Biomaterial Interfaces
Room 202 - Session BI+MC-MoA

Characterization of Biomaterial Interfaces

Moderator: L. Hanley, University of Illinois at Chicago

2:00pm **BI+MC-MoA1 Surface Tools for the Characterisation of Biomaterials**, ***M.C. Davies***, *S.J.B. Tendler, C.J. Roberts, P.M. Williams, S. Allen,* University of Nottingham, UK **INVITED**

Understanding the interfacial chemistry of biomaterials has long been a goal in the search for optimum biocompatibility. The interfacial environment has a major control on materials properties and the exploitation of nanosurface engineering, to tailor the optimum surface behaviour and function has made a significant impact in the biotechnological and biomedical sciences over the last decade. This talk will explore the role of advanced surface tools for the characterisation of modern biomaterial materials and review the limitations and advantages of different approaches, highlighting fruitful areas for future activity.

2:40pm **BI+MC-MoA3 Electrochemical SPR for Biomaterial Applications**, ***R.M. Georgiadis***, Boston University, usa; *R.J. Heaton,* Boston University

Electrochemical SPR is the combination two powerful surface specific techniques which together provide the basis for many surface modification and detection schemes with new applications to biomaterial interfaces. Although the general effect of an applied electrochemical potential on the surface plasmon resonance response from a simple metal surface is well known, the response from more complex interfaces has not been studied in detail. Yet, such studies are crucial for many applications such as quantifying binding at interfaces in the presence of an applied electrochemical field. We show that very useful information can be obtained directly from the potential dependent SPR optical data: the potential of zero charge can be determined in the presence or absence of immobilized organic or biomaterial films and the specific adsorption of anions can also be distinguished. We report results for a series of modified interfaces including self assembled monolayer films and for thiol bound DNA oligomer films in various electrolytes.

3:00pm **BI+MC-MoA4 Characterization of Surface Modified Microporous PTFE Biomembranes using Surface Charge, Topography and Chemistry Studies**, ***I.D. Baikie***, *B. Lägel,* Robert Gordon University, UK

Functionalised microporous PTFE membranes have many applications involving cell growth and adhesion such as skin grafting and cell scaffolds. Key factors in promoting cell growth are the chemistry and topography of the surface, however a much overlooked surface parameter is that of surface charge. Using a multitip scanning kelvin probe (SKP)@footnote 1@ we have performed surface potential/charge topographies of bare and surface modified bio-membranes prior to Human Skin Fibroblast growth. Additionally surface characterization with SEM and XPS provided topography and chemistry information on the top-most layers. Subsequent video-microscopy growth data indicates an extraordinary correlation between a regime of homogeneous negative surface charge profiles and confluent HSF films. Indeed the optimum growth surface displays two dimensional charge transport characteristics. Up to now little work has been performed on the electrical properties of modified polymers due to the difficulties in obtaining accurate surface potential data. The SKP features a truly noninvasive charge imaging measurement mode and we anticipate many future applications both in monitoring biomaterials and biological interfaces. @FootnoteText@ @footnote 1@I. Baikie, P.J.S. Smith, D.M. Porterfield and P.J. Estrup, Rev. Sci. Instrum. 70, 1842 (1999).

3:20pm **BI+MC-MoA5 Enhanced TOF-SIMS Imaging of a Micropatterned Protein by Stable Isotope Protein Labeling**, ***A. Chilkoti***, Duke University; *A. Belu,* Physical Electronics; *Z.P. Yang,* Imation Inc.; *R. Aslami,* Duke University

Patterning of biomolecules on surfaces is an increasingly important technological goal. Because the fabrication of biomolecule arrays often involves step-wise, spatially resolved derivatization of surfaces, spectroscopic imaging of these arrays is important in their fabrication and optimization. Although imaging time-of-flight secondary ion mass spectrometry (TOF-SIMS) is a powerful method for spatially resolved surface analysis of organic molecules on surfaces, TOF-SIMS images of micropatterned proteins on organic substrates can be difficult to acquire because of the lack of high intensity, protein specific molecular ions that are essential for imaging under static conditions. In contrast, low mass ions are of suitable intensity for imaging, but can originate from different chemical species on the surface. A potential solution to this problem is utilize stable-isotope labeled proteins, an approach that has heretofore not been explored in TOF-SIMS imaging of micropatterned proteins and peptides. In order to investigate the feasibility of stable isotope enhanced TOF-SIMS imaging of proteins, we synthesized @super 15@N-labeled streptavidin by metabolic labeling of the protein during expression from a recombinant gene. The spatial distribution of streptavidin bound to biotin micropatterns, fabricated on a polymer and on a self-assembled monolayer on gold, was imaged by TOF-SIMS. Imaging of high intensity, low m/z secondary ions (e.g., C@super 15@N@super -@ and C@super 15@NO@super -@) unique to streptavidin, enabled unambiguous spatial mapping of the micropatterned protein with a lateral resolution of a few microns. TOF-SIMS imaging of micropatterned @super 15@N-labeled streptavidin also illustrated the exquisite sensitivity of TOF-SIMS to low fractional coverage of protein (0.5 nm effective thickness) in the background regions of the protein micropattern.

3:40pm **BI+MC-MoA6 Quantitative Chemical Mapping of Lipid-protein Langmuir-Blodgett Layers by Laser-SNMS**, ***N. Bourdos***, *F. Kollmer, R. Kamischke, H.-J. Galla, A. Benninghoven,* Westfälische Wilhelms-Universität, Germany

Quantitative molecular mapping of chemically modified or functionalized surfaces is still an important challenge in surface analysis. We demonstrate that analyzing sputtered neutrals may be a big step forward in the quantitative mapping of laterally structured overlayers of organic molecules or biomolecules, respectively. We studied samples consisting of phospholipids and a small 34-residue peptide, the surfactant protein C (SP-C). These overlayers are phase-separated into a fluid and condensed phase. They were prepared on Au substrates with the Langmuir-Blodgett (LB) technique and investigated using a combined SIMS/SNMS instrument equipped with a reflectron-type TOF analyzer, a 30 keV Ga+ primary ion source, and an excimer laser for resonantly enhanced multiphoton post-ionization of neutrals. Laser-SNMS was applied for the first time to study LB layers. The SP-C clearly engenders typical amino acid-specific neutral fragments, by which it can be identified and localized on a surface. Most of them result from the cleavage of the COOH group, e. g., CH@sub4@N, C@sub 4@H@sub 8@N or C@sub 5@H@sub 12@N. The small CN is not typical of a certain amino acid but the entire molecule. It is the most intense peptide-based secondary particle and therefore gives excellent maps of SP-C-rich domains formed in the overlayer. It is possible to calculate the protein content in a lipid layer by histogram evaluation. The yields and damage cross-sections calculated from TOF-SIMS measurents indicate that the lateral resolution may be far below instrument limitations (beam focus). A quantitative comparison of the secondary particle emission from SP-C-rich and SP-C-free domains (on the same substrate) allowed some insight into the process of secondary ion and neutral generation from the molecular overlayer as well as from the substrate.

4:00pm **BI+MC-MoA7 Probing the Spatial Organization of Mixed Lipopeptide/Phospholipid Monolayers : Complementarity of AFM and XPS**, ***Y.F. Dufrene***, Universite Catholique de Louvain, Belgium; *M. Deleu, P. Jacques,* Faculte Universitaire des Sciences Agronomiques de Gembloux, Belgium; *P. Thonart,* Centre Wallon de Biologie Industrielle, Belgium; *M. Paquot,* Faculte Universitaire des Sciences Agronomiques de Gembloux, Belgium

Surfactin is a surface-active bacterial lipopeptide, with important biological properties, which is known to interact with lipid membranes. To gain insight into the spatial organization (miscibility, molecular orientation) of mixed surfactin/dipalmitoyl phosphatidylcholine (DPPC) monolayers, the morphology and chemical composition of mixed monolayers transferred on mica were determined by atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS), respectively. AFM topographic and friction images revealed phase-separation for mixed monolayers prepared at 0.1, 0.25 and 0.5 surfactin molar ratios. The step height measured between the surfactin and the DPPC domains was about 1.2 nm, pointing to a difference in molecular orientation: while DPPC had a vertical orientation, the large peptide ring of surfactin was lying on the mica surface. These data were in excellent agreement with the monolayer properties at the air-water interface and with computer simulation data. The N/C atom concentration ratios obtained by XPS for pure monolayers were consistent with two distinct geometric models: a random layer for surfactin and for DPPC, a layer of vertically-oriented molecules in which the polar headgroups are in contact with mica. XPS data for mixed systems were accounted for by a combination of the two pure monolayers, considering respective surface coverages that were in excellent agreement with those measured by AFM. Finally, exciting new possibilities offered by dynamic AFM imaging modes (force modulation, phase imaging) to

investigate the film nanomechanical properties will be presented. This work demonstrates the complementarity of AFM imaging and XPS analysis to directly probe the molecular organization of multicomponent monolayers.

4:20pm **BI+MC-MoA8 Detection of Intact Biomolecules with Matrix-Enhanced ToF-SIMS**, ***D.G. Castner***, *P. Kingshott, J. Nesiba, S.L. Golledge, B.D. Ratner,* University of Washington

An improved understanding of the interfacial interactions between biomolecules and surfaces is important for the successful design of the next generation of biomaterials. This study combines the high surface sensitivity and chemical specificity of ToF-SIMS with the 'soft' ionization capabilities of MALDI. Model peptides with beta-sheet and alpha-helix structures were used in conjunction with MALDI matrix molecules [2,5-dihydroxybenzoic acid (DHB) and sinapinic acid (SA)] to facilitate generation of molecular ions with the SIMS Cs+ ion source. The positive ToF-SIMS spectra from the beta-sheet peptide incorporated into crystals of DHB show peaks representative of sodium-adduct ions of the peptide (M-H+Na+) (m/z 1096.7). The spectrum from the bulk beta-sheet contains only fragment ions and no molecular ions, suggesting that there is a synergistic effect in producing sodium adduct molecular ions when both Na and matrix molecules are present. When sodium is eliminated from the system, peaks that can be assigned to the M+ ion (m/z 1074.7) can still be detected. Molecular ions from the alpha-helix peptide were also detected when DHB was present. The use of SA as matrix failed to generate peptide molecular ions, suggesting the matrix-specific nature of this MALDI-SIMS technique. Imaging-SIMS indicated that the peptides are incorporated within the DHB matrix crystals, but are not fully incorporated into the SA crystals. This shows the importance of good mixing between the peptides and matrix molecules for detection of intact molecular ions.

4:40pm **BI+MC-MoA9 Molecular Orientation of Annealed Artificial Joint Polymers: Characterization by Soft X-ray Absorption**, *S. Sambasivan,* SUNY Stony Brook; ***D.A. Fischer***, National Institute of Standards and Technology; *M. Shen,* University of Maryland; *J.A. Tesk, S. Hsu,* National Institute of Standards and Technology

For the past 30 years ultra-high molecular weight polyethylene (UHMWPE) has remained the dominant polymer in artificial joints due to its outstanding wear resistance properties. It has been recognized that wear of UHMWPE contributes to the loosening of the implants and is the main cause for the failure of long-term implants. Hence, there is an urgent need to understand the mechanism and the surface morphology leading to wear and failure of the artificial joint. Hubbard et al.@footnote 1@ and Hastings et al.@footnote 2@ have demonstrated that the molding and annealing the UHMWPE at safe elevated temperatures resulted in increased mechanical strength. Also, cross-linking of UHMWPE has been demonstrated to reduce wear significantly. We have previously measured molecular orientation as a function of wear motion.@footnote 3@ Molecular orientation in biomaterials is thought to be critical in characterizing the precursors of wear and the production of debris during the wear process. While the link between molecular orientation and wear has not been clearly established, molecular orientation has been recognized as an important parameter in wear resistance. This study examines the change of molecular orientation caused by annealing UHMWPE. Our technique utilizes soft x-ray absorption spectroscopy at a synchrotron beamline to non-destructively characterize the molecular orientation of the UHMWPE surface layer. Current methods of inferring or deducing orientation are not accurate and often rely on staining and cutting specimens.@FootnoteText@ @footnote 1@Hubbard et al., Trans. 25th Soc. For Biomaterials, 325(1999). @footnote 2@Hastings et al., Trans. 25th Soc. For Biomaterials, 328(1999). @footnote 3@Fischer et al., Trans. 25th Soc. For Biomaterials, 351(1999).

5:00pm **BI+MC-MoA10 Titanium-Alginic Acid Chemistry of Adhesion Using X-ray Photoelectron Spectroscopy**, ***R.A. Brizzolara***, David Taylor Research Center, NSWC

The interfacial chemistry between alginic acid and a titanium surface has been examined using x-ray photoelectron spectroscopy (XPS). This study is motivated by the effort to mitigate effects of seawater biofouling on heat transfer surfaces via materials or surface modification strategies. Alginic acid is a predominant adhesive in bacterial biofilms, and titanium is a common material in naval ship cooling and piping systems. XPS has been used to quantify the alginic acid adsorbed to the titanium surface from aqueous solution. The experiments were performed at various solution pH's to examine the effect on alginic acid adsorption of changing the charge state of the ionizable groups on the alginic acid and of the titanium surface. The effects of ions in the solution were investigated by performing the alginic acid adsorption in the presence of calcium chloride. To separate the effects of the carboxyl and hydroxyl moieties present in alginic acid, XPS has also been used to measure caproic acid (carboxyl) and glucose (hydroxyl) adsorption as a function of solution pH. High-resolution XPS spectra have been utilized to separate the various carbon and oxygen chemistries present, and angle-resolved XPS spectra and advancing contact angle measurements were used to elucidate molecular orientation effects. Atomic force microscope (AFM) images were obtained to determine adsorbate morphology and surface coverage. These data will be interpreted in light of potential alginic acid - titanium adsorption mechanisms such as hydrogen bonding and anion exchange. This information regarding the biofilm-surface chemical interaction will be useful in developing fouling resistant surfaces. The NSWC Carderock Division In-House Laboratory Independent Research Program supported this work.

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