Tuesday Afternoon, October 30, 2012

Biomaterial Interfaces
Room: 23 - Session BI+AS-TuA

Characterization of Biointerfaces

Moderator: L. Meagher, CSIRO Materials Science and Engineering, Australia

2:00pm **BI+AS-TuA1 Surface Characterization Meets Cells and Proteins**, ***B.D. Ratner***, University of Washington **INVITED**

Surfaces such as Ni(100) and Si(100) have been extensively studied and each has been found to be more complicated than simple geometric models would suggest. In this context, consider more mobile surfaces than these precisely defined crystal surfaces that are comprised of 20 amino acids integrated into hundreds of different proteins. Also, these surfaces may contain lipids and complex saccharide structures. It should be apparent that these surfaces can be staggeringly complex, and yet, as with surfaces in general, they efficiently catalyze complex reactions. But they do this at room temperature and atmospheric pressure in a way that makes life possible. For these reasons, the ability to characterize such surfaces will certainly lead to advances in surface design and surface functionality. Tools taken from the "surface science tool chest" can be applied in special ways to complement the tools developed by biologists for molecularly characterizing such surfaces. This talk, primarily focused on electron spectroscopy for chemical analysis (ESCA) and secondary ion mass spectrometry (SIMS), will start with analysis of amino acids and peptides, move to adsorbed protein films and finally consider complex surfaces such as decellularized extracellular matrices and cell monolayers.

2:40pm **BI+AS-TuA3 Using Binary Solvent Mixtures Produces High Graft Density Poly (Ethylene Glycol) Layers**, *A.R. Arcot*, Aalto University, Finland, *S. Zhang*, *R.L. Meyer*, *R. Ogaki*, Aarhus University, Denmark, ***P. Kingshott***, Swinburne University of Technology, Australia

The success of PEG based non fouling surfaces depends on several factors such as graft density [1] and nature of head group-substrate interaction. [2] The 'grafting-to' technique though simple, often results in low pinning density when compared to 'grafting-from' technique. [3] This limitation of 'grafting-to' technique can be overcome by grafting under reduced solubility conditions. [4] We demonstrate a simple and versatile way to coat surfaces with PEG at high graft density using binary solvent mixtures, where a poor and a good PEG solvent are mixed with the PEG. The addition of poor solvent decreases the hydrodynamic radius of PEG molecules and hence results in thicker films due to diminished steric repulsion (Supplementary Fig 1a and 1b). The 'good' and 'poor' solvent pair was chosen based on solubility parameter distance calculated from Hanson solubility parameters. [5]

The PEG thiol films on gold formed from acetone-ethanol mixtures were analyzed using x-ray photoelectron spectroscopy (XPS), ellipsometry and atomic force microscopy. The PEG film gets thicker with more ethanol, which is a poorer solvent for PEG (Supplementary table 1). The high resolution sulfur 2p spectra confirmed the absence of precipitate particles. Grafting under high ionic strength conditions used by Kingshott et al. was used as reference for comparison. [4] The PEG thiol films were exposed to fetal bovine serum (FBS) and it was observed that thicker films could resist protein adsorption better than thin films that were formed from high solubility conditions. This method of using binary solvent mixtures can be extended to any polymer-substrate system by choosing appropriate 'good-poor' solvent pair. To demonstrate this point we also studied 5 kDa PEG silane films grafted using acetone-diethyl ether solvent mixture.

References:

1. L. D. Unsworth, H. Sheardown and J. L. Brash, Biomaterials (30), 5927-5933 (2005).

2. P. Kingshott, J. Wei, D. Bagge-Ravn, N. Gadegaard and L. Gram, Langmuir (17), 6912-6921 (2003).

3. N. Luo, J. B. Hutchison, K. S. Anseth and C. N. Bowman, Macromolecules (7), 2487-2493 (2002).

4. P. Kingshott, H. Thissen and H. J. Griesser, Biomaterials (9), 2043-2056 (2002).

5. B. A. Miller-Chou and J. L. Koenig, Progress in Polymer Science (8), 1223-1270 (2003).

3:00pm **BI+AS-TuA4 Adsorption Behavior of Serum Albumin on Nanocrystalline Apatites**, ***K. Fears***, *D. Burden*, *C. Love*, U.S. Naval Research Laboratory, *D. Day*, Missouri University of Science and Technology, *T. Clark*, U.S. Naval Research Laboratory

The adsorption behavior of bovine serum albumin (BSA) on nano-crystalline hydroxyapatite (HA) and strontium apatite (SrHA) microspheres, derived from borate glasses, was assessed using circular-dichroism spectroscopy (ECD). Numerous reports have shown that surfaces which present nano-sized features can exhibit better cellular response than surfaces with features in the micron regime. The microspheres were incubated in BSA solutions (40 mg/mL; ~64% helix; ~1% sheet) to determine if BSA adsorbed in a fundamentally different manner than on bioinert yttria-alumina-silicate (YAS) spheres that induced minimal conformational changes (~56% helix; ~4% sheet). On the apatite spheres, BSA loss a substantial amount of its helical structure and strained disulfide bonds were detected. However, the protein density on the SrHA spheres was 50% lower than on the HA spheres, indicating that BSA has a higher affinity for irreversible adsorption on HA. 5,5'-Dithio-bis-(2-nitrobenzoic acid), was used to selectively modify free thiols post-adsorption, indicating that solvent-accessible free cysteines were present on the apatite spheres, despite the absence of a reducing agent. Subsequent BSA molecules, or other proteins in vivo, could potentially form intermolecular disulfide bonds leading to increased adhesion of proteins or support the formation of macroscopic protein structures.

4:00pm **BI+AS-TuA7 Quantitative Characterization of Cells in Biofilms and on Surfaces**, *A.C. Areias*, *C. Sousa*, *G.P. Mendes*, University of Minho, Portugal, *P. Mack*, Thermo Fisher Scientific, UK, *S. Lanceros-Méndez*, University of Minho, Portugal, ***D.Y. Petrovykh***, International Iberian Nanotechnology Laboratory, Portugal

Films of cells on solid substrates are encountered in a variety of biological and biomedical environments, including cells in biofilms that spontaneously colonize medical devices and multilayers of cells filtered from suspensions for analysis. Understanding the chemical properties of cells in such films is important for providing clues about the behavior of the cells or about the effects of treatments that had been applied to the cells. Similarly to other types of surface-based systems, the characterization of cells on solid substrates poses several analytical challenges. In particular, the small number of cells on each sample, the interference from surface interactions, and the absorbance of the substrate material prevent the characterization of cells on surfaces by the standard optical methods that are used in solution. We show that protocols similar to those used for preparing samples for electron microscopy can be adapted to prepare biofilm samples for characterization by X-ray photoelectron spectroscopy (XPS). Modern XPS instruments also provide the functionality required for characterization of these complex samples, for example, sample charging on insulating substrates can be efficiently and consistently compensated. Finally, the Ar cluster ion beam technology that recently became available on XPS instruments provides additional capabilities for a more detailed characterization of cells in biofilms, which typically have thicknesses larger than the sampling depth of XPS. We characterized several types of fixed and dried cell samples, including biofilms and cells filtered from suspensions, to compare different preparation protocols and to identify qualitative and quantitative parameters that can be reliably obtained from XPS analysis of such films of cells. We will present the results of our comparative analysis and possible applications of our methodology for characterization of cells in biological and biomedical experiments.

4:20pm **BI+AS-TuA8 Antimicrobial Multilayers and Their Analysis by Laser Desorption Postionization Mass Spectrometry**, *M. Blaze*, *C. Bhardwaj*, *A. Akhmetov*, ***L. Hanley***, University of Illinois at Chicago

Bacterial biofilms are structured communities of microbes encapsulated within a self-developed polymeric matrix which adhere to surfaces and display genetic expression distinct from freely floating bacteria. Biofilms are frequently found to populate medical devices, leading to significant problems of infection in the first few days after implantation. Polyelectrolyte multilayers are developed for the delayed delivery of antibiotics to inhibit biofilm growth on biomedical devices [1]. Ten layers each of chitosan and alginate are prepared on a gold substrate, then infused with a novel antibiotic compound. This antibiotic-infused multilayer is found to inhibit the growth of *Enterococcus faecalis* bacterial biofilms on membranes over an 18 hour exposure. Laser desorption postionization mass spectrometry (LDPI-MS) is used to characterize the antibiotic after synthesis [2]. LDPI-MS analysis shows that the antibiotic survives sterilization of the multilayer surface, but <1% of the antibiotic remains after exposure to the biofilm.

[1] M. Blaze M.T., L.K. Takahashi, J. Zhou, M. Ahmed, G.L. Gasper, F.D. Pleticha, and L. Hanley, Anal. Chem. 83(2011) 4962.

[2] A. Akhmetov, J.F. Moore, G.L. Gasper, P.J. Koin, L. Hanley, J. Mass Spectrom. 45 (2010) 137.

4:40pm **BI+AS-TuA9 Combining Colloidal Probe Atomic Force and Reflection Interference Contrast Microscopy to Study the Mechanics of Biopolymer Films**, ***R.P. Richter***, CIC biomaGUNE, Spain; Joseph Fourier University, France; Max Planck Institute for Intelligent Systems, Germany, *S. Attili*, CIC biomaGUNE, Spain; Max Planck Institute for Intelligent Systems, Germany, *V. Borisov*, Institut Pluridisciplinaire de Recherche sur l'Environnement et les Materiaux, France

Highly solvated polymer films have naturally evolved as multifunctional interfaces in biological systems, e.g. as mucosal films, cellular coats or bacterial biofilms. Surface-confined polymer films are also becoming increasingly popular as biomaterials and in various (bio)technological applications. The mechanical response of such polymer films is not only important for functional performance, but it can also provide valuable information about the film's internal organization, interactions and dynamics.

Here, we present a method that combines colloidal probe atomic force microscopy (AFM) and reflection interference contrast microscopy (RICM) to measure the mechanical properties of thin and solvated polymer films. When analyzing such films, a fundamental problem in colloidal probe AFM experiments is to determine the distance at closest approach between the probe and the substrate on which the film is deposited. By combining AFM and RICM *in situ*, forces and absolute distances can be measured simultaneously, and experimental drifts that otherwise would pass unnoticed can be corrected (1).

We used the combined setup to quantify the compressive mechanics of films of end-grafted hyaluronan (HA brushes) (2). Hyaluronan is a polysaccharide that plays a vital role in the organization and function of pericellular coats and extracellular matrices in vertebrates, and that is also attractive for biomedical applications. We show that HA brushes can swell dramatically as a function of ionic strength or upon binding of the cartilage proteoglycan aggrecan. Detailed comparison of the experimental data with polymer theory reveals that hyaluronan is a prototype of a strongly charged, semiflexible polyelectrolyte with intrinsic excluded volume (3).

The novel combined AFM/RICM setup should be broadly applicable to quantify the mechanical properties of soft hydrated polymer films with precise control of probe-sample separation. The generated data on HA brushes represent a valuable reference for future quantitative studies of more complex HA-rich films and to refine theories of polyelectrolyte brushes of strongly charged and intrinsically stiff polyelectrolytes.

References:

(1) Attili and Richter *Langmuir* **2012,** 28:3206;

(2) Richter et al. *J. Am. Chem. Soc.* **2007,** 129:5306;

(3) Attili et al. *Biomacromolecules* **2012,** in press.

5:00pm **BI+AS-TuA10 Surface Modification of Silicone Hydrogels through Adsorption of Diblock Copolymers**, ***Y.J. Huo***, *S.S. Perry*, University of Florida

The interaction between an ethylene oxide-*block*-butylene oxide (EOBO) copolymer surfactant and the surfaces of four silicone hydrogel (SH) contact lenses—PureVision® (PV), O_2OPTIX® (O_2), ACUVUE® Oasys ® (AO), and Biofinity® (BF)—was investigated using angle-resolved X-ray photoelectron spectroscopy (AR-XPS) following treatment in test solutions containing various concentrations of EOBO. The nature of this interaction was further understood by quantifying the amount of eluted EOBO from each lens following the same treatment using ultra performance liquid chromatography (UPLC). The elution study revealed a large disparity in the amount of EOBO uptake by the different samples following each solution treatment. The XPS results, however, suggested that the amount of EOBO retained on the surface of the lenses demonstrated a largely different trend. For example, AO and BF displayed little evidence of signal at binding energies characteristic of the EO blocks, whereas O_2 and PV exhibited a clear EO signature. The correlation between the elution and XPS results highlights the difference in the interaction mechanism of the EOBO copolymer with different lenses. For lenses such as O_2OPTIX®, this interaction is predominantly bound to the surface; for ACUVUE® OASYS®, however, EOBO was uniformly distributed through the lens structure.

5:20pm **BI+AS-TuA11 Microfluidic Devices for High-Throughput Quantitation in Biology: From Biophysics to Diagnostics**, ***S. Maerkl***, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland **INVITED**

Microfluidic devices promise to have a significant impact on human health, particularly in diagnostics and drug development. We have developed a suite of microfluidic devices for high-throughput protein biochemistry and applied them to a wide range of applications spanning from protein biophysics to diagnostics, drug development, and vaccine development. Here I will discuss a novel approach to obtaining hundreds of kinetic rate measurements of bimolecular interactions on a single microfluidic device. In a second example I will present a generic microfluidic platform capable of quantitating biomarkers from a wide variety of samples in high-throughput and ultra-low cost, which could ultimately supersede the classical ELISA assay. We applied this platform to vaccine development by quantitating the activation of dendritic cells in response to a large panel of binary adjuvant combinations.

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