Tuesday Morning, October 16, 2007

Applied Surface Science

Room: 610 - Session AS+BI+NS-TuM

Surface Analysis and Related Methods for Biological Materials

Moderator: J. Soares, University of Illinois at Urbana-Champaign

8:00am **AS+BI+NS-TuM1 Nano-bio Chemical Image of Single Cells and Tissues for Bio-medical Applications**, ***D.W. Moon***, *T.G. Lee, J.Y. Lee*, Korea Research Institute of Standards and Science **INVITED**

Biochemical imaging of cells and tissues is a basic infra-technology in various bio-medical applications. Instead of conventional labeling methodology for biomolecular imaging with fluorescent dyes, label-free single cell and tissue biochemical imaging methodologies such as a nonlinear optical technique, coherent anti-Stokes Raman scattering (CARS) and an ion beam sputtering analysis technique, Secondary Ion Mass Spectrometry (SIMS) using cluster ion beams were developed. They were used to measure in a complementary manner 2D or 3D biochemical images of various cells and tissues such as Hella cells, adipogenic stem cells, fat liver tissues, cancer tissues, and skin tissues. Preliminary results will be discussed on the following issues. 1) Interactions of fibroblasts with native and denatured collagen thin films were studied with CARS and SIMS. It was extended to study the interactions of fibroblasts with 500 nm nano-fibers and 5 μm micro-fiber made of 40% poly (glycolic acid) (PGA) and 60% collagen. 2) Photoaging effects of skin by UV radiation were studied with SIMS, which showed significant changes in the biochemical imaging of amino acids representing collagen fibers and lipid molecules. 3) It was shown that SIMS imaging of colon cancer tissues has some potential to develop personalized cancer therapy with new drugs. Finally, the present status and future prospects of nano-bio technology based on laser, mass spectrometry, and nanoprobe for biochemical imaging of single cells and tissues at KRISS will be discussed for practical applications in bio, medical, and pharmaceutical researches.

8:40am **AS+BI+NS-TuM3 SIMS Imaging of Polymer Membranes and Single Cells**, ***G. Jiang***, *R. Michel, D.J. Responte, L. Mayorga, K. Greenland, T.N. Davis, T.A. Horbett, D.G. Castner*, University of Washington

The ability to obtain 3-D images of drug distributions in polymers can provide information about drug loading and release profiles. Likewise 3-D images of biological species (lipids, proteins, sugars, etc.) in cells can provide information about the distribution of those species within the cell. With the advent of C_{60} cluster ion beam sources, it is now possible to use time-of-flight secondary ion mass spectrometry (ToF-SIMS) to examine these important biological problems. This study used a dual beam approach (C_{60}^+ for sputtering and Bi_1^+ or Bi_3^+ for analysis) to generate 3-D images from drug (dipyridamole) loaded polyurethane (PEU) films cast onto glass and single cells (yeast and monocytes) adsorbed onto porous polycarbonate (PC) membranes. 3-D images were successfully obtained from all samples. For PEU films without the drug, the intensity of organic fragment ions from the PEU remained constant until the PEU/glass interface was reached, then decreased as the intensity of fragments from the glass increased. In the initial stages of sputter profiling drug loaded PEU films, the intensity of the drug peaks decreased while the intensity of the PEU fragments increased. Then intensities from both components remained relatively constant until the PEU/glass interface was reached. Molecular ions from the drug were readily detected throughout the entire PEU film. ToF-SIMS 2-D and 3-D images of single yeast (size ~ 5 microns) and monocyte (size ~ 10 microns) cells were obtained for cells adsorbed onto the surface of the PC membrane and within the pores of the PC membrane. Fragments from biological species from these cells (e.g., phospholipid at m/z = 184) could be detected in the ToF-SIMS images. These results indicate the possibility of 3-D chemical state mapping of single cells and other biomedical samples with the spatial resolution of a few microns.

9:00am **AS+BI+NS-TuM4 Surface Energy Control Within Copolymer Libraries Synthesised as Micro Arrays for Biological Screening**, *M. Taylor, A.J. Urquhart*, The University of Nottingham, UK, *D.G. Anderson, R. Langer*, Massachusetts Institute of Technology, *M.C. Davies*, ***M.R. Alexander***, The University of Nottingham, UK

There is currently much interest in polymer microarrays in the field of high-throughput materials development.[1,2] Although combinatorial material synthesis is relatively advanced, methods for characterising the surface chemical properties of such libraries are less well developed. We report on methods to characterise the surface chemistry and surface energy of 480 polymers on a microarray formed using on-slide copolymer synthesis. We used X-ray Photoelectron Spectrometry and Time of Flight Secondary Ion Mass Spectrometry to provide surface chemical information from each spot. Water and diiodomethane contact angle measurements were made from individually dosed picolitre volume droplets to estimate surface energy of each copolymer formulation.[3] Such arrays provide extra challenges for characterisation due to the large sample numbers, small sample size and increased data volume. Here, we will focus on the correlations determined between the monomer structures and the surface energy. The information XPS and SIMS can provide on the actual surface chemistry is presented and contrasted to the bulk surface chemistry. We highlight the ability to tune the surface energy using certain polymerised monomer combinations by varying their relative concentrations. This has great utility in controlling the biological response to polymer surfaces.

[1] J. A. Hubbell, Nature Biotechnology 2004, 22, 828.
[2] D. G. Anderson, S. Levenberg, R. Langer, Nature Biotechnology 2004, 22, 863.
[3] Taylor, M.; Urquhart, A. J.; Zelzer, M.; Davies, M. C.; Alexander, M. R., Picolitre water contact angle measurement on polymers. Langmuir Letters (2007, in press).

9:20am **AS+BI+NS-TuM5 Surface Characteristics of Listeria Monocytogenes Mutants with Variable Pathogenicity Levels**, ***N.I. Abu-Lail***, *B.-J. Park*, Washington State University

Despite being an important food-borne pathogen, L. monocytogenes in fact comprises a diversity of strains with varying virulence. Whilst many strains of L. monocytogenes have pathogenic potential and can result in disease and mortality, others have limited capability of establishing infections and relatively avirulent. Although very important, the question of how the composition of the bacterial surface and the properties of bacteria vary between strains that have different level of virulence at the molecular level needs to be answered. To answer this question, interaction forces between five different L. monocytogenes mutants that vary in their virulence and a model surface of silicon nitride were investigated using atomic force microscopy (AFM). Adhesion measurements between the strongest Listeria mutant and silicon nitride reveled that although both surface polysaccharides and surface proteins contributed significantly to the total adhesion, polysaccharides contribution (1.0 ± 0.2 nN) was larger than that of proteins' contribution(0.38 ± 0.1 nN). Adhesion forces were also dependent on the pH value of the solution, temperature, and media type. Experiments on intermediate virulence mutants and avirulent mutants are currently ongoing. Successful completion of these experiments will improve our understanding of the main molecular differences between virulent and avirulent strains of L. monocytogenes. Such findings would be very important, because it will allow for the first time and at a molecular level, to define a criteria that can distinguish virulent L. monocytogenes' strains from avirulent ones and therefore reduce unnecessary recalls of food products and help in preventing disease outbreaks.

9:40am **AS+BI+NS-TuM6 First Observation of Charge reduction and Desorption Kinetics of Multiply Protonated Peptides Soft Landed onto Self-assembled Monolayer Surfaces**, ***O. Hadjar***, *J.H. Futrell, J. Laskin*, Pacific Northwest National Laboratory

Soft-landing (SL) of hyperthermal ions onto semiconductive surfaces is a promising approach for highly-selective preparation of novel substrates using a beam of mass-selected ions. In addition, controlled deposition of complex ions onto surfaces presents a new approach for obtaining molecular level understanding of interactions of large molecules and ions with a variety of substrates relevant for biology and catalysis research. In this work we present a first study of the kinetics of charge reduction and desorption of peptide ions soft-landed onto a fluorinated self-assembled monolayer (FSAM) surface at hyperthermal energy (40 eV). An in situ 8 keV Cs^+ secondary ion mass spectrometry (SIMS) in a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer is used. Doubly protonated peptide ions are produced by electrospray ionization, mass-selected and transferred to the surface. The experiment allows the Cs^+ beam to merge with the peptide ion beam on the surface facilitating real time soft landing process monitoring. The surface is regularly probed using SIMS

during and after ion soft-landing. All peptide-related peaks in SIMS spectra show a gradual increase during the soft-landing. Rapid decay of the $[M+2H]^{2+}$ signal accompanied by increase of the $[M+H]^{+}$ signal is observed after soft-landing is stopped. The $[M+H]^{+}$ signal maximizes 2-3 hours after the end of the soft-landing and shows a relatively slow time decay at longer delay times. Several peptide fragments followed a very different kinetics behavior showing very slow, almost linear decay after soft-landing. We attribute this time signature to fragments that originate from neutral peptide molecules on the surface. Other peptide fragments show a mixed behavior suggesting that they are formed from different charge states of the soft-landed peptide ions. Our results demonstrate for the first time that various peptide-related peaks follow very different kinetics, signatures for doubly protonated, singly protonated and neutral peptides retained on the surface. The experimental results are in excellent agreement with a simple kinetic model that takes into account charge reduction and desorption of different species from the surface. The kinetic modeling allowed us to obtain for the first time desorption and charge exchange rate constants for different peptide species on the surface.

10:40am **AS+BI+NS-TuM9 Ultra Fast Mid Infrared Spectroscopic Imaging for Biomedical Applications**, ***J. Phillips***, *H. Amrania, J. Plumridge, M. Frogley*, Imperial College London, UK

We discuss the potential biomedical applications for a unique infrared spectroscopic micro-imaging system. A table top tuneable solid state laser has been coupled to a commercial infrared microscope to create a unique mid-IR imaging tool. By integrating with a modified high resolution infrared camera that has previously only been available to the military market, we have constructed a broadband imaging system capable of performing diffraction limited spatially resolved spectroscopy of biological specimens. The narrow line-width of the laser allows us to take spectra at a resolution of 20cm-1. A polymer film sample with a micron scale structure has also been imaged in reflective mode to resolve details down to 8 microns in size. We also discuss results from spectrally imaging cancerous cervical tissue samples. The high peak power of the laser (10MW) offers signal to noise levels previously unobtainable with stand-alone laboratory based commercial instruments. This coupled with a short pulse duration will for the first time enable time resolved imaging at a 100psec resolution.

11:00am **AS+BI+NS-TuM10 X-ray Spectromicroscopy and Ion Spectroscopy to Evaluate a Blend of Poly(L)lactic Acid and Fluorine End-capped Poly(L)lactic Acid**, ***D. Wells***, *J.A. Gardella*, University at Buffalo

Blending polymers is a versatile method for tuning the physical and chemical characteristics of a material such as strength, thermal stability, optical properties, and degradation rates. As the field of nanomaterials continues to grow it is essential to be able to evaluate the microstructure of polymeric materials as well as to characterize the chemistry that occurs at the interfaces of blended polymer films. Two techniques capable of such analysis are scanning transmission X-ray microscopy (STXM) and imaging time of flight secondary ion mass spectrometry (ToF-SIMS). STXM is a spectromicroscopy technique, that is, it combines both imaging and chemical spectral information. Recent advancements in cluster primary ion sources for ToF-SIMS have extended the range of its applications. The system of primary interest in this work is a blend of poly(L)lactic acid (PLLA) with fluorine end-capped poly(L)lactic acid (F-PLLA). This material has potential as a drug delivery device whose degradation could be controlled by changing the ratio of hydrophobic F-PLLA to hydrophilic PLLA. It is known that the fluorine containing component will preferentially surface segregate.[1] By reducing the concentration of F-PLLA we predict that we can create lateral surface segregation as well as vertical segregation. Both STXM and ToF-SIMS generate images containing chemical information and are useful to evaluate lateral phase segregation. Our intent is to use these two techniques as the primary means to evaluate the effects of changing the ratio of F-PLLA to that of pure PLLA.

[1]Won-Ki Lee, I. L., Joseph A. Gardella Jr., Synthesis and Surface Properties of Fluorocarbon End-Capped Biodegradable Polyesters. Macromolecules 2001, 34, (9), 3000-3006.

11:20am **AS+BI+NS-TuM11 Influence of Molecular Environment on ToF-SIMS Detection of Bio-Active Molecules on Self-Assembled Monolayers**, ***Z. Zhu***, Pacific Northwest National Laboratory

Bio-active molecules can be immobilized on solid substrates to form a monolayer or sub-monolayer. Because interactions between bio-active molecules are typically special, this structure is very useful in bio-recognition. So far, it has been widely used in bio-analysis or disease diagnosis. Alkanethiol self-assembled monolayer (SAM) on Au substrate is one type of commonly used solid substrate due to its versatile surface properties. During the last decade, time-of-flight secondary ion mass spectrometry (ToF-SIMS) has proven one of the most convenient techniques to detect sub-monolayer of organic molecules on alkanethiol SAMs. We have earlier described the possibility of quantitative detection of peptide molecules on COOH-terminated SAMs. However, we found that molecular environment greatly affect the signal intensity. For example, Au+ signal from -S(CH2)2(CF2)9CF3 film is much stronger than Au+ signal from S(CH2)11CO2H film. Therefore, quantitative comparison of the density of bio-active molecules on different SAMs by ToF-SIMS is difficult unless effect of molecular environment can be quantitatively considered. In this work, a number of bio-active molecules were deposited on -S(CH2)11CH3, -S(CH2)10OH, -S(CH2)10CO2H, and -S(CH2)2(CF2)9CF3 films with similar density, and ToF-SIMS measurements were made. Two major factors are found to affect SIMS signal intensity. Firstly, electron-attraction organic functional groups are found to enhance positive ion signals but depress negative ion signals. For example, positive ion signals are enhanced on -S(CH2)2(CF2)9CF3 film but negative ion signals are depressed. In addition, active H-atoms such as those from COOH groups are able to enhance signal of positive molecular ions since they are normally protonated.

11:40am **AS+BI+NS-TuM12 Advances in Organic Depth Profiling Using Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) under Optimized Ion Beam Conditions**, *H.-G. Cramer, T. Grehl*, ION-TOF GmbH, Germany, *N. Havercroft*, ION-TOF USA Inc., *F. Kollmer, R. Moellers,* ***E. Niehuis***, *D. Rading*, ION-TOF GmbH, Germany

Depth profiling of inorganic materials has been one of the most important applications of SIMS in general, and more recently also of TOF-SIMS. In contrast, depth profiling of organic materials has always suffered from the fact that high-mass molecular information, to a large extent, is rapidly lost under high-dose sputtering conditions. With the advent of cluster ion beams, however, more and more examples of successful organic depth profiling have been presented, such as C_{60} profiling of PMMA, PLA, etc. On the other hand, it also became obvious that the projectiles and conditions commonly used were not successful for profiling of every organic material analyzed. In this paper we used the so-called dual beam mode of depth profiling to start a systematic investigation of organic depth profiling with a TOF-SIMS instrument. Similar to the case of inorganic profiling, we found the dual beam mode beneficial because sample erosion and the sample analysis are decoupled and can be independently optimized. We applied different primary projectiles, such as C_{60}, Bi_n cluster ions, O_2 and Cs with a wide range of impact energies to a variety of organic specimens. The results will be discussed with respect to the specificity of the detected ions, their yields, the damaged and removed sample volume per primary ion, and classical figures of merit such as depth resolution.

12:00pm **AS+BI+NS-TuM13 Fragment Free Mass Spectrometry for Bio-Molecular Surfaces with Size Selected Cluster SIMS**, ***J. Matsuo***, *S. Ninomiya, K. Ichiki, Y. Nakata, T. Aoki, T. Seki*, Kyoto University, Japan

Polyatomic and cluster ions have been utilized for bio-molecular analysis as the primary ion beam for SIMS. Enhancement of sputtering and secondary ion yields, and the capability for depth profiling of bio-materials have been reported for cluster ions, and are due to the effects of multiple collisions and high-density energy deposition of such ions on solid surfaces. In bio-molecular analysis, not only molecular ions, but also fragment ('daughter') ions are usually observed in the mass spectra, and this makes interpretation of the spectrum difficult. Therefore, reducing fragment ions is very important especially for practical applications. These phenomena strongly depend on cluster size, which is a unique parameter, and one of the fundamental questions is what size of cluster ion is most appropriate for bio-SIMS. To date there have been very few studies on the effect of size on secondary ion emission from bio-molecules. We have examined the size dependence of the secondary ion emission from amino acid, sugar and small peptide films with large cluster ion (N>100) by using the double deflection technique. When the total energy of the cluster ion is fixed, the secondary ion emission (SI) yield of molecular ions increases with size due to the non-linear effect. However, when the cluster size is too large, the SI yield is gradually diminished, because the energy per atom becomes too low to emit secondary ions. The maximum molecular SI yield from amino acid film was obtained for Ar clusters with the size of a few hundred at the energy of 20keV. The ratio of fragment ions to molecular ions was also measured as a function of cluster size. The ratio decreases quite rapidly with increasing the cluster size. When the cluster size was larger than 1000, very few fragment ions were observed in the mass spectrum. In this case, each incident Ar atom has kinetic energy of a few eV, which is comparable to the bonding energy of peptides. Ultra-low energy SIMS can be realized by using large cluster ions. The size effect in secondary ion emission and damage cross-section will be discussed.

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