Biomaterial Interfaces
Room 2014 - Session BI+AS-ThA

Biomolecule-Surface Characterization I
Moderator: J.S. Shumaker-Parry, University of Utah

2:00pm **BI+AS-ThA1 Nanostructured Titanium Surfaces for Bone Biotemplating Applications**, ***K.C. Popat***, *T. Desai,* University of California, San Francisco

A major goal in orthopaedic biomaterials research is to design implant surfaces which will enhance osseointegration in vivo. Several micro as well as nanoscale architectures have been shown to significantly affect the functionality of bone cell i.e. osteoblasts. In this work nanotubular titania surfaces fabricated by a simple anodization process were used as templates for culturing osteoblasts. The size of nanotubes can be controlled by varying the voltage and the time of anodization. Marrow stromal cells were isolated from rat and were seeded on nanotubular titania surfaces along with control surfaces. Cell adhesion, proliferation and viability were investigated for up to 7 days of initial culture culture. The cells were then provided with differentiation media to induce matrix production. The alkaline phosphatase activity and matrix production were quantified using a colorimetric assay and X-ray photoelectron spectroscopy (XPS) for up to 4 weeks of culture (3 weeks after providing differentiation media). Further, scanning electron microscopy (SEM) was used to investigate osteoblast morphology on these nanotubular surfaces.

2:20pm **BI+AS-ThA2 Nonlinear Optical Studies of Transmembrane Polypeptide Incorporation into Supported Bilayer Membranes**, ***D. Levy***, *K.A. Briggman,* National Institute of Standards and Technology

The structure and organization of proteins in biological membranes play critical roles in cellular functions related to recognition and signal transduction. Supported bilayer membranes (SBMs) have been developed as model membrane systems for the characterization of biomolecular interactions at cell surfaces. In the present study, the nonlinear interface-specific technique of vibrational sum-frequency spectroscopy (VSFS) is used to characterize the thermal phase transition for both single and binary component lipid layers in SBMs. The incorporation of alpha-helical transmembrane polypeptides into SBMs has also been characterized by VSFS and infrared spectroscopy to determine the insertion kinetics, structure and orientation of the polypeptides into the various SBMs.

2:40pm **BI+AS-ThA3 Analysis of In-Vitro Biomineralization Processes Quantitatively by Quartz Crystal Microbalance (QCM)- and Transmission Electron Microscopic (TEM)- Explorations**, ***U. Plate***, *Ch. Mentrup, H.J. Hoehling,* Universitaet Muenster, Germany

The primary crystallites of different developing hard tissues describe an apatitic structure with crystal lattice fluctuations representing an intermediate state between amorphous and fully crystalline. Some non-collagenous proteins (NCPs) bound immobilized at the surface of collagen type I are implicated in the initiation and regulation of crystal formation and growth. In the investigations we have induced synthetic biomineralization processes. Collagenous matrices were reconstituted in-vitro and Phosvitin, a Phosphoprotein from an eggshell, were used (concentration 6 mg/ml in 0,3M Na@sub 2@CO@sub 3@). Phosvitin was cross-linked to collagen type I fibrils (concentration 0,25 mg/ml in 0,05M HAc) with Divinylsulfon (DVS). Dynamic in-vitro biomineralization processes at this matrices, pure collagen and Collagen-DVS-Phosvitin, were induced by contacting their surfaces with defined inorganic Ca- and PO@sub 4@-solutions. Qualitative and quantitative measurements were achieved in an in-vitro model for Quartz Crystal Microbalance (QCM)- and Transmission Electron Microscopic (TEM)- explorations. To locate the organic matrix for QCM- and TEM- measurements statistically, the Au-surfaces on the quartz- and TEM-grids were functionalised with thiols containing chain-length carboxylic-acid groups, in the experiences 10 µmM carboxylic-acid thiols with a mercaptopropyl group. This carboxylic-acid groups form self assembling monolayers (SAMs) and are utilized for the modification of an Au-surface to introduce carboxylic groups on it. Then the carboxylic groups are converted to amines of biomaterials. To induce biomineralisation processes 2,2 mM CaCl@sub 2@- and 1,3 mM K@sub 2@HPO@sub 4@-solutions were pumped along the matrices in defined time intervals with a peristaltic pump. The experiments were carried out in a chamber under native conditions (T = 37°C, pump velocity of the Ca-phosphate solutions of 1,62 ml/min, comparable to the fluid flux of blood in capillaries.).

3:00pm **BI+AS-ThA4 Study of the Interfacial Water Structure on Sulfobetaine-Terminated Thiolate Self-Assembled Monolayers**, ***M.J. Stein***, *B.D. Ratner,* University of Washington

The foreign body response to prosthetic devices limits the extended use of virtually all medical implants and biosensors. Non-specific protein adsorption is believed to be a key determinant of this response. To circumvent or control these reactions, our initial study utilized a zwitterionic sulfobetaine thiol, structurally similar to taurine (HS(CH@sub 2@)@sub 11@N(CH@sub 3@)@sub 2@@super +@CH@sub 2@CH@sub 2@CH@sub 2@SO@sub 3@@super -@), and diluted it with hydrophobic and hydrophilic thiols to determine whether the nonfouling ability of the sulfobetaine self-assembled monolayers (SAMs) could be enhanced by either an improved packing of its bulky headgroup or through an increase in the internal hydrophilicity of the thiol monolayer. In our current study, we hypothesized that the diluted groups that were previously shown to be the most nonfouling would exhibit more structured water (~3200 cm@super -1@) versus free water (~3400 cm@super -1@)) . For this study, attenuated total reflectance (ATR) was utilized to characterize changes in the water peak signal intensity through a full time-series of dilutions at multiple temperatures. Initial results have shown that a trend is present that mirrors the earlier protein adsorption results and that this trend follows a time-dependant pattern.

3:20pm **BI+AS-ThA5 Multi-Technique Characterization of Lipid/PEG Interactions and Oligonucleotide Microarrays**, ***H.J. Griesser***, University of South Australia, Australia*; K. Vasilev, B. Thierry, K. Bremmell, S. Griesser, P.-C. Nguyen,* University of South Australia*; P. Hale, P. Pigram,* LaTrobe University, Australia **INVITED**

This contribution will discuss two recent studies utilizing multi-technique characterization of surfaces by both vacuum spectroscopic methods and in contact with aqueous solutions. The first study aimed to investigate why PEG graft surfaces have produced excellent protein resistance in vitro but disappointing outcomes in vivo. Our hypothesis was that a possible reason involves attractive interfacial interactions with lipids that then provide a platform for subsequent protein adsorption. Using three different proein-resistant PEG coatings it was indeed found that two of them gave measurable lipid adsorption. Using lipid molecules that were neutral, positively charged, or negatively charged, and aqueous media of various ionic strengths, we explored the possible role of electrostatic interactions. Interaction force measurements using the AFM colloid probe method showed purely repulsive steric forces on approach, but on retraction adhesive forces were observed in some cases. A key issue is to differentiate between interfacial forces that emanate from the substrate and 'shine through' the PEG graft layers, and forces associated with the PEG layer itself. The second study involves the fabrication of micro-patterned surfaces and their use for oligonucleotide and protein microarrays. Using a mask with circular holes we plasma polymerize arrays of dots consisting of thin layers of plasma polymers that carry reactive groups (aldehyde, amine, or epoxy) suitable for covalent immobilization of end-functionalized oligonucleotides or proteins. XPS imaging using a Kratos Ultra unit with a DLD clearly showed the arrays of dots on perfluoropolymer substrate and the immobilization of biomolecules. An IonTOF ToF-SIMS unit with a Bi3+ beam was used to analyze immobilized oligonucleotides. Clearly identifiable peaks were observed with masses up to 2,500 Da and higher, from fragments as large as containing five nucleotides. Different oligonucleotides could be distinguished by the distinct fragmentation patterns.

4:00pm **BI+AS-ThA7 Effects of Annealing and Sample Processing Methods on Surface Molecular Orientation of Ultra-high Molecular Weight Polyethylene**, ***S. Sambasivan***, *D.A. Fischer, M.C. Shen, J.A. Tesk, S.M. Hsu,* National Institute of Standards and Technology

Ultra-high molecular weight polyethylene (UHMWPE) has remained the dominant polymer in artificial joints due to its outstanding wear resistance properties. It has been have demonstrated in the past that the molding and annealing the ultra-high molecular weight polyethylene (UHMWPE) at a safe elevated temperature resulted in increased mechanical strength. Also, cross-linking of UHMWPE has been shown to reduce wear significantly. This novel study utilizes resonant absorption of linearly polarized soft x-rays at a synchrotron beamline to characterize the molecular orientation of the UHMWPE surface layer (top 10 nm) which is understood to be a precursor to wear. Carbon-K-edge x-ray absorption measurements were done on the UHMWPE samples, which were annealed in nitrogen atmosphere. Effects of annealing and cross-linking on the wear characteristics were also examined. It was found that the degree of orientation after annealing the

sample at 130°C in nitrogen, the average molecular orientation in UHMWPE decreased significantly (about 80% reduction) compared to the un-annealed UHMWPE. These studies show a promising new insight into how UHMWPE wears and will aid in the development of new materials for artificial joints. In addition to the annealing and cross-linking studies, it was observed that routine surface preparation methods such as molding, polishing and microtoming also induced surface molecular orientation to various degrees.

4:20pm **BI+AS-ThA8 NanoTribological Studies on the Mechanisms of O-Linked Glycosylated Proteins in the Boundary-Lubrication of Articular Cartilage**, *S. Zauscher, N.I. Abu-Lail,* ***D. Chang****, F. Guilak,* Duke University*; G. Jay,* Brown University

The diarthroidal (synovial) joints of the body enable locomotion and activity while withstanding millions of loading cycles, which may be several times body weight. Recent macroscopic tribological experiments and biochemical analyses suggest that heavily glycosylated proteoglycans, encoded by gene proteoglycan 4 (PRG4) and expressed by synoviocytes in synovial fluid as lubricin and by chondrocytes on the superficial zone of articular cartilage as surface zone protein (SZP), provide boundary lubrication in cartilage in the absence of interstitial fluid pressurization. We will present results from nanotribo-mechanical measurements on model surfaces and cartilage, combined with other surface specific physicochemical measurements that shed new light on the mechanisms by which lubricin/SZP provides lubrication and wear protection in diarthroidal joints. Our results suggest that the role of effective boundary lubricants in mediating friction in articular joints is largely one of wear protection of surface asperities, maintaining the surfaces in a nonadhesive mode, and causing shear dissipation in the biopolymeric boundary lubricant layer, even at the cost of attaining "high" coefficients of friction (COF ~ 0.15). Lubricin's ability to form intermolecular disulfide bonds appears to be critical for its ability to develop large steric repulsion forces. Our results contribute significantly to the understanding of the conformation and physico-chemical function of mucinous glycoproteins on biological interfaces.

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