Biomaterial Interfaces

Room: 4C - Session BI-TuP

Biomaterials Interfaces Poster Session

BI-TuP1 Investigating the Adhesion of Biomolecules on Plasma Polymerized Thin Films, ***R. Foerch***, *S. Brueninghoff, E.-K. Sinner, W. Knoll, R. Berger*, Max-Planck-Institut for Polymer Research, Germany

The controlled adhesion of biomolecules on surfaces has become a major topic of interest in biomaterial surface design. The interest in this has been triggered by a broad range of biomaterial applications. For example, those that rely on proteins and cells immobilized on solid surfaces for the development of new implant materials and tissue regeneration. It is generally accepted that both the surface morphology and the surface chemistry need to be in concert to ensure optimum conditions for biomaterial adhesion. However, the surface interactions are very complex and are not completely understood. Further, there are only very few analytical tools available that deliver reliable, real time insights into the biomaterial/ surface interactions. We present recent results in which plasma polymerization techniques have been used to prepare "model" surfaces that can be conditioned towards the adhesion of a particular mammalian cell line (P19 progenitor cells). Techniques such as Surface Plasmon Resonance (SPR), Micro-Cantilever Sensor technology (MCS) and standard optical microscopy have been used to study in real time the adsorption of proteins and P19 cells on such surfaces. Correlations to the chemical environment at the interface are made.

BI-TuP2 Tailoring Surface Properties of Spider Silk Protein Films for Biomaterial Applications, ***P.A. Johnson***, *H. Zhang, C. Skinner, T.G. Martinez-Servantez*, University of Wyoming

Spider silk is well known for its unique, outstanding material properties. Dragline spider silk in particular is one of the strongest natural materials with a high degree of elasticity. These properties, together with its inherent biodegradability and biocompatibility, make it a promising biomaterial for tissue engineering applications. Though the best material properties are derived from native dragline spider silk, producing spider silk naturally is not practical to generate sufficient quantities for biomedical applications. Recombinant spider silk proteins have been successfully produced in bacterial expression systems as well as in goat's milk. In our studies we compared the surface properties and biological responses of native major ampullate silk from N. clavipes, the major ampullate proteins produced in E. coli, and the major ampullate proteins produced in goats' milk. Thin films were cast from hexafluoroisopropanol and then treated with 90% methanol. The films with and without methanol treatment were characterized by AFM, SEM and contact angle analysis. The untreated films initially had very different surface properties, but after methanol treatment the contact angles and surface roughness converged to similar values. Once processes for generating consistent films were established, the biological response of the films was determined. Protein adsorption studies were conducted via mass sensitive techniques (QCM-D). Cellular responses were established to determine cell adhesion, cell morphology and cell proliferation. The films were protein resistant and inhibited cell adhesion. Therefore, to promote cell attachment and growth the spider silk films were modified with cell binding peptides.

BI-TuP3 Self-Assembly of Biomolecules at Surfaces, Characterized by NEXAFS, ***X. Liu***, *F. Zheng*, University of Wisconsin-Madison, *A. Jürgensen*, Canadian Synchrotron Radiation Facility, Synchrotron Radiation Center, *V. Perez-Dieste*, Universitat Autònoma de Barcelona, Spain, *D.Y. Petrovykh*, University of Maryland and Naval Research Laboratory, *N.L. Abbott, F.J. Himpsel*, University of Wisconsin-Madison

Surface science has made great strides towards tailoring surface properties via self-assembly organic molecules. It is now possible to functionalize surfaces with complex biomolecules, such as DNA and proteins. However, probes of the chemical orbitals involved in this process have remained scarce. Here we show how NEXAFS (Near Edge X-ray Absorption Fine Structure spectroscopy) can be used to characterize the assembly of biological molecules at surfaces in atom- and orbital-specific fashion. The technique is illustrated by self-assembled monolayers with customized terminal groups. Applications are demonstrated by DNA oligonucleotides and Ribonuclease A[1], a small protein containing 124 amino acids. The N 1s absorption edge is particularly useful for characterizing DNA and proteins, because it selectively interrogates the π^* orbitals in nucleobases and the peptide bonds in proteins. Information about the orientation of molecular orbitals is obtained from the polarization dependence. Quantitative NEXAFS models are developed to explain the polarization dependence in terms of molecular orientation and structure.

[1]Xiaosong Liu et al., Langmuir 22, 7719 (2006).

BI-TuP4 Adsorption of an S-layer Bacterial Protein by Total Internal Reflection UV Absorption Spectroscopy, ***M.A. Bratescu***, Nagoya University, Japan, *D.B. Allred*, Nagoya University, Japan, and University of Washington, *N. Saito*, Nagoya University, Japan, *M. Sarikaya*, University of Washington, *O. Takai*, Nagoya University, Japan

Surface-layer (S-layer) proteins from many species of bacteria and archaea self-assemble into two-dimensional supramolecular arrays and form specific space groups. Although S-layer proteins are now used as molecular and nanoscale templates for nano- and bio-nanotechnology, the fundamental bases of assembly and ordered organization are still under study. The purpose of our research is to characterize adsorption specificity, surface interactions, and assembly of S-layer proteins on solid surfaces. We use absorption of UV evanescent light produced by multiple total internal reflections in a quartz IRE sensor which is sensitive to a depth of a few tens of nanometers. The S-layer proteins were prepared as described in Ref. [1], starting from a cell culture of Deinococcus radiodurans. A final concentration of protein in sodium dodecyl sulfate was estimated to be 0.5 mg mL^{-1}. For experiments, the protein solution was diluted in deionized water to a concentration of 0.2 mg mL^{-1}. A comparative analysis of the S-layer protein adsorption was performed on different functionalized surfaces (amino- and hydroxyl- terminated) or surfaces deposited with noble metals (platinum and gold). The amino-terminated monolayer was obtained by dipping freshly cleaned quartz into a 1 wt % solution of (3-aminopropyl) trimethoxysilane in toluene for 3 hours at 60 ° C. The hydroxyl-terminated surface was obtained by photochemical exposure of quartz to VUV radiation at 172 nm. The noble metals were deposited by magnetron sputtering. S-layer protein absorption bands were found at 274 nm and 307 nm, characteristic of tryptophan and tyrosine residues, which have UV absorption bands in the spectral region 270 - 350 nm. The strongest absorption was obtained when S-layer proteins were adsorbed on an amino-terminated surface. Time dependence of protein adsorption will be correlated with data obtained by atomic force microscopy analysis of S-layer proteins on different solid surfaces to derive a better understanding of the adsorption process.

[1]D.B. Allred, M. Sarikaya, F. Baneyx, D.T. Schwartz, Nano Lett., 5 (2005) 609.

BI-TuP5 Adsorption Kinetics and Dynamics of Fibrinogen with Surface Interactions, ***N. Saito***, *Y. Sato, O. Takai*, Nagoya University, Japan

The control of blood coagulation on biomaterial surfaces is an urgent issue in the medical field. The main process occurring in blood coagulation is the polymerization of fibrinogen into erythrocyte-trapping fibrin. Other plasma proteins are also involved in initiating this process. The multi-interactions among proteins and material surface govern the process. The molecular structure of fibrinogen as a free molecule has been investigated in detail. However, its adsorption state on a biomaterial surface has yet to be elucidated. Recently, the adsorption state of fibrinogen on various substrates has been the subject of intensive investigation employing atomic force microscopy (AFM). Such use of AFM has been crucial in the study of molecular biology on surfaces. In this study, the adsorption kinetics and dynamics of fibrinogen with the interaction of heparin onto hydrophobic and hydrophilic surfaces is investigated with AFM analysis and QCM (quartz crystal microbalance). CH3-terminated self-assembled monolayer (SAM) and SiOH-terminated substrates were utilized as the hydrophobic and hydrophilic surfaces. Fibrinogen adsorption proceeded following Langmuir type. On the process, the orientation of fibrinogen on the sample surface was changed. Heparin did not promote desorption of fibrinogen but the adsorption on the hydrophilic surface though it is well known as an anti-coagulation factor. On the other hands, heparin promoted the adsorption on the hydrophobic surface. The differences were discussed based on 3-body interactions analysis.

BI-TuP6 In situ Analysis of Fibrinogen on Self-Assembled Monolayers by Evanescent Optical Spectroscopy, ***M. Matsuno***, *T. Ishizaki, O. Takai, N. Saito*, Nagoya University, Japan

Fibrinogen is one of proteins in blood plasma and plays a great important role on blood coagulation. Many researchers have investigated the adsorption of fibrinogen on various substrates in order to develop inactive biomaterials for fibrinogen. However, the coagulation mechanism has not been understood yet, since it is due to interactions among many factors, ex. other proteins and ions. The research on fibrinogen adsorption from molecular viewpoints has been required. In this study, we aimed to

understand fibrinogen adsorption on OH-terminated, CH_3-terminated, NH_2-terminated and poly(ethylene glycol) (PEG) surfaces using an evanescent optical spectroscopy, an atomic force microscopy (AFM) and a zeta potentiometry. The evanescent optical spectroscopy allows us to detect few fibrinogens on surfaces with high-sensitivity. A quartz glass was used as a substrate, which worked as an optical waveguide. A vacuum ultraviolet (VUV) lamp with a wavelength of 172 nm irradiated the quartz substrates. The surface changed to OH-terminated surface. CH_3-terminated surface was prepared from n-octadecyltrichlorosilane (OTS) dissolved in toluene through liquid phase method. NH_2-terminated and PEG surfaces were prepared from n-(6-aminohexyl)aminopropyltrimethoxysilane (AHAPS) and 2-methoxy[polyethyleneoxy]propyltrimethoxysilane (MPEOPS) through a vapor phase method. Dried fibrinogen was dissolved in phosphate buffered saline (PBS). The concentrations of fibrinogen were adjusted to 0.1, 1 and 10μM. Fibrinogen adsorption process was monitored by the evanescent optical spectroscopy. An absorption peak was observed at wavelength of ca. 280nm, which is attributed to tyrosine and tryptophan in fibrinogen. The change of the intensity against time was determined by types of surfaces. In order to reveal the difference, the fibrinogens on the surfaces were observed by AFM. In addition, zeta potentials of the fibrinogen and the sample surfaces were obtained in order to elucidate the effect of electrostatic interaction among them on adsorption. Finally, we propose a kinetic model of the adsorption.

BI-TuP7 Determining the Surface Release Kinetics of KGF Protein from a Biodegradable Polymer Film, ***S.A. Burns***, *J.A. Gardella Jr.*, SUNY Buffalo

Biodegradable polymers are of interest in developing strategies to control protein drug delivery. The protein that was used in this study is Keratinocyte Growth Factor (KGF) which is a protein involved in the re-epithelialization process. The protein is stabilized in the biodegradable polymer matrix during formulation and over the course of polymer degradation with the use of an ionic surfactant Aerosol-OT (AOT) which will encapsulate the protein in an aqueous environment. The release kinetics of the protein from the surface of the polymer matrix requires precise timing which is a crucial factor in the efficacy of this drug delivery system. Determining the release kinetics was accomplished by a two fold method. The first step was to measure the accumulation of the surfactant and protein at the surface of the polymer film. X-ray Photoelectron Spectroscopy (XPS) was used to measure the surface concentration of the surfactant and polymer using the unique elemental composition of these compounds. The surfactant has been identified from the polymer matrix using the sulfur region while protein identification utilizes the nitrogen signal. Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) was used in the same capacity to identify the molecular ion peak of the surfactant and polymer and use this to determine surface concentration. The surfactant molecular ion peak was observed in the positive and negative mode at m/z 467 and 422, respectively. These peaks were determined to be [AOT + Na+]+ and [AOT-Na+]-. These methods are used to identify the surfactant and protein from the polymer matrix and are used to measure the rate of surface accumulation. The second step was to compare this accumulation rate with the release rate of the protein into an aqueous solution during the degradation of the biodegradable film. This rate is compared to fluorescence spectroscopy measurements that were done using the autofluorescence of the protein that has been released into an aqueous solution. This study was done to determine the release kinetics of an unmodified biodegradable system containing only a polymer, surfactant, and protein. One method that is currently being used to tune the release rate of the polymer is micro-patterning. The pattern will determine the rate at which the polymer degrades and the rate at which protein is released.

BI-TuP8 Protein Encapsulation in Organo-Functionalized Mesostructured Silica and Titania, *C.T. Burns, S.Y. Choi,* ***M.A. Firestone***, Argonne National Laboratory

Interfacing biomolecules to inorganic frameworks is essential for fabricating robust, functionally integrated biocomposites that may prove useful in a wide range of technologies including biocatalysis, biosensors or protein-based devices. Our work is directed at developing means to integrate biomolecules (e.g., proteins) into 'active' mesostructured inorganics. These active frameworks serve to both improve the mechanical stability (robustness) of the proteins and to facilitate communication with the encapsulated guests. Toward that end, we have synthesized and characterized a variety of photoactive mesoporous silicas and have examined the encapsulation of soluble proteins within them. Specifically, we have both carried out post-synthesis modification of the silica pore surface and prepared organosilicas in which spiropyran groups are homogeneously incorporated throughout the walls of the mesoporous silicas as a means to introduce photo- and redox active moieties within the frameworks. Protein incorporation within mesostructured titania thin films has also been achieved. The synthetic strategies used to prepare these materials, and details of the characterization of the frameworks and the biocomposites will be presented.

BI-TuP9 Dynamic Visible Spectroscopic Ellipsometry Studies of Protein Adsorption and Conformational Change, ***S. Sarkar***, *L. Castro, D.W. Thompson, A. Subramanian, J.A. Woollam*, University of Nebraska, Lincoln

Protein adsorption onto surfaces continues to be widely researched, as it is an everyday occurrence either improving or impeding the quality of life. Often investigated are kinetics of adsorption. In the present work we monitored protein adsorption to surfaces with different chemistries. Modified chitosan surfaces and silicon wafers were used. Spin-cast Chitosan was cross-linked and activated with diepoxides and selected chemical ligands (n-butyl amine, t-butyl amine, n-octyl amine, 2,4,6 Tris and 2-t-Ethyl(butyl amine)) and anti-human albumin molecule. These surfaces were created to preferentially adhere to human serum albumin (HSA). Protein solutions consisting of HSA, immunoglobulin and fibrinogen dissolved in phosphate buffered saline were used to study adsorption processes. Solutions were introduced onto modified chitosan using a fluid cell, and dynamic data optically modeled to obtain protein adsorption profiles. Chitosan is a well known hydro-gel, and spun cast chitosan is anisotropic. Our EMA-based optical model accounts for this anisotropy. A thermodynamic adsorption model was formulated which accounts for changes in protein surface binding capacity during dynamic conformational changes. The model assumes irreversible binding of proteins and a process that is not diffusion limited. The adsorption profiles were then modeled and the parameters compared. Our studies indicate typical protein adsorption processes differ from a simplistic Langmuir model. It also describes changes in protein binding rates during a given adsorption cycle. We propose these regions of data result from surface bound protein conformation changes.

BI-TuP10 Performance and Properties of Poly(N-isopropylacrylamide) Based Switchable Coatings, ***M.A. Cole***, University of South Australia, *H. Thissen,* CSIRO Molecular and Health Technologies, Australia, *N.H. Voelcker,* Flinders University, Australia, *H.J. Griesser*, University of South Australia

Surface modification of biomedical and biotechnological devices using thin polymeric coatings is a popular method employed to alter the interactions of synthetic materials with biomolecules and cells from surrounding biological media and environments. Advancements in this field have been made largely from an interdisciplinary approach combining surface modification and polymer science with biomaterials science and biological studies. Recent research into stimuli-responsive or switchable materials has focused on means to control protein-material and cell-material interactions with respect to directing the spatial location, temporal location and biological function of biomolecules. Controlling the interfacial interactions of biological components is of interest for a wide range of biomedical/biotechnological applications including microarrays, biosensors, drug delivery, cell sheet engineering and 'lab on a chip' devices. As part of ongoing research we report our findings on the grafting and properties of stimuli-responsive coatings incorporating poly(N-isopropylacrylamide) (pNIPAM). Thin films of pNIPAM were prepared via 'grafting to' and 'grafting from' techniques and investigated at temperatures above and below the lower critical solution temperature (LCST) of approximately 32 °C. Switching of coatings between hydrated and collapsed states below and above the LCST was investigated with respect to the degree of change in phase transition and the ability to induce protein adsorption. Surface analysis was carried out using X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and time of flight secondary ion mass spectrometry (ToF SIMS). Adsorption of model proteins, lysozyme and bovine serum albumin were investigated using a quartz crystal microbalance (QCM), optical waveguide lightmode spectroscopy (OWLS), TOF SIMS, and AFM. Results from switching and protein adsorption experiments show the transformation of pNIPAM coatings between low fouling (protein resistant) and fouling (protein adsorbent) states. Colloid probe force analysis of pNIPAM coatings reveal considerable changes in protein-pNIPAM interactions at different temperatures. The present study is expected to assist the development of switchable coatings for biomedical and biotechnological applications.

BI-TuP11 Molecular Dynamics Parameterization for Electrostatic Interactions between Proteins and Biomaterial Surfaces, ***G. Collier***, Clemson University, *B.R. Brooks,* National Institutes of Health, *S.J. Stuart, R.A. Latour*, Clemson University

Since protein-biomaterial interactions govern the biocompatibility of implanted materials, controlling biocompatibility through material design must begin with the study of protein-biomaterial interactions at the atomic level. All-atom molecular dynamics simulation provides an excellent approach to investigate this type of problem. However, current molecular

dynamics simulation methods and parameters are generally not designed to accommodate the unique types of atomic interactions that exist for the case of a protein interacting with a functionalized surface. To address this problem, we have begun adapting the molecular modeling community's range of tools to develop a set that is specifically designed for the simulation of the adsorption behavior of proteins to functionalized surfaces. Protein adsorption behavior is predominantly governed by nonbonded interactions, with electrostatic effects representing the strongest type of these interactions and the type that is most difficult to accurately represent. In an effort to establish the most appropriate method of treating electrostatic interactions for the simulation of adsorption processes, we are evaluating the calculated differences in ion distribution over a charged surface using a variety of nonbonded interaction techniques. Our 4.5 x 4.3 x 10.0 nm^3 model system is comprised of a 150 mM NaCl aqueous solution with TIP3P water over a 50% deprotonated COOH-SAM surface (pK_a = 7.4) with Na^+ counterions. Nanosecond-scale molecular dynamics simulations are then conducted to model the structure of the electric double layer over the surface using a series of different methods to represent the electrostatic interactions of the system, including particle-mesh Ewald, radial cutoffs, isotropic periodic sum, and anisotropic periodic sum methods. The results of these simulations are then compared to the analytical solution of the ion distribution based on the Poisson-Boltzmann equation to gauge the accuracy of each of the different simulation methods. Within each different method, the parameters defining the limits of atomic interactions, such as interaction cutoff distances in the case of radial cutoffs, have been varied to establish a balance between computational cost and simulation accuracy. The results of this study will establish the most efficient and accurate method for the representation of nonbonded electrostatic interactions for the simulation of protein-surface interactions.

BI-TuP12 Photogenerated Surfaces for Carbohydrate-Protein Interactions, ***O. Ramstrom***, Royal Institute of Technology, Sweden, *M. Yan*, Portland State University

Photogenerated bioactive surfaces have been developed following a perfluorophenylazide-based, double ligation strategy. Gold-plated quartz crystal microbalance crystals were initially coated with different polymers, either covalently or non-covalently, and the resulting surfaces were subsequently used as substrates for photoinsertion of active photoprobes. A range of different polymers were evaluated, where high biocompatibility and durability could be shown. The photoprobe insertion yielded a range of different carbohydrate-based recognition motifs presented at the surfaces, which were subsequently used as sensor recognition elements in a flow-through quartz crystal microbalance instrumentation. A series of different carbohydrate-binding proteins (lectins) were analyzed, and selectivity and affinity of protein binding could be efficiently monitored. The results clearly show the predicted protein selectivities, demonstrating the applicability of the approach.

BI-TuP13 Microlens Array Patterning of Glass and Silicon for Protein Bioarray Formation, *M.R. Linford, M.C. Asplund, R. Gates, F. Zhang,* ***G. Saini***, Brigham Young University

Microlens arrays (MLAs) are powerful tools for surface modification. These commercially available optics offer a wide range of shapes and packings for their microlenses. Here we describe the use of MLAs for patterning monolayer-coated glass and silicon substrates. In this process, a nanosecond pulse of laser light is directed through a MLA. In the region where the microlenses focus the light onto the surface the protective monolayer is removed leaving a pattern of spots. A wide variety of proteins, including ferritin and ferritin analogs, adsorb directly onto these spots. A reactive polymer will also adsorb to these spots and undergo subsequent reactions that are typical of bioconjugate chemistry. This process is further demonstrated with 266 nm light on glass, where glass is the substrate material of choice for bioarrays.

BI-TuP14 Protein Adsorption Correlated with Surface Properties of Copolymer Libraries Synthesised as Microarrays, ***M. Taylor***, *A.J. Urquhart,* University of Nottingham, UK, *D.G. Anderson, R. Langer,* Massachusetts Institute of Technology, *M.R. Alexander, M.C. Davies*, University of Nottingham, UK

Combinatorial methods have become increasingly popular as a means of material development, allowing rapid discovery and optimisation of new materials. Micro patterned combinatorial material libraries have been shown to be a useful method of screening materials for a number of biological applications. Protein adsorption to surfaces underpins biological response and is therefore of great importance in both implantation and tissue culture situations. Adsorbed proteins effectively translate the structure of a surface into a biological language that ultimately influences the way cells adhere and function. Hence, understanding why and how different proteins adsorb to different surfaces and the effect this has on cell adhesion and growth is of major importance. In this abstract, we report on the adsorption of fluorescently labelled fibronectin to a spatially patterned micro-arrayed library of 480 novel copolymers designed to illicit a range of surface phenomena. Using partial least squares models, protein adsorption has been related to the data generated from the high-throughput surface analysis of the array, including surface chemistry (ToF-SIMS and XPS) and wettability (contact angle, surface energetics), as well as the data derived from the screening of the adhesion and proliferation of the chicken embryonic stem cells to the copolymer library. Interesting correlations between surface phenomena and biological response have been derived from the large data sets, information that will provide important pointers for controlling cellular interactions with such polymeric surfaces.

BI-TuP15 On the Thermodynamics of Protein Adsorption Processes, ***J.C. Hower***, *Y. He, S. Jiang*, University of Washington

While significant advances in biocompatible and environmentally benign materials have been made, one of the remaining challenges is to understand surface resistance to protein adsorption. Significant experimental efforts have produced only a small number of nonfouling materials and coatings. Moreover the mechanisms of protein resistance are poorly understood and a majority of new material breakthroughs are made fortuitously. Molecular simulations can aide in material development. By simulating in-silico, one can perform costly experimentation after candidates are selected by initial screening. Molecular simulations also provide access to interactions at the protein-surface-solution interface. We have performed extensive work quantifying the repulsive forces that nonfouling surfaces generate on proteins and analyzing the cause of these forces. Yet, the thermodynamic criterion of adsorption or resistance is the change in free energy as a protein approaches a surface. In this work, molecular simulations were used to calculate the free energy change as model peptides in solution approach surfaces of varying nonfouling ability and to develop simulation-based evaluation criteria. The simulations were supported by protein adsorption experiments. By combining simulations and experiments we verified our simulations and evaluated the relative influence of the surface and hydrating water on the process. This combined approach provides feedback on our simulation parameters and a deeper understanding of the mechanisms of protein resistance and adsorption. Our research has demonstrated a strong link between surface hydration and non-fouling ability. Thus simulations and experiments were conducted to evaluate the hydration of functional moieties representing a wide range of nonfouling abilities. The extent of hydration of biologically relevant functional groups, like oligo-ethylene glycol and sugar alcohols, was evaluated by calculating the partial molar volume change due to hydration. This data was then compared to protein adsorption to self-assembled monolayer surfaces presenting the same functional groups. By using a simple measurement of hydration, it is possible to rapidly screen candidate non-fouling moieties. By combining molecular simulations and experimental techniques, we are able to develop a fundamental description of the interactions present at the molecular and macro scale. This in turn supports rational material design based on desired molecular function.

BI-TuP16 Correlation of Changes in Protein Bioactivity Post-Adsorption to Adsorbed Orientation and Conformation, ***K.P. Fears***, *R.A. Latour*, Clemson University

It has long been known that proteins rapidly and irreversibly adsorb onto biomaterial surfaces upon contact with bodily fluids. The structure and bioactivity of the adsorbed protein layer are recognized to be critical factors that influence subsequent cellular responses; however, the molecular mechanisms involved are mostly unknown. The bioactivity of an adsorbed protein could be inhibited due to adsorption-induced conformational changes, orientation effects causing the active site to be sterically blocked, or a combination of both. We have developed experimental methods to measure the bioactivity of an adsorbed protein layer and probe protein orientation and/or adsorption-induced conformational changes. Alkanethiol self-assembled monolayers (SAMs), with different surface chemistries, were used as model surfaces and standard spectrophotometric bioactivity assays were used to measure the percent of protein bioactivity retained post-adsorption. The secondary structure of the adsorbed protein layers was determined using circular dichroism (CD) and compared to the native structure of the proteins. Solvent accessible tryptophan residues were successfully modified using 2-hydroxy-5-nitrobenzyl bromide (Koshland's Reagent) and quantified via spectrophotoscopy. The specific locations of the modified residues are being determined by mass spectrometry to further assess the adsorbed orientation and tertiary structure of the proteins for correlation with changes in their bioactivity.

BI-TuP17 Screening Protein-Surface Interactions with Surface Gradients and TIRF, ***Y.-X. Ding***, *B. Wright, V. Hlady*, University of Utah

Macroscopic surface gradients with negative to neutral surface charges were created by reacting 3-mercaptopropyltrimethoxysilane (MTS) with fused silica and selectively oxidizing surface-bound MTS by controlled UV

exposure. XPS analysis and contact angle titrations of the MTS gradients showed that UV oxidation of the MTS sulfhydryl group converts it into a charged sulfonate-like moiety. The MTS gradients were also characterized by AFM in topography, adhesion, and friction modes. The reactivity of unoxidized sulfhydryl groups in the gradient region was assessed by quantitative fluorescence microscopy. The MTS gradients were then used to screen protein interactions with model surfaces. Adsorption kinetics of three human blood proteins, albumin, IgG and fibrinogen, were measured at 1% of their plasma concentrations using a dual channel total internal reflection fluorescence (TIRF) technique. For each protein, two binding experiments were carried out using the same surface gradient sample. In one TIRF channel the solitary binding of one of the fluorescently labeled plasma proteins was observed, and in the other the adsorption of the same was observed from a mixture with the other two unlabeled proteins. The TIRF experiments were later quantified using autoradiography. The adsorption behavior of the three proteins along the sulfhydryl-sulfonate surface gradient was analyzed by taking into account the convective/diffusive transport processes. The on- and off-binding rate constants were obtained as a function of the gradient position by fitting the experimental data to a simple model. Because the unoxidized MTS sulfhydryl can be further derivatized, these techniques have potential to be used as screening tools to study protein-surface interactions on a wide variety of gradient surface chemistries.

BI-TuP18 Detection of Lp-PLA2 as a Biomarker for Atherosclerosis Using Superquenching, ***S. Chemburu***, *Y. Wu*, University of New Mexico, *K. Ogawa, K. Schanze*, University of Florida, *D. Whitten, G.P. Lopez*, University of New Mexico

Lipoprotein associated phospholipase A2 (Lp-PLA2) is being recognized as a new biomarker for the prognosis and diagnosis of atherosclerotic patients. Lp-PLA2 cleaves the sn-2 acyl bond of glycerol-phospholipids yielding a fatty acid and a lysophospholipid as byproducts, which play an important role in the generation of pro-inflammatory moieties. The assays that have been developed for quantifying its catalytic activity or its concentration are time consuming and involve tedious experimental procedures. We have developed a simple bead based fluorescent assay for the quantification of the catalytic activity of Lp-PLA2. Using the layer-by-layer coating of surfaces approach, borosilicate glass beads (5uM dia) were coated with a cationic fluorescent conjugated polyelectrolyte poly(phenylene ethynylene) (PPE). The polymer-coated beads were then covered by a layer of an anionic lipid bilayer that is a natural substrate for PLA2. The lipid bilayer acted as a barrier protecting the fluorescence of PPE from being quenched by anthraquinone disulfonate (AQS). Upon the addition of PLA2, the hydrolysis of the lipid bilayer is catalyzed exposing the PPE to AQS and hence the fluorescence of PPE is turned off. The decrease in fluorescence quenching of the PPE in the presence of the lipid bilayer by AQS has been termed as frustrated superquenching and the authors have used this to develop a simple assay for the quantification of Lp-PLA2 activity.

BI-TuP19 Nanoscale Patterning of Photosynthetic Light Harvesting Proteins, ***N. Reynolds***, *S. Janusz, J. Olsen, C.N. Hunter, G.J. Leggett*, The Univ. Of Sheffield, UK

Here we present the patterning of light harvesting 2 (LH2) complexes from the photosynthetic bacterium Rhodobacter sphaeroides. LH2 complexes consist of circular arrays of bacteriochlorophyll and carotenoid molecules, held together by a cylindrical assembly of polypeptides. As many as 100 LH2 complexes are organised in the membrane to form an interconnected energy transfer networks comprising thousands of bacteriochlorophyll molecules that absorb photons, channelling the excitation energy down an energy gradient towards the reaction centre (RC), leading to a charge separation that drives subsequent biosynthetic reactions in the cell. When removed from the photosynthetic membrane, LH2 complexes retain the ability to absorb light, and they emit the energy as fluorescence. This property has been utilised in order to gain insight into the biological functionality of the LH2 after immobilization. Alkanethiol SAMs on gold surfaces have been used in conjunction with photolithographic techniques to produce patterned assemblies of LH2. Selective exposure of alkanethiols to UV light (wavelength 244 nm) leads to their photo-oxidation to alkylsulfonates, which may be displaced by a second thiol in a solution-phase process. The adsorption of LH2 onto SAMs with a variety of functional groups has been measured in order to determine which surfaces resist non-specific adsorption. In contrast to plasma proteins, which adsorb strongly to most surfaces, simple patterns consisting of hydrophilic and hydrophobic regions may be used effectively to pattern LH2. Covalent attachment to carboxylic acid groups using carbodiimide activation methods is an effective means of immobilising LH2 at the surface. Fluorescence spectroscopy measurements of proteins immobilized by attachment to patterned SAMs have confirmed that biological function is retained, leading to the observation of absorption spectra qualitatively identical to those of complexes in solution. Nanoscale chemical patterns have been fabricated using scanning near-field photolithography (SNP), in which a scanning near-field optical microscope coupled to a UV laser is used to selectively expose regions of a SAM. Using SNP, lines of carboxylic acid functionalised thiols as small as 70 nm have been fabricated in monolayers of perfluorinated thiols, and used to form LH2 structures with a width of less than 100 nm.

BI-TuP20 Molecular Simulation Studies of Protein Interactions with Phosphorylcholine Self-Assembled Monolayers, ***Y. He***, *J.C. Hower, S. Chen, M.T. Bernards, S. Jiang*, University of Washington

We performed molecular simulations to study the interactions between a protein (lysozyme) and phosphorylcholine(PC) self-assembled monolayers (SAMs) in the presence of explicit water molecules and ions. The all-atom simulations were performed to calculate the force generated on the protein as a function of its distance above the SAM surfaces. The structural and dynamic properties of water around PC-SAM surfaces were analyzed. These properties were also compared with those for the oligo(ethylene glycol) (OEG) SAM systems. Results show that the water molecules above the PC-SAM surfaces create a strong repulsive force on the protein as it approaches the surfaces. Further studies show that the dynamics of the water molecules are significantly slowed around both the PC-SAM and OEG-SAM surfaces as compared with that of bulk water, suggesting that the PC-SAM surface generates a tightly bound, structured water layer around their head groups, similar to the OEG-SAM surface. Our results also show that the PC-SAM surface is holding water more strongly than the OEG-SAM surface. The water molecules in the hydration layer of the PC-SAM surface stay longer and reorients slower than those in the hydration layer of the OEG-SAM surface. The most significant difference observed between these two surfaces is that the dipole of non-hydrogen bonded interfacial water molecules reorients much slower on PC-SAM than on OEG-SAM, which can be due to the zwitterionic nature of PC head groups. The application of PC-SAM in bio-lubrication studies will also be discussed.

BI-TuP21 First Direct Observation of Membrane Lipid Asymmetry Induced by Polypeptide Association, ***N. Biswas***, *J.C. Conboy*, University of Utah

Cellular membranes of eukaryotic cells are characterized by a heterogeneous distribution of phospholipids, which is key to many physiological functions. While some membranes (such as the endoplasmic reticulum) are symmetric, others (as the plasma membrane) are asymmetric with phosphatidylethanolamine and phosphatidylserines being primarily localized in the inner leaflet. The loss of asymmetry in plasma membranes has been thought to have direct relevance to numerous physiological and pathological events, such as phagocytosis and cell apoptosis. Although much is know about the biosynthesis of phospholipids, little is known about their mechanism of translocation and even less is known about the mechanism which produces or maintains lipid asymmetry in membranes. The present study shows for the first time, spontaneous development of asymmetry in a DSPC-d_{70}/DSPS (1,2-distearoyl-sn-glycero-3-phosphocholine /1,2-distearoyl-sn-glycero-3-[phospho-L-serine] planar supported lipid bilayer, in the presence of a positively charged polypeptide (polylsyine). The negatively charged DSPS molecules selectively localize in the top leaflet of the bilayer due to the electrostatic association with the polypeptide. The highly surface specific, second order nonlinear spectroscopy, sum frequency vibrational spectroscopy (SFVS), has been used since it offered the opportunity to study the kinetics of unlabeled lipids that were not modified by a fluorescent or spin-probe.

BI-TuP22 Smart Polymers and Cancer Cell Culture: Investigating The Effects of Film Deposition and Cell Culture Parameters on Cellular Behavior, ***J.A. Reed***, *E. Romero, A. Wandinger-Ness, H.E. Canavan*, University of New Mexico

Cell/surface interactions are mediated via the extracellular matrix (ECM). Many important proteins extend into the ECM, such as epidermal growth factor receptor (EGFR). EGFR is often up-regulated in cancer cells, resulting in uncontrollable cell growth and metastasis, which makes them key candidates for in vitro cancer therapeutic models. However, the methods traditionally used to detach cells from surfaces destroy the integrity of proteins in the ECM, resulting in damaged protein and receptors, making them incompatible with the solution-based analysis. In this work, we describe the use of a thermo-responsive polymer, poly(N-isopropyl acrylamide) or pNIPAM, for non-destructive release of cells into suspension. Many cell types, ranging from fibroblasts to epithelial cells, have demonstrated cell sheet detachment on pNIPAM surfaces. However, there is presently very little data on primary cells, cell culture parameters, or the method of film fabrication. To optimize the pNIPAM substrates, pNIPAM films were prepared using different methods (e.g., dissolution in silica vs. plasma deposited films) and compositions (10-40 wt% pNIPAM). The surface chemistry of the resulting films was characterized using X-ray

photoelectron spectroscopy (XPS), film thickness was ascertained via interferometry and XPS, and the thermo-responsivity was determined by contact angle analysis. The cell releasing properties of the films was characterized by incubating baby hamster kidney (BHK) cells to confluency, introducing a variety of solvents (e.g., PBS vs. serum free media) below the LCST, and observing the rate of detachment via microscopy. We find that plasma deposited films exhibited the best detachment behavior when rinsed with PBS and allowed to detach in 4C serum free media.

BI-TuP23 Development of Antimicrobial Materials Based on Surface-Active Biocides in a Resin Matrix, ***R.A. Brizzolara***, NSWC, Carderock Division, *J.H. Wynne*, Naval Research Laboratory, *J. Jones-Meehan*, Department of Homeland Security

The goal of this work is to develop a surface that demonstrates biocidal activity with less than 0.5% biocide concentration in the bulk material. The incorporation of an amphiphilic biocide as a surface-active component in a resin matrix is being investigated as a means of achieving a higher biocide concentration at the surface (and therefore better biocidal efficacy), while maintaining low bulk biocide concentrations. The amphiphilic quaternary ammonium compounds containing both aliphatic and oxyethylene moieties afforded greatest biocidal activity. These molecules were synthesized via simple condensation of a tertiary dimethyl-N-alkylamine with the corresponding halo-oxyethylene. Purity was confirmed using 1H- and 13C-NMR. The biocide-containing urethane coating was dried on glass slides for microbiological analysis and on gold surfaces for x-ray photoelectron spectroscopy (XPS) analysis. Microbiological analysis was performed to determine biocidal efficacy of coating formulations against S. aureus (Gram-positive bacterium). 10 µL of bacterial culture was placed on the surface of the slide. After 2 hours, the slide surface was swabbed. Swab contents were resuspended in appropriate neutralization media, serial dilution was performed with plating on LB agar plates. XPS was used to determine the quantity of biocide at the coating surface compared to the bulk to determine the segregation of biocide to surface, and to correlate surface concentration of biocide to the coating's biocidal efficacy. The N1s binding energy shift between nitrogen in quaternary ammonium and nitrogen in polyurethane was used to differentiate biocide from polyurethane. The XPS analysis demonstrated surface enrichment of two quaternary ammonium-based biocides in a polyurethane coating by approximately a factor of 10 compared to the bulk. The quaternary ammonium-containing materials exhibited increased killing of - S. aureus cells compared to the control (polyurethane containing no biocide). These results indicate that the use of surface-active biocides can result in significant biocidal efficacy with small bulk biocide concentrations. This work was sponsored by the DARPA Defense Sciences Office.

BI-TuP24 Immobilization of Live Salmonella on Abiotic Surfaces for AFM Investigation, ***Z.Y. Suo***, *R. Avci, L. Kellerman, X.H. Yang, D.W. Pascual*, Montana State University

High-resolution AFM images of gram-negative pathogenic Salmonella typhimurium reveal the morphological features of bacterial cells, including CFA/I fimbriae with a diameter of ~3 nm, flagella with a diameter of ~11 nm, and the extracellular polymeric substance surrounding the bacteria. The fine details of the CFA/I fimbriae and the lipopolysaccharides decorating them are clearly resolved when imaged with ultrasharp tips in tapping mode. For studies in liquid, however, it is necessary to immobilize bacterial cells through some sort of "leash," or cross-linker. Live S. typhimurium and their adhesins were successfully immobilized through interactions between bacterial surface antigens and their corresponding antibodies covalently linked to a substrate. Cells immobilized in this way remain viable for hours in PBS buffer and are capable of regenerating if incubated in a growth medium. Immobilized live S. typhimurium cells were imaged in PBS buffer in contact mode and force-volume mode. This approach opens up new fields of investigation, such as quantification of adhesin-receptor interactions, affinity mapping and patterning of bacterial cells on surfaces, which will be discussed in our presentation.

BI-TuP25 Development of Multi-Phasic Scaffolds for Ligament Tissue Engineering via Melt Electrospun Polyurethanes: Cytotoxicity of Melt Electrospun Aliphatic Polyurethane Fibers, ***A. Karchin***, *J.E. Sanders*, University of Washington

Towards the goal of developing electrospun polyurethane (PU) scaffolds for tissue engineering applications, the cytotoxicity of basic aliphatic PUs based on (CH2)4-content diisocyanates, polycaprolactone and 1,4-butane diamine or 1,4-butanediol were tested. These biodegradable polymers were chosen due to their general biocompatibility, excellent mechanical properties, and designed so that in vivo degradation products can be cleared through normal metabolic pathway. Electrospinning from melt, compared to from solution, is an attractive manufacturing process as it allows for the formation of small diameter fibers while eliminating the use of solvents which can be cytotoxic. A two-tiered experimental design was employed to determine the suitability of the specific PUs for use as tissue engineering scaffolds from a biocompatibility perspective. First, the effect of atmosphere, temperature and time at elevated temperature on the polymer cytotoxicity was assessed. Second, an investigation into the relationship between melt electrospinning and cytotoxicity was explored by performing cytotoxicity tests on electrospun meshes. These experiments are useful as a guide for subsequent development of the novel electrospun biohybrid enzymatically biodegradable PUs into a tissue engineering scaffolds.

BI-TuP26 "Smart" Biopolymer for Reversible Stimuli-Responsive Platform of Cell-Based Biochips, ***K. Na***, *O. Kim, J. Jung, J. Lee*, Seoul National University, Korea, *J.W. Park, T.G. Lee*, Korea Research Institute of Standards and Science, *J. Hyun*, Seoul National University, Korea

In the presentation, we describe the genetical synthesis of lysine tagged ELP (ELP-K) with inverse phase transition temperature (Tt) of 30? for fabricating a thermo-responsive culture surface. For the micropatterning of ELP-K a removable polymer template was microcontact-printed on the glass surface derivatized with epoxide. After conjugating ELP-K onto the glass surface followed by dissolving a polymer template, highly resolved ELP-K micropatterns were efficiently created on the surface. The successful micropatterning of the polypeptide was confirmed using ELP-K conjugated with fluorescence dye by confocal microscopy as well as atomic force microscopic images. TOF-SIMS images of ELP-K micropatterns verified the highly resolved ELP microstructure on the surface.

BI-TuP27 Growth Behavior of Fibroblast Cell in Culture Medium Containing Nanoparticles, ***S. Fujita***, *T. Ishizaki, N. Saito, O. Takai*, Nagoya University, Japan

1. Introduction Nanoparticles of metal alloy, metallic oxides, semiconductors and ceramics have unique properties compared with bulk materials since they have high reactivity and catalysis by the large specific surface area. Thus, nanoparticles have been paid attention in the various fields and utilized as electric devices, biomedical materials and cosmetics. On the other hand, it has been reported that nanoparticles could have a harmful effect on human body. For example, nanoparticles such as soot in exhaust fumes are inhaled into lung and cause pulmonary and cardiac diseases. In some recent researches, it is reported that nanoparticles could activate an adverse reaction in the body because they pass through cell walls and move through the blood or lymph vessel. However, nobody knows their interactions with the body in detail. In this study, we aimed to investigate the influence of the nanoparticles on the cell growth and assess the risk of nanoparticles. 2. Experimental procedure Au, Pt, and Ag nanoparticles were synthesized by a wet reduction process. Citric acid was used as a reducing agent. Mouse fibroblast cells (NIH-3T3) were cultured in medium (DMEM, pH: 7) containing the nanoparticles in humidified atmosphere containing 5.0% CO_2 at 37°C for 3 days. The cultured cells were counted using blood cell counting chamber and observed with phase-contrast microscope and transmission electron microscope (TEM). 3. Result Only Ag nanoparticles prevented the cells from growing onto the culture dish. Number of cells after the culture for 72 hours decreased with the increase of concentration of Ag nanoparticles. TEM images showed that the Ag nanoparticles were engulfed into a cell tissue. In addition, the ingested Ag nanoparticles were aggregated around the nuclear. However, such aggregation is not the reason that the cell cannot be cultured in the medium containing Ag nanoparticles, because Au and Pt nanoparticles were also aggregated around the nuclear. At least, Ag nanoparticles are toxic to cells. We believe that nanoparticles may provide us many types of risk; damage of DNA, disappear of proteins and so on. We must investigate the mid- and long-term influence of the nanoparticles on human being.

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