Biomaterial Interfaces
Room: Hall 3 - Session BI-TuP

Biomaterial Interfaces Poster Session

BI-TuP1 Flash Networking Poster: Simple Method Toward Lignin Based Surface Coatings, ***Patrick Burch***, *P.B. Messersmith*, University of California at Berkeley

Lignin is the second most abundant biopolymer on earth. Millions of tons are produced annually as a byproduct of the paper industry but yet its use is limited to mostly low-value applications, such as concrete additives or fuel for paper mills. Significant research efforts are devoted towards the disruption of lignin into its monomers with the aim to create high-value applications. This task remains challenging due to the heterogeneity of lignin and the harsh conditions needed to degrade the covalent bonds linking the monomers together. Our approach instead takes advantage of this intrinsic stability of lignin by further polymerizing lignin building blocks on surfaces to form organic coatings. These coatings consequently alter the surface properties of these substrates in a simple, scalable, versatile and renewable fashion.

BI-TuP2 Flash Networking Poster: Characterising Hydrogel Chemistry Through Low Temperature ToF-SIMS, ***Michael Taylor***, *D. Scurr*, The University of Nottingham, UK, *M. Lutolf*, Ecole Polytechnique Fédérale de Lausanne (EPFL), Swtizerland, *L. Buttery*, *M. Zelzer*, *M.R. Alexander*, The University of Nottingham, UK

Over the last decade the beneficial properties of hydrogels as artificial cell culture supports have been extensively investigated[1]. Certain synthetic hydrogels have been proposed to be similar in composition and structure to the native extracellular matrix of the stem cell niche, their *in vivo* cell habitat, which is a powerful component in controlling stem cell fate[2]. The stem cell differentiation pathway taken is influenced by a number of factors. When culturing cells within or upon hydrogels this choice can be strongly dependent on the underlying 3D hydrogel chemistry which strongly influences hydrogel-cell interactions[3]. The interrelationship between hydrogel chemistry and that of biomolecules in controlling cellular response ideally requires analysis methods to characterise the chemistry without labels and often in 3D. Time-of-flight secondary ion mass spectrometry (ToF SIMS) has the potential to be utilised for through thickness characterisation of hydrogels. The frozen-hydrated sample format is well suited to minimise changes associated with dehydration or the chemical complexity of 'fixation', a challenging aspect in vacuum analysis conditions[4]. Frost formation can occur in the ambient atmosphere preventing ready depth profiling of the frozen hydrogels. We develop a simple method to remove this frost by blowing with gas prior to entry into the instrument which is shown to produce remarkably good profiles on a poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel film where a model protein, lysozyme, is incorporated to demonstrated how biomolecule distribution within hydrogels can be determined. A comparison of lysozyme incorporation is made between the situation where the protein is present in the polymer dip coating solution and lysozyme is a component of the incubation medium. It is shown that protonated water clusters $H(H_2O)_n^+$ where n=5-11 that are indicative of ice are detected through the entire thickness of the pHEMA and the lysozyme distribution through the pHEMA hydrogel films can be determined using the intensity of characteristic fragment secondary ions. Early stage data from more complex gel systems will be presented to determine the limitation of this approach.

BI-TuP3 Flash Networking Poster: Molecular-Level Insights into the Wet Adhesion Mechanisms of the Lady Beetle (*Cocinella septempunctata*), ***James Fowler***, Oregon State University, *J. Franz*, Max Planck Institute for Polymer Research, Germany, *S. Gorb*, University of Kiel, Germany, *T. Weidner*, Max Planck Institute for Polymer Research, Germany, *J.E. Baio*, Oregon State University

Humans have always marveled at the ability of insects to cling and climb along virtually any surface – whether it's vertically up a wall or upside-down supporting masses orders of magnitude greater than their own. Many insects have adapted to a range of environmental surfaces by evolving a wet adhesion process that combines an expansive array of hairy contacts on their feet, known as setae, and an adhesive fluid that forms contact between the setae and a substrate. Previous studies of this adhesion mechanism have focused almost exclusively on the mechanical and kinematic aspects of adhesion, and not on the molecular interactions at the fluid – substrate interface. In the work presented here, we probe the molecular interactions between the adhesive fluid taken from lady beetles (*Cocinella septempunctata*) on two model substrates (deuterated-PMMA and deuterated-polystyrene) with vibrational sum frequency generation (SFG) spectroscopy and scanning electron microscopy (SEM). High-resolution SEM images of individual seta-fluid footprints on both sets of surfaces indicate localized water-oil emulsion de-wetting with no sign of distinct patterning within the footprint. SFG spectra collected at the C-H stretching region (2800-3100 cm^{-1}) contain peaks at 2848 cm^{-1} and 2867cm^{-1}, characteristic of symmetric CH_2 and CH_3 stretches, respectively. For the fluid on both the PMMA and polystyrene, we observe a large ratio of the 2867/2848 cm^{-1} peaks suggesting a well-ordered hydrocarbon monolayer with methyl groups oriented normal to the substrate. Spectra at the amide I stretching region (1500-1800 cm^{-1}) collected from the PMMA-fluid sample contain a single peak at 1700 cm^{-1} indicating the presence of ordered free fatty acids; however, this signal is absent from the polystyrene-fluid spectra. Combined, this set of SFG spectra demonstrate that during adhesion to a polar surface, fatty acids within the fluid form a highly ordered layer at the substrate surface. While on a non-polar surface, the mechanism changes and some other hydrocarbon species present within the fluid orders at the interface.

BI-TuP5 Flash Networking Poster: Structured Noble Metal Nanosurfaces for Biosensing and Bioanalysis (1): Controlling Galvanic Displacement Reaction for Creation of Silver Nanostructures, ***Shingo Yoneda***, Toyo University, Japan, *T. Okamoto*, RIKEN, Japan, *H. Vieker*, *A. Beyer*, *A. Gölzhäuser*, Bielefeld University, Germany, *H. Takei*, Toyo University, Japan

There has been an increasing interest in creating silver nanostructures for optical analytical techniques such as surface-enhanced Raman spectroscopy. Galvanic displacement reaction, exploiting the difference in ionization tendency of different metals, can be used for such purposes, but previously there was not much room for controlling the final morphology of nanostructures when bulk metal was simply treated with silver nitrate solution. However, recently it was reported that use of copper colloids rather than bulk copper allows finer structural controls. We have also found that surface-adsorbed copper structures can be transformed to silver nanostructures with various morphologies. The size and amount of copper were crucial for the morphology of the final structures. Recently we have also begun experimenting with addition of polyvinylpyrrolidone (PVP) to the silver nitrate solution because PVP was known to affect the morphology of the silver nanostructures with the conventional galvanic displacement reaction using bulk metal. Here we describe its results.

Our base metal nanoparticles are formed by evaporation of a base metal on surface-adsorbed monodisperse SiO_2 nanospheres. Variable parameters are the metal deposition thickness and the nanosphere diameter. We treated these base metal nanoparticles with an aqueous silver nitrate solution containing PVP. In this experiment, we investigated effects of changing the concentration of PVP, the molecular weight of PVP (K15, K30, K90), and the deposition thickness of copper (10, 30, 60 nm). For evaluation of the structures, we observed their morphologies with SEM and obtained Raman spectra of 1 mM rhodamine 6G, R6G.

Use of PVP leads to silver nanostructures with different morphologies. When PVP is used, massive silver nanostructures are formed. When not, however, silver nano-filaments are formed instead. It was also confirmed that the form of silver nanostructres is dependent on the molecular weight of PVP used and the deposition thickness of the original base metal. When these nanostructures are used as a substrate for SERS, the enhancing effect becomes greater when prepared with PVP.

BI-TuP7 Preparation of Bioglass-ZrO_2 Functionalized Coatings by EPD, ***Ana Arizmendi-Morquecho***, *M.A. Aguilera-Bustos*, Centro de Investigación en Materiales Avanzados, Mexico, *A.C. Chávez-Valdez*, Consultant, *M.S.D. Sánchez-Domínguez*, *M.S.V. Sánchez-Vázquez*, Centro de Investigación en Materiales Avanzados, Mexico

Surface functionalization is an important tool for many technical and biomedical applications. The modification of a nanoparticle surface can alter its adhesion characteristics, improve the dispersion in matrices or enhance catalytic properties. In this work, strategies for surface functionalization of inorganic materials with a special focus on zirconia (ZrO_2) synthetized nanoparticles as reinforcement for bioglass ceramic matrix are discussed. The density of functionalization in nanoparticles using different organosilanes molecules was measured by Si–O and hydroxyl (–OH) bonds. The reactivity between organosilanes and the surface of nanoparticles was measured by electrostatic potential using molecular simulations with Gaussian software. This simulation showed the regions from silane molecules with the lowest electrostatic potential representing the sites were the metallic oxides are attached. The morphology of nanoparticles and functionalization layer was characterized using TEM-EDS, FTIR and TGA. Due to the low stability of bioglass in organic

suspensions, the modification of its surface was necessary using functionalized nanoparticles which allowed the preparation of Bioglass-ZrO_2 reinforced composites. Suspensions were prepared using ethanol as dispersing media. Bioglass suspensions showed poor stability, while suspensions with Bioglass and functionalized nanoparticles showed improved stability. The formation of bonds between bioglass particles and functionalized ZrO_2 nanoparticles allowed the dispersion of the material which is necessary for the preparation of coatings by Electrophoretic Deposition (EPD). The Bioglass and Bioglass-ZrO_2 coatings were characterized by SEM-EDS and XRD. A uniform coating was obtained with well dispersed ZrO_2 nanoparticles. These coatings using functionalized ZrO_2 as reinforcement can improve the mechanical properties as well as the biocompatibility of the composite.

BI-TuP9 Flash Networking Poster: Gold Nanoparticle-Delivered RNA Genetic Control Devices, ***Michael Newton***, *J.M. Carothers, D.G. Castner*, University of Washington

Gold nanoparticles (AuNPs) promise to offer a minimally toxic, easily modifiable, and high payload carrying drug or biologic delivery agent. Despite this promise and the emphasis placed on their intrinsically high surface area to volume ratio, surface functionalized AuNP conjugates in biomedical applications often lack detailed surface characterization. Consequently, there is little experimental validation of a surface attached ligand's orientation or conformation. Nucleic acids are one such ligand and biologic diagnostic or therapeutic agent currently being investigated. RNA-based logic circuits responsive to small molecules, endogenous proteins, and miRNAs have been demonstrated to diagnose the state of a cell and implement a programmed therapeutic outcome. A truncated RNA circuit such as this could benefit from local targeting and concentrated delivery on a nanoparticle platform and prove more effective. Proper tools and techniques to characterize biomedical AuNP conjugates would inform their design and result in better understanding the observed cellular uptake, efficacy, toxicity, and clearance.

Short single-stranded DNA oligo's on AuNPs have been used to sense a complementary nucleic acid, or as a capture strand to facilitate assembly of larger constructs. Additionally it is often recognized that the method of attachment and incorporation of spacers will play a factor in the assembled conjugate's performance. A thiol attachment to Au is often used with a polynucleotide spacer for simple assembly and increased performance. These investigations typically neglect to consider other types of spacers like ethylene glycol chains of various lengths. We will compare the polynucleotide and ethylene glycol spacers used individually and jointly in a single-stranded DNA capture oligo conjugated AuNP in terms of particle surface characterization and nucleic acid functionality. This will be realized through X-ray Photoelectron Spectroscopy and Localized Surface Plasmon Resonance in conjunction with standard bulk characterizations like Dynamic Light Scattering, Electrophoresis, and Transmission Electron Microscopy, and through incorporation of strand-displacement and RNA aptamer nucleic acid devices with distinct programmed fluorescent outputs from a specific small molecule or oligo input.

BI-TuP11 Flash Networking Poster: Exhaled Breath Analysis of Ammonia Gas using Colorimetric Attenuated Total Reflectance Spectroscopy, ***MariaAntoaneta Bratescu***, *K. Isawa*, Nagoya University, Japan, *T. Kiguchi*, Shibaura Institute of Technology, Japan, *O.L. Li, N. Saito*, Nagoya University, Japan

The identification of exhaled breath volatile organic compounds represents a metabolic biosignature with the potential to recognize some diseases. There are various techniques to analyze exhaled breath gases, as spectrometry, gas chromatography, and spectroscopy. The recent trend towards breath analysis instruments has led to the development of fully integrated prototypes of point-of-care devices.

In this research we develop a miniature sensor using attenuated total reflectance spectroscopy to detect breath gases in the range of hundreds ppb. A 0.2 mm thick, 20 mm wide, 65 mm long, fused silica plate with 60° beveled edges (Shin-Etsu Quartz Products, Inc.) was used as the internal reflection element (IRE).[1] The IRE surface was coated with the chemical sensor specific to one of the gases from the breath, embedded in a polymer, using a layer-by-layer electrostatic assembly method. The evanescent light produced by multiple total internal reflections penetrates a few tens of nanometers in the film leading to a high detection sensitivity.

For ammonia gas detection Poly(diallyldimethylammonium chloride) (PDDA, Mw: 200000-350000, 20 wt% in H_2O) and tetrakis(4-sulfophenyl)porphyrin (TSPP, Mw: 934.99) were used as polymer and chemical sensor, respectively.[2,3] The detection consists in measuring the decrease of light intensity of the Soret band of the porphyrin molecule around 483 nm under the chemical reaction with ammonia. First we recorded absorption spectra through the IRE using a broadband visible light source and a spectrograph for different film conditions and ammonia concentrations. Then the broadband light source and the spectrograph were replaced with a 450 nm light emitting diode (LED) and a photodiode (PD). The ammonia sensing capability of the porphyrin film was tested in both liquid and gas phases and a 500 ppb detection limit was found. We studied the ammonia gas detection limit in dependence with the number of layers of the chemical sensor and the polymer deposited on the IRE surface. AFM measurements show the surface modifications after the ammonia gas adsorption on the chemical sensor film. Stability, reversibility, and the humidity influence will be discussed.

[1]M. A. Bratescu, et al., Attenuated total reflectance spectroscopy of coumarin organosilane molecules adsorbed on a fused silica surface, Applied Surface Science, 257, 1792, 2010.

[2]K. S. Suslick, et al., An optoelectronic nose for the detection of toxic gases, Nature Chemistry, 1, 562, 2009.

[3]S. Korposh, et al., Sensor and Materials, 21, 179, 2009.

BI-TuP12 Flash Networking Poster: Structured Noble Metal Nanosurfaces for Biosensing and Bioanalysis (3): Surface-Enhanced Fluorescence Detection with Cap-shaped Silver Nanoparticles, ***Miki Ebisawa***, *T. Kawakami, H. Takei*, Toyo University, Japan

OBJECTIVE

Surface-enhanced fluorescence, SEF, is a highly sensitive method for detecting fluorescent molecules using a noble metal structure. We used cap-shaped silver nanoparticles that were fabricated by vacuum deposition of silver onto a dense layer of silica nanoparticles immobilized on a glass slide. While there is much in common with surface-enhanced Raman spectroscopy, SEF requires that molecules be placed at a certain distance away from the metal surface in order to avoid quenching. To satisfy this requirement, we have evaluated the effect of introducing a dielectric layer on the substrate surface. Furthermore we have investigated a number of techniques for patterning different antibodies or DNA fragments as we are targeting immunoassay and DNA diagnosis for the application of SEF, up to several dozens of different target molecules at the same time.

METHODS

Cap-shaped silver nanoparticles prepared with the above method were soaked in a silane coupling agent (tetraethylorthosilicate, TEOS) to form a dielectric layer. With DNA measurements, we used a probe DNA (19 mer) and FITC-labeled target DNA (13 mer). After immobilization of the probe DNA, the target DNA was made to hybridize. We examined two methods for patterning. One way was to prepare polymer fibers at whose end silver nanoparticles were formed. Fibers modified with different capture molecules were bundled together to be used for measurements. As a second method, after a substrate surface was uniformly modified by a single type of capture molecule, the molecule was locally destroyed: a "subtractive" method. For destruction, ozone treatment or additional deposition of silver was used.

RESULTS

From results by a microarray scanner, we confirmed that the signal intensity of the fluorescent modified DNA from a silver nanoparticle substrate with a dielectric layer was more than doubled compared with the control substrate, demonstrating the effect of a dielectric layer. As for the ozone cleaning, the treatment of the substrate enhanced the signal intensity if applied prior to adsorption of the target molecules; if afterward, the same treatment was found to diminish the signal, as we expected. However, the effect of additional silver deposition was unexpected. We assumed that the signal would be diminished by additional deposition several nm in thickness, but on the contrary the signal was enhanced by additional deposition, the signal intensity being maximized when the thickness was 150 nm. In the future, we will examine this phenomenon in greater detail, with a hope of obtaining a fundamental insight into the mechanism of SEF.

BI-TuP13 Flash Networking Poster: Structured Noble Metal Nanosurfaces for Biosensing and Bioanalysis (2): Localized Surface Plasmon Resonance Sensor Operating in the Near-IR Regime, ***Takumi Miyashita***, *N. Bessho*, Toyo University, Japan, *T. Okamoto*, Riken, Japan, *H. Vieker, A. Beyer, A. Gölzhäuser*, Bielefeld University, Germany, *H. Takei*, Toyo University, Japan

Noble metal nanoparticles possess an optical extinction peak due to localized surface plasmon resonance, LSPR. The peak shifts when the refractive index in the immediate vicinity changes, a useful property for making biosensors. While there are numerous ways to prepare noble metal nanostructures, we find that evaporating a noble metal on a surface coated with a monolayer of monodisperse silica nanospheres is a convenient method. With the above method, it is easy to form highly dense nanostructures, with the extinction peak in excess of O.D. 2. The spectrum can be readily modified by varying the sphere diameter and metal deposition thickness. While this method has been used by a number of groups, we found that resulting structures possess not only a peak in the

visible but also an additional peak in the near-IR. The latter peak has four times the sensitivity of the peak in the visible.

There are many ways to use LSPR sensors other than the traditional antigen-antibody monitoring. One has to do with improving a colorimetric diagnostic method. Often it is implemented in the form of immunochromatographic assays, ICA. It is, however, used mostly only for qualitative assays, such as detection of flu. We believe that bestowing it with a quantitative detection capability as well as an increased dynamic range would further boast the use of ICAs. In some colorimetric assays, an enzyme is used to generate a colored product. By using the alkaline phosphatase-NBT/BCIP system, here we describe various techniques for maximizing the interaction between the enzymatic reaction product and the sensor surface. Another application is detection of nanoscopic gas bubbles. If bubbles form on the sensor surface, even with the size of 5 nm or less, it should lead to a detectable shift of the peak. We have decided to test this concept on detection of useful microorganisms from soil samples obtained below the ocean bed where very few are expected. Our strategy is to look for catalase which is secreted by practically every life form. When catalase, captured on the sensor, reacts with H_2O_2, oxygen bubbles form so that the presence of microorganisms can be potentially detected by monitoring bubble formation. Another set of experiment involves monitoring of vascular endothelial cells in the presence of elevated amounts of glucose. We have cultivated VE cells on our LSPR sensor. It is our intention to use the sensor signal for monitoring changes in the morphology of cells induced by glucose, leading to a better understanding of diabetes.

BI-TuP14 Flash Networking Poster: Exploration of Conformational Changes of Nucleic Acids as a Function of Interactions with Histone-mimic Nanoparticles using All-atom Simulations, ***Yaroslava Yingling***, *J.A. Nash*, North Carolina State University

Nucleic acid based nanotechnology and gene therapy approaches depend on the compaction, or packaging, of the nucleic acids DNA and RNA. Though there has been much experimental work on the interactions of DNA with proteins, the atomic details of DNA-nanoparticle binding remain to be comprehensively elucidated. Even less is known about the binding of double stranded RNA with cationic molecules. Here, we report the results of a comprehensive large scale all-atom molecular dynamics simulation investigation of the binding ligand-functionalized gold nanoparticles (NPs) binding to the nucleic acids double stranded DNA and RNA as a function of NP charge and solution salt concentration. Our simulations show that low charge NPs bind to DNA and cause little distortion of the DNA helix, however, nanoparticles with charges of +30 or higher cause DNA to bend and wrap in a way similar to nucleosome. Moreover, shape of the NP ligand corona plays an essential role in quality of DNA wrapping. The nanoparticles cause different behavior with short segments of RNA in that they are not able to induce bending for even the most highly charged nanoparticles in 0.1M NaCl. To compact RNA, a combination of highly charged nanoparticles with low salt concentration is required. Results from this paper can be used for future design of efficient NP vectors for gene delivery and other biomimetic materials.

BI-TuP17 Flash Networking Poster: Nanoscale Structures in Live Cells Visualized through High Resolution Imaging and Mechanical Property Mapping, ***Bede Pittenger***, Bruker, *H. Schillers*, Univ. Muenster, *A. Slade, J. Shaw, S. Hu, I. Medalsy, T. Mueller*, Bruker

Nanoscale structures on or just beneath the cell surface often strongly influence cell function. Atomic Force Microscopy allows measurement of both topography and mechanical properties of these structures on live cells at resolutions far below the diffraction limit. When integrated and synchronized with optical microscopy (including fluorescence, confocal microscopy, and super-resolution imaging) AFM provides new methods of studying the relationship between cell structure and function in near-physiological conditions.

One class of these nanoscale structures is the microvillus -- a structure commonly found on epithelial cells. Epithelial cell function is coupled to the density of microvilli and degradation can cause malabsorption and diarrhea [1]. Observing the both tiny and very flexible structures such as microvilli on the apical surface of a live cell has been very challenging because the native microvilli structures are displaced and deformed by the interaction with the AFM probe.

Another class of nanoscale structure within cells is the actin fibril. These structures make up the actin cytoskeleton and are thought to play an important role in many types of cancer [2]. AFM allows observation of the actin fibrils, their position and their stiffness. PeakForce Tapping (with PeakForce QNM) provides fast, high resolution, quantifiable maps of the distribution of the actin fibrils in the cytoskeleton.

In this talk we will present the first images of microvilli on the membrane surface of living kidney cells obtained by AFM. Because the data was collected with PeakForce Tapping, it is possible to compare the response of the microvilli to different applied forces, and observe the effect of force on microvilli structure. Finally, we will also present mechanical maps of live MDCK cells showing the distribution and stiffness of individual actin fibrils.

[1] E. Cutz, J.M. Rhoads, et al., N. Engl. J. of Med. 320, 646 (2009).

[2] M. F. Olson and E. Sahai, Clin. Exp. Metastasis 26, 273 (2009).

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