing detectable differences between hybridized and unhybridized films. These results contribute to the understanding of DNA films and help to establish a foundation for the investigation of more complex biomolecules *in situ* using SFG spectroscopy.

9:40am **BI1-WeM6 Surface Characterization of Mixed DNA/Mercaptoundecanol Assembly on Gold**, ***N. Vandencasteele***, *L. Árnadóttir, J.E. Baio, T.M. Weidner, L.J. Gamble*, University of Washington

The hybridization efficiency of DNA microarrays and biosensors is determined in part by variables such as the density and orientation of the single stranded DNA oligomers used to build the devices. In order to better understand the chemistry on the microarray surfaces, and therefore improve their response, we study model surfaces of DNA adsorbed on gold with x-

ray photoelectron spectroscopy (XPS), near-edge x-ray absorption fine structure (NEXAFS) spectroscopy and time-of-flight secondary ion mass spectrometry (ToF-SIMS) to characterize the surface order and structure. We previously showed that varying the amount of diluent molecule, and thus the probe density, affects the hybridization efficiency of a 20mer thiolated single stranded DNA probe and the target.[1,2] In this study we compare the density and orientation of a 40mer single-stranded thiolated DNA (HS-ss-DNA), when varying amounts of the diluent molecule 11-mercapto-1-undecanol (MCU) are adsorbed. DNA is detected on the surface using XPS (N1s and P2p peaks) and ToF-SIMS analysis. ToF-SIMS results for various backfill times and various backfilling media are compared using PCA analysis. In the first set of experiments the MCU backfill reaction takes place in water (ultra pure 18 MΩ), in the second set of experiment it takes place in sodium Tris EDTA buffer solution (STE). We showed that when the co-adsorption reaction is done with a solution of MCU in water, the amount of MCU that incorporates into the surface saturates at 18h and the amount of DNA on the surface remains relatively constant. When the same experiment is carried out using a MCU solution in STE buffer the MCU binding removes the surface bound DNA and all of the HS-ss-DNA is removed from the surface after 2h, suggesting a better hydration of the DNA in the STE medium. The improved hydration of the DNA increases it's mobility in the solution allowing an easier access of the MCU to the gold surface. Angle dependent NEXAFS spectra taken for DNA layers after different MCU backfill times in water showed the highest degree of orientational order after a 30 min MCU backfilling step. The data also indicate that the DNA nucleotide base rings are ordered parallel to the surface. Hybridization efficiencies of the 40 mer DNA layer with various densities will be compared with surface plasmon resonance (SPR).

(1) Gong, P.; Lee, C.-Y.; Gamble, L. J.; Castner, D. G.; Grainger, D. W. *Analytical Chemistry* 2006, *78*, 3326-3334.

(2) Lee, C.-Y.; Gong, P.; Harbers, G. M.; Grainger, D. W.; Castner, D. G.; Gamble, L. J. *Analytical Chemistry* 2006, *78*, 3316-3325.

Biomaterial Interfaces
Room: Taos A - Session BI2-WeM

Proteins & Peptides on Surfaces
Moderator: P. Kingshott, Aarhus University, Denmark

10:40am **BI2-WeM9 The Surface Mediated Assembly of Small Biomolecules Relevant to Prebiotic Chemistry**, ***D.J. Frankel***, *L. Donlon*, Newcastle University, UK

11:00am **BI2-WeM10 Stabilization of Proteins by Sol-Gel Encapsulation and Study of its Interactions with the Host Matrix**, ***S. Sammeta***, *G. Doroudian, G.P. Lopez, E.Y. Chi*, University of New Mexico

Utilization of biological molecules such as proteins in biosensing and biofuel cell applications is one of the most innovative research fields. A major challenge that needs to be overcome in these applications is the adequate stabilization of proteins. Porous silica material made by the sol-gel process provides a promising host matrix for the encapsulation of proteins to increase their stability. The main objective of this study is to elucidate protein-host matrix interactions for the stabilization of proteins. In our study, lysozyme and cytochrome c were used as model proteins. We have developed two novel vapor exposure sol-gel techniques to encapsulate proteins using tetra methyl orthosilicate (TMOS) as a precursor: (I) in-situ protein exposure method, where a buffer containing a protein is directly exposed to TMSO vapor, and (II) buffer exposure method, where a buffer was first exposed to TMOS vapor and then a protein was added. Additionally, organically-modified glasses are used to study the effect of host-matrix hydrophobicity on protein structure and stability. Circular dichroism and high resolution derivative UV spectroscopy are used to evaluate the structure and thermal stability of encapsulated proteins. The effect of protein concentration and sucrose (a model osmolyte) on the structure and stability of encapsulated proteins are characterized. Cytochrome c retained native-like structures while lysozyme becomes partly unfolded when encapsulated in the silica matrix. Entrapment generally increases the thermal stability of proteins. Proteins encapsulated via the in situ technique are found to have higher thermal stability compared to those encapsulated using method II. In general, method II gave rise to proteins with more secondary structure. However, proteins in this matrix are less resistant to thermal denaturation. In addition, there are concentration-dependent decreases in protein secondary structure when encapsulated by method II. We believe that the trends are due to protein adsorption onto silica which causes denaturation. Osmolytes shift the protein native state ensemble towards more compact conformations, thereby increasing the conformational stability of proteins. We observed enhanced secondary structure of cytochrome c with the addition of 0.5 M sucrose and this enhanced structure and stability is preserved when the protein is encapsulated in silica gel in the presence of sucrose. The effect of increasing the host-matrix hydrophobicity by incorporating alkyl group substituted alkoxysilanes into the silica matrix on protein structure and stability will be presented.

11:20am **BI2-WeM11 ToF-SIMS Imaging to Characterize DNA Microarray Surfaces**, ***L.J. Gamble***, *N. Vandencasteele, L. Arnadottir, D.G. Castner,* University of Washington, *D.W. Grainger*, University of Utah

Commercial DNA array slides are commonly made by microprinting techniques. These nanoliter doplets evaporate within seconds and this fast drying may contribute to heterogeneous spots and inconsistent results. Successful development and optimization of DNA-functionalized surfaces for microarray and biosensor applications requires a better characterization of immobilized DNA. In this work, time-of-flight secondary ion mass spectrometry (ToF-SIMS) is applied to the study of spotted DNA surfaces on commercial microarray slides. ToF- SIMS results are directly compared to fluorescence images. Maximum Autocorrelation Factor (MAF) image analysis, a technique independent of the scaling of the raw data, is used to analyze the ToF-SIMS images. An IONTOF TOF.SIMS 5-100 instrument using a Bi source is used to analyze the samples. Immobilized DNA probes with 10, 20 and 40μM DNA concentrations as well as different Cy3 label concentrations are spotted on a commercially available microarray polymer slide. The effect of the spotting solution concentration as well as the amount of Cy3 label on spot uniformity is studied. MAF analysis of the ToF-SIMS image for a 20μM DNA spot shows that the areas seen as having high fluorescence intensities are related to higher concentrations of phosphate groups (from the DNA backbone) as well as sulfates and peaks with masses corresponding to the DNA bases. A comparison of MAF analysis of ToF-SIMS images for different DNA spotting concentrations indicates that the concentration of the spotting solution has an effect on the uniformity of the spot.

11:40am **BI2-WeM12 Protein Nanopatterning for Studying Cell Adhesion**, ***S. Kristensen***, *J. Malmström, J. Lovmand, M. Duch, D.S. Sutherland*, Aarhus University, Denmark

Synthetic materials are often used for biomedical applications. Interaction of cells with the interfaces and tissue components determine the biological outcome of the device. Knowledge about the interaction between the cells and biointerfaces is hence of importance in area such as biomaterials, tissue engineering and cell culture. The interaction of the cells with its surroundings is mediated at the molecular and macromolecular level. Specific interaction with the extracellular matrix components or macromolecules in the outer membrane of adjacent cells provides signaling and communication pathways. Here patterns of extracellular matrix protein are used to study the development of cellular adhesion complexes.

Protein nanopatterns at the 100-3000 nm scale and with lateral ordering between independent ligands and controlled lateral mobility has been made by using a nanoscale chemical contrast of Au patches in a background of SiO_2 by colloidal lithography. The nanostructured surfaces are made by depositing a triple polyelectrolyte layer (PDDA/PSS/PAX) at Au substrates. Latex particles self assemble at the surface governed by electrostatic forces followed by SiO_2 evaporation and removal of the particles. The generated short range ordered arrays were further modified by octadecylmercaptane adsorption. The samples were subsequently treated with PLL-*g*-PEG for 30 min. followed by adsorption of fibronectin for 2 h. Myoblast cell(C2C12) or MDA-MB-435 cells were added to the samples and allowed to adhere to the surfaces for 6 h or 24 hours.

Fibronectin distribution at the nanopatterned surfaces was studied via liquid AFM showing that protein were adsorbed preferentially on the alkane thiol patches. SEM images showed that protein was patterned over large areas. Protein patterns of several other proteins such as Osteopontin, Vitronectin and Laminin were also demonstrated. Fluorescent microscopy showed that cells adhered to the patterns of size from 200nm and up. Small focal complexes were observed at the 200nm structures which were not linked to the actin cytoskeleton. For 500nm and 1000nm patchs cell showed small focal adhesions connected to thin actin fibers and the adhesions were limited to individual patches.

We utilize colloidal lithography to fabricate protein patterns of size from nano to micro scale and from different proteins. The patterned areas are of a sufficiently large area to carry out large scale cellular characterisation in terms of adhesion morphology and differentiation. The protein patterning makes it possible to limit the length of developing focal adhesions to single patches and hence alter the cells ability to generate forces, spread and move.

Biomaterial Interfaces
Room: Taos - Session BI-WeA

Proteins & Peptides on Surfaces
Moderator: H.E. Canavan, University of New Mexico

2:00pm **BI-WeA1 Interaction of Amphiphilic Antimicrobial Peptides with Phospholipid Membranes, Bacteria, and Cells**, ***M. Malmsten***, Uppsala University, Sweden **INVITED**

Due to increasing resistance development, antimicrobial peptides (AMPs) are receiving increasing attention since these may provide rapid and broad-spectrum response to a host of pathogens. In addition, some of these peptides provide also strongly antiinflammatory responses, and are therefore promising in therapies of both acute and chronic inflammation. Critical for their antimicrobial action is the interaction between AMPs and bacteria membranes, where significant current efforts are directed to identifying peptides being potent antimicrobials, yet simultaneously displaying low toxicity. In inflammation, additional aspects are of importance, including interaction with lipopolysaccharide and other bacterial components. In our efforts to adress these and other challenges in the development of such peptides to practical therapeutics, our research addresses various aspects of interaction of AMPs with lipid membranes, bacteria, and cells. Focusing on endogenous peptides generated during normal microbial infections, we combine basic biophysical investigations on various aspects of AMP-membrane interactions with modern biotechnological tools for peptide design, and with biological experiments including bacteria, cells, and various animal models. Some recent examples of the work done in these contexts will be provided, aiming at synthesizing biophysical and biological aspects of these peptides.

2:40pm **BI-WeA3 Curcumin Offers Neuroprotection by Inhibiting amyloid-β Insertion into Membranes**, ***A. Thapa***, *B. Gilver, E.Y. Chi*, University of New Mexico

Alzheimer's disease (AD) is a major cause of dementia in elderly people, affecting 5 million people in USA alone. AD is caused by the abnormal accumulation of aggregated amyloid beta peptides (39 to 43 amino acid residues) in the brain. Amyloid beta peptides are proteolytic products of the amyloid precursor protein of unknown function. Unfortunately, there are no cures available for this disease. However, there are several mechanisms purposed to cause and cure AD. The lipid membrane has been shown to mediate the fibrillogenesis and toxicity of Alzheimer's disease amyloid beta (Aβ) peptide. Several reports have linked the insertion of Aβ peptide into membranes as a possible mechanism of neurotoxicity. We hypothesized that small molecules capable of preventing the insertion of Aβ into membranes may ameroliate Aβ toxicity. Therefore, we investigated the effect of curcumin, a naturally occurring anti-inflammatory and antioxidant compound that suppresses oxidative damage, inflammation, cognitive deficits, and amyloid accumulation, on Aβ40 induced toxicity and in Aβ40 insertion into 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG) monolayer using surface pressure insertion isotherms and fluorescence microscopic techniques. We found that curcumin attenuates Aβ40 induced neuronal toxicity by inhibiting the insertion of Aβ into membranes possibly by interacting with membranes. Our data also demonstrated that neuroprotective action of curcumin in Aβ induced toxicity does not exclusively come through oligomerization inhibition, indicating that c urcumin-membrane interaction but not curcumin-Aβ is associated in curcumin mediated neuroprotection. Altogether, our study suggests that curcumin-like small molecules inhibitors of Aβ insertions into membranes could be potential target to cure AD.

3:00pm **BI-WeA4 Nonspecific Protein Adsorption Requires Large Adhesive Domains on the Surface**, ***L. Shen***, *X.-Y. Zhu*, University of Texas, Austin

We study the dynamics of protein adsorption using nm – mm scale patterns involving hydrophobic domains in hydrophilic matrices. We report the discovery of a critical requirement on the sizes of adhesive pads for protein adsorption: the area of each adhesive pad must be more than two orders of magnitude larger than the footprint of a protein molecule before irreversible adsorption occurs . We attribute this to the minimal surface area sampled by a mobile protein molecule in a precursor state before irreversible adsorption occurs.

4:00pm **BI-WeA7 Study of Adsorption and Orientation of $FnIII_{7-10}$ Fibronectin Fragment on Self-Assembled Monolayers using Time of Flight Secondary Ion Mass Spectrometry**, ***L. Árnadóttir***, *J. Brison, L.J. Gamble*, University of Washington

Protein adsorption and orientation plays a critical role in many biomedical applications. Fibronectin (FN) is an extracellular matrix protein that is involved in many cell processes such as adhesion, migration and growth. The orientation and conformation of FN adsorbed onto surfaces can therefore play a critical role on cell-surface interactions. In this study, the adsorbed orientation and conformation of the 7-10 fragment of FNIII was studied on three different model surfaces (self-assembled monolayers (SAMs) of C_{11} alkanethiols on Au with $-CH_3$, $-NH_2$, and -COOH functional groups). X-ray photoelectron spectroscopy (XPS) was used to quantify the amount of protein adsorbed on the different surfaces and time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to characterize their orientation and conformation. A trehalose coating was also used to inhibit the conformation changes due to the dehydration of the sample. With the help of principal component analysis (PCA), the peaks which are responsible for the variance observed between the spectra relative to the protein adsorbed on the different surfaces could be identified. Because the surface sensitivity of the ToF-SIMS technique is lower than average protein size, these changes in the spectra reflect differences in the conformation and the orientation of the FN fragment. Comparison of trehalose protected and unprotected samples show a significant difference in the ratio between hydrophilic and hydrophobic amino acids. The results suggest that the more hydrophilic amino acids stay on the outside of the trehalose protected protein while the more hydrophobic ones get exposed to the protein air interface upon drying. Comparison of the trehalose protected fragment on $-CH_3$ and -COOH terminated SAMs show more intense signals of Arg and Asp on the -COOH surface and more intense Val, Pro and Leu signals on the $-CH_3$ SAMs. The detection of these different amino acids for the protein on the different SAMs suggests that the fragment might partly denature upon adsorption to the hydrophobic surface.

4:20pm **BI-WeA8 Temperature Controlled Dehydration of Protein Films: Time-of-flight Secondary Ion Mass Spectrometry Study of Conformational Mobility of Proteins in Vacuum**, ***H.P. Bui***, *T.P. Beebe, Jr.*, University of Delaware

Once a biomaterial is implanted, a film of protein will adsorb onto the surface and it is this protein film that will dictate how the biomaterial will interact with the surrounding cells and tissue. Two strategies to increase the success rate of biomaterials are to passivate the surface so that it is resistant to protein adsorption, or to activate the surface to obtain a desired cell response. One method of activation is the grafting of one or more proteins onto the surface that direct specific cellular interactions. However a thorough understanding of the protein film's composition, conformation and orientation is needed in the development of these advanced biomaterials. Time-of-flight secondary ion mass spectrometry's (TOF-SIMS) high surface specificity, analytical sensitivity and ability to provide long-range molecular information can be used to probe protein composition, conformation and orientation. In this study, we demonstrate that the use of temperature-programmed dehydration and principal component analysis can be used as a method of determining the conformation and orientation of protein films. We have found that TOF-SIMS is sensitive to the dehydration of the protein film and the accompanying conformational changes

4:40pm **BI-WeA9 Structure and Function of von Willebrand Factor on Glass, Polystyrene, and Tissue Culture Polystyrene**, ***E. Hillenmeyer***, *R.A. Penkala, W. Thomas, D.G. Castner*, University of Washington

von Willebrand Factor (vWF) is a blood-soluble clotting protein responsible for binding platelets through the glycoprotein 1b (GP1b) receptor on the platelet surface. vWF can become activated and bind platelets when bound to exposed collagen in blood vessels or when vWF experiences increased shear.

vWF can also bind platelets when adsorbed to synthetic surfaces and participate in clot formation, which is not desirable for blood-contacting biomaterials. There is evidence that surface properties can influence vWF adsorption. Previous studies showed differences in protein topography (1) and conformation (2) when vWF was adsorbed on mica (1), octadecyltrichlorosilane modified glass (1,2), and collagen VI (2). However, studies were not performed to relate adsorption differences to vWF function.

To more fully characterize the adsorption properties of vWF, we adsorbed the platelet binding domain of vWF (A1 domain) to three surfaces: polystyrene, tissue culture polystyrene, and glass. Protein structure was investigated using x-ray photoelectron spectroscopy (XPS) and time of

flight secondary ion mass spectrometry (ToF-SIMS). Protein function was tested by measuring platelet binding in a physiologically relevant flow assay.

Using nitrogen as a marker of protein, XPS showed similar amounts of vWF A1 adsorbed to the three surfaces. However, the flow assay showed significantly different platelet binding to vWF A1 on each surface, as measured by platelet rolling velocity. Rolling velocity was highest on glass, indicating lowest platelet binding. The slowest rolling velocity was observed on polystyrene, indicating the highest level of platelet binding. ToF-SIMS data was analyzed using principle component analysis (PCA). PCA showed separation of the three surfaces with adsorbed vWF A1, indicating conformational differences between the proteins on each surface.

These studies show that surface properties influence structure and function of adsorbed vWF domains. Although there was a similar amount of protein on each surface, protein function was different. Polystyrene, the most hydrophobic of the surfaces, appeared to have the strongest activating effect on vWF. ToF-SIMS studies showed conformational differences, suggesting that conformational differences contribute to the observed functional differences.

Understanding the structure and function of adsorbed vWF gives insight into how vWF behaves on biomaterial surfaces and how this might affect platelet binding. Characterizing vWF adsorption also allows *in vitro* behavior to be more accurately related to *in vivo* thrombosis events.

Raghavachari. *Colloids Surf B* (2000) 19:315.

Kang. *Thromb Res* (2007) 119: 731.

5:00pm **BI-WeA10 Secondary Structures of Soft- and Reactively Landed Multiply Charged Protein Ions**, ***Q. Hu***, *P. Wang, J. Laskin*, Pacific Northwest National Laboratory

Soft- and reactive landing of mass-selected ions enables highly selective preparation of uniform thin films of a variety of complex molecules on surfaces. We previously demonstrated that conformationally-selected peptide arrays can be prepared using SL of peptide ions onto self-assembled monolayer (SAM) surfaces. In this work we studied the secondary structures of protein ions soft- and reactively landed onto SAM surfaces using infrared reflection absorption spectroscopy (IRRAS). Different charge states of ubiquitin were generated by electrospray ionization (ESI). The structure of the low charge state corresponds to the pseudo-native state of the protein and the high charge state corresponds to an unfolded state. Inert CH_3-terminated SAM (HSAM) and hydrophilic COOH-terminated SAM (COOH-SAM) were used as soft- landing targets. Detailed analysis of IRRAS spectra, especially the amide I band, provides valuable information on the secondary structures of the immobilized protein species. This technique allows us to study the effect of the initial conformation and the properties of the surface on the secondary structure of immobilized proteins. Secondary structure of ubiquitin ions reactively landed onto SAM of N-hydroxysuccinimidyl ester terminated alkylthiol on gold (NHS-SAM) was also studied by IRRAS, and the reaction rate was determined from the depletion of the strong asymmetric carbonyl stretching band of the NHS group.

5:20pm **BI-WeA11 Analysis of Unspecific Protein Adsorption onto Polymer Materials using Radioactive Labeling, Atomic Force Microscopy and ELISA**, ***M. Holmberg***, *X.L. Hou*, Technical University of Denmark

In this study radioactive labeling is used in combination with ELISA measurements and Atomic Force Microscopy (AFM) analysis to investigate aspects of unspecific protein adsorption onto polymer materials. The radioactive labeling is a setup in which different proteins are labeled with isotopes that emit gamma radiation with different energies. This makes it possible to detect several proteins simultaneously onto the same sample and thus to investigate competitive protein adsorption and how the presence of some proteins influence the adsorption of others. Results from protein adsorption onto polymer materials using the radioactive labeling setup have shown adsorption levels higher than expected for monolayer adsorption and suggest the existence of protein multilayers on some surfaces. Results from fibrinogen adsorption onto surfaces that are pre-adsorbed with albumin show that fibrinogen can adsorb on top of albumin and that exchange of already adsorbed albumin is not a dominant process during the competitive adsorption with fibrinogen. Preliminary results on QCM-D (quartz crystal microbalance with dissipation monitoring) also strengthen the idea of the existence of an interface between polymer surface and protein solution in which proteins interact with both each other and the surface in a matrix structure that have multilayer character. To further illustrate the impact on (or lack of) unspecific protein adsorption using blocking buffers and pre-adsorption of proteins, we show results from ELISA measurements of unspecific protein adsorption onto TCPS (tissue culture polystyrene) and PS (polystyrene). We can detect large difference in adsorption level between proteins with and without a HIS tag (six histidines) onto TCPS, but not onto PS, with the HIS tagged proteins showing much higher adsorption onto TCPS compared to the same protein without a HIS tag. Furthermore, low or none impact on the level of adsorption of these HIS tagged proteins is observed when the TCPS surfaces are blocked with BSA (bovine serum albumin). We are combining quantitative results from radioactive labeling (and QCM-D) with AFM analysis performed in liquid to obtain data regarding homogeneity and topography of adsorbed protein layers. Furthermore, ELISA is used as a supplementary technique to acquire more knowledge regarding unspecific adsorption of proteins onto polymer materials. The obtained information is of importance when evaluating interactions between proteins and biomaterials.

5:40pm **BI-WeA12 Surface Interactions of GG-X-GG and X_n Oligopeptides with Inorganic Substrates**, ***K.P. Fears***, *J.L. Kulp, T.D. Clark, D.Y. Petrovykh*, Naval Research Laboratory

The adsorption behavior of model GG-X-GG and X_n oligopeptides on Au and native Si oxide substrates was investigated to elucidate the contributions of different amino acids (AAs) to peptide-surface interactions. The manner in which peptides and proteins interact with surfaces is of critical importance in many biological and technological systems. The mechanisms underlying surface adsorption of proteins, however, are poorly understood, largely due to the inherent complexity of natural proteins. Accordingly, in this work simple model peptides were chosen to systematically examine the interactions between natural AAs and inorganic surfaces. Surface interactions of a series of AAs were probed by incubating inorganic substrates in aqueous solutions of model GG-X-GG pentapeptides, in which an AA of interest was flanked with Gly. The effects of cooperative adsorption were also examined using model X_n oligopeptides (n = 5, 10). The amount of peptides that *irreversibly* adsorbed on each substrate was quantified by X-ray photoelectron spectroscopy (XPS), the resulting systematic data revealed several trends in surface adsorption of oligopeptides as a function of their composition and length. On the negatively-charged, hydrophilic native SiO_x layer of a Si wafer, only peptides containing positively-charged residues (Lys and Arg) and polar residues (Ser and Thr) adsorbed at significant levels. Peptides adsorbed more readily on Au-coated Si wafers, on which the maximum surface coverage was ca. 3 times greater than that on the native SiO_x. For a particular AA (X), adsorption tended to increase, sometimes dramatically, with increasing units of X ($GG\text{-}X\text{-}GG < X_5 < X_{10}$). In pairs of AAs having side chains that only vary by alkyl chain length (L and V, Q and N, R and K, E and D), the AA with the longer alkyl chain adsorbed more readily, although this trend diminished with the increasing number of X residues.

In Situ Microscopy and Spectroscopy Topical Conference
Room: Acoma - Session IS+BI+AS-WeA

In Situ Microscopy/Spectroscopy – Biological Interfaces
Moderator: M. Grunze, University of Heidelberg, Germany

2:00pm **IS+BI+AS-WeA1 Adsorption and Phase Transition of Liposomes via Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy**, ***M.R. Hernandez***, *T.C. Ng, E.N. Towns, B.C. Walsh, D.P. Land*, University of California at Davis

Liposomes are becoming increasingly prevalent as an important part of drug delivery systems in modern medicine, however a better understanding of the physical characteristics is needed. In this study we present our results on the stability and adsorption of liposomes formulated from dipalmitoylphosphatidylcholine (DPPC) via attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. The phase transition temperature of liposome formulations of pure DPPC, DPPC and cholesterol, and DPPC, cholesterol, and 1,2-Distearoyl-phosphatidylethanolamine-methyl-polyethyleneglycol -2000 (DSPE-mPEG2000) is determined using a temperature dependant study (25°C to 50°C) and been found to occur abruptly around 41°C for pure DPPC liposomes and exhibit gradual temperature changes from 35°C to 43°C for the other two liposome formulations. We have studied the adsorption characteristics of different formulations of liposomes with both hydrophobic and hydrophilic surfaces created by different self-assembled monolayers, and will present a new method for studying an in vitro way of studying the adsorption of different formulations of liposomes onto a surface of immobilized proteins. Knowing the stability of liposomes and liposome-protein adsorption characteristics allows for a better understanding of their use and design of future formulations in drug delivery systems.

2:20pm **IS+BI+AS-WeA2 Synchrotron Based Infrared Imaging at the Diffraction Limit**, *J. Nasse,* University of Wisconsin-Milwaukee, *C. Gohr, A. Rosenthal,* Medical College of Wisconsin, ***C. Hirschmugl***, University of Wisconsin-Milwaukee

A new mid-infrared beamline (IRENI) extracting a large horizontal swath of radiation (320 hor. x 25 vert. mrads^2) to homogeneously illuminate a commercial IR microscope equipped with an infrared Focal Plane Array (FPA) detector has recently been commissioned at the Synchrotron Radiation Center in Stoughton, WI. This new facility provides the opportunity to obtain chemical images with diffraction-limited resolution, for all wavelengths in the mid-IR concurrently, in minutes. The design of this facility and an initial application will be presented.

IRENI combines a bright IR synchrotron source to an FTIR microscope with a multi-element detector for wide-field imaging as opposed to the common dual-aperture geometry with raster scanning that is available at most synchrotron IR beamlines. The swath of radiation from the SRC is extracted as 12 beams and recombined into a 3 x 4 bundle of beams that is refocused onto a sample plane of an infrared microscope illuminating 40 x 60 micron^2 sample area. The sampled spatial resolution is defined by both the magnification after the sample and the FPA pixel size. Here, a 74x Schwarzschild objective achieves effective geometric pixel sizes of 0.54 x 0.54 micron^2, which is approximately $\lambda/4$ for even the shortest wavelength of 2 μm. This spatial oversampling provides adequate information to obtain concurrent, diffraction-limited images across the entire spectral range. In addition, the spectral quality is excellent, since the high density, stable, broadband flux from the synchrotron achieves high quality spectra for 0.54 x 0.54 micron^2/pixel using similar measuring times as table-top instruments that image 5.5 x 5.5 micron^2/pixel.

The presence of calcium-containing crystals, including calcium pyrophosphate dihydrate (CPPD) and hydroxyapatite-like basic calcium phosphate (BCP), in synovial fluids plays a major role in cartilage degeneration in osteoarthritis. Models of calcium crystal formation tend to produce small, sparse crystals embedded in debris enriched in proteins, lipids, and carbohydrates, which interfere with many identification techniques. Synchrotron FTIR imaging circumvents difficulties in identifying these crystals and also allows for characterization of the surrounding matrix. We present results from well-characterized models of calcium crystal formation that demonstrate our ability to both identify crystals *in vitro* and characterize the matrix surrounding these crystals.

This work has been done with support from an NSF Major Research Instrumentation grant (DMR-0619759) and the Synchrotron Radiation Center, which is also supported by NSF (DMR-0537588).

2:40pm **IS+BI+AS-WeA3 Proteins and Lipids at Liquid/Solid Interfaces: *In situ* Studies by Neutron Reflectometry and Infrared Spectroscopy**, *M. Strobl, M. Kreuzer,* University of Heidelberg, Germany, *M. Reinhardt, R. Steitz,* Helmholtz Zentrum Berlin, Germany, *M. Grunze,* ***R. Dahint***, University of Heidelberg, Germany **INVITED**

Proteins and lipids at liquid/solid interfaces are of crucial importance in the design of biofunctional interfaces. For example, adsorbed protein layers determine the biocompatibility of implants and may control bacterial adhesion. Upon surface contact, proteins commonly undergo structural changes, which will alter their activity and biological function. In combination with lipids, proteins are valuable model systems to mimic cell membrane function. Thus, in order to improve our understanding of biofunctional interfaces, a strong need exists to develop surface analytical tools, which facilitate *in situ* characterization on a molecular level.

Due to its *in situ* capability, non-destructive character and the short wavelength of neutron beams, neutron reflectometry offers a very attractive approach to the analysis of layer structures on the nanometer scale. It provides detailed information on the amount of adsorbed species as well as on the thickness, density and hydration of the absorbate. In combination with surface sensitive infrared spectroscopy (ATR-FTIR), additional information is obtained on specific molecular groups of the adsorbate as well as on molecule conformation.

We will report on the set-up of a new time-of-flight neutron reflectometer at the Helmholtz Center Berlin, which is especially adapted to biological samples and, for the first time, facilitates simultaneous *in situ* ATR-FTIR characterization. Dedicated sample environments have been developed to study biological films as a function of applied pressure, shearing forces and temperature. As a potential application, we discuss the phase behavior and stability of immobilized oligolamellar lipid bilayer films under load and shear, which are important in bio-lubrication and the search for advanced implant materials, such as artificial joints. A second example will focus on the impact of surface chemistry and structure on the activity of immobilized proteins.

4:00pm **IS+BI+AS-WeA7 Biological Imaging with Coherent X-rays: The Lens-less Approach to High Resolution**, ***A. Beerlink***, Universität Göttingen, Germany **INVITED**

Understanding molecular functions in complex environments such as biological cells or novel composite materials are a prerequisite for the advancement of nano and biomedical sciences. They require a combination of high spatial resolution, quantitative contrast and

full compatibility with environmental conditions, such as aequous media. To this end, the potential of x-ray imaging is not yet fully developed, but currently undergoes rapid progress. While classical x-ray microscopy based on Fresnel zone plates has matured and provides useful structural information in a growing range of applications, this technique is severely limited by the nanostructuring process of the lenses. In

recent years, novel lens-less approaches for imaging have emerged, where the object functions are reconstructed from the measured intensities

either in the far-field regime, or under near-field conditions (propagation imaging). We present experiments using x-ray quasi point sources to illuminate the sample in combination with digital recording of the resulting diffraction patterns. One focus is the applicability towards biological samples, for which the imaging properties of the different coherent microscopy approaches will be compared. In this context, recent results obtained with ultrabright femtosecond pulses provided by the free electron laser FLASH will be presented and accessible information complementary to synchrotron imaging will be discussed.

4:40pm **IS+BI+AS-WeA9 Dielectric Constant and Polarization of Biomolecules Determined by Torsional Resonance Nanoimpedance Microscopy**, ***K. Kathan-Galipeau***, *S.U. Nanayakkara, P.A. O'Brien, B.M. Discher, D.A. Bonnell*, University of Pennsylvania

We have developed a new technique, torsional resonance nanoimpedance microscopy (TR-NIM), that allows for the measurement of frequency-dependent local transport properties on soft materials. AFM measurements at torsional resonances provide a key advantage: the ability to achieve low-force scanning while maintaining the tip in the near-field. As a result, it is possible to measure impedance between the tip and sample without damaging the sample.

This technique has been used to determine the resistance, capacitance, and dielectric properties of a novel class of biomolecules. These redox active molecules, known as maquettes, consist of dimers and tetramers of alpha helix polypeptides and provide a convenient functional alternative to natural proteins. Maquettes are capable of binding a range of cofactors; this study examines the properties of iron and zinc porphyrins. Maquettes serve as a benchmark for integrating electronics with biologically inspired materials that possess unique characteristics, such as electron-transfer capability, the possibility of gating redox activity, optoelectronic functionality, and nanometer size.

In order to determine the behavior of these functional biomolecules on electrodes, PDMS stamping was used to create stripes that alternate between maquettes and bare graphite. Stamping also allowed us to control the assembly of the redox-active maquettes from multilayers to horizontally oriented monolayers (maquettes laying down) and vertically oriented monolayers (maquettes standing up) by varying the stamping time and the concentration of the maquettes in organic solvents.

Interestingly, we observe that the resistance decreases with increased height of maquettes, which is explained in terms of the configurations of the molecules on the electrode. The dependence of local impedance on exposure to optical radiation revealed an increase in capacitance and decrease in resistance when the maquettes are exposed to 425 nm light. This is true for both zinc and iron porphyrin cofactors. We attribute the decrease in resistance to photoactivated current. The increase in capacitance is due to an increase in the polarizibility of the maquettes.

5:00pm **IS+BI+AS-WeA10 Dynamic Observation of Phospholipid Model Cell Membranes and Particles by STM and Vibrational Spectroscopy**, ***T. Yamada***, RIKEN, Japan, *S. Matsunaga,* The University of Tokyo, Japan, *T. Kobayashi,* RIKEN, Japan, *M. Kawai*, The University of Tokyo, Japan

Scanning tunneling microscopy (STM) and other surface-scientific techniques can be utilized to explore the microscopic dynamics of biological molecules in the context that the techniques are applicable for solid surfaces immersed in aqueous solutions. We devised STM and vibrational spectroscopies to make usable for molecular monolayers at solid-liquid interface. We attempted to observe phospholipid layers formed on octanethiol-terminated gold (111) single-crystalline substrates placed in aqueous buffer solutions (in situ STM). By in situ STM we could observe dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), a relatively short kind of lipid, forming a fluidic monolayer. A crystalline phase of this monolayer was observed by applying an electrode potential compatible with the

membrane potentials of real cells. Furthermore, mixed lipid layers have been examined by STM [1]. We found some nanometer-scale raft structures (phase-separated domains), which are functionally characteristic for real cell membranes. We also studied phospholipid particles suspended in buffer solutions. Suspensions were prepared from a phosphcholine (PC) and an ethanolamine (PE), consisting of nanometer-scale phospholipid particles with narrow size distribution. In situ STM revealed particles with a diameter ~ 10 nm (named "minimal lipid particles (MLP)"), forming a monolayer along the Au(111). It is known that some categories of antibiotics selectively attack lipids contained in germ cell membranes and disintegrate the whole cells. We chose "duramycin", a 19-residued peptide antibiotic, which specifically binds PE. When the total concentration of phospholipid was controlled between 100 μM and 500 μM, a layer of MLP was discerned. During STM scanning, 7 μM of duramycin solution was added into the suspension, and the PC+PE MLP became fragile and seemed to be scratched by the tip, ending up with a widespread multilayer. This sort of highly leveraged effect of duramycin is characteristic in the action of antibiotics [2]. These works demonstrated the advantage of STM in monitoring the live nanometer-scale reactions of biological entities, which have not been recognized experimentally so far. We expect more application of STM in physiological investigation in cell biology.

[1] S. Matsunaga et al., *Electrochem. Commn*. **9** (2007) 645.

[2] S. Matsunaga et al., *Langmuir* **25** (2009) 8200.

5:20pm **IS+BI+AS-WeA11 Rapid In-Situ Assessment for Microbes on Simultaneously Prepared Plate with Substrate and Zirconium Based Thin Film Metallic Glasses (TFMGs)**, ***P.T. Chiang***, I-Shou Univ./Fooyin Univ. Hospital, Taiwan, Republic of China, *G.J. Chen, H.H. Liu, Y.H. Shih,* I-Shou Univ., Taiwan, Republic of China, *J.P. Chu,* National Taiwan Univ. of Science and Technology, Taiwan, Republic of China, *J.S.C. Jang*, National Central Univ., Taiwan, Republic of China

ZrAlNiCuSi TFMGs could modify the stainless steel's surface with high hardness, scratch-adhesion capabilities. Zr-based TFMGs' smooth surface could decrease and prolong the lag phase of microbes' growth for at least 24 hours.

The actual numbers of pathogenic bacteria might be underestimated by conventional methods due to sublethal injury, malnutrient's and other physiological factors which reduce bacterial viability. Moreover, these methods would limit the real-time quantitative detection and easily cause contaminations with bias.

Rapid comparisons in the same culture condition are obtained on a simultaneously prepared plate with substrate and Zr based TFMGs. By utilization of GFP plasmid (pGLO) into HB101 with 10mM arabinose induction, we could measure the intensity of green fluorescence by LAS-3000 fluorescent detector to setup the real-time monitor system for observation of bacterial growth on TFMGs' surface.

This integrated method was time-saving, cost-effective and simple. The serially rapid in situ monitor of the microbial growth will emerge as a novel tool to realize the TFMGs or other materials' antimicrobial properties.

Biomaterial Interfaces
Room: Taos - Session BI1+NS-ThM

Replicating Biological Environments and Processes
Moderator: E.O. Reimhult, University of Natural Resources and Applied Life Sciences, Switzerland

8:00am **BI1+NS-ThM1 Biofunctionalized Micro- and Nano-cup Arrays by Plasma Polymer Templating**, ***R. Ogaki***, *M.A. Cole, D.S. Sutherland, P. Kingshott*, Aarhus University, Denmark

We present a novel fabrication method for creating an array of 'cups' on the micro- and nano scale by using a combination of plasma polymerization (pp), self assembled monolayers (SAMs) and colloidal lithography (CL).The method uses polystyrene (PS) particles that are first self-assembled into a hexagonal close-packed (HCP) structure onto a desired substrate over a large area via the lift-off method. The assembled particles are reduced in size by plasma etching and a plasma polymer is deposited into the interstitial spaces between the particles. The particles are subsequently removed by ultrasonication, forming an array of plasma polymerized cups with controllable sizes (through particle choice) and chemistries (through plasma monomer choice). The plasma polymer does not coat the contact region between the particles and the substrate. Thus a chemical pattern is generated, in our case, when SAMs are assembled onto the exposed substrate region. This provides a platform for site specific immobilization of biomolecules and cells with a diversity of chemistries possible. The method can be extended to other types of coatings such as those from physical vapor deposition (PVD), prior to the removal of the particles. As a result, up to three different chemistries can be presented on the array, with the first chemistry on the uppermost surface, the second chemistry on the internal wall and the final chemistry on the particle-substrate contact region inside the cup. The structural and chemical success of the cups and the patterns are determined by scanning electron microscopy (SEM), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), time of flight secondary ion mass spectrometry (ToF-SIMS). The method is adaptable to create micro- and nano-sized cups by the appropriate choice of particle sizes. The versatility of the method to tune the cup sizes and the potential to incorporate up to three chemistries is apparent, thus the presented fabrication method could be potentially utilized for immobilizing a range of multiple biological cells and molecules of different sizes inside the cups for applications such as multi-functional biosensors or for carrying out specific reactions inside the cups for biological studies.

8:20am **BI1+NS-ThM2 Fabrication of Functional Hydrogel Nano-structures for Biomolecule Immobilization**, ***R.T.S. Lam***, *J.-W. Jang, P.L. Stiles, S.R. Nettikadan*, NanoInk Inc.

Hydrogels have been used extensively for tissue engineering scaffolds and other biomedical applications because of their unique three-dimensional cross-linked polymer network that provide structural support while endowing an environment similar to natural tissue. Fabrication of hydrogels in submicron scale is greatly desirable; however structures with well-defined organization and high uniformity are not easily achievable by using traditional methods. Herein, we have demonstrated the printing of thiolated PEG hydrogels on a glass chip with precise control over the architecture and feature size using dip-pen nanolithography (DPN) techniques. This direct and reliable method for generating hydrogel patterns may serve as useful tools to explore cell-substrate interactions. In addition, conjugating different proteins through the free thiol functional groups in hydrogels is a promising approach of functionalizing the substrate with different biomolecules. This can be used as a platform for high throughput screening of protein-cell interaction studies. We have shown specific immobilization of thiol-reactive rhodamine red maleimide molecules on to the hydrogel patterns. Yellow-fluorescent was observed exclusively at the patterned area. By adjusting the ratio between the two PEG hydrogel precursors, we should be able to fine-tune the number of free thiol functional groups in the hydrogels, and hence the density of conjugated biomolecules. These hydrogels with different composition can be also printed simultaneously by DPN techniques to create gradient pattern in a single array. In a nutshell, our studies has combined the top-down approach of generating 3D nanostructures surfaces with controlled surface chemistry which creates an ideal interface for solving various fundamental questions in the field of cell biology.

8:40am **BI1+NS-ThM3 Engineering Cell Behavior in Microfabricated Substrates: Adding Dimensionality to the Sensory Toolbox**, ***M.H. Textor***, *M. Ochsner, V. Vogel,* ETH Zurich, Switzerland, *M.L. Smith*, Boston University **INVITED**

The physical properties of the local cell microenvironment regulate cell behavior in concert with soluble or matrix bound signaling molecules. *In vivo*, these properties are defined by a fibrillar ECM and adjacent cells and have implications for human health and disease. Our understanding of their role in regulating cell physiology resulted from technological advances which led to reductionist cell culture systems with tunable substrate stiffness, ligand density, or cell adhesive area and shape in two dimensions (2-D). Most of these studies were performed on flat, 2-D culture surfaces where studies have shown that these properties regulate a seemingly endless variety of observable cell responses. Regulating these processes with engineered cell culture platforms might prove useful in tissue engineering or regenerative medicine applications where a specific cell phenotype needs to be stimulated or maintained.

The extent to which observations made in 2-D Petri dishes can be transferred to predict cell behavior in a 3-D environment is a focus of current research. However, only a limited number of studies investigated the different microenvironmental parameters as a function of dimensionality, often with no or limited control of cell shape and substrate stiffness, and thus cannot be directly compared to observations made on (patterned) 2-D culture systems.

The focus of this talk is to demonstrate how the surface area of adhesive contact and substrate rigidity differentially regulate actin cytoskeleton assembly in 2-D versus 3-D environments, and how this impacts cell phenotype and function. PDMS polymeric substrates (compatible with inverted stage microscopy) for the organization of single cells in engineered quasi-3-D microenvironments were fabricated presenting arrays of microwells of different shape/aspect ratios, and stiffness (typically 1 MPa to 10 kPa Young's Modulus). The walls and bottom of wells were backfilled with extracellular matrix proteins such as fibronectin or mobile lipid bilayers.

On rigid substrates cytoskeleton assembly within single fibroblast cells was found to occur in 3-D microwells at shapes that inhibited stress fiber assembly in 2-D. In contrast, cells did not assemble a detectable actin cytoskeleton in soft 3-D microwells (20 kPa), but did so on flat, 2-D substrates that were otherwise equivalent. These data indicate that neither cell shape nor rigidity are orthogonal parameters directing cell fate. The sensory toolbox of cells seems to integrate mechanical (rigidity) and topographical (shape and dimensionality) information differently when cell adhesions are confined to 2-D or occur in a 3-D space.

9:20am **BI1+NS-ThM5 Uniform Spheroid Formation Using a "Smart" Polymer**, ***J.A. Reed***, *J.P. Freyer, H.E. Canavan*, University of New Mexico

Spheroids are small (~50-1000 μm diameter) sphere-shaped aggregates of cells that have been developed as 3D models for tumors. In addition to providing a model that more closely approximates the microenvironments of tissues and tumors than 2D cultures, spheroids can be more easily controlled than tests preformed on animal models. Current approaches for spheroid formation result in spheroids with a wide size distribution (>25% standard deviation), requiring the use of secondary sorting to obtain a uniformly-sized population. To increase the efficacy of these models for drug discovery in cancer therapeutics, it is necessary to develop an efficient way to fabricate a large number of uniform spheroids. Using a thermoresponsive polymer, poly(*N*-isopropyl acrylamide) (pNIPAM), cell aggregates of reproducible size and cell density can also be obtained. In this work, we pattern pNIPAM on an anti-fouling substrate to direct cell attachment, and ultimately cell sheet detachment for uniform spheroid formation. Using plasma polymerization, pNIPAM is patterned on Pluronic® F-127, which is nonfouling, to form 300μm diameter reversibly cell adhesive "islands" in non-fouling Pluronic® F-127 "seas." EMT6 cells are grown to confluence on the islands in 2 days, at which time their growth media is exchanged to stimulate cell detachment to form 70 spheroids of ~100μm from each 35mm diameter substrate. To verify pattern fidelity, X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), atomic force microscopy (AFM), and contact angle goniometry are used. In addition to developing a novel technique for the formation of tumor analogs, we also find that use of the larger surface area: volume ratio accelerates the speed of cell release.

9:40am **BI1+NS-ThM6 Parylene Peel-Off Technology: A Tool for Nano- and Microengineering Biological Environments**, ***C.P. Tan***, *B.R. Cipriany, B.R. Seo, D.J. Brooks, E.M. Chandler, C. Fischbach, D.M. Lin, H.G. Craighead*, Cornell University

Spatial manipulation of biomolecules and cells on a surface with nano- and micrometer scale precision is important in engineering biological microenvironments for tissue engineering, micro total analysis systems (biosensors, microfluidics and microarrays), and fundamental biophysical studies. We present Parylene Peel-Off, a simple and adaptable tool that can be used to improve current patterning/engineering of biological environments. In this work, we describe the fabrication process for creating a polymer (parylene-C) template to serve as a stencil for printing nano- and microscale regions of nucleic acids, proteins, lipids and cells. Afterwards, the parylene template can be easily peeled away to yield arrays of highly uniform biomolecular features in a large area format. We demonstrate the use of our Parylene Peel-Off technology to micropattern tumor cell arrays, for investigations into the role of cell-cell interactions in angiogenesis and cancer progession. By combining Parylene Peel-Off with current inkjet printing technologies, we have also generated multi-component, combinatorial protein arrays with array feature sizes down to 90nm. We anticipate that Parylene Peel-Off will be useful for enabling high-resolution studies of subcellular biological processes, integrating biochemical functionalities with miniaturized sensors, and engineering cellular and tissue microenvironments. Beyond basic science, our Parylene Peel-Off technology can be a useful tool to pattern chemically sensitive materials that are difficult to manipulate on the nano-scale, improve drug screening, and enable current inkjet printing technologies to extend their resolution to the sub-micrometer scale.

Biomaterial Interfaces
Room: Taos A - Session BI2+NS-ThM

Quantitative Sensing at Biointerfaces
Moderator: E.O. Reimhult, University of Natural Resources and Applied Life Sciences, Switzerland

10:40am **BI2+NS-ThM9 Membranes on Solid Surfaces**, ***A.P. Shreve***, Los Alamos National Laboratory **INVITED**

Lipid assemblies on solid substrates provide a means of integrating biological and non-biological systems. They serve as a basis for a number of technological applications, particularly biological sensing and imaging platforms. They also provide important materials for the study of fundamental biophysical processes, and additionally are a platform for the study of the structure and dynamics of low-dimensional complex fluids. Working with a number of collaborators [1-4], we have been investigating how lipids interact with variously textured and functionalized solid surfaces, with an emphasis on the use of optical microscopy and spectroscopy as means of interrogating structure, dynamics and function within membrane assemblies on surfaces. Selected examples from recent and ongoing work will be discussed, including the use of diffusion measurements to infer the nature of membrane interactions with nanotextured surfaces, preparation and characterization of controlled multi-bilayer architectures, study of how electrostatic interactions with surfaces affect the structure and asymmetry of membranes, the interaction of functionalized nanoparticles and nanomaterials with membranes, and the development and characterization of multicomponent membranes on patterned nanoporous and nanostructured substrates. All of these topics are related to long-term interests in the application of solid-substrate supported lipid assemblies in sensing and characterization of biological systems.

[1] T.H. Yang, C.K. Yee, M.L. Amweg, S. Singh, E.L. Kendall, A.M. Dattelbaum, A.P. Shreve, C.J. Brinker, A.N. Parikh, "Optical detection of ion-channel-induced proton transport in supported phospholipid bilayers," *Nano Letters* **7** (2007) 2446. [2] A.E. Oliver, E.L. Kendall, M.C. Howland, B. Sanii, A.P. Shreve, A.N. Parikh, "Protecting, patterning, and scaffolding supported lipid membranes using carbohydrate glasses," *Lab on a Chip* **8** (2008) 892. [3] A.P. Shreve, M.C. Howland, A.R. Sapuri-Butti, T.W. Allen, A.N. Parikh, "Evidence for leaflet-dependent redistribution of charged molecules in fluid supported phospholipid bilayers," *Langmuir* **24** (2008) 13250. [4] J.H. Werner, G.A. Montaño, A.L. Garcia, N.A. Zurek, E.A. Akhadov, G.P. Lopez, A.P. Shreve, "Formation and dynamics of supported phospholipid membranes on a periodic nanotextured substrate," *Langmuir* **25** (2009) 2986.

11:20am **BI2+NS-ThM11 2010 AVS Albert Nerken Award Lecture - AlGaN/GaN High Electron Mobility Transistor Based Sensors for Bio-Applications**, ***F. Ren****, *S.J. Pearton, B.H. Chu, C.Y. Chang,* University of Florida*, W.J. Johnson,* Nitronex*, A. Dabiran, P.P. Chow*, SVT Associates **INVITED**

It is highly desirable to have a programmable, single chip sensor with an array of sensors for different purposes that is handheld and capable of wireless communication. This kind of sensor can be very useful for environmental, safety, and biomedical applications. For example, the sensor can be programmed in the doctor office for specific uses to give it to patients to use at home, transmitting the sensing results to their doctor directly to monitor the effectiveness of prescribed medicines. Thus, patients can get better, prompt and adequate health care. It can also reduce the number of unnecessary visits to the emergency room and the cost of the national health system. AlGaN/GaN high electron mobility transistor based sensors are good candidates for low cost, handheld, and wireless chemical and biomedical sensor due to their excellent thermal as well as chemical stability and sensitivity to the changes of ambient. We have demonstrated AlGaN/GaN HEMT based individual sensors for protein, DNA, kidney injury molecules, prostate cancer, pH values of the solutions, pH in the exhaled breath condensate, and mercury ions with specific surface functionalizations. Recently, we integrated ZnO nanorods with AlGaN/GaN HEMT to detect glucose. This approach offers a possibility of integrating AlGaN/GaN HEMT based sensors with ZnO nanorod sensors on a single "smart sensor chip" for bio-sensing applications.

***** Albert Nerken Award Winner

Nanometer-scale Science and Technology
Room: La Cienega - Session NS+BI-ThA

Biomolecular Templates & Bioinspired Nanomaterials

Moderator: B. Borovsky, St. Olaf College

2:00pm **NS+BI-ThA1 Bio-functionalization of Nanopatterned Surfaces and their Integration with DNA Nanostructures**, ***M. Palma***, *J.J. Abramson, E. Penzo, A. Gorodetsky, R. Wang, M.P. Sheetz, C. Nuckolls, J. Hone, S.J. Wind*, Columbia University

The ability to control biomolecules on surfaces with nanometer resolution is of great interest in the field on nanoscience and nanotechnology. DNA nanoarrays, in particular, are of interest in the study of DNA-protein interactions, for biodiagnostic investigations and as a tool to drive self-organization of nanomoieties on surfaces. In this context, achieving a highly specific nanoscale assembly of oligonulceotides at surfaces is critical.

Here we describe different strategies to control the immobilization of single- and double-stranded DNA, as well as DNA nanostructures (DNA "origami"), on nanopatterned surfaces, with features down to the sub-10nm regime.

Using electron-beam and nanoimprint lithography we fabricated sub-10nm metal dots arranged in multiple configurations on Si or glass substrates. We have developed strategies for the selective bio-functionalization of these patterns, at the single nanodot level: each step of the biochemical functionalization has been monitored by Fluorescence Microscopy. The bio-functionalization approach used allowed for the formation of non-sterically hindered DNA nanodomains where the dsDNA attached to the dots maintains its native conformation, as confirmed by restriction enzymes studies. This allowed us, moreover, to follow the activity (at surfaces) of a restriction enzyme in real time and at the nanoscale: the monitoring of protein-DNA interactions with such biological nanoarrays will be discussed.

We will highlight the broader utility and application of such nanopatterned surfaces for the self-organization of DNA nanostructures. In-situ hybridization between the complementary strands on DNA nanostructures and on functionalized nanodots has been achieved, resulting in the ordered placement of the origami on the dot patterns, as demonstrated by Atomic Force Microscopy (AFM) imaging, both in liquid and in air.

Finally, we will discuss the application of DNA origami as functional scaffolds for the assembly of different nanomoieties (e.g Au nanoparticles and carbon annotubes): highly complex arrangements can be created with high resolution and high throughput, opening the possibility for the realization of electronic devices at the molecular scale.

2:20pm **NS+BI-ThA2 De Novo Nanostructure Design: from Protein Folding to Self-Assembly-Templated Nanomaterials**, ***M. Ryadnov***, National Physical Laboratory, UK

Rational design of self-assembled nano-to-micro scale structures offers an efficient tool for molecular nanotechnology improving our ability to engineer nanostructured materials at whim. Such attention is driven by the need for approaches leading to specialist nanostructures whose properties can relate to particular biological functions. Critical in this respect becomes the hierarchical nature of self-assembly rendering the process a "bottom-up" strategy in challenging different levels of nanostructural complexity [1].

Generic protein folding motifs are proving to be instrumental for prescriptive nanoscale engineering. Of particular demand are nanostructures which can be made functionally and architecturally amenable in cellular environments. In this report, bioinspired nanoscale designs based on peptide self-assembling systems possessing antimicrobial [2], cell-supporting [3], encapsulating[4] and tuneable morphological[5] properties will be discussed.

1. Ryadnov, M. G. (2009) *Bionanodesign: Following the Nature's touch.* RSC Publishing, 250 pp.

2. Ryadnov, M. G., Mukamolova, G. V., Hawrani, A. S., Spencer, J. & Platt, R. (2009) RE-coil: An antimicrobial peptide regulator. *Angew. Chem. Int. Ed. 48*, 9676-9679.

3. Ryadnov. M. G., Bella, A., Timson, S. & Woolfson, D. N. (2009) Modular design of peptide fibrillar nano- to microstructures. *J. Am. Chem. Soc.*, 131, 13240-13241.

4. Ryadnov, M.G. (2007) A self-assembling peptide polynanoreactor. *Angew Chem Int Ed Engl. 46*, 969-972 .

5. Ryadnov, M. G. & Woolfson, D. N. (2003) Engineering the morphology of a self-assembling protein fibre. *Nature Mater.*, *2*, 329-332.

2:40pm **NS+BI-ThA3 Rare Earth Nanoparticles to be used as Both Fluorescent Probes and MRI Contrast Agents**, ***L. Axelsson***, *M. Ahrén, L. Selegård, F. Söderlind,* Linköping University, Sweden, *P. Nordblad,* Uppsala University, Sweden, *M. Lindgren,* Norwegian University of Science and Technology, Norway, *K. Uvdal*, Linköping University, Sweden

Nanotechnology continuously explores new fields, and nanomedicine presents an entirely new research area with unlimited possibilities. For the last 20 years, gadolinium complexes have been used clinically as contrast enhancing agents for Magnetic Resonance Imaging (MRI). Simultaneously, Quantum Dots (QDs), with its excellent photostability and high quantum yield, are developed to replace organic fluorophores for medical diagnosis. The aim of this study is to develop nanoprobes that possess both the magnetic properties suitable for a contrast agent, and luminescent properties.

We have designed a novel nanomaterial of gadolinium oxide nanoparticles doped with europium ($Eu:Gd_2O_3$) or terbium ($Tb:Gd_2O_3$). Using nanoparticles, the local signal intensity in MRI can be increased compared to Gd complexes with only one Gd ion per complex. When introducing luminescent europium or terbium ions into the gadolinium oxide nanocrystal, fluorescent properties are added, creating a bifunctional nanocrystal. In addition to the favorable size for biomedical applications, nanoparticle contrast agents can bring advantages such as longer rotational correlation time to obtain increased relaxivity, and surface-coating possibilities for attaching targeting molecules. This will enable tailored design of a new generation of contrast agents. We present highly crystalline, 5 nm large nanoparticles, showing typical Eu^{3+} or Tb^{3+} fluorescence with a long luminescent lifetime. The strength of both europium and terbium ions is the suitable properties for excitation in an ordinary confocal microscope, which makes them promising as components when designing nanoprobes for cell studies. Relaxation measurements show relaxivity ratios in the same range as the pure Gd_2O_3 nanoparticles. The nanoparticles present a promising bifunctional core material, acting as a platform when developing advanced nanoprobes for future applications in biomedical imaging.

3:00pm **NS+BI-ThA4 Plasma Polymerized Amino Acids used for Bio-Assisted Fabrication of Nanostructures**, ***R. Jakubiak***, Air Force Research Laboratory, *K. Anderson,* Georgia Institute of Technology, *J. Slocik,* UES, Inc., *M. McConney,* Georgia Institute of Technology, *J. Enlow,* UES, Inc., *T. Bunning, R. Naik,* Air Force Research Laboratory, *V. Tsukruk*, Georgia Institute of Technology

Plasma-enhanced chemical vapor deposition (PECVD) allows deposition of conformal, ultrathin, and uniform polymer coatings from gaseous, liquid or solid precursors onto a variety of materials. Our process uses a modified afterglow plasma reactor operated at room temperature where plasma polymerization occurs downstream from plasma generation. This allows controllable retention of the precursor's functionality needed for surface-induced biomineralization on soft or delicate substrates that cannot withstand high temperature or multiple wet-chemistry treatments. Amine-functionalized substrates, derived from the plasma polymerization of L-tyrosine, enabled biomineralization of gold nanoparticles from a solution of gold chloride. Templated gold nanoparticle coatings were formed by the placement of a shadow mask on the substrate during plasma deposition creating a micropatterned plasma polymerized tyrosine film. Subsequent gold chloride exposure created a gold nanoparticle network replica of the initial micropattern. Similar processing conditions were used to biomineralize titania on highly order three-dimensional structures.

3:40pm **NS+BI-ThA6 Molecular Shuttles for 'Smart Dust' Biosensors, Active Self-Assembly, and Protein-Resistant Coatings**, ***H. Hess***, Columbia University **INVITED**

Biomolecular motors, such as the motor protein kinesin, can serve as biological components in engineered nanosystems. Initially, a nanoscale transport system termed molecular shuttle has been explored by others and us as a model system. The development of this system has revealed a number of challenges in engineering at the nanoscale, particularly in the guiding, activation, and loading of these shuttles. Overcoming these challenges requires the integration of a diverse set of technologies, and continues to illustrate the complexity of biophysical mechanisms.

A proof-of-principle application of the developed technologies is a "smart dust" biosensor for the remote detection of biological and chemical agents, which is enabled by the integration of recognition, transport and detection into a submillimeter-sized microfabricated device.

The application of nanoscale forces introduces an interesting element into self-assembly processes by accelerating transport, reducing unwanted connections, and enabling the formation of non-equilibrium structures. The formation of nanowires and nanospools from microtubules transported by kinesin motors strikingly illustrates these aspects of motor-driven self-assembly.

Finally, a critical aspect of the design of these hybrid systems is the controlled adsorption of proteins. In pursuit of this goal of controlled adsorption, we have utilized kinesin motors as probes of residual protein adsorption to non-fouling coatings and achieved the detection of a few adsorbed molecules per square micrometer (adsorbed mass on the order of pg/cm^2). Furthermore, we have developed a Random Sequential Adsorption model which successfully explains residual protein adsorption as the result of randomly occuring "bald" spots on a surface covered with PEG-chains.

4:20pm **NS+BI-ThA8 Probing Biomineralization Protein Interactions with Hydroxyapatite Using SFG and NEXAFS Spectroscopy**, ***T.M. Weidner***, *M. Dubey, N.F. Breen, J. Ash, J.E. Baio,* University of Washington*, C. Jaye, D.A. Fischer,* National Institute of Standards and Technology*, G.P. Drobny, D.G. Castner*, University of Washington

The structural integrity of hydroxyapatite (HAP) in tooth enamel is maintained through the saliva environment that is supersaturated with calcium and phosphate salts. The biomineralization protein statherin adsorbs onto HAP surfaces with high binding affinity. It regulates HAP growth and prevents the buildup of excess HAP on the tooth surface by inhibiting spontaneous calcium phosphate growth. Owing to the importance of the underlying physiological processes and a general interest in biomineralization mechanisms, the binding of statherin to HAP has attracted significant interest in the biomaterials community. Sum frequency generation (SFG) spectroscopy can probe protein orientation and secondary structure at the solid-liquid interface and we have recently shown it can address specific protein regions with atomic resolution when combined with isotopic labeling.[1] Near edge X-ray absorption fine structure (NEXAFS) spectroscopy can give valuable information about the structure and binding chemistry of proteins on surface. We have combined both techniques to characterize the structure of the binding domain of statherin, SN15, a short peptide with 15 residues (Ac-DSSEENKFLRRIGRFG-OH) adsorbed onto a model HAP surface. Protein adsorption was verified using X-ray photoelectron spectroscopy and time-of-flight secondary ion mass spectrometry. SFG confirmed a loosely helical secondary structure of SN15 on HAP. Deuteration was used to specifically probe the orientations of the hydrophobic leucine and isoleucine side chains with SFG in situ. Side-chain orientations were determined using ratios of the symmetric and asymmetric CD_3 stretching modes. The leucine chain was tilted 120° from the surface normal (pointing towards the surface) and the isoleucine was tilted 5° from the surface normal. For the first time, element labels were employed to probe individual side chain orientations with NEXAFS spectroscopy. Para- and perfluorination of the phenylalanine rings F7 and F14 allowed us to precisely measure their orientations using angle dependent NEXAFS data. The tilt angles from the surface normal were determined to be 26° for F7 and 35° for F14.

[1] T. Weidner, N. F. Breen, K. Li, G. P. Drobny, D G. Castner, submitted.

4:40pm **NS+BI-ThA9 Assembly of Nanoparticles for Patterning and Functional Materials in Nature's Way at Liquid-Liquid Interfaces**, *L. Isa, E. Amstad, M.H. Textor,* ***E.O. Reimhult***, ETH Zurich, Switzerland

An interesting aspect of the self-organization in Nature resulting in precise patterning of hierarchically structured materials is that the "synthesis" and patterning of the materials occur at liquid amphiphilic interfaces such as membranes. That particles can be organized and change the properties of liquid interfaces has long been known and explored as e.g. Pickering emulsions in foams, food processing and other large-scale, bulk materials applications. However, self-assembly of nanometer-sized colloids with defined surface properties at liquid-liquid interfaces is also a process with huge potential for the fabrication of controlled two-dimensional nanoscale structures and patterns as well as "nanomaterials". This is due to three key factors: a) the particles are trapped at the interface, but b) retain lateral mobility and c) exhibit specific interactions, which when properly understood and controlled lead to assembly of controlled structures. We have recently explored both how the oil-water interface can be used for unprecedented control of the assembly of nanoparticle patterns and transferred to substrates for low-cost nanolithography, and how tailored core-shell nanoparticles with functional cores can be assembled at such interfaces.

I will describe how self-assembly at the liquid-liquid interface (SALI) can be used for the deposition of non-close-packed crystalline arrays of NPs for lithographic masks and the physical control parameters for the successful application of this method. Our approach allows us to control the spacing of particles in a wide range; we have demonstrated reproducible and homogeneous patterns with spacing between 3 to 20 particle diameters using colloids from 40 to 500 nm over chip-sized areas. The use of bimodal size distributions at controlled ratios also allows for induced phase separation and thus hierarchically ordered patterns to emerge.

By optimization of a simple Schäfer-type deposition setup and the choice of the proper oil phase, the particle patterns can be transferred to a substrate with few limitations. We will demonstrate use of the deposited particle patterns to fabricate a range of nanostructures for electrochemical and nanoplasmonic biosensing which previously could not be fabricated by particle lithography, and this at a fraction of cost to other available patterning techniques.

5:00pm **NS+BI-ThA10 Characterization of Folate Receptor Targeting Drug Loaded PLGA-Lipid Hybrid Nanoparticles**, ***S. Sandoval***, *A. Liberman, J. Yang, S. Aschemeyer, L. Zhang, W.C. Trogler, A.C. Kummel*, University of California, San Diego

The response rate of breast cancer to first line chemotherapies is encouraging, but 20-30% of patients develop chemoresistance to these drugs, and consequently, have a cancer reoccurrence 7-10 months after their last treatment. Chemoresistance is believed to be due to drug efflux proteins responsible for the removal of many commonly used anti-neoplastic agents. One possible way to overcome these drug efflux pumps is to give higher doses of chemotherapy, but high doses of such agents commonly lead to chemocytotoxicity. Targeted PLGA-Lipid hybrid nanoparticle (NP) drug delivery systems have been developed that can deliver high doses of chemotherapy agents specifically to breast cancer cells. A practical cancer targeting drug delivery system will reduce the overall amount of chemotherapy agents given to patients for a given amount of targeted NPs endocytosed by cancer cells. Biodegradable NPs were synthesized using a novel nano-precipitation lipid-polymer hybrid platform which also allows for the encapsulation of hydrophobic chemotherapy drugs within the NPs. Using this method, drug free NPs have been shown to have an average diameter size of 81.78 nm (PDI: 0.25), while single loaded NPs, with Paclitaxel or Doxorubicin, show an average size between 72.33 to 89.64 nm (PDI: 0.242 to 0.339), signifying that the synthesis technique creates consistent sub 100nm particles. The majority of all NPs show a zeta-potential value of > -30 mV consistent with the NPs having sufficient repulsive interaction to be mono-dispersed in solution under physiological conditions. Folate receptors are often over expressed on the surfaces of cancer cells; therefore, folic acid was incorporated to the surface of these NPs as a cancer targeting ligand. Previous studies have shown that HeLa cells, a cervical cancer cell line, over expresses folate receptors. Immunofluorescence studies show that folic acid coated PLGA-Lipid hybrid NPs are readily endocytosed by HeLa cells compared to non-targeted NPs. Cytoxicity studies will determine the increased effectiveness of drug delivery with targeted PLGA-Lipid hybrid NPs vs. untargeted PLGA-Lipid hybrid NP in cell lines and in animal models.

5:20pm **NS+BI-ThA11 Controlled Surface Modification of Ultra-stable Superparamagnetic Iron Oxide Nanoparticles**, ***E. Amstad***, *M.H. Textor, E.O. Reimhult*, ETH Zurich, Switzerland

Biocompatibility, magnetic properties and ease of synthesis renders iron oxide nanoparticles (NPs) attractive for many especially biomedical applications such as magnetic resonance (MR) contrast agents, triggered drug release and cell separation. Good NP stability under physiologic conditions and controlled surface chemistry are key to successful application not only in the biomedical field but also for assembly into various materials.

NPs with close control over the interfacial chemistry and good stability at high salt concentrations and elevated temperatures can only be achieved if dispersants are irreversibly bound to the NP surface. The dispersant binding affinity is determined by its anchor group. Low molecular weight dispersants which consist of one high affinity anchor covalently linked to poly(ethylene glycol) (PEG) spacers have been proven well suited to sterically stabilize Fe_3O_4 NPs. However, we found that electronegatively substituted catechols such as nitrocatechols vastly outperform the well-known and often used catechol anchors such as DOPA and dopamine. Because of the optimized binding affinity of nitrocatechols, PEG-nitrocatechol coated Fe_3O_4 NPs remained stable under physiologic conditions up to 90 °C whereas e.g. PEG-dopamine stabilized Fe_3O_4 NPs started to agglomerate below body temperature.[1] Further investigations showed that the optimal binding affinity of nitrocatechols to Fe_3O_4 is closely related to a redox reaction between Fe^{2+} located at the Fe_3O_4 NP surface and nitrocatechols, which leads to electron delocalization in the adsorbed catechol ring, and a close to covalent bond of nitrocatechols to Fe_3O_4 surfaces. Irreversible binding of PEG-nitrocatechols to Fe_3O_4 NPs allowed us to closely control and investigate the influence of dispersant layer thickness by varying the nitrocatechol-PEG molecular weight. Furthermore, NPs could easily be functionalized by co-adsorbing differently end-functionalized dispersants on the Fe_3O_4 NP surface.[2]

In summary, nitrocatechols have a close to optimal binding affinity to Fe_3O_4 surfaces. This optimized binding affinity not only leads to ultra-stable PEG-nitrocatechol coated superparamagnetic Fe_3O_4 NPs but also allows for close control over the hydrodynamic diameter and interfacial chemistry, factors which crucially determine NP performance especially in biomedical applications.

[1] E. Amstad, T. Gillich, I. Bilecka, M. Textor, E. Reimhult, *Nano Letters* **2009**, *9*, 4042.

[2] E. Amstad, S. Zurcher, A. Mashaghi, J. Y. Wong, M. Textor, E. Reimhult, *Small* **2009**, *5*, 1334.

Biomaterial Interfaces
Room: Southwest Exhibit Hall - Session BI-ThP

Biomaterial Interfaces Poster Session

BI-ThP1 Temperature Gradient Device for Investigation of Cell Detachment from Thermoresponsive Surfaces, ***M.A. Cooperstein***, *H.E. Canavan*, University of New Mexico

Poly(*N*-isopropyl acrylamide) (pNIPAM) undergoes a phase change in a physiologically relevant temperature range that leads to cell release. Above its lower critical solution temperature (LCST, ~32^{o}C), pNIPAM is relatively hydrophobic, and when grafted to a surface, it takes on a packed conformation. There have been numerous studies on the conformation change of pNIPAM across its LCST. Although it is known how pNIPAM chains tethered to a substrate behave when the temperature is changed, no study has probed the influence of a temperature gradient on the behavior of cells attached to the polymer. In this work, we present the investigation of cell detachment from pNIPAM-grafted surfaces resting on a temperature-gradient device. The polymer was deposited on the surface using plasma polymerization. This deposition technique creates a conformal, sterile film that is compatible with any surface chemistry, including the transparent well plates required for these experiments. Prior to their use for cell culture, it is imperative to characterize the pNIPAM films for film thickness, surface chemistry, and thermoresponse, as the surface characteristics determine cell attachment and detachment. The characterization was performed via interferometry, X-ray photoelectron spectroscopy (XPS), and contact angle measurements. Using a device fabricated in our laboratory, we studied whether there is a gradual progression of cellular detachment from the polymer along the temperature gradient, or if there is an abrupt step-change in the detachment. This work will have valuable insights regarding the optimal temperature for cell detachment from pNIPAM.

BI-ThP2 Protein Resistant Oligo(ethylene glycol) Terminated Self-Assembled Monolayers of Thiols on Gold by Vapor Deposition in Vacuum, *L. Kankate,* University of Bielefeld, Germany, *H. Großmann,* Johann Wolfgang Goethe-University, Germany, *U. Werner,* University of Bielefeld, Germany, *R. Tampé,* Johann Wolfgang Goethe-University, Germany, *A. Turchanin,* ***A. Gölzhäuser***, University of Bielefeld, Germany

Protein resistant oligo(ethylene glycol) terminated (OEG) self-assembled monolayers (SAMs) of thiols on gold are commonly used for suppression of nonspecific protein adsorption in biology and biotechnology. The standard preparation for these SAMs is the solution method (SM) that involves immersion of the gold surface in an OEG solution. Here we present the preparation of 11-(mercaptoundecyl)-triethylene glycol ($HS(CH_2)_{11}(OCH_2CH_2)_3OH$) SAMs on gold surface by vapor deposition (VD) in vacuum. We compare the properties of SAMs prepared by VD and SM using X-ray photoelectron spectroscopy (XPS), polarisation modulation infrared reflection absorption spectroscopy (PM-IRRAS) and surface plasmon resonance (SPR) measurements. VD and SM SAMs exhibit similar packing density and show a similar resistance to the nonspecific adsorption of various proteins (bovine serum albumin, trypsin, and myoglobin) under physiological conditions. A very high sensitivity of the OEG SAMs to X-ray radiation is found, which allows tuning their protein resistance. These results show a new path to *in situ* engineering, analysis and patterning of protein resistant OEG SAMs by high vacuum and ultra high vacuum techniques.

[1] L. Kankate, H. Großmann, U. Werner, R. Tampé, A. Turchanin, A. Gölzhäuser, *Biointerphases* 5, 30 (2010)

BI-ThP3 Spontaneous Micro-Fluidic Flow Driven by Marangoni Effect, ***Y.C. Hu***, *Y.H. Lin, Y.C. Ou*, National Applied Research Laboratories, Taiwan, Republic of China

This article presents an innovative new type of micro-fluidic chips; the marangoni effect is used as the driving force of the fluidics, while the fluidics is confined in ”Surface tension-confined micro-fluidics” chips for regularly transportation. "Surface tension-confined micro-fluidics” is an open structure micro-fluidic chip, there is no micro-channels, only hydrophobic and hydrophilic coating patterns on the chips to confined fluidic flow. Normally the fluidics is driven by surface tension force, since there is no micro-channels structure, there is no way to use conventional pumps. In this study we propose a initiative driving force source that can be applied in "Surface tension-confined micro-fluidics " chips；low surface energy surfactants (such as alcohol) after atomization is sprayed on local area of the fluidics so that the fluidics surface can render surface energy gradient, according to the marangoni effect liquids with higher surface energy will attract liquids from lower surface energy area and generate fluidic flow, while the fluidic flow is confined along the hydrophilic pattern channel, with carefully designed patterns one can control the fluidic flow to a designed location. The surfactants spraying nozzle can be moved to any position so that one can easily control the fluidic flow just by simply move the spraying nozzle along the pattern channel. If one want to separate the fluidic flow in to two parts, just move the spraying nozzle to the middle area of the fluidic flow, it will be cut into two parts. By reciprocating oscillate the spraying nozzle one can disturb the fluidics and enhance the mixing efficiency.

This innovative micro-fluidic chip has the following advantages: the process is simple, low cost, easy to integrate and it is easy to use.

BI-ThP4 Printable Biological Ink on Gelatin for Self Crosslinking Wound Dressings, ***M. Yanez***, *T. Boland*, University of Texas at el Paso

The printable biological ink on gelatin is a biopolymer that could be use in a handheld, portable device, such as a ink jet of printer and become a substrate for advanced wound care. Skin is very affected by when burned, and also by diabetic venous ulcers. Currently, some skin substitutes exist that for treatment of diabetic foot ulcers, but these products are really expensive. Based on this we want to create a low cost wound care material that helps not only people with diabetic foot ulcers and venous leg ulcers, but also with burned skin. We selected sodium alginate because it is a FDA approved carbohydrate that has many applications for tissue regeneration and cell therapy, and it is very compatible with human body. Oxidation of sodium alginate has been investigated to obtained alginate dialdehyde (ADA). Alginate dialdehyde is a fast acting self-crosslinking degradable polymer, when applied over collagen or gelatin. We investigated, the degree of oxidation, the degree of cross-linking and mechanical properties of the materials. The degree of crosslinking was determined by trinitrobenzene sulphonic acid assay maintaining constant ADA concentration in borax or PBS buffer and varying concentration of gelatin in borax or PBS. An increase of the degree of oxidation of sodium alginate, an increase in the cross-linking and a decrease of the gelling time was observed with increasing ADA concentration. We investigated the effect of ADA concentration on viscosity and found that at concentrations of about 10% ADA, the solutions are viscous. We will present data on the use of ADA solution as an ink in a printing device and the effect of printed ADA to cross-link gelatin. In general, control over the concentrations of ADA as well as the spatial dispensing via printing should allow us to generate wound dressings of tunable properties. The use of a portable device makes this solution attractive to low resource settings. Future work will include testing the wound dressing in a small animal model and investigating the effect of adding keratinocytes as well as endothelial cells to the material on healing and wound contraction.

BI-ThP5 Carbohydrate-functionalized Surfaces: Analysis by a Multi-Method Approach, ***P.M. Dietrich***, BAM Federal Institute for Materials Research and Testing, Germany, *T. Horlacher,* Max Planck Institute of Colloids and Interfaces, Germany, *T. Gross, T. Wirth,* BAM Federal Institute for Materials Research and Testing, Germany, *R. Castelli,* Max Planck Institute of Colloids and Interfaces, Germany, *A. Lippitz,* BAM Federal Institute for Materials Research and Testing, Germany, *P.H. Seeberger,* Max Planck Institute of Colloids and Interfaces, Germany, *W.E.S. Unger*, BAM Federal Institute for Materials Research and Testing, Germany

Carbohydrate microarrays formed by hundreds of different sugars, covalently or non-covalently bound, have enabled an emerging field of applications in the last decade, i.e. in diagnostics or high-throughput analysis.[1-5] Saccharide microarrays are valuable tools to investigate interactions with other molecules since many glycans are involved in fundamental biological processes.

Carbohydrate-based microarrays are commonly prepared by covalent attachment of chemically modified saccharides that bind selectively to a functionalized solid support such as gold, glass or polymers. Arrayed structures of spotted sugars can be printed with standard robotic microarray printers.

For future applications a reliable surface chemistry combined with an advanced surface analysis is required for improved microarray qualities and performances.

Herein, we report on the combined XPS, NEXAFS and ToF-SIMS surface analysis of carbohydrate functionalized gold and glass surfaces containing synthetic glycans. Advanced characterization of spot shape, size and chemical composition across a spot surface are provided by surface sensitive methods as ToF-SIMS and XPS, used in their imaging modes.

References

[1] T. Feizi, F. Fazio, W. Chai, C.-H. Wong, *Curr. Opin. Struct. Biol.* **2003**, 13, 637.

[2] D. M. Ratner, E. W. Adams, M. D. Disney, P. H. Seeberger, *ChemBioChem* **2004**, 5, 1375.

[3] S. Injae, P. Sungjin, L. Myung-ryul, *Chem.--Eur. J.* **2005**, 11, 2894.

[4] J. L. De Paz, P. H. Seeberger, *QSAR Comb. Sci.* **2006**, 25, 1027.

[5] T. Horlacher, P. H. Seeberger, Chem. Soc. Rev. 2008, 37, 1414.

BI-ThP6 Enzymeless Flow Injection Analysis of 2,4,6-Trichlorophenol Based on Preoxidation by Ammonium Cerium (IV) Nitrate, ***J.S. Wang***, National Applied Research Laboratories, Taiwan, Republic of China

Enzymeless flow injection analysis of 2,4,6-trichlorophenol (2,4,6-TCP) based on preoxidation by ammonium cerium (IV) nitrate is present in this work. A preoxidation scheme is applied for 2,4,6-TCP determination without any enzyme treatment. This preoxidation scheme can provide a determination method with low cot and non-conductive polymerization reaction of 2,4,6,-TCP. In proposed scheme, the 2,4,6-TCP is oxidized to 2,6-dichloro-1,4-benzoquinone by ammonium cerium (IV) nitrate and the 2,6-dichloro-1,4-benzoquinone can be detect at low reduction potential. The linear range of 2,4,6-TCP determination was 0.4 to 750 mM with correlation coefficient (R^2) 0.9999 and the estimated detection limit (S/N=3) was 40 nM which were demonstrated by flow injection analysis. Twenty consecutive successive detect of 100 mM 2,4,6-TCP showed the relative standard deviation was 1.56 %. Several 2,4,6-TCP structure-like compounds were studied as interferences including 2,4-dichlorophenol, 2-chlorophenol, phenol and 4-aminophenol. No obviously influences were observed. Two water samples which were collected from local farm and pool were adopted as analytical application. The recoveries of two water samples are 105.2 % and 107.5%, respectively. An easy operation and enzymeless treatment detection scheme of 2,4,6-TCP is illustrated at this work.

BI-ThP7 Evaluation of Electrochemical Impedance Spectroscopy with an ITO Culture Chip, ***S.-Y. Hsiao***, Instrument Technology Research Center, NARL, Taiwan, *Y.-S. Lin,* Hungkuang University, Taiwan, *Y.-P. Lu,* Instrument Technology Research Center, NARL, Taiwan, *C.-Y. Lin,* Taipei Medical University, Taiwan, *H.-S. Huang,* Instrument Technology Research Center, NARL, Taiwan, *H.-M. Huang,* Taipei Medical University, Taiwan, *D.-C. Chen,* National Yang-Ming University, Taiwan, *T.-S. Liao, J.-S. Kao*, Instrument Technology Research Center, NARL, Taiwan

An electrochemical impedance spectroscopic (EIS) instrument with indium-tin oxide (ITO) culture chip module and lock-in impedance readout module was developed. The lock-in impedance readout module achieves impedance measurements with a small, bio-harmless AC signal. Moreover, transparent ITO culture-chip module is experience friendly; other optical inspections can be applied on the chip. The impedance readout module is designed to perform in a range from 1 to 10 kHz; the phase measuring errors are within 5.1% in that range. Typical examples of PBS solutions, BSA proteins and cell culturing tests are discussed in experiments. Higher concentration levels of PBS produced lower impedance. Higher concentration level of BSA solution also produced lower impedance. Furthermore, experiments of 0.25% BSA dissolved in sDDW and 1X PBS show that the mediums influence system impedance. Moreover, the log phase (period of cell proliferation) of B16F10 cell culture tests ended at 9, 16, 23, and 65 h for seeded cell densities of 5×10^4, 1×10^5, 2×10^5, and 4×10^5/mL, respectively. Finally, cultures are incubated and doped to demonstrate the monitoring of cell proliferation.

An EIS using microelectrode arrays has gained much attention as a promising, label free, fast, and real-time method for cellular analysis. The electric cell substrate impedance sensing (ECIS; Applied BioPhysics) and real time cell electronic sensing (RT-CES; Roche Applied Sciences) systems are widely applied in measuring cell proliferation, attachment and spreading, motility, toxicology, barrier function, wounding, and migration. The sensing chips are opaque and the influence of solutions is generally disregarded. However, transparent chips for optically related detection are required in many applications. And, in fact, chemical additives for biochemical treatments induce impedance variations.

This study develops an EIS instrument with an ITO culture chip module and lock-in impedance readout module. The instrument can apply and extract bio-harmless small signals by lock-in technology. Experimental results prove impedance readout module performance. The ITO culture chip module electrodes are connected via the electrolyte medium and the route of cell/tissue. Typical examples of the effects of solutions and cell culturing tests are discussed. A higher level of PBS causes lower impedance. Further study of solution ingredients will improve the measurement accuracy and results analysis. The ESI instrument with an ITO culture chip module and lock-in impedance readout module can be applied in other conductive liquid sample investigations.

BI-ThP8 Molecular Interactions between Lubricin and Type II Collagen Surfaces: Adsorption, Adhesion, Steric Repulsion, and Boundary Lubrication, ***D.P. Chang***, *F. Guilak,* Duke University, *G.D. Jay,* Brown University / Rhode Island Hospital, *S. Zauscher*, Duke University

Although many studies have tried to elucidate the lubrication mechanisms that occur in articular cartilage, the molecular details of how constituents of the synovial fluid interact with cartilage surfaces and mediate cartilage to cartilage interaction still remain poorly understood. One of the major constituents of the synovial fluid that is thought to be responsible for boundary lubrication is the glycoprotein lubricin; however, details of the molecular mechanisms by which lubricin carries out its vital functions still remain largely unknown. Here, we examine i) the molecular details in which lubricin interacts with type II collagen, the main component of cartilage that provides structural integrity and tensile strength, ii) whether collagen structure can affect lubricin binding and change the adhesive interactions and boundary lubrication between collagen surfaces. We found that lubricin adsorbed strongly onto denatured, amorphous and fibrillar collagens surfaces. Furthermore, we found large repulsive interactions, between the collagen surfaces in presence of lubricin, increase with increasing lubricin concentration. Lubricin attenuated the large friction and also the long-ranged adhesion between the fibrillar collagen surfaces. Interestingly, lubricin mediated the frictional response between the denatured and native amorphous collagen surfaces equally, and showed no preference on the supramolecular architecture of collagen. We speculate that in mediating interactions at the cartilage surface, an important role of lubricin is to attach to the cartilage surface and provide a protective coating that maintains the contacting surfaces in a sterically repulsive state.

BI-ThP9 A Study of the Effect of Solvent-based Sterilization on the Reversible Adhesion of Biological Cells to a Thermoresponsive Surface, ***L.J. Pawlikowski***, *V.J. Eriacho, H.E. Canavan*, University of New Mexico

Poly(*N*-isopropyl acrylamide) (pNIPAM) is a thermoresponsive polymer that is widely used in bioengineering applications, including tissue culture engineering, single cell adhesion/detachment, and biofouling prevention. Although there are many ways to treat surfaces with pNIPAM, plasma polymerization is one of the more adaptable ways for coating surfaces for the use in tissue culture experiments. While plasma polymerization creates a sterile environment useful for tissue culture, occasionally, additional sterilization techniques must be used. Some sterilization methods include using UV light to sterilize the surfaces, and the use of different solvents, such as ethanol. To date, there have been no studies on the effect of these sterilization techniques on the reversible adhesion of biological cells on pNIPAM treated surfaces. In this work, we investigate the effect of different sterilization techniques (e.g., ethanol and UV light) on the thermoresponsive nature of pNIPAM. Substrates were coated using plasma polymerization (ppNIPAM), after which they were sterilized using solvents, and characterized to determine if the solvents changed the thermoresponsive nature of the polymer. X-ray photoelectron spectroscopy (XPS) and interferometry were used to determine the surface chemistry and thickness of our ppNIPAM surfaces. Goniometry was used to confirm the thermoresponsive nature of our surfaces. Finally, we tested the adhesion and detachment of cells on the surfaces using bovine aortic endothelial cells (BAECs). We found that the use solvents as sterilizing agents does have an effect on the thermoresponsive nature of pNIPAM (as demonstrated by the decreased detachment of cells from the surfaces), even when the pNIPAM film's chemistry appears unaffected.

BI-ThP10 Probing the Molecular Interactions at Bio-Inorganic and Bio-Organic Interfaces using X-ray Photoelectron Spectroscopy, ***R.A. Rincón***, *K. Artyushkova, D. Ivnitski,* University of New Mexico, *M. Eby,* Universal Technology Corporation, *H.R. Luckarift, G.R. Johnson,* Air Force Research Laboratory, *P. Atanassov*, University of New Mexico

BI-ThP11 Polysomes Interaction with Self-Assembled Monolayers, *L. Marocchi, L. Lunelli,* Center for Materials and Microsystems - FBK, Italy, *G. Viero,* CiBio – University of Trento, Italy, *F. Piras,* Center for Materials and Microsystems - FBK, Italy, *N. Arseni, A. Provenzani,* CiBio – University of Trento, Italy, *C. Pederzolli,* Center for Materials and Microsystems - FBK, Italy, *A. Quattrone,* CiBio – University of Trento, Italy, ***M. Anderle***, Ict - Pat, Italy

The cellular machinery dedicated to the synthesis of the polypeptide chain translated from the mRNA template is the ribosome. Clusters of translating ribosomes held together by mRNA form the so-called polysome. The study of such supramolecular assemblies has interests both in structural and functional aspects as addressed in this study. As consequence the analyses of polysomal mRNA are emerging as good estimators in directly representing cell phenotypes. Since traditional methods of polysome purification are still a time consuming and laborious procedures, high throughput polysomal purifications are attracting growing interest.

Recently, more modern approaches have been developed, based on the specific affinity between genetically modified ribosomes and functional surfaces. However, native ribosomes are required for several analytical applications. This work is a first step in developing a convenient and fast strategy to purify native polysomes thanks to their adhesion ability to appropriate substrates. We studied the interaction between functionalized gold surfaces and polysome fractions purified from human cultured cells or ribosomes derived from rabbit reticulocyte lysate. Surfaces endowed with different chemical properties were obtained using functional thiols able to form self assembled monolayers on gold substrates. Substrate surface properties were studied with XPS, ToF-SIMS and AFM. The interaction of rabbit ribosomes with the surfaces was assessed with AFM and confocal microscopy.

Despite different imaging approaches have been used to visualize bacterial and eukaryotic polysomes, human native polysomes have never been observed in physiological conditions. Moreover nothing is known about the formation of the mRNA-ribosomes macroassembly. Here we present the very first AFM images of native polysomes from mammalian cell lines adherent to surfaces. For the first time to our knowledge, we could observe ribosome aggregates associated to RNA, providing the imaging of polysome assemblies. Our imaging approach suggests the existence of a complex pattern of conformations and reveals novel levels of polysomal organization.

BI-ThP12 Adsorption of a Therapeutic Monoclonal Antibody on Surfaces Characterized by X-Ray Photoelectron Spectroscopy and Atomic Force Microscopy, ***K.L. Steffens***, *J.R. Wayment*, National Institute of Standards and Technology

As the number of FDA-approved monoclonal antibody therapeutics increases, the need to understand and control aggregation and surface adsorption of these therapeutics becomes more critical. Aggregation of protein therapeutics is a concern, because aggregates may lead to serious immunological responses in patients. Currently, the cause of protein aggregation is not clearly understood. Recent evidence from several studies has suggested that the interaction of proteins with surfaces may influence solution phase aggregation processes. Therefore, investigation of these effects is critical in order to reduce or eliminate aggregation in biopharmaceutical products. In this study, we investigate the interaction of Rituxan™, a therapeutic monoclonal antibody, with various surfaces including alkanethiol self-assembled monolayers on gold, polyethylene glycol on gold, gelatin on gold, and bare glass. Both as-prepared and heat-stressed (aggregated) protein solutions were investigated with size exclusion chromatography (SEC) before and after contact with the surfaces to measure the presence of small antibody aggregates (monomers to trimers). The presence of larger antibody aggregates formed in solution was assessed by performing AFM of protein solutions deposited and dried on mica. Protein adsorption on the various surfaces was measured using x-ray photoelectron spectroscopy (XPS) to probe the surfaces before and after exposure to the protein solutions. In addition, the standard overlayer model was used to estimate the thickness of adsorbed protein layers. Results show that adsorption of the protein is highly dependent on the surface character as well as on the presence of solution phase aggregates.

BI-ThP13 Univariable Synthetic Material for the Study of Cell Response to Substrate Rigidity, ***A.T. Leonard***, *J.R. Funston,* University of New Mexico*, K.N. Cicotte,* Sandia National Laboratories*, M.N. Rush, E.L. Hedberg-Dirk*, University of New Mexico

There is a strong indication that the mechanics of a substrate plays an important role in many cellular functions. Native human soft tissue has elastic modulus in the range of 0.01 to 1000 kPa. The synthetic materials commonly used to fabricate cell culture platforms with varied moduli, including polyacrylamide and polydimethylsiloxane, are limited in applicability due to a restricted range of achievable moduli and/or surface chemistry instabilities. The copolymer network of n-octyl methacrylate (nOM) and diethylene gycol dimethacrylate (DEGDMA) offers attractive material properties that overcome these limitations. In our laboratory, co-polymer networks were fabricated with 3 to 33 wt% DEGDMA. The compressive modulus was 25±2 kPA for the 3% DEGDMA network and increased with increasing DEGDMA fraction to 4700±300 kPa at 33 wt%. The networks demonstrated consistent surface wettability over the range of gel formulations examined as determined by goiniometry. Surface interrogation with x-ray photoelectric spectroscopy (XPS) at the two extremes of formulations, 3 and 33 wt.% DEGDMA, showed similar elemental and chemical bond compositions. The formulation 3% DEGDMA had elemental composition of 82.1±2.0 % Carbon 1s and 16.8±0.7 % Oxygen 1s. The higher DEGDMA composition of 33 wt% DEGDMA showed 83.2±0.8 % Carbon 1s, 15.4±0.2 % Oxygen 1s. High resolution carbon XPS indicated similar ratios of ether, ester and alkyl groups at the two extremes of DEGDMA compositions. The murine osteoblastic cell line MC-3T3 was used as a model for cell attachment and viability at six and 72 hours, respectfully. Scanning electron spectroscopy was used to visualize the long range nano and micro surface topology. Atomic force microscopy was used to map as well as quantify the surface roughness for each of the formulations. Our results indicate nOM/DEGDMA substrates can vary in modulus over three orders of magnitude while maintaining comparable chemical and topographical surface features. These networks are the first that allow for the study of the effects of material mechanics without the interference of other material properties.

BI-ThP14 In Vitro Cytotoxicity Studies of Antimicrobial Conjugated Polyelectrolytes, ***K.N. Wilde***, *L. Ding, Y. Wang, E. Ji, T.S. Corbitt, L.K. Ista, D.G. Whitten, H.E. Canavan*, University of New Mexico

An estimated 19,000 deaths and $3-4 billion in health care costs per year in the U.S. are attributed to methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Infected individuals inevitably touch a wide variety of surfaces. Therefore, making these surfaces antimicrobial would reduce or prevent the spread of potentially untreatable strains of bacteria. Current sterilization and disinfection techniques tend to be temporary and surface-specific, and require constant vigilance on the part of medical and support staff. Certain conjugated polyelectrolytes (CPEs) with arylene ethynylene repeat unit structure have been demonstrated to exhibit dark and light-activated antimicrobial activity. Both in solution and anchored to a support, these polymers have been effective at killing Gram-negative bacteria, specifically *Pseudomonas aeruginosa* strain PAO1 and *Cobetia marina*. This light-activated antimicrobial activity enables their use in a wide range of potential applications. However, until recently, it was unknown if the CPEs would exhibit similar biocidal activity toward mammalian cells. In this study, bovine aortic endothelial cells were exposed to two different CPEs for increasing periods of time, from 10 minutes to 24 hours, in both light and dark conditions. The relative cytotixicity was then assessed using a live/dead fluorescence assay, and imaged via epi-fluorescence microscopy. While CPEs demonstrate biocidal activity toward *P. aeruginosa* strain PAO1 and *C. marina*, these polymers do not appear to be toxic toward mammalian cells when the cells are exposed to the polymers in both light and dark conditions. Further work is underway to evaluate cytotoxicity at concentrations above the micromolar concentrations tested to date, to correlate mammalian test conditions to bacterial test conditions, and to include an epithelial cell line. Because membrane disruption is a common mechanism of action for antimicrobial agents, molecular dynamic simulations will also be performed to model polymer insertion into a hydrated zwitterionic phospholipid bilayer. These combined data are important to determine how to best incorporate CPEs into antibacterial household and health care products.

BI-ThP15 Aptamer-based Protein Recognition using CMOS Single-Photon Detector Arrays for Time-Resolved Analysis, *L. Pasquardini,* Center for Materials and Microsystems - FBK, Italy*, M. Benetti,* University of Trento, Italy*, L. Lunelli,* Center for Materials and Microsystems - FBK, Italy*, D. Iori,* University of Trento, Italy*, L. Pancheri, F. Borghetti, L. Gonzo,* Center for Materials and Microsystems - FBK, Italy*, G.F. Dalla Betta,* University of Trento, Italy*, D. Stoppa,* ***C. Pederzolli***, Center for Materials and Microsystems - FBK, Italy

There is a high demand for convenient methodologies for detecting and measuring the levels of specific proteins in biological samples because their detection, identification and quantification can be very complex, expensive and time consuming. Biosensors are interesting tools offering certain operational advantages over standard photometric methods, especially with respect to sensibility, duration, ease-of-use, cost, simplicity and portability. Regarding this research field we are developing a monolithic silicon biochip suitable for detecting traces of proteins in biological fluids that are not detected by conventional immunoassays. Particularly in this contribute we discuss the performances of SPAD (Single-Photon Avalanche Diode) detector arrays fabricated in CMOS technology utilized within a lab-on-chip system consisting of a micro-reactor (MR) array for bioaffinity assays based on fluorescence markers. In a typical fluorescence lifetime experiment a pulsed laser is used to excite the fluorescent dyes and the emitted light is revealed by means of high sensitivity detectors. The utilized SPAD detector module, having a total area of 600 x 900 μm2 per MR, can be arranged to build a small pixel array to be directly coupled to the MRs. No emission filters are needed, since the ultra-short laser pulse is cut off in the time domain. Every module consists of a 10x10-SPAD sub-array, where each SPAD cell is equipped with dedicated active quenching and recharging circuit. A memory has also been implemented in order to enable only low dark count rate (DCR) SPADs, so that a total DCR of about 100kHz can be achieved for the whole photosensitive area. Two time-windows have been implemented in this architecture, with a time width that can be set within the range 500ps-10ns with a resolution of 500ps.

The biofunctional layer is based on a dual-site binding strategy with aptamers, that are short single-stranded DNA folded into well-defined three-dimensional structures to form binding pockets and cleft for the

specific recognition and tight binding of the molecular target. The biological model chosen for the present work is the protein thrombin (factor IIa). Two aptamer sequences able to bind to different sites of this protein are used. The first aptamer, anchored to substrate, specifically immobilizes the target protein to the sensor surface, while the second sequence, carrying a fluorescent molecule, allows target detection.

BI-ThP16 Adsorption of Beta-Helical Peptides, ***J.L. Kulp***, *K.P. Fears, T.D. Clark*, Naval Research Laboratory

This poster will describe surface adsorption of beta-helical scaffolds derived from peptides composed of alternating D- and L-amino acids. The monomeric and stable conformation of beta-helical peptides in solution provides a well-defined starting point for discriminating the changes in secondary structure of peptides induced by surface adsorption. NMR, CD, VCD, and XPS results will be presented.

BI-ThP17 Surface Characterization of Baked-on Siliconized Vials for Biopharmaceuticals, ***G. Torraca***, *A. Vance, P. Masatani, L. Wong, B. Eu, M. Pallitto, M. Ricci, Z.-Q. Wen*, Amgen, Inc.

Protein formulations have been found to interact with the glass container surface, resulting in protein adsorption, glass delamination, or glass dissolution. To mitigate surface interactions, alternative primary containers with chemically modified surfaces are actively investigated. One container coating technology that was studied utilized a method of coating the vial interior with a dilute emulsion of polydimethysiloxane (PDMS) and then baking the vials to form a durable layer. The baked-on layer is meant as an isolation layer that insulates the drug product from the glass surface to prevent glass degradation as well as product interactions. Baked-on siliconized vials from two primary container vendors were examined for changes in surface properties over a 6-mo stability timecourse. To better understand and compare representative samples, three analytical techniques were employed to study the interior surface of the vials. These were Polarized Light Microscopy, X-ray Photoelectron Spectroscopy (XPS) which is also referred to as Electron Spectroscopy for Chemical Analysis (ESCA) and Contact Angle Measurement. The baked-on PDMS layer of the vial container was determined to be incompatible with the formulations that were studied. The coating characteristics and the robustness of these coatings from each vendor are discussed.

BI-ThP18 Accurate Delivery and Detection of Single Biomolecules Using Sub-Attoliter Pipettes on a Confocal/AFM Microscope, ***Y. Hu***, *S. Tadayyon, R. Taylor, J. Dechene, P.R. Norton*, University of Western Ontario, Canada

The development of novel techniques to accurately deliver, manipulate, and detect single molecules is one of the most interesting but challenging subjects in modern biotechnology and molecular biology. Most of the recent studies are focused on electrochemical methods using glass micro/nano pipettes that are mechanically pulled and an electrical field used to move molecules through microchannels. However, "single-molecule delivery" has not yet been achieved.

We report on our recent studies of single molecule delivery using a sub-attoliter reservoir formed at the end of what is essentially an NSOM optical fiber, mounted on a combined AFM/confocal fluorescence microscope. The reservoir is formed by chemical etching and focused ion beam (FIB) milling and is then coated with Al to confine the light for fluorescence detection in an NSOM geometry, and for the expulsion of the solution containing the molecule of interest. The volume of the reservoir can be in the range of attoliters by control of the etching and/or milling conditions. Initial experimental results using fluorescein dye indicates that when the solution concentration is low enough, single molecule delivery is achievable. Further study on cell labeling is discussed.

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BI-ThP19 Exploring Critical Parameters in Biointerface Design using Plasma-Modified Polymers, ***S.H. North***, NUARI-Institute for Advanced Sciences Convergence, *E.H. Lock, J. Wojciechowski, C.R. Taitt, S.G. Walton*, U.S. Naval Research Laboratory

The development of new transducing substrate materials, such as polymers, has spurred a growing interest in assessing the influences of surface topography and chemistry of the abiotic layer on the bio-functionality of the biotic layer. However, it is a challenge to differentiate the contributions of surface roughness and surface chemistry to biointerface functionality, as most surface modification methods tend to alter *both* at the same time. In this work, we discuss a dry and wet chemistry approach to biointerface development on polymeric substrates aimed at minimizing changes in the surface morphology. Specifically, an NRL developed electron beam-generated plasma processing system was used to functionalize the surface of polystyrene microtitre plates, which were then silanized to covalently immobilize biomolecules. Electron beam plasmas are unique in that they deliver high flux of low energy (< 5 eV) ions to substrate surfaces enabling selective, chemical alteration of the top few nanometers of any material *without* significant morphological modification. Thus, reactive hydroxyl groups, that make the characteristically inert material amenable to silane chemistry, can be introduced while maintaining substrate morphology. Silanization is an easy, controllable, and conformal covalent immobilization method that supports a wide variety of bio-immobilization schemes. Biomolecules covalently immobilized using this combined dry-wet chemistry approach retained functionality and demonstrated attachment efficiency comparable (and in some cases superior) to specialized commercial microtitre plates. We have used a combination of complementary surface analytical techniques to evaluate the relationships between the physical and chemical properties of the treated polymer surfaces, with particular emphasis on attachment of a functional silane layer, which is a critical parameter for efficient covalent bio-immobilization. Based on our results, we conclude that the development of novel interface materials with superior transducing capabilities is contingent on the deeper understanding of the complex physicochemical interactions between substrate polymer and the chemical/biological components attached to it.

Biomaterial Interfaces
Room: Taos - Session BI+MN-FrM

Sensors & Fluidics for Biomedical Applications
Moderator: S.L. McArthur, Swinburne University of Technology, Australia

8:20am **BI+MN-FrM1 Release of Biomolecues from a Photovoltaic Device for Targeted Drug Delivery**, ***S.L. Ambure***, University of Texas at el Paso, *D. Terreros,* Texas Tech Univerity Health Sciences Center, *T. Xu*, University of Texas at el Paso

Introduction

An important goal of targeted drug delivery is to minimize the exposure of normal tissues to the drugs while maintaining their therapeutic concentration in diseased parts of the body. However, current methodologies are not yet ideal for such goal; therefore, new strategies for targeted drug delivery are needed. A photovoltaic device (PD) is a system that converts lights into electricity as well as induces charge transfer by photovoltaic effect. Motivated by such unique property, we have hypothesized that a PD can serve as a new drug delivery system to carry chemotherapeutic drugs and release them upon external photo stimulation, such as near Near-Infrared (NIR) light or Laser source. Taking advantage of repulsion between a photovoltaic device and a substance is proposed to serve as a new drug delivery method. In this study, we have investigated if the if the charged molecules can be effectively released from the PV device upon photon stimulation.

Methodology

As proof of principle, we have first experimented coating of commercially available photovoltaic devices with positively charged poly-l-lysine and negatively charged bovine serum albumin (BSA) tested the release of the molecules upon photo stimulation. These molecules were physically absorbed onto the surface of the PDs before exposed to an IR LED illuminator, which was used as an external light source. Moreover, the pure glass slide was used as a control of the device with non-photovoltaic effect for drug delivery.

Results and Discussion

During the series of experiments, we have found that, the PD has a capability to release the charged drugs by photovoltaic effect. Compared to no light stimulation, the positively charged poly-l-lysine and the negatively charged BSA when exposed to IR illuminator for 3 hours were released about 2.0 folds and 2.1 folds respectively. Moreover, in the control group (pure glass slide with no photovoltaic effect), there was no significant release of both poly-l-lysine and BSA when exposed to the IR illustrator.

Conclusion

These data showed that the new PD can effectively carry either positively or negative charged molecules on its surface and release them upon external photo stimulation, which suggests the PD has potential to be used as a new drug delivery system to carry cancer chemotherapeutic drugs. Further experiments are planned on the micro-fabricated photovoltaic devices (Size 300μm to 500 μm). With this project a new approach for targeted drug delivery with micro- photovoltaic devices will be developed.

(*Proprietary: an IP application based on this study is pending)

8:40am **BI+MN-FrM2 Enzymatic Activity Enhancement on Nano-structured TiO_2 Platforms by Ru doping for Biosensor Applications**, ***R.R. Pandey***, Centre for Cellular and Molecular Biology, India, *K.K. Saini,* National Physical Laboratory, India, *M. Dhayal*, Centre for Cellular and Molecular Biology, India

Devices based on nanomaterials platforms are emerging as a powerful tool for ultrasensitive sensors for the direct detection of biological and chemical species. In this work, we will report the preparation and the full characterization of Ru doped TiO2 nanostructured platforms those have been used for electrochemical detection of enzymatic activity for biosensor applications. Ru doped TiO2 platforms were prepared by sol-gel deposition onto conducting substrates and as test model cholesterol oxidase, urease and gucose oxidase were loaded onto the nanostructured platforms. Cholesterol /Urease/Glucose oxidase immobilized onto Ru doped TiO2-based nanostructured surfaces exhibited a pair of well-defined and quasireversible voltammetric peaks in CV measurements. The electron exchange between the enzyme and the electrodes was greatly enhanced in the Ru doped TiO2 nanostructured environment. The electrocatalytic activity of cholesterol, uresae and glucose oxidase embedded on Ru doped TiO2 electrodes had enhanced significantly toward detection of low concentrations of cholesterol, urea and glucose.

9:00am **BI+MN-FrM3 A Microfluidic Single Cell Isolation Device for Ensemble Measurements of Viral Hemorrhagic Fever Pathogenesis in Macrophages**, *M.W. Moorman, J.B. Ricken, R.F. Renzi, R.P. Manginell, C.S. Branda, O.A. Negrete,* ***C.D. James***, *B.D. Carson*, Sandia National Laboratories **INVITED**

Arenaviruses are a particular class of viruses that cause lethal hemorrhagic fever in humans, and a fundamental problem in understanding their pathogenicity is that many effects of viral infection are not mediated directly by the virus itself (primary immune response) but by the response of the immune system (secondary immune response). Thus, population level experiments on cells make it difficult to elucidate the timing of signaling events during pathogenesis in order to lay the groundwork for improved antiviral therapeutics, vaccines, and biological countermeasures. Our objective here is to deconvolute the pathogenic response by isolating and infecting individual macrophage host cells followed by real-time measurements of response-critical cytokines. We have developed a microfluidic cell isolation platform that can trap up to 150 individual host cells in fluidically isolated microchambers. The chip design eliminates chamber-to-chamber fluidic communication, thus signaling molecules that are secreted from infected cells are prevented from interacting with uninfected cells. This configuration allows us to differentiate between primary and secondary immune response when compared to bulk cell population level studies. The chip is made with a three level reactive-ion etch process in silicon that produces trapping features that place cells adjacent to an anodically-bonded coverslip to permit high-resolution confocal imaging. The microfluidic device is operated with a custom pressure controller system that permits computer-controlled delivery and routing of up to 10 different reagents. Currently, we are using experimental and computational techniques to identify the mechanisms by which arenaviruses provoke lethal cytokine production in host cells. This is accomplished with a fluorescent reporter fusion construct that we developed to measure the cytoplasm-to-nucleus translocation dynamics of a transcription factor. This construct allows us to assess the early (< 1 hour) response of host cells to viral infection and when combined with a second reporter construct for real-time monitoring of cytokine induction, we are also able to monitor a late (>1 hour) immune response event. Initial studies using a viral mimic challenge showed oscillation of the transcription factor in and out of the nucleus over the first several hours of pathogen exposure, and a rapid 2X increase in cytokine induction over the first five hours post-infection. Future work will use a live Pichinde virus to examine transcription factor (NFkappaB) translocation and cytokine (TNFalpha, IFNbeta) induction dynamics.

9:40am **BI+MN-FrM5 Elastomeric Microparticles that Exhibit Negative Acoustic Contrast in Bioassays**, ***K.W. Cushing***, *M.E. Piyasena, B.A. Lopez, N. Carroll, T. Woods, D.N. Petsev, S. Graves,* University of New Mexico, *G.P. Lopez*, Duke University

The development of more sensitive and rapid medical assays is imperative to decreasing time-to-diagnosis in diseased state individuals, and thus to improving patient outcomes. Enhanced detection limits afford the ability to detect the presence of low concentrations of biomarkers that may be present during the early-onset stages of a disease. Reduction in sample preparation requirements can further decrease assay time and the expertise level of the user. Our research aims to develop a sensitive and rapid bioassay platform using elastomeric capture microparticles (ECμPs) coupled to an acoustic sample preparation chamber and a flow cytometer. ECμPs possess unique physical and mechanical properties enabling the separation of ligand-bound ECμPs from biological particles (e.g., red blood cells) within a collected fluid sample (e.g., whole blood) by placing them under acoustic pressure. ECμPs have acoustic properties (negative contrast) that allow their positioning separately from many biological particles, which typically exhibit positive contrast, (e.g., cells) within an acoustic pressure field. Hence, an in-line acoustic sample preparation instrument can be used to separate unwanted biological particles (along with interfering soluble molecules) from ligand-bound ECμPs within biological fluids, The acoustic field will also concentrate the ECμPs, and so combined with removal of unwanted biological particles, much higher analysis rates of ECμPs may be possible. Since the acoustic sample preparation system operates continuously and can be mounted to feed separated ECμPs directly into a flow cytometer, this approach may decrease sample preparation time. Our studies show that simple emulsion polymerization methods using commercially available silicone precursors can be used to easily form elastomeric microparticles that exhibit negative contrast.

10:00am **BI+MN-FrM6 Optimization of Biosensors using Selective Chemistry**, ***O. Seitz***, *P.G. Fernandes,* University of Texas at Dallas*, H.C. Wen,* Texas Instruments, Inc*., H.J. Stiegler, R.A. Chapman, E.M. Vogel, Y.J. Chabal*, University of Texas at Dallas

There is currently a strong need to develop sensitive and reliable biosensors, based on electronic detection, such as field-effect transistors (FET). Most of the focus has been on improving sensitivity by decreasing the FET channel size, using nanowires instead of similar devices on planar silicon. Issues of silicon functionalization, important for device reliability have been mostly ignored.

In this work, we present a robust approach to functionalize the channel region of a SOI wafer, thus achieving better reliability and sensitivity to very low analyte concentrations. The process leads to attachment of active SAM on oxide-free (H-terminated) silicon through formation of a Si-C bond on the channel. Combining IR absorption (IRAS) and X-ray photoelectron (XPS) spectroscopies, photoluminescence, atomic force microscopy (AFM) and electrical measurements, we find that this configuration results in a stable device where the active SAM is more strongly attached to the Si than silane molecules do on oxides. This functionalization is achieved by immersion in carboxylic acid (COOH)-terminated alkene molecules to functionalize the H-terminated channel. After processing, XPS and IRAS confirm that the channel remains oxide-free, that the packing of the SAM on the channel is dense. Photoluminescence measurements confirm the high quality of the interface on the channel where non-radiative recombination (interface states) is not detected. The AFM pictures confirm that active molecules attach to the channel (imaged by attachment of nanoparticles). Electrical measurements, on these improved devices, indicate excellent response for both pH and protein sensing with sensitivity at least as good as the one of similar structure with a uniform SAM functionalization (i.e. using oxidized Si channels).

10:20am **BI+MN-FrM7 Nanopatterned Pores on a Gel-supported Membrane For On-chip Sample Preparation in Surface Plasmon Resonance Sensing**, ***G.R. Marchesini***, Joint Research Centre, Italy*, S. Rebe Raz,* Wageningen University, the Netherlands*, M.G.E.G. Bremer,* RIKILT – Institute of Food Safety*, P. Colpo, G. Giudetti,* Joint Research Centre, Italy*, W. Norde,* Wageningen University, the Netherlands*, F. Rossi*, Joint Research Centre, Italy

10:40am **BI+MN-FrM8 Stability and Selectivity of Biorecognition Proteins Immobilized on Diamond Surfaces**, *R.J. Hamers,* ***C.J. Stavis***, University of Wisconsin-Madison*, A. Radadia, R. Brashir,* University of Illinois at Urbana-Champaign*, J.A. Carlisle,* Advanced Diamond Technologies*, W.P. King,* University of Illinois at Urbana-Champaign*, H. Zeng*, Advanced Diamond Technologies

The use proteins, such as antibodies, for the detection of target biological species in water supplies or with *in situ* medical diagnostics will require immobilization of these proteins on surfaces that resist non-specific adsorption and maintain the protein's activity over time. Ultrananocrystalline diamond (UNCD) thin films are a promising material that may address several major challenges for the next generation of biosensors including detection of cellular mass loading, stability throughout multiple uses and regenerations of the sensor surface, and use at elevated temperatures.

We are currently investigating the chemical functionalization of diamond thin films with antibodies for selective recognition and detection of biological cells, using E. coli as a model system. Infrared spectroscopy and X-ray photoelectron spectroscopy measurements have been performed to characterize the covalent attachment of antibodies to the surface and to quantitatively characterize the antibody surface attachment via the N(1s) and S(2p) levels. To determine the factors controlling selectivity and stability, we have performed time-dependent cell capture studies and have correlated the time-dependent changes in cell capture efficiency with corresponding measurements of the surface composition. These measurements are used to establish whether long-term stability and selectivity for biomolecular recognition is limited by loss of the ligands directly linked to the diamond substrate, by removal of the biological layer, or by alteration of the antibody structure. Infrared measurements of the Amide I band is particularly useful in characterizing changes in the antibody secondary structures. These studies provide important fundamental insights into the chemical factors that control biological interactions at surfaces and provide guidance on efforts to make ultra-stable biological sensing platforms.

11:00am **BI+MN-FrM9 Saturated Ionic Conductance at Low Electrolyte Concentration Through Solid-State Nanopores**, ***P. Waggoner***, *H. Peng, S. Harrer, B. Luan, S.M. Rossnagel*, IBM T.J. Watson Research Center

It has been observed that ion currents pass through biased nanopores in electrolyte solutions with bulk conduction properties until the concentration of ions decreases beyond a certain level. At this critical point, the conductance of the pores saturates at a constant value despite further decreasing the concentration of ions, an effect that has been attributed to charge shielding effects of surface charges within the pore. Below the critical concentration, the Debye length associated with screening the surface charges becomes larger and larger compared to the size of the pore and effectively cuts off bulk conduction. However, the conductance of the pore is not affected by the continuing growth of the space-charge region with the Debye length. In the following we present a model describing the nanopore-electrolyte system with an analytical solution that explains the experimentally observed behavior for five different salt solutions at low concentrations, including KCl, KCl in 50% glycerol, Tris-EDTA buffer, phosphate buffered saline, and $CaCl_2$. Conduction through the nanopore at low ion concentrations is analogous to hole extraction in reverse biased diodes and is related only to the diffusion of the minority carrier into the space charge region where it is then accelerated through the nanopore. These results also have important implications for solid-state nanopores being applied for DNA detection and sequencing technologies.

11:20am **BI+MN-FrM10 Fabrication of *in situ* Oligonucleotide Arrays by Inkjet Printing and their use in Gene Assembly**, ***I. Saaem***, *J. Tian*, Duke University

In our studies, we utilized an inkjet based *in situ* oligonucleotide synthesis platform that uses salvaged printheads from commercial printers. The platform utilizes standard four-step phosphoramidite chemistry with some modifications in order to synthesize oligonucleotides on functionalized substrates. A sensitive pressurization system is used to ensure print quality and an on-board vision system enables substrate registration and synthesis monitoring. Using this platform we synthesized oligonucleotide on prepatterned functionalized plastic slides. Such patterned substrates help in proper droplet formation and fluid mixing on the surface while mitigating satellite and irregular drops, which can lead to cumulative synthesis errors. Functional integrity of synthesized oligonucleotides was confirmed by hybridization with complementary strands. Being able to hot emboss microfluidic structures directly onto plastic slides in combination with the ability to generate arbitrary sequences provides diagnostic capabilities as well as the means to harvest pools of cheap oligonucleotides on demand. Importantly, our combination of technologies has allowed formation of genes and large DNA constructs by amplifying oligonucleotides off of the synthesized arrays and assembling them in the on-chip chambers.

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Walton, S.G.: BI-ThP19, 26
Wang, J.S.: BI-ThP6, **24**
Wang, P.: BI-WeA10, 15
Wang, Q.: BI1-TuA2, 9
Wang, R.: NS+BI-ThA1, 20
Wang, Y.: BI-ThP14, 25
Wayment, J.R.: BI-ThP12, 25
Wehbe, N.: BI2+AS-TuA12, 10
Weidner, T.M.: BI1-WeM6, 12; BI2+AS-TuA8, 10; NS+BI-ThA8, **21**
Wen, H.C.: BI+MN-FrM6, 28
Wen, Z.-Q.: BI-ThP17, 26
Werner, U.: BI-ThP2, 23
White, J.D.: MB+BI-MoM5, 3
Whitten, D.G.: BI-ThP14, 25
Wilde, K.N.: BI-ThP14, **25**
Wilker, J.W.: MB+BI-MoM5, **3**
Willemsen, P.R.: MB+BI+AS-MoA3, 4
Williams, P.: BI1-TuA1, 9
Wind, S.J.: NS+BI-ThA1, 20
Wirth, T.: BI-ThP5, 23
Wojciechowski, J.: BI-ThP19, 26
Wong, L.: BI-ThP17, 26
Woods, T.: BI+MN-FrM5, 27
Wu, Y.: BI1-TuA2, **9**

— X —

Xu, T.: BI+MN-FrM1, 27; IJ+BI+MN-MoM8, 1; IJ+BI+MN-MoM9, 2

— Y —
Yamada, T.: IS+BI+AS-WeA10, **16**
Yanez, M.: BI-ThP4, **23**
Yang, J.: BI1-TuA1, 9; NS+BI-ThA10, 21
Yarin, A.L.: IJ+BI+MN-MoM1, **1**
Ys, H.: BI1-TuA3, 9
— Z —
Zauscher, S.: BI-ThP8, 24
Zeng, H.: BI+MN-FrM8, 28
Zhang, L.: NS+BI-ThA10, 21
Zhu, X.-Y.: BI-WeA4, 14
Zychowicz, M.: BI-TuM2, 6