Tuesday Afternoon Poster Sessions

Biomaterials
Room: Exhibit Hall B2 - Session BI-TuP

Biointerfaces and Surfaces I

BI-TuP1 Computer Simulation of Water Near Structureless Model Surfaces and Self-assembled Monolayers: Interfacial Behavior and Hydration Forces, ***T. Hayashi***, *A.J. Pertsin, M. Grunze*, Heidelberg Universität, Germany

The hydration forces between both structureless and structured surfaces are calculated using the grand canonical Monte Carlo technique. Primary attention is given to large surface-to-surface separations (40 Å and more), where the oscillations of the hydration force have practically decayed. For simulations of structureless surfaces, both orientation independent and strongly directional potentials are employed. Our results show that water confined between hydrophobic surfaces experiences a capillary evaporation at surface-to-surface separations less than about 58 Å. At larger separations, hydrophobic attraction due to a density depression in the confined region is observed. In cases of hydrophilic surfaces, the sign and magnitude of hydration force are found to be strongly dependent on the presence of orientation dependent terms in the water-surface potential. Simulations of structured surfaces were performed with methoxy tri(ethylene glycol) terminated alkane thiol self-assembled monolayers (SAMs) on the Au(111) and Ag(111) substrates. Although both of the SAMs show a typical hydrophobic behavior similar to that observed with structureless hydrophobic surfaces, there are substantial differences in their interaction with water. The simulation results are discussed in the context of the experimentally observed protein adsorption properties and surface force behavior between the SAMs.

BI-TuP2 Permittivity Responsive Interface for Biosensor in Tissue Engineering, ***P.O. Bagnaninchi***, *M. Tabrizian*, Mc Gill University, Canada

We have designed micro-porous host medium which fit with requirements for permittivity changing interface. The main application is the assessment of the growth of cells in micro-porous matrix for tissue engineering. These biointerfaces will be used with evanescent wave transducer or Inter Digital Capacitance since they are based on the change of permittivity in a surrounding medium. The complex biological medium is heterogeneous and our approach to describe the macroscopic dielectric behaviour of this mixture in terms of the bulk properties of the constituents is based on and derived from the effective medium theory; which is valid as long as the wavelength of electromagnetic wave is larger than the size of heterogeneities. Our method allow us to design permittivity responsive matrix and to improve the sensitivity of the biosensor in order to assess in a non-destructive way the growth of cells in micro-porous matrix. In this study the biointerface is characterized by complex permittivity measurement over a wide frequency range with the aid of a dielectric probe and network analyser.

BI-TuP5 Interaction of Poly(L-Lysine)-g-poly(ethylene glycol) with Negatively Charged Supported Phospholipid Bilayers, ***F. Rossetti***, Swiss Federal Institute of Technology, *I. Reviakine*, University of Houston, *G. Csucs, S.M. De Paul, J. Vörös, N.D. Spencer, M. Textor*, Swiss Federal Institute of Technology

The goal of the study presented in this poster is to develop a general, one-step method for modifying the surfaces of different types of vesicles. The proposed idea is to coat negatively charged phospholipid or polymeric vesicles with functionalised Poly(L-Lysine)-g-poly(ethylene glycol) (PLL-g-PEG), a polyelectrolyte with a positively charged backbone and protein-resistant ("stealth"[1]) side chains. Negatively charged supported phospholipid bilayers (SPBs) were used to investigate the adsorption behaviour of PLL-g-PEG as a function of charge density. The main investigation methods used were the Quartz Crystal Microbalance (QCM-D, including measurement of dissipation) and fluorescence microscopy. The SPB system used consisted of mixtures of a zwitterionic phospholipid (dioleyl phosphatidyl choline - DOPC) and a negatively charged phospholipid (dioleyl phophatildyl serine - DOPS) in a range from 0 (neutral SPB, used as a control) to 18 mol-%. Effects of the buffer composition on the adsorption process (with particular attention to the ionic strength) and the presence or absence of Ca^{2+} ions, which were found to be crucial for the formation of an SPB from DOPC:DOPS vesicles, were also considered.

[1] Woodle, M.C., Lasic D.D., Biochim. Biophis. Acta, 1992, 1113, 171-199.

BI-TuP6 Derivatization and Surface Characterization of poly(2-hydroxyethyl methacrylate) for Oriented Protein Immobilization, ***J.L. Schwartz***, *S.M. Martin, D.G. Castner, C.M. Giachelli, B.D. Ratner*, University of Washington

When biomaterials are placed in the body they are immediately covered with a random layer of mixed proteins. This may trigger the foreign body reaction leading to collagenous tissue encapsulating the biomaterial. To circumvent this, many schemes have been developed to pre-immobilize a protein or peptide onto the surface intended to subdue the inflammatory response. The goal of this study is to immobilize osteopontin (OPN) in a single orientation such that the active site is presented to the surface for optimal cellular interaction. Proteins can be immobilized via a primary amine from a lysine unit, but due to the number and distribution of lysine in OPN there is no control over the final orientation. A different approach is to immobilize OPN engineered with a polyhistidine tail, which can interact with the coordination sites of a divalent metal ion. The recombinant six-histidine (His-tag) sequence does not hinder protein activity and can be placed at a characteristic site on the protein of interest. The divalent metal ion, Ni^{2+}, binds tightly to nitrilotriacetic acid (NTA), a metal chelating agent covalently attached to poly(2-hydroxyethyl methacrylate) (pHEMA) via a N,N-carbonyldiimidazole (CDI) intermediate reaction step. The choice of pHEMA as the immobilizing substrate was due to the abundance of hydroxyl groups on the surface as well as low non-specific protein adsorption. These surfaces were characterized by XPS and ToF-SIMS before and after chemical derivatization as well as after each reaction step. Derivatization with fluorine containing molecules was used to probe hydroxyl, carboxyl, and imidazole carbamate availability with XPS. The amount of protein immobilized to pHEMA was quantified by ^{125}I radiolabeled OPN and the protein was tested for retained biological activity.

BI-TuP9 Polysaccharide Adsorption on Hydrophobic and Hydrophilic Surfaces, ***K.T. Queeney***, *C. Royce*, Smith College

The interaction of extracellular polysaccharides with solid substrates plays an important role in the adhesion of bacterial cells to a variety of natural and synthetic surfaces. We have used a combination of surface infrared spectroscopy and atomic force microscopy to investigate the fundamental chemical interactions that govern the adsorption behavior of such polysaccharides. The adsorption of xanthan gum, a model polysaccharide, is studied on silicon and silica surfaces that have been modified to present a range of both surface wettability and chemical functionality. Adsorption is monitored both in- and ex-situ, using a custom-designed cell for infrared spectroscopy at silicon/aqueous interfaces. Simultaneous studies of the adsorption characteristics of the constituent monosaccharides of xanthan provide a way to assess the importance of effects such as polymer conformation and polymer-polymer interactions in the adsorption process. For instance, while monosaccharides exhibit similar adsorption characteristics on hydrophilic, silanol-terminated silica and hydrophobic, hydrogen-terminated silicon, xanthan exhibits a marked preference for the hydrophobic surface. The importance of adsorbate-adsorbate interactions in the adsorption of both poly- and monosaccharides is explored through analysis of spectral evolution from mono- to multilayer regimes.

BI-TuP10 Plasma Treatment of Plastics to Reduce Water Adhesion and Bio-fouling, ***M.J. Neumann***, *P.J.A. Fackler, D.N. Ruzic*, University of Illinois at Urbana

Polymers have become a part of our everyday lives for use in a wide range of applications. This is due to polymers exhibiting high strength with little weight, wide range of flexibility, ease of formability, and economics of production. However, processes designed to achieve the desired surface properties of a polymer can comprimise the overall bulk material. The ability to alter the surface of the polymer while leaving the underlying bulk material unchanged has a large potential for development in the area of biomaterials. By modifying the surface of a polymeric material so as to impede water adhesion, the ability of bacterial and cell growth and hence, infection, can be minimized on those surfaces that are incorporated into biological systems. Surface modification was accomplished via plasma processing in a commerical size plasma etching device which achieves plasma densities and and electron temperatures up to 10^{11} cm^{-3} and 4 eV. The desired degree of change is controlled by macroscopic external controls, rather than invasive internal modifications. This process lends itself well for use in exisiting plasma processing systems. Water contact measurements have been taken before and after treatment of HDPE that show a change from a pretreatment of 85^{o} to post treatment of near 0^{o} and 0^{o} angles, which corresponds to a dramatic change in surface energy of the polymer. Video of the surface interaction with water shows drops rolling off

the material. Applications of the process includ intubation tubes, blood vials, shunts, splints, and many other biomedical devices.

BI-TuP11 Comparison of Polystyrene and Teflon-AF as Model Surfaces for Hydrophobic Adsorption and Biocompatibilization, ***L. Feller***, *N. Tirelli, S.M. De Paul, J.P. Bearinger, A. Napoli, J.A. Hubbell, M. Textor,* Swiss Federal Institute of Technology

The motivation of this investigation is to produce materials for applications in biosensors and biomedical materials via surface modification based on hydrophobic interactions. For this purpose we have used the physical adsorption of amphiphilic poly(propylene sulfide)-block-poly(ethylene glycol) (PPS-PEG), from a polar solvent (methanol, water). Upon deposition on a hydrophobic surface, these polymers display the biocompatible, protein-repellent PEG as the top layer and are believed to maintain this architecture when placed in either water-based model electrolytes or a physiological environment. We have chosen to apply this method of surface modification to two hydrophobic materials, poly(styrene) and Teflon-AF. These materials have already been used in biomedical applications but which require a surface biocompatibilization to reduce unfavorable foreign-body reactions. Both polymers can be produced in form of thin films (20-50 nm thickness) via spin-coating. We studied the properties of thin films of these two hydrophobic surfaces and their behavior in subsequent PPS-PEG adsorption studies. The thickness and uniformity of spin-coated surfaces are sensitive to rotation speed and concentration. For optical waveguide lightmode spectroscopy (OWLS) investigations it is necessary to have an optically transparent homogenous thin layer of ideally 12 nm thickness. We produced a series of both poly(styrene) and Teflon-AF layers of various thicknesses and characterized their homogeneity and thickness using an Atomic Force Microscope (AFM). Layer stability was characterized by dynamic contact angle measurements. The adsorption of PPS-PEG block copolymers through hydrophobic interactions was subsequently studied by OWLS. PPS-PEG demonstrated good adsorption on both surfaces, showing complete and stable coverage under physiological conditions. Preliminary experiments have also shown that the deposited layers strongly decrease the protein adsorption on such substrates.

BI-TuP12 In-situ Single-Molecular Detection of Antibody-Antigen Binding by Tapping-Mode Atomic Force Microscopy, ***L. Li***, *S. Chen, S. Jiang*, University of Washington

Ever since its invention atomic force microscopy (AFM) has been widely used in biotechnology and biomedical research, including imaging, force mapping and sensor application. In this work, we have performed studies on AFM-based single-molecule detection. Target molecules are detected by directly comparing two tapping-mode AFM topographical images at the same location before and after exposing an immobilized antibody to a solution containing its antigen, or vise visa. Two pairs of antigen/antibody systems were investigated: chorionic gonadotropin (hCG) and monoclonal antibody (MAb) to hCG, goat anti-hCG and MAb to goat immunoglobulin (IgG). Antibody molecules are chemically immobilized on uniform mixed self-assembled monolayers (SAMs) terminated with COOH and OH, which allow the detection of the individual antigens, antibodies, and antigen/antibody complexes. The advantages of the in-situ detection at the same location include the detection of antigen/antibody binding at single-molecule resolution and the distinction of non-specific interactions from specific ones. This AFM-based immunoassay is more sensitive and reliable.

BI-TuP13 Transformation of a Single Peptide Molecule Measured with Atomic Force Microscopy, ***M. Kageshima***, *S. Takeda,* National Institute of Advanced Industrial Science and Technology, Japan, *A. Ptak,* Poznan University of Technology, *C. Nakamura, S.P. Jarvis, H. Tokumoto, J. Miyake*, National Institute of Advanced Industrial Science and Technology, Japan

Transformation of protein molecule is a fundamental process in various function of the molecule. Such a transformation is considered to accompany substantial rearrangement of intramolecular hydrogen bonds. In a peptide molecule in an α-helix form, breaking of hydrogen bonds takes place as it is unfolded by a tensile force along its helical axis and results in variation in the longitudinal stiffness of the molecule. Therefore, in order to understand the unfolding process in a single-molecule scale, measurement of the variation in stiffness and the energy dissipated during refolding process is indispensable. In the present study this measurement was implemented by AFM with magnetic modulation technique. An end of a single $C_3(AEAAKA)_6C$ peptide molecule was picked up with the AFM probe and was stretched. The AFM cantilever was modulated with an AC magnetic force with a frequency of 500 Hz via a magnetic particle on its backside. The amplitude and phase shift in the AC component of the cantilever deflection were measured simultaneously with the DC force. The dissipation during one cycle of oscillation and the variation in the stiffness of the molecule was calculated from the measured amplitude and phase. The contribution by the liquid in the measured dissipation was calculated from the amplitude signal and was subtracted. From the dissipation change during the unfolding process, the dissociation energy per one hydrogen bond was determined. Thus, it is shown that both the conservative and the dissipating processes taking place in a single molecule during its transformation can be measured with this technique.

BI-TuP14 Selective Photocatalysis by Means of Molecular Recognition, ***Y. Paz***, Technion- Israel Inst. of Technology, Israel

Titanium dioxide is known to be a non-selective photocatalyst for the treatment of polluted air and water. An approach for obtaining selectivity, thus facilitating its use for the mineralization of hazardous, non-biodegradable contaminants is presented hereby. This approach is based on the construction of molecular recognition sites (MRS) anchored on inert domains in the vicinity of photoactive sites. These MRS are designed to physisorb target molecules and to "shuttle" them to the photocatalytic sites. Care is taken to prevent the photocatalytic degradation of the MRS, since (as we have found before) the photoinduced oxidizing species are, in principle, able to attack molecules anchored in the vicinity of titanium dioxide domains. Here we present several examples of selective photocatalysis by means of molecular recognition, based on the trapping of target molecules on thiolated cyclodextrins sites, followed by surface diffusion and photodegradation of the contaminants. The prospects and limitations of this approach will be discussed.

BI-TuP15 Reflex Arc on a Chip - Directed Neuron Growth, ***M. Poeta***, *G. Jacob, M. Das, P. Molnar, J. Hickman*, Clemson University

The reflex arc is one of the simplest controls systems in the body. Yet it rivals the most complex man-made systems in complexity. The reflex arc is a controls loop consisting of a muscle fiber innervated by a motoneuron. A dorsal root ganglion (DRG) innervates both cells, completing the loop and providing feedback. Our group will look at building this system on a Micro Electrical Mechanical System (MEMS) chip. Currently, we are investigating the directed growth of motoneurons on substrates. We have created patterns of Self Assembled Monolayers (SAMs) on glass cover slips. The patterns have two geometric variables: somal (cell body) adhesion site diameter and axon (signal sending process) track width. The patterns are made of diethylenetriamine (DETA), a SAM cytophilic to motoneurons. The cover slips were then backfilled with tridecafluoro-1,1,2,2-tetrahydrooctal-1-trichlorosilane (13F), a SAM cytophobic to motoneuron growth. Photolithographic techniques are used to create the patterns. We are determining the geometric parameters (somal adhesion site diameter, axon track width) that are most conducive to motoneuron growth. We also will present electrophysiological characteristics of the motoneurons and relate changes in electrical activity to parameters in the neuron local environment.

BI-TuP17 Explorations of the Influence of Electrostatic Interactions on Surface-Peptide Binding by Matrix-Assisted Laser Desorption / Ionization Mass Spectrometry, *G.R. Kinsel,* ***J. Zhang***, *R.B. Timmons, M. Li*, University of Texas at Arlington

Protein-surface interactions play an important role in a variety of fields. The mechanism of these interactions remains unclear, however, due to the extraordinary complexity of the protein-surface interface and the wide range of chemical and morphological properties that may be present. The use of well characterized surfaces and peptides with well-defined properties can alleviate some of these problems and allow the systematic study of the influence of various surface or protein properties on the protein-surface binding interactions. In the present studies surfaces having well characterized chemical and morphological properties have been created by plasma polymerization of allyl amine or vinyl acetic acid leading to surfaces with high contents of amine or carboxylic acid functional groups respectively. Bradykinin, angiotensin I, and buccalin are three small peptides with similar molecular weights but various primary sequences leading to systematic changes in the peptide pI from 12.0 to 6.9 to 3.8 respectively. It is shown that these peptides have increasing binding affinity for plasma polymerized vinyl acetic acid modified PET surfaces, but decreasing binding affinity for plasma polymerized allyl amine modified PET surfaces. These trends may be attributed to electrostatic interactions between the peptides and the chemical groups on the plasma modified surfaces. This interpretation of the observed effects can be further explored by altering the pH of the solution in which the binding interactions take place. For example, it is found that as the acidity of the solution is increased binding of the acidic peptide to the basic surface is reduced, consistent with the peptide being neutralized in low pH solutions. Additional studies that explore the effect of solution pH on peptide surface binding interactions have been performed and are interpreted in terms of the changing electrostatic properties of the peptide and surface.

BI-TuP18 Realisation of Biosensor Interfaces by Surface Reactions on Silanised Tantalum Pentoxide, ***W. Laureyn***, *R. De Palma, F. Frederix, K. Bonroy, J.-M. Friedt, K.-H. Choi, A. Campitelli*, IMEC, Belgium, *G. Maes*, KULeuven, Belgium

Affinity biosensors allow the detection of affinity based interactions between bio-molecules, e.g. in antibody-antigen recognition. The presence of antigens in an analyte can be verified by their binding to complementary antibodies, immobilised onto a biosensor surface. Tantalum pentoxide (Ta_2O_5) is regarded as a promising material for the realisation of affinity biosensors, especially for impedimetric biosensing, because of its high dielectric constant and chemical stability. To date, the main method for the immobilisation of proteins to oxide surfaces has involved reactions with short-chain trialkoxysilanes, leading to heterogeneous and less effective biosensor interfaces. Alkyltrichlorosilanes, on the contrary, generate well-structured Self-Assembled Monolayers (SAMs), when produced under the proper conditions. Unfortunately, most polar functional groups ideal for protein immobilisation (COOH and NH_2) have to be generated from non-polar precursor alkyltrichlorosilanes, after SAM-formation of the latter. In this contribution, several approaches for the introduction of polar functional groups on Ta_2O_5 , silanised with alkyltrichlorosilanes, will be presented. A novel surface reaction for the introduction of COOH and NH_2 groups on SAMs of bromoalkyltrichlorosilane is evaluated and compared to the oxidation of allylalkyltrichlorosilane and the reduction of cyanoalkyltrichlorosilane respectively. The proposed surface reaction consists in a nucleophilic substitution of the bromine termination with functional thiol compounds. The silanisation of Ta_2O_5 and the subsequent surface reactions are characterised by means of contact angle measurements, XPS, infrared spectroscopy and cyclic voltammetry. Finally, the immobilisation of IgG, on the generated functional Ta_2O_5 surfaces, and the subsequent binding of anti-IgG are monitored by means of Quartz Crystal Microbalance and Atomic Force Micrososcopy.

Authors Index

Bold page numbers indicate the presenter