Tuesday Afternoon Poster Sessions

Biomaterial Interfaces
Room: Hall 3 - Session BI-TuP

Biomaterial Interfaces Poster Session I

BI-TuP2 Formation of Stable Microbubbles by Encapsulation in Silica, ***K. Staggs***, University of New Mexico, *G. Gupta,* Los Alamos National Laboratory, *M. Tartis,* New Mexico Tech, *G.P. Lopez*, University of New Mexico

A new technique for stabilization of supramolecular assemblies (termed soft petrification) has been applied to the stabilization of microbubbles. Commonly used as ultrasound contrast agents, most types of microbubbles are inherently unstable. Current procedures require many types of microbubbles to be filled with hydrophobic, high molecular weight gases such as octafluoropropane and perfluorobutane. Special equipment including syringe pumps and sealing devices are required, because most microbubbles must be formed under these types of gases. This requirement limits the conditions under which microbubbles can be studied. They cannot be studied on a bench top open to atmosphere. Once microbubbles have undergone soft petrification, they can be studied in a wide range of environments. In soft petrification of air filled lipid coated microbubbles, a vapor deposition technique encapsulates the microbubbles in a thin silica shell. After undergoing soft petrification, it was observed that microbubble stability under adverse conditions is significantly improved. These adverse conditions include pressures up to 120 psig and temperatures up to 80°C. Those values are double the values found for microbubbles without the silica shell. Other unique characteristics of these microbubbles include the ability to withstand temperatures well below freezing, without loss of size. Air filled microbubbles have a bench top life span of approximately four hours before encapsulation. Following encapsulation, it is observed that the same microbubbles have a bench top lifespan of up to several months. Several formulations were characterized and unprecedented air filled microbubble studies are facilitated by this technique.

BI-TuP3 Synthesis of Grafted PNIPAAm Surfaces Using ATRP in Presence of Air for Cell Adhesion Studies, ***P. Shivapooja***, *L.K. Ista, S. Mendez, G.P. Lopez*, University of New Mexico

Poly(N-isopropylacrylamide) (PNIPAAm) is a thermoresponsive polymer that exhibits a change in relative hydrophobicity above and below its lower critical solution temperature (LCST ~ 32^0C). This stimuli responsive polymer when grafted onto surface can act as a biofouling resistant coating as it has the property of formation and collapse of hydrogen bonding with water molecules below and above the LCST. Atom transfer radical polymerization (ATRP) has been proven to yield well-defined polymer but in rigorously deoxygenated environment, for example in a Schlenk line or glove box. Matyjaszewski *et al.* group developed activators regenerated by electron transfer (ARGET) that allow ATRP in presence of limited amount of air *[Langmuir 2007, 23, 4528-4531]*. We report grafted PNIPAAm brushes synthesized by this relatively simple method of ARGET ATRP which does not require an oxygen-free environment using a small amount of reducing agent together with a catalyst under homogeneous conditions. We evaluated the effect of the amount of reducing agent and reuse of monomer solution on the grafting thickness. The grafted PNIPAAm surfaces have been characterized by FTIR, XPS, contact angle measurements and ellipsometry. We anticipate using these grafted PNIPAAm surfaces for cell adhesion studies above and below the LCST.

BI-TuP4 Undercovering the Extracellular Matrix with Thermoresponsive Microgels, ***J.A. Reed***, University of New Mexico, *R.K. Shah, T. Angelini, D.A. Weitz,* Harvard University, *H.E. Canavan*, University of New Mexico

The extracellular matrix, or ECM, remains a hidden biological interface between a cell and a substrate. One method for examining proteins, such as those in the ECM, is flow cytometry (FC), which is a fast, high throughput method of quantification. Since FC is a solution technique, it is necessary to dissociate the cells from the surface. Traditional cell harvesting methods, such as enzymatic digestion and physical scraping, damage the ECM proteins as well as the cell morphology. Recently it has been shown that a thermoresponsive polymer, poly(N-isopropyl acrylamide) or pNIPAM, can be used to harvest a sheet of cells without damaging the integrity of the ECM. Above its lower critical solution temperature (LCST), this polymer is relatively hydrophobic, and mammalian cells grown on pNIPAM-grafted surfaces act in a similar fashion as those grown on typical tissue culture polystyrene (TCPS): they proliferate into a confluent sheet. Below the LCST (i.e., room temperature) the polymer physically changes, becoming more hydrophilic and swelling. Furthermore, when the temperature of the cell culture is dropped below the LCST of the polymer, the cells will detach as an intact cell sheet. These intact cell sheets, however, are not ideal for investigation in a flow cytometer, which requires individual cells for quantification. In this work, we developed a novel method for single cell detachment using pNIPAM microgels on the order of 20-200 micrometers. After fabrication, these gels were examined to ensure pNIPAM's characteristic thermoresponse was maintained, followed by cell culture on the gels. From these results, we conclude that these microgels have the capability of giving us access to this buried biological

BI-TuP5 Facile C-terminal Attachment of Proteins on Surfaces by Hydrazine-Intein Chemical Reactions, ***P. Yang***, *S. Marinakos, A. Chilkoti*, Duke University

Protein immobilization on surfaces is important in many areas of research, including their biophysical characterization, affinity chromatography, and heterogeneous immunoassays. We describe a new site-specific C-terminal immobilization of proteins on surfaces to control the orientation and attachment point of the protein. Our strategy was to exploit intein chemistry and the related hydrazine attack reaction. To provide-proof-of principle of this approach, a tripartite thioredoxin-intein-elastin-like polypeptide (ELP) fusion was synthesized in E. coli from a plasmid-borne gene. In this fusion protein, the C-terminus of the target protein thioredoxin was fused with the N-terminus of an intein, a self-splicing protein domain. An ELP was fused to the C-terminus of the intein to facilitate purification of the fusion protein because ELP fusions allow simple purification of proteins from cell lysate by isothermal, salt-triggered phase transition of the ELP. Incorporation of the intein at the C-terminus of the target protein provided an unnatural peptide bond between the target protein and the intein, which is selectively reactive towards nucleophiles such as hydrazine. In order to immobilize the target protein –thioredoxin– a hydrazine-functionalized surface on a protein-resistant background was fabricated by modifying a gold-coated glass substrate with a mixed self-assembled monolayer consisting of a hydrazine-terminated hexa(ethylene glycol) thiol and a tri(ethylene glycol)thiol. We demonstrate that the hydrazine groups on the surface were able to directly attack the unnatural peptide bond between the target protein and the intein, liberating the thioredoxin from the tripartite fusion and covalently attaching it to the surface. As a result, the target protein was site-specifically immobilized on the planar substrates with uniform orientation. This technique provides many advantages over other site-specific immobilization methods, including faster reaction kinetics, higher surface density, and the ability to easily purify the protein prior to immobilization though a simple, one-step non-chromatographic process that exploits the phase transition of the ELP.

BI-TuP6 Fabrication and Characterization of Non-toxic and Non-Biofouling Plasma-Polymerized Polyethylene Glycol Thin Films, ***C. Choi***, *K.S. Kim, D. Jung,* Sungkyunkwan University, Rep. of Korea, *D.W. Moon, T.G. Lee*, KRISS, Rep. of Korea

Polyethylene glycol (PEG) is a key molecule in the fabrication of non-biofouling surface for various biological applications such as biochips and tissue engineering. In particular, plasma-polymerized PEG (PP-PEG) thin films have many practical uses due to their strong adhesion onto any solid substrates. In this work, we fabricate PP-PEG thin films by using the capacitively coupled plasma chemical vapor deposition (CCP-CVD) method and non-toxic PEG200 molecules as a precursor. The surfaces of the PP-PEG thin films were characterized by using contact angle measurement, X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), Fourier transform infrared (FT-IR) spectroscopy and time-of-flight secondary ion mass spectrometry (TOF-SIMS). Our results show that PP-PEG thin film surfaces deposited at low plasma power were very similar in chemical composition to the PEG polymer surfaces. In addition, these PP-PEG surfaces showed excellent non-biofouling property and biocompatibility during *in vitro* and *in vivo* tests. These results indicate that our PP-PEG thin films would be useful for practical biological applications.

BI-TuP7 Optimization of Elastin-Like Polypeptide Fusions for the Purification of Membrane Proteins by Inverse Transition Cycling, ***T. Thapa***, *S. Simons, E. Chi,* University of New Mexico, *A. Chilkoti,* Duke University, *G.P. Lopez*, University of New Mexico

Low column efficiency is a common problem associated with the affinity purification of surfactant solubilized membrane proteins synthesized in recombinant and cell free expression systems. Elastin-like polypeptide (ELP) tags, which have been designed to allow non-chromatographic purification of soluble proteins, offer a potential means to enable facile large-scale purification of detergent solubilized recombinant membrane proteins. However, the phase transition temperature (T_t) of ELPs is sensitive

to the addition of cosolutes and many detergents increase the T_t of ELPs to temperatures greater than the thermal denaturation temperature of many proteins that are fused to the ELP, hence prohibiting their use for protein purification. To identify detergents that would satisfy the dual and potentially conflicting requirements of stabilizing membrane proteins fused to an ELP, we screened different detergents with respect to their effect on the T_t of ELP[$V_5A_2G_3$-180]. We found that dodecyl maltoside (DDM), a detergent that is commonly used to solubilize recombinantly expressed membrane proteins, did not significantly alter the phase transition characteristics of ELPs or their structure as probed by a temperature-programmed turbidity assay and circular dichroism spectroscopy. Our results clearly indicate that DDM does not affect the inverse transition cycling of ELPs and therefore may be useful to purify membrane proteins which are otherwise difficult to extract and purify by affinity chromatography.

BI-TuP8 Self-assembly of Proteins on Well-defined Sapphire Surfaces, ***T. Isono***, *K. Yamazaki, T. Ogino*, Yokohama National University, Japan

Non-specific adsorption is a very serious issue when biomolecules are immobilized on solid surfaces. Non-specific adsorption should be suppressed especially in application of solid surfaces to biosensors or implant devices. We have tried to control protein adsorption on step-controlled sapphire surfaces. Because sapphire surface is chemically stable in liquid as well as in air, it is a suitable material for bioapplication. To control interactions between the sapphire surfaces and protein molecules, we designed surface atomic structures. In this study, we report on self-assembly of several kinds of protein molecules by using well-defined sapphire surfaces. Single crystalline sapphire (0001) surfaces were used for protein adsorption. By a high temperature annealing, the sapphire surfaces are covered with bunched steps accompanied with crossing steps when the miscut direction slightly tilts from the stable direction of atomic steps. These surfaces were cleaned by a mixture of sulfuric acid and hydrogen peroxide. We call this surface oxidized surface. Two domains, which exhibit different hydrophilicity and charge density from each other, coexist on this surface. Center regions of the terraces (domain A) are relatively hydrophobic and weakly charged, and the others (domain B) hydrophilic and negatively charged. To control the surface chemical properties, the oxidized surfaces were slightly etched by phosphoric acid. We call this surface etched surface. Protein molecules were physically adsorbed on the oxidized and the etched surfaces in a buffer solution. Protein adsorption patterns were observed by atomic force microscopy in the same buffer solution. When the oxidized surfaces were immersed in a solution of ferritin molecules whose surfaces exhibit a negative charge, ferritin molecules were selectively adsorbed on the domain A by the electrical repulsion between the domain B and the molecules. On the other hand, avidin molecules whose surfaces exhibit a positive charge were selectively adsorbed on the domain B by the attractive interaction. These protein molecules were adsorbed on the sapphire surfaces depending on the surface charges. However, small amount of avidin molecules were adsorbed on the hydrophobic domain A. Generally, it is easier for protein molecules to be adsorbed on hydrophobic surfaces than hydrophilic ones. To make the domain A hydrophilic, the oxidized surfaces were etched using phosphoric acid. When the etched surfaces were immersed in a solution of avidin molecules, avidin molecules were selectively adsorbed without non-specific adsorption on the domain A.

BI-TuP10 Lubricin Prevents Degenerative Changes in Articular Cartilage Structure and Morphology, ***J. Coles***, Duke University, *C. Cha*, Brown University, *M. Warman*, Boston Children's Hospital, *G. Jay*, Brown University, *F. Guilak, S. Zauscher*, Duke University

Lubricin is a mucin-like glycoprotein which contributes to boundary lubrication in joints and is also thought to have a role in protecting cartilage surfaces. Direct studies of joint protection by lubricin have been difficult but a lubricin null mouse has been developed recently, providing completely lubricin-free cartilage for study. We have shown that atomic force microscopy can be used for measurements of interfacial friction in the boundary lubrication regime and use this technique to measure friction directly on cartilage not expressing lubricin. We further use atomic force microscopy and histology to characterize stiffness and surface and subsurface morphology of these joints. While friction measured directly on lubricin null cartilage was only slightly lower than on wild type cartilage, surface structure and mechanical integrity were altered significantly. Lubricin null cartilage surfaces were significantly rougher, stiffness did not develop normally, and glycosaminoglycan (a core structural component of cartilage) concentration near cells was lost as joints developed. While reduction of friction is likely an important factor in lubricin's role in protecting cartilage, our measurements on lubricin null cartilage suggest that lubricin may additionally protect cartilage through other mechanisms.

BI-TuP11 Plasma Polymerization Induced Structural Modification of PCL, ***A.M. Sandstrom***, *L. Grøndahl, J.J. Cooper-White*, University of Queensland, Australia

Cellular response to tissue culture scaffolds is thought to be directed by chemical and topographical cues from surfaces which bind biological motifs recognized by cell receptors. Plasma polymerization (PP) is frequently used to functionalize surfaces for improved biocompatibility. It has been reported that the type and distribution of functional groups created on a surface is dependent on plasma parameters. However, in addition to changing chemistry, modification of plasma parameters is also likely to introduce topographical variation to the sample. The aim of this work was to elucidate the combined effects of plasma power and treatment time on the surface chemistry and topographical features of the substrate.

Scaffolds [5% poly(ε-caprolactone) (PCL)/1,4-dioxane] were prepared using thermally induced phase separation. Samples were plasma polymerized in a custom-built radio frequency reactor using heptylamine (HA) at 20W and 50W for 30s and 60s. Additional substrates were prepared by spincoating PCL on Si wafers. For each of the four treatment conditions, surface homogeneity was confirmed across the wafers and scaffold sections via XPS. Surface topography was investigated on wafers by AFM, and scaffolds were examined using SEM.

Disappearance of the PCL carboxyl (C=O) peak following PP for all treatment conditions suggests complete coverage (>10nm) of the top surface of all substrates by PPHA. The N/C ratio was slightly higher on the Si wafer than the scaffold. Treatment was homogenous across individual samples. Slight chemical functionality shifts were found between samples, which may reflect intrinsic plasma differences or post-PP oxidative variation.

Polymer aggregates formed on Si following PPHA treatment at 20W, but were diminished at 50W. Topographical changes were more pronounced on PPHA PCL-coated Si. Although spherulite size variation was minimal between untreated semi-crystalline PCL and PPHA PCL on Si for most treatments, complete recrystallization of the base polymer was observed at 50W 60s. Change in surface roughness was evidenced by disappearance of well-defined fibrous domains as treatment power and time increase for all samples, except at 50W 60s when distinct spherulites re-emerged.

Highly reactive HA recombination led to aggregate size reduction on Si, whereas PPHA on PCL appeared to affect structural organization of the substrate. Scaffold morphology also changed following PPHA, as more energetically favored fibrous extensions in the porous region of the surface were observed by SEM. These results may be used in conjunction with cellular studies to tune PPHA reactions on scaffolds as required for support of various cell types.

BI-TuP12 Molecular Dynamics Simulation of Interactions between Structured Peptides and Functionalized Solid Surfaces, ***G. Collier***, *R.A. Latour, S.J. Stuart*, Clemson University

The chemical and physical interactions between proteins and biomaterial surfaces govern the biocompatibility of those materials when introduced into a living system. Therefore, the possibility of controlling biocompatibility on a molecular level through the strategic design of biomaterials begins with the study of these interactions at the atomic level. Without an accurate molecular description of the way that proteins interact with biomaterials, biomaterial design is primarily relegated to a trial-and-error approach. To address this issue, we have begun to evaluate the applicability of existing all-atom molecular simulation methods and force fields to the unique situation of simulating the interaction of structured peptides with solid biomaterial surfaces.

Today's most advanced molecular modeling tools do not include parameterization options for molecular systems interacting with solid biomaterial surfaces, so we have undertaken a variety of pilot studies to guide the development of our methods for use in more complex simulations. These pilot studies include nanosecond-scale simulations of structured peptides interacting with charged and uncharged self-assembled monolayer (SAM) surfaces, ion distributions over a charged SAM surface, peptide-peptide interaction studies, peptide stability studies, analysis of environmental changes resulting from peptide conformation changes, and analysis of the role of water molecules in the peptide-surface adsorption process. The adsorption and stability studies are being conducted using multiple molecular modeling force fields, enabling a comparison of their performance and usefulness in the simulation of these unique systems. The majority of this work has been conducted using replica-exchange molecular dynamics (REMD) techniques in order to optimize conformational sampling, resulting in the most efficient path toward structural data that can be compared with experimental results.

The results from these studies are enabling us to assess the suitability of currently available molecular simulation methods and force fields and are providing us with a better understanding of peptide-surface interactions at the atomic level.

BI-TuP13 ToF-SIMS Study of Fibronectin Orientation on Self-Assembled Monolayers, ***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 extra cellular 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 have a critical effect on cell-surface interactions. In this study the adsorbed orientation and conformation of the 7-10 fragment of FN ($FNIII_{7-10}$) was studied on four different model surfaces (self-assembled monolayers (SAM) of C_{11} alkanethiols on Au, $-CH_3$, $-NH_2$, -COOH and -OH terminated SAM). The $FNIII_{7-10}$ fragment incorporates both the Arg-Gly-Asp (RGD) FN receptor binding motif and the PHSRN synergy site which participate in the RGD binding. The effect of different surface chemistries on binding and adsorption configuration was investigated using X-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectrometry (ToF-SIMS). XPS showed significantly higher protein coverage on the hydrophobic $-CH_3$ terminated surface than the hydrophilic and negatively charged –COOH terminated surface. Both XPS data and SIMS peak intensities for certain amino acid were used to verify the adsorption process and monolayer coverage. Full coverage was determined by a plateau in SIMS peak intensities with increasing protein exposure. A principal component analysis (PCA) of the ToF-SIMS data on surfaces with similar sub-monolayer protein coverage was then used to determine changes in the spectra that differed when the fragment was adsorbed on various surfaces. These results are related to different orientation or conformations of the fragment on the different surfaces.

BI-TuP14 Wear-Rate Behavior of Hydroxyapatite-Coated Titanium, ***A.F. Jankowski***, Texas Tech University

Calcium phosphate-based ceramics are used in medicine and dentistry due to excellent biocompatibility with human tissues. The formation of hydroxyapatite coatings with a porosity gradient on the surface of metal implants can provide a surface suitable for the in growth of connective tissue. A need exists to both protect the metal substrate against corrosion and provide sufficient mechanical adhesion. Hydroxyapatite coatings have been produced by the reactive, radio-frequency sputtering of stoichiometric $Ca_{10}(PO_4)_6(OH)_2$ targets using planar magnetrons onto titanium-coated silicon substrates. A fully dense and thin initial-coating layer appears to avoid cracking, improves substrate adhesion, and provides corrosion protection (J. Mater. Res. 16, 2001, 3238). The effect of varying the wear rate is now evaluated on the mechanical behavior of sputter deposited hydroxyapatite coatings.

BI-TuP15 Asymmetric Hybridization Behavior Exhibited by DNA Probes Containing Surface-Attachment Ligands and Self-Complementary Sequences, *S.M. Schreiner, D.F. Shudy*, University of Wisconsin, ***D.Y. Petrovykh***, Naval Research Laboratory and University of Maryland, College Park, *A. Opdahl*, University of Wisconsin

We demonstrate the impact that intra-molecular and nucleotide-gold interactions have on conformation of surface-immobilized DNA probes and their hybridization behavior. We take advantage of a method based on the intrinsic affinity of adenine nucleotides for gold (Opdahl et al., PNAS, **104**, 9–14, 2007) to immobilize block-oligonucleotides having sequences that follow a $d(A_k\text{-}T_m\text{-}N_n)$ pattern: a block of k adenines [$d(A_k)$], followed by a block of m thymines [$d(T_m)$], and a block of n (arbitrary) nucleotides [$d(N_n)$]. Such block-oligos assemble on gold via the d(A) blocks, which allow a high degree of control over DNA surface coverage and conformation. We characterize immobilization and hybridization of these DNA probes using x-ray photoelectron spectroscopy (XPS) and surface plasmon resonance (SPR) imaging, specifically to compare two 15-nucleotide N_n sequences: 15 thymines (T15) and a realistic arbitrary sequence (P15). For A15-T5-P15 probes, the hybridization efficiency at the P15 end is enhanced 2-fold compared to that at the A15 end. For A15-T20, which forms a hairpin in solution, we observe a larger asymmetry of hybridization efficiencies when immobilized on gold: while the T15 end is highly accessible for hybridization, the A15 end has virtually no hybridization activity. A thiolated version of the same probe (A15-T20-SH), in contrast, exhibits little asymmetry and overall low hybridization efficiency with either A15 or T15, consistent with an immobilized structure of a stable hairpin. Additional experiments, whereby a displacement thiol is added to reduce DNA-gold interactions, are used to support our inferences about the role played by intra-molecular and surface interactions in immobilization and hybridization of DNA probes.

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