Tuesday Evening Poster Sessions, October 26, 1999

Biomaterial Interfaces Group
Room 4C - Session BI-TuP

Poster Session

BI-TuP1 Characterization of Adsorbed Protein Films by Static Time of Flight Secondary Ion Mass Spectroscopy (ToF-SIMS), ***M.S. Wagner***, *J.B. Lhoest, D.G. Castner,* University of Washington

The development of effective biomaterials requires knowledge about the composition and structure of the adsorbed protein film that is formed upon implantation of a biomedical device in the body. Static Time of Flight Secondary Ion Mass Spectroscopy (ToF-SIMS) is a powerful technique for probing the molecular structure and composition of the outermost layers of a surface, making it an ideal technique for characterizing adsorbed protein films. Previous static ToF-SIMS studies of adsorbed protein films have shown the data obtained are complex fragmentation patterns arising from the amino acids present in proteins. To efficiently utilize all the information in these complex spectra requires multivariate analysis methods. We have used Principle Component Analysis (PCA) and Partial Least Squares (PLS) regression of the static ToF-SIMS data to characterize the composition of adsorbed protein films on mica and Teflon surfaces. PCA was used to differentiate the pure films of various plasma proteins (albumin, fibronectin, fibrinogen, etc.). PLS regression was used to quantitate the surface composition of protein films resulting from the competitive adsorption of binary protein mixtures comprised of albumin/fibronectin and albumin/Immunoglobulin G. For the albumin/fibronectin mixtures it was found that solutions containing more than 20% fibronectin produced adsorbed protein films that appeared to be pure fibronectin. The static ToF-SIMS results could be quantified by calibrating them with radiolabeled protein adsorption results. The overall aim of this research is to generate of a ToF-SIMS spectral database that can be used to determine the composition and structure of multicomponent adsorbed protein films with static ToF-SIMS.

BI-TuP2 Immobilization of Proteins on Self-Assembled Monolayers by Electrostatic Interactions, ***Y. Du***, *S.S. Saavedra,* University of Arizona

Immobilization of proteins onto solid substrates has been studied widely because of the importance of biomolecular films molecular devices. Adsorption of protein molecules onto solid surfaces by physical interactions is technically simple immobilization methods. However, in order to form highly ordered protein films, a single type of site-directed binding must be employed. In this study, sulfonated silane films were prepared on Si substrates. The resulting anionic surface was used to electrostatically adsorb positively charged proteins, such as horse heart cytchrome c. The formation of the sulfonated silane films was supported by XPS measurements, contact angle measurements, and ellipsometry measurements. The nature of the electrostatic adsorption of protein molecules and the protein film structure were investigated using a variety of surface spectroscopic techniques.

BI-TuP3 Adsorption Studies of Barnacle Cement Proteins from Balanus Improvisus, ***K. Mjorn***, *F. Hook, H. Elwing,* Goteborg University, Sweden

Marine specimens such as barnacles and mussels anchor themselves to solid surfaces with biological glues that are curable under water. The glue used by barnacles is called cement, which is a clear proteinous liquid. When a barnacle is dislodged from a surface under certain conditions, such cement is secreted. The adsorption of fresh cement from the barnacle Balanus improvisus without prior purification has been studied on an electrically inert non-polar methylterminated thiolated gold surface and polar gold surface by a combined use of Surface Plasmon Resonance (SPR) technique and an extended version of Quartz Crystal Microbalance (QCM-D) technique. With SPR, which is an optical surface sensitive method, we can determine the adsorbed amount of protein at the surface due to the difference in refractive index between water and the protein. With QCM-D, an acoustic method, we can determine the degree of bound water in the protein layer and the structural flexibility of the proteins. The adsorbed mass was almost twice as great for the hydrophilic gold surface compared to the mass adsorbed on the hydrophobic surface showed with SPR. The amount coupled water did not differ in a great extent between the two surfaces. This indicates that the flexibility and the amount adsorbed water did not vary much with the chemistry of the surface, in contrast to the mussel adhesive protein, Mepf-1, showed by C. Fant et al.@footnote 1@ The behavior of the cement monolayer could then be followed with these two methods when the buffer conditions where changed, e.g. ionic strength and pH. The influence of this was also followed with 2D SDS-PAGE, where the separation of fresh cement was compared to treated cement. How and at which circumstances the cement is cured is under investigation. @FootnoteText@ @footnote 1@C. Fant, K. Mjorn, H. Elwing and F. Hook, Adsorption behavior and enzymatic induced cross-linking of mussel adhesive protein (submitted).

BI-TuP4 Soft X-ray Spectromicroscopy Studies of Protein-Polymer Interactions, ***A.P. Hitchcock***, *T. Tyliszczak, Y.M. Heng, R. Cornelius, J.L. Brash,* McMaster University, Canada*; H.W. Ade,* North Carolina State University

Soft X-ray microscopy and micro-spectroscopy has considerable potential for characterizing the interaction of biological molecules with the surfaces of polymers used for biomaterials applications such as blood contact. We are exploring the use of scanning transmission X-ray microscopy (STXM) and various surface-sensitive X-ray microscopies - photoelectron emission microscopy (PEEM), scanning photoelectron microscopy (SPEM), and electron yield detection in STXM - for characterizing phase segregation at surfaces of polymers used for biomaterials applications, and for investigating specificity of cell and protein interactions with polymer surfaces. Transmission mode (STXM) has been used to study multilayer albumin adsorption on a polyurethane thin film which had micron-scale phase segregation. C 1s and N 1s STXM images and spectra recorded before and after protein exposure demonstrate that C 1s absorption spectroscopy differentiates protein, hard segment (urea), and soft segment (polyether) polyurethane signals and can locate proteins relative to an underlying phase segregated polymer. The strong C 1s -> @pi@*(C=O) excitation at 288.3 eV was found to be sufficiently well separated from the spectral features of the underlying polyurethane that considerable sensitivity to protein is achieved. The results clearly demonstrate that NEXAFS microscopy is relevant to biomaterials problems and that spatial segregation of protein does occur on phase separated polymers. Results to date suggest that albumin prefers the aromatic hard segment rather than the aliphatic soft segment regions of polyurethanes. The status of efforts to develop surface sensitive detection using total electron yield in STXM will also be discussed. X-ray microscopy is carried out at the Advanced Light Source (supported by DoE under contract DE-AC03-76SF00098), supported financially by NSERC (Canada).

BI-TuP5 A Circulating Flow Surface Plasmon Resonance System for Measuring Protein-Vesicle Binding Affinities, ***J. Shumaker-Parry***, *L.S. Jung, M.H. Gelb, C.T. Campbell,* University of Washington

We have developed a circulating flow surface plasmon resonance (SPR) system for measuring equilibrium binding of proteins to surface-bound vesicles. Traditional methods for measuring interfacial binding of proteins to membranes often require the use of a non-physiological reporter group such as a tryptophan residue. The addition of a tryptophan has been shown to perturb binding in some cases. The fluorescence-based methods also lack the sensitivity to measure high binding affinities (K@sub d@ < 10@super-7@M). Other methods that rely on centrifugation are plagued by protein loss through non-specific adsorption on tube surfaces. Our circulating flow SPR system measures equilibrium binding constants rapidly without a spectral probe and can be used to quantify values of K@sub d@ down to 10@super -10@M. The sensing surface contains a high density of vesicles immobilized via biotinylated lipids that bind to biotin sites in a streptavidin monolayer. Fluorescence studies using dye-filled vesicles show that the vesicles remain intact when attached to the surface. We have used the surface-bound vesicles to measure K@sub d@ values for phospholipase A@sub 2@ with phospholipid vesicles. A circulating flow system is used to introduce the protein to the vesicle surface and the protein concentration can be adjusted by adding protein or buffer to a reservoir. A binding isotherm is generated by performing equilibrium titrations and is used to obtain the K@sub d@ value directly without the need to fit association and dissociation rate curves. We have shown that vesicles provide a more biologically relevant surface for measuring binding affinities than planar hybrid bilayer membranes.

BI-TuP6 Adsorption Behaviour and Enzymatic Induced Cross-linking of the Mussel Adhesive Protein, Mefp-1, ***C. Fant***, *H. Elwing, F. Hook,* Goteborg University, Sweden

The blue mussel produces a family of 3, 4-dihydroxyphenylalanine (DOPA) containing proteins that allow the organisms to attach themselves to solid surfaces with high adhesive strength. We have studied the adsorption behaviour of the mussel adhesive protein, mefp-1, to a non-polar CH@sub 3@-terminated thiolated gold surface and to a polar silicone dioxide (SiO@sub 2@) surface. Cross-linking of these monolayers was induced by

catechol oxidase, which catalyses the transformation of DOPA to a highly reactive o-quinone participating in cross-linking. The adsorption and the cross-linking of mefp-1 was studied with respect to changes in the mass and viscoelastic properties of the protein adlayer. This has been possible by combined use of the Surface Plasmon Resonance (SPR) and an extended version of the Quarts Crystal Micro Balance (QCM-D@super TM@). The SPR response are proportional to the adsorbed mass (m). QCM-D measures change in mass uptake as a change in frequency (f) of a piezo electric quartz crystal. The change in frequency is proportional to the adsorbed mass including trapped water. In addition, the QCM-D technique allows simultaneous measurement of the energy dissipation (D) related to the rigidity/viscoelasticity of the adlayer. The results suggest that the protein layer formed on the polar surface is rigidly attached. In contrast, the layer formed on the non-polar surface is flexible, and it contains a large amount of bound water. Upon cross-linking, the dissipation decreased more on the non-polar surface compared to the hydrophilic surface. This demonstrates that the combination of these techniques provides unique information with respect to rigidity and water content of the adsorbed protein. The combination of these two techniques also make it possible to study the cross-linking of mefp-1, which is important knowledge for the development of a medical glue.

BI-TuP7 Thin Films of Functionalized Poly(Ethylene Gylcol) for the Specific Attachment of Proteins, ***S.N. Xia***, *H.B. Lu, C.T. Campbell, D.G. Castner,* University of Washington

The formation of ultrathin monolayers (10-30 angstroms thickness) of functionalized poly(ethylene gylcol) (PEG) oligomers and PEG-grafted siloxane polymers on gold surfaces has been investigated using x-ray photelectron spectrsocopy (XPS), static time of flight secondary ion mass spectromety (ToF SIMS), and surface plasmon resonance (SPR). PEG chains (MW 2000 to 5000) with an orthopyridyl-disulfide (OPSS) terminal group could be directly attached to a gold surface via formation of a gold-thiolate bond. By varying the type of terminal group at the other end of the PEG chain, the PEG monolayer can inhibit protein adsorption (OCH3), selectively attach proteins with surface cysteines (OPSS) through the formation of disulfide bonds, or selectively attach proteins with surface lysines (N-hydroxysunccinimide, NHS) through the formation of amide bonds. PEG monolayers that inhibit protein adsorption can also be formed from adsorption of siloxane polymer chains grafted with both methoxy-terminated ethylene gylcol and dialkyl disulfide side chains onto gold surfaces. The ability of the polymeric monolayers to specifically attach proteins with suface lysines was accomplished by replacing some of the methoxy-terminated PEG side chains with NHS-terminated PEG side chains.

BI-TuP8 A Novel Method for Characterization of Cell Surface Interactions, ***S. Kihlman,*** Chalmers University of Technology, Sweden*; C. Fredriksson, M. Rodahl,* Q-Sense AB, Sweden*; D.M. Steel,* Göteborg University, Sweden*; B. Kasemo,* Chalmers University of Technology, Sweden

A real-time in vitro technique to probe cell-surface interactions at biomaterial interfaces (e.g., contact surface, adhesion proteins, membrane and cytoskeleton modifications of the cell), would be very useful. In this study, we have explored the potential of a new method to characterize cell-surface interactions in liquid environments by dynamically monitoring the Quartz Crystal Microbalance (QCM) response due to adhering cells. The method is based on a new technique of simultaneously extracting resonant frequency and dissipation factor of the QCM. This is called QCM-D. It has here been applied to study the adhesion process of Chinese hamster ovary cells to polystyrene surfaces in serum-containing and serum-free environment at 37°C. The results show that: (i) adhesion of small colonies of a few hundred cells can readily be detected and characterized using the QCM-D technique, and (ii) the method of combining QCM data from changes in resonant frequency and dissipation factor gives new, relevant information about the character of the cell adhesion on surfaces and appears to be sensitive to properties of the surface (such as wettability) as well as the presence of serum-proteins on the surface.

BI-TuP9 The Role of Angiogenesis at Biomaterial Interfaces, ***P.C. Stephans,*** University of Washington*; R.B. Vernon, E.H. Sage,* Hope Heart Institute*; P.S. Stayton,* University of Washington

When a biomaterial is implanted, a relatively avascular fibrotic layer forms around the device. For devices such as sensors, this can inhibit the proper function of the device because the sensor only samples the microenvironment of the capsule. However, if the vascularity of the tissue adjacent to the device is increased, then the sensor can continue sampling a more representative environment. This problem has led to an interest in angiogenesis, the growth of new blood vessels from an existing vasculature, and how it is altered by various material properties. We are studying three material factors that may affect angiogenesis. The first, porosity, is based upon previous in vivo work that demonstrated a correlation between porosity and the number of vascular structures located close to the material. The second is coating the material with molecules, such as extracellular or matrix proteins. For example, we are currently immobilizing a peptide from SPARC that has shown angiogenic activity in vivo. The third factor is the release of soluble agents in the context of a material. Various soluble factors have been identified that are angiogenic, but now we can study the factor release from a material to determine if the angiogenic response can be stimulated within the material. To facilitate these studies, we are developing in vitro assays to study angiogenesis in the context of a biomaterial. For example, we have developed an assay to measure migration, a crucial step in angiogenesis. A teflon fence is used to grow endothelial cells to confluence in a well-defined area on the material. Once the fence is removed, the migration on various surfaces can be measured. The macrophage, a key player in angiogenesis, can also be incorporated into the assay to determine the effect of cytokine secretion on the endothelial cells. By studying the materials with these assays, we hope to gain a better understanding of the cell-material interactions that guide angiogenesis.

BI-TuP10 A Biosensor for In Vitro Monitoring of Cancer Cell Adhesion Behaviour, ***G. Nimeri,*** Gothenburg University, Sweden*; C. Fredriksson,* Q-sense AB, Sweden*; R. Hultborn, H. Elwing,* Gothenburg University, Sweden

A quartz crystal microbalance and dissipation (QCM-D) sensor, allowing simultaneous resonant frequency (f) and energy dissipation (D) measurements, was used to monitor cancer cell adhesion behaviour (attachment, spreading, and death) in vitro. This method is a mechanical sensor based on a minute (1nm) oscillation in the ultrasound frequency range. By measuring changes in f, the technique can be used to monitor the contact area of the cells with the substrate. Changes in D, which reflect energy losses as a result of friction in the system, provide information related to the internal structure (e.g., stiffness of the cytoskeleton). These two parameters offer new real time information regarding the status of cultured cells in vitro without interruption. The QCM-D signals were monitored for FADU cancer cells (human carcinoma squamous cells) for 8-24 hours. Cells were injected into a specially designed measurement chamber, filled with a minimal essential buffer and kept at 37 degrees C. The deposition and consecutive behaviour on the sensor surface, pre-coated with a tissue culture quality polystyrene overlayer, was then followed. Cell numbers from a few thousand up to a hundred thousand cells on a 1 cm2 surface were studied. The results show that the cells adhere and form a settled layer on the surface with relatively stable baselines in f and D after 4-8 hours. These baseline values indicate that a cultured layer of cells would provide contributions in f and D which can be monitored e.g., during cell growth or treatment. Preliminary results on the adhesion behaviour of non-treated and cells irradiated with 4 Gy (normal tumor treatment) show that the signals are distinctly different. The indications of differences in behaviour are considerably earlier than current methods based on growth rates (DNA staining etc).

BI-TuP11 The Effects of Surface Chemistry and Adsorbed Proteins on Monocyte/Macrophage Adhesion to Surfaces, ***M. Shen***, *T.A. Horbett, Y.V. Pan, B.D. Ratner, K.D. Hauch,* University of Washington

Adherent macrophages play a central role in inflammatory responses to implanted biomaterials. Human monocyte adhesion to surfaces was therefore studied to determine the effects of surface chemistry, adsorbed proteins, and adhesion time. The surface chemistry of a series of commercially available modified polystyrene (PS) surfaces, fluorinated ethylene-propylene polymer (FEP), and plasma-polymerized-tetraglyme (PPT) coated FEP was analyzed by ESCA. The surfaces were preadsorbed with plasma, serum, fibrinogen, fibronectin, or albumin. Human monocytes in 10% serum were allowed to adhere to the surfaces for 2 hours or 1 day. Adhesion was measured by an LDH method. After 2 hours, monocytes adhered to most surfaces under all conditions examined. Adhesion was greater on charged hydrophilic TCPS, Plastek C, or Primaria than on hydrophobic PS, Plastek A, Plastek B, or FEP. Adhesion was lowest on uncharged hydrophilic PPT-coated FEP or Costar's Ultra Low Attachment hydrogel, which were also shown to resist fibrinogen adsorption. Monocyte adhesion was greater on surfaces preadsorbed with fibrinogen or fibronectin than on surfaces preadsorbed with albumin. However, 2-hour adhesion to surfaces preadsorbed with serum was similar to surfaces preadsorbed with plasma, despite the lack of fibrinogen. Preadsorption of dilute plasma or serum increased adhesion to TCPS but did not increase adhesion to PS. After 1 day, monocyte adhesion decreased and was lowest

to surfaces without preadsorbed proteins. One-day adhesion was greater on plasma than on serum preadsorbed surfaces and was much greater on fibrinogen or fibronectin than on albumin preadsorbed surfaces. Overall, monocytes adhered to all surfaces and preadsorbed fibrinogen and fibronectin significantly promoted monocyte adhesion. Non-fouling surfaces that minimize protein adsorption may minimize overall macrophage adhesion and activation.

BI-TuP12 Human Monocyte/Macrophage Adhesion to Biomaterials Surfaces, ***J. Shan***, *T.A. Horbett, C.M. Giachelli,* University of Washington

The biocompatibility of synthetic implants is thought to be related to their attractiveness to monocyte/macrophages. The hydrophobicity and protein adsorption capabilities of an implant surface are believed to influence the adhesion of monocytes and macro phages. Therefore, a range of materials varying widely in hydrophobicity and protein adsorption characteristics were evaluated for monocyte/macrophage adhesion in vitro. Freshly isolated human peripheral blood monocytes were cultured on surfaces in the p r esence of autologous serum from 2 hour up to 8 days. The surfaces includes polystyrene (PS), tissue culture treated polystyrene(TCPS), human fibrinogen (Fbg) preadsorbed to PS (Fbg-PS), BSA preadsorbed PS (BSA-PS), plasma polymerized hexaflurocarbon (C 3F6) coated PS, plasma polymerized triglyme coated PS(triglyme), fluoro-ethylene-propylene (FEP) copolymer, and plasma polymerized tetraglyme coated FEP(PEO-FEP). Monocyte adhesion was determined by measuring the LDH activity of lysates. Of these surfaces, t he most hydrophobic is C3F6 coated PS, the most hydrophilic surface was PEO-FEP, which had less than 5ng/cm2 adsorption of Fbg from 1% human plasma. Adhesion to most PS based surfaces was similar for periods up to 24 hours. However, monocyte adhesion to T CPS was much lower by 24 hours. Monocyte adhesion to PEO-FEP at earlier hours was about 1/2 to 1/3 of the level achieved on PS and FEP, respectively. After the 8 day period, macrophage adhesion to the surfaces ranged from none on TCPS and Fbg-PS, low on B SA-PS, to significantly higher on PS, C3F6, triglyme and PEO-FEP. Adhesion to PEO-FEP after the 8-day period was similar to that on PS, C3F6, and triglyme, however macrophage morphologies differed among surfaces. Possible explanations (including non-protein mediated mechanisms) for monocyte/macrophage adhesion to PEO-film will be included in the poster presentation.

BI-TuP13 Adhesion of Endothelial Cells to Patterned OTS Surfaces, ***S.R. Webb***, *T.B. Boland, D.R. Weinbrenner,* Clemson University

Thromboresistance is necessary for a biomaterial in vascular applications to maintain proper function. One approach to thromboresistance is to line the interior wall of artificial vascular grafts with endothelial cells.This study examines the effects of surface chemistry and adhesion of endothelial cell membranes to proteins. Hence, understanding these two factors will facilitate applications to reduce thrombogenesis. Cell response to patterned materials was examined by employing highly organized monolayers of self-assembled octadecyltrichlorosilane (OTS) on a silicon wafers. Pure OTS monolayers are poor substrates for cell growth, most likely because of the denaturing of serum proteins near the surfaces. The OTS surfaces were exposed to an electron beam, which selectively oxidized the organic film, creating a variety of oxygenated species at the surface. The monolayers and the patterned surfaces were analyzed by ellipsometry, water contact angle, electron spectroscopy for chemical analysis (ESCA) and AFM. Bovine heart endothelial cells were cultured. Cells were inoculated at 1 x 106 cfu/mL and cultured in twelve well plates in the presence of pure and patterned OTS surfaces. To insure the cells were not toxic to the OTS surfaces three cytotoxicity test were performed. The first was a live dead assay in which calcein green was used to determine if the cells were viable. The second test was MTS to determine if the cells were proliferating. The last test to determine how cytotoxic the surfaces were to the cell was BCA or total protein test. All three tests proved that the patterned surface was not toxic to the cells. The attachment, spreading and growth of the cells on the surfaces will also be presented. The cell spreading on the etched surfaces suggests that the cells may be able to attach more firmly to the extracellular proteins on the etched pattern. The results from this cell growth study will aid in designing micro-patterned surfaces for cell-based biosensors.

BI-TuP14 Microcontact Printing of Protein Patterns to Direct Cellular Response, ***T.C. McDevitt***, *M.D. Garrison, T.R. Kyriakides, M. Scatena, P. Bornstein, C.M. Giachelli, P.S. Stayton,* University of Washington

In order to control the cellular response at a biomaterials interface, we are applying micropatterning techniques to spatially arrange extracellular matrix (ECM) ligands on surfaces. Cell function has previously been related to cell shape and cytoskeletal architecture, thus we are investigating how microcontact printing of proteins can be used to modulate these parameters. Various combinations of ECM proteins can be patterned to create heterogeneous, ordered surfaces that selectively promote cell adhesion, spreading, and intracellular signaling events. Designated adhesive and non-adhesive regions are dictated by the choice of particular ECM proteins which possess these characteristics and cell attachment is confined by the features of the micropatterning. Our aim is to use micropatterning as a tool to recreate an ordered, complex surface that more accurately resembles the true ECM, stimulates a specific and desirable cellular response, and thus induces a more favorable interaction with the biomaterial.

BI-TuP15 Surface Functionalization Strategies for Miniature Multichannel Biosensing, ***H.B. Lu***, *J. Homola, C.T. Campbell, B.D. Ratner, S.S. Yee,* University of Washington

Biosensor development has advanced towards highly integrated, multichannel array configurations with more detection power and faster speed. Robust surface functionalization methods for immobilizing sensing molecules and making non-fouling surfaces are needed. Also, precise spatial control of surface functionalization is valuable. In this presentation, various surface functionalization methods including orthogonal self-assembly (OSA), masked plasma polymerization and protein contact printing are introduced. The potential of these methods for precisely immobilizing sensing biomolecules to specific surface regions, or creating a non-fouling area using passivation molecules, is discussed. We used a dual-channel surface plasmon resonance (SPR) sensor with a thin tantalum oxide (Ta2O5) overlayer configuration for demonstrating the feasibility of these surface functionalization methods, as well as for further developing a miniaturized multichannel biosensor. The high refractive index dielectric Ta2O5 overlayer covers part of the gold surface to excite part of the incident light in a higher wavelength and thus produce a second SPR dip besides the original gold SPR dip. To use this second SPR dip as an internal reference channel, the surface functionalization methods were used to deliver biosensing molecules (e.g. antibodies) or passivation molecules (e.g. oligo(ethylene glycol) or bovine serum albumin) to the gold or the Ta2O5 surface respectively. The optical nature of such an SPR sensor configuration requires precise delivery of molecules to these two surface regions in order to separate signal from the two channels. Therefore, it provides an excellent tool for demonstrating the spatial control ability of the surface functionalization methods used. The surface functionalization strategies described in this paper should have general applicability for developing miniaturized multichannel biosensors in other formats as well.

BI-TuP16 Kinetics of Vesicle Adhesion and Fusion, ***K. Glasmästar***, *F. Höök, C.A. Keller,* Chalmers University of Technology and Göteborg University, Sweden*; V.P. Zhdanov,* Chalmers Univ. of Tech., Göteborg Univ., Sweden and Institute of Catalysis, Novosibirsk, Russia, Sweden*; B. Kasemo,* Chalmers University of Technology and Göteborg University, Sweden

Vesicle adhesion and fusion are essential in many cellular processes and in the formation of supported membranes. Because of their similarity to natural membranes, they play an important role in the development of biosensors and in model studies of membrane-mediated processes. We have studied the kinetics of adsorption of small unilamellar lipid vesicles on carefully prepared SiO@sub 2@ surfaces and their subsequent fusion to form a lipid bilayer, using surface plasmon resonance (SPR) and a new quartz crystal microbalance (QCM-D) technique. With the latter the energy dissipation (D) and the resonance frequency (f) of the QCM oscillator are measured. The lipid mass adsorbed at the surface and the mass of water trapped by the adsorbed layer are measured as changes in f. The SPR technique is only sensitive to the amount of lipid adsorbed. Since one of the primary differences between lipids adsorbed as vesicles or as a bilayer is the water trapped within and between the vesicles, the combination of the two techniques provides a detailed picture of how a lipid bilayer forms on the SiO@sub 2@ surface. In addition the two types of adsorption have very different viscoelastic properties, which are reflected in the D factor. The formation of a lipid bilayer on a SiO@sub 2@ surface is a two-stage process. First a layer of intact vesicles adsorbs on the surface. Then at a sufficiently high surface concentration the vesicles begin to break and form a fluid bilayer. From detailed QCM-D and SPR measurements at different vesicle concentrations in the liquid phase, we find that (i) the adsorption is irreversible, (ii) it is rate limited by bulk diffusion, (iii) vesicle to bilayer transformation starts at a critical surface coverage, after which (iv) further adsorption from the bulk phase drives the vesicle to bilayer transformation. The kinetics of these events is further elucidated by Monte Carlo simulations, employing different mechanistic models for the kinetics.

BI-TuP17 Thiopeptide-Tethered Lipid Bilayers for the Incorporation of the Enzyme Complex Cytochrome c Oxidase, ***H.D. Lauer,*** Max-Planck-Institut for Polymer Research, Germany*; E.K. Schmidt,* Lab. for Exotic Nanomaterials Frontier Research Program, Japan*; R. Naumann,* Max-Planck-Institut for Polymer Research, Germany*; A. Offenhäusser,* Max-Planck-Institut for Polymer Reseach, Germany*; W. Knoll,* Max-Planck-Institut for Polymer Research, Germany

Lipid bilayers form the basic structure of biological membrane due to which membrane proteins are able to carry out their specific functions. Membrane proteins have precisely designed moving parts whose mechanical actions are coupled to chemical events. This coupling of chemistry and movement is the reason why membrane proteins play the predominant role in most biological processes. Without a better understanding how proteins operate, it is very difficult to apreciate cell biology. Therefore in the last few years a number of model systems have been developed to create a biomimetic system. To retain the functionality of an incorporated membrane protein, the lipid bilayer should be fluid and the membrane/protein-complex has to be to some extent spatially decoupled from the substrate. Without this decoupling from the surface, the protein denatures. A number of spacers are well known to decouple the membrane/protein-complex from the gold substrate and preserve a thin water reservoir between lipid and the gold.@footnote 1, 2, 3@ We present here a biomimetic system utilising thiopeptides as spacer molecules chemisorbed onto a gold surface and covalently attached to lipid layer.@footnote 4@ This system is used to incorporate the membrane protein cytochrome c oxidase, a key enzyme in the cell respiration. Optical and electrochemical characterisation methods are used to obtain more information about the architecture and the operation of the membrane/protein-complex. @FootnoteText@ @footnote 1@ Cornell, B.A. et al., Nature, 387, 1997, 580 @footnote 2@ Vogel, H. et al., J. Phys. Chem., 99, 1995, 1038 @footnote 3@ Evans, S.D. et al., Langmuir, 13, 1997, 751 @footnote 4@ Naumann, R., et al., Angew. Chem., 107, 1995, 2168.

BI-TuP18 Functionalized Glycolipids for Model Biomembrane, ***S. Schiller,*** Max-Planck-Institut for Polymer Research, Germany*; H. Kunz,* University of Mainz, Germany*; W. Knoll,* Max-Planck-Institut for Polymer Research, Germany

Fluid Model Biomembranes are important tools for general investigations of membrane properties and have many potential applications in the pharmaceutical industry e.g. for analyte screening using competitive immuno assays with membrane-integral receptors. The most advanced of these systems are tethered lipid bilayer membranes (tBLMs) on gold or silicon. tBLMs developed so far often show poor electrical properties compared to BLMs. Some systems insufficiently decouple the lipid bilayer from the surface and do not provide optimal tether properties in terms of hydrophilicity of the submembrane and stable membrane support, which is necessary for the incorporation of membrane proteins. An alternative route uses functionalized carbohydates as tether molecules to meet some of these requirements. Several carbohydrate systems are already under investigation, polysaccharides, especially dextran, cellulose and agar are used to provide a hydrophilic, soft and gel like support. Regio- and stereospecific manipulations in small and defined areas are difficult in such large polymer systems. We therefore focus on small oligosaccharides and their functionalized derivatives and the synthesis of a complex multicomponent system with specific lateral spacer molecules and several glycolipid tether sytems. Important components are glycolipids functionalized with anchor groups for surface attachment and spacer saccharides. The possibility to introduce side functionalities to multifunctional saccharides opens a wide range of variations, for example the functionlization with fluorescent probes at different positions along the tether chain or variation of physical properties by varying side groups. The approach we present here consists of the use of several saccharides and different lipids to learn more about the fundamental properties of these systems. Further research will be the synthesis of the complex system described above and the challenging study of complex membrane processes.

BI-TuP19 Force Differentiation Assay: A New Approach Sensing, ***M. Natesan,*** GeoCenters*; C. Yanavich,* Nova Research*; S. Metzger,* Geocenters*; G.U. Lee,* Naval Research Laboratory

Biomolecular interactions have recently been measured at the single molecule scale using microscopic techniques such as atomic force microscopy, optical tweezers and micropipettes. The force required to rupture a specific interaction has been found to be a characteristic of the structure of the molecular interaction and the rate at which it is stressed. We have used these insights to develop a new approach to molecular detection based on sensing force. Force detection has two advantages, it has single molecule sensitivity and the magnitude of the force can be used to differentiate specific from nonspecific interactions. We will describe two force based diagnostic techniques that apply a magnetically derived force to an antibody-antigen sandwich using a micron size particle.

BI-TuP20 Solid Supported Lipid Bilayers by Fusion of Mixed Thiolipid/Lipid Vesicles onto Gold, ***H. Wieder,*** *S. Lingler,* Max-Planck-Institute for Polymer Research, Germany*; J. Mack,* Institute for Organic Chemistry, Germany*; A. Offenhäusser,* Max-Planck-Institute for Polymer Reseach, Germany

Recently, planar solid supported lipid membranes have become a frequently used model system for biological membranes. Their big advantage to other model systems is the relatively high stability to mechanical and electrochemical stress in addition to the accessibility by a variety of surface sensitive techniques. These features make them an interesting system for biosensing applications. In this work supported lipid mono- and bilayers tethered to Au-electrodes by thiolipids are being investigated using surface plasmon resonance spectroscopy (SPR), impedance spectroscopy (IS), cyclic voltammetry (CV), reductive desorption and contact angle measurements. Following work on hybrid bilayer membranes,@footnote 1@ we are now looking at systems with and without laterally diluted hydrophilic spacers of different length to provide a hydrophilic reservoir between membrane and support. The dependence of the membrane properties on different parameters in the preparation by self-assembly, LB-techniques and vesicle fusion are being investigated. Into the membranes noted above transmembrane proteins are reconstituted and checked for their functional activity. @FootnoteText@ @footnote 1@Lingler S., Rubinstein I., Knoll W., Offenhäusser A. (1997) Fusion of Small Unilamellar Lipid Vesicles to Alkanethiol and Thiolipid Self-Assembled Monolayers on Gold, Langmuir 13, 7085-7091.

BI-TuP21 Incorporation of Membrane Proteins into Lipid Bilayers Supported on a SiO@sub 2@-surface., ***A. Persson,*** *F. Höök, J Rydström, B. Kasemo,* Chalmers Univ. of Tech. and Göteborg Univ., Sweden

Transmembrane proteins are difficult to study in their native state, since detergents are used for the solubilization of transmembrane proteins, which might influence the protein properties and function. One way to circumvent this is to incorporate the proteins into lipid membranes deposited on solid supports. The focus of this project is to combine spontaneous formation of lipid membranes on solid supports, known to occur on SiO@sub 2@, with incorporation of transmembrane proteins. Understanding and mastering of this process has important implications for the development of biosensors and biomaterials, for investigations of the respiratory chain, studies of the photosynthesis, and in neurobiology. Small unilammellar vesicles (SUV's) form a lipid bilayer on hydrophilic SiO@sub 2@-surfaces.@footnote 1@ This process is most likely a two-stage process, where initially intact vesicles adsorb at the surface at low coverages. When a certain surface-concentration of SUV's is reached, the vesicles break and form a fluid bilayer. The protein containing SUV's studied in this work seem to behave in the same way. The SUV's were prepared by sonicating different phospholipids in buffer, and the proteins were incorporated with detergent. The vesicle and protein adsorption is studied with a new QCM-D technique where the frequency shift (mass adsorbed on the surface) and the energy dissipation shift (reflecting the viscoelastic properties of the overlayer) are measured simultaneously.@footnote 2@ The presence of the proteins in the supported bilayer is, after deposition, directly measured using the QCM-D technique, combined with a secondary process using specific monoclonal antibodies to the membrane bound proteins. The preparation procedures and the first results are presented. @FootnoteText@ @footnote 1@Keller, C. A., et al. 1998. Biophys. J. Vol 75 p.1397 @footnote 2@Rhodahl, M., et al. 1995. Rev. Sci.Instrum. Vol 66 p.3924.

BI-TuP22 A Dissimilatoric Nitrate Reductase as a Signal Transducer in a Tethered Membrane FET Architecture, *H. Borcherding,* ***Th. Hettmann,*** *S. Diekmann, P. Steinruecke,* IMB Jena e.V., Germany

Nitrate reductase is a key enzyme in the anaerobic denitrification of nitrate to elementary nitrogen. Nitrate is reduced to nitrite in the catalytical @alpha@-subunit of the enzyme. The dissimilatoric enzyme from Pseudmonas stutzeri also comprises a membrane-integral @gamma@-subunit which is involved in the transfer of electrons from the the bacterial quinole pool to the site of nitrate reduction. This makes the enzyme a valuable functional unit for reconstitution in lipid bilayers. We developed a suitable purification protocol for nitrate reductase from Ps. stutzeri . The enzyme is reconstituted in phospholipid vesicles which are used for the

reconstitution of the enzyme in a tethered bilayer covering the gate of a field effect transistor. By generation of a nitrate-specific transmembrane potential, it should be possible to design a nitrate-specific FET biosensor. Data will be given on the suitability of our approach to create a new type of biosensor.

BI-TuP23 On Compressional Wave Velocity in Animal Bone by Compensated Ultrasonic Timer Technique (CUTT), ***S. Mohiuddin***, King Saud University, Saudi Arabia

Ultrasonic propagation properties play a vital role, as they throw light on molecular architecture and cellular assembly of the living system, in understanding the physiological reality of the system which is involved in the life processes. The present investigation on compressional wave velocity of cancellous bone (Scapula and rib) and compact bone (femur) by adopting compensated ultrasonic timer technique, reveals that in cancellous bone tissue (scapula and rib), ultrasonic compressional wave velocity is the same, but it is relatively more than that of compact bone (femur). Considering the values of compressional wave velocity obtained for scapula, rib and femur, when measured at different places of the same sample along its axis, there exit no definite relations between the parameters related to the composition the bone and the parameters concerned with the propagating ultrasonic wave in the tissue. The compressional wave velocity in bovine scapula, rib and femur bones is more than those reported for soft tissues and hard calcified derivatives of integuments. It is interesting to note that the velocity in the bone is high when compared to other tissue of the same animal.

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