Biomaterial Interfaces
Room: Southwest Exhibit Hall - Session BI-ThP

Biomaterial Interfaces Poster Session

BI-ThP1 Temperature Gradient Device for Investigation of Cell Detachment from Thermoresponsive Surfaces, ***M.A. Cooperstein**, H.E. Canavan*, University of New Mexico

Poly(*N*-isopropyl acrylamide) (pNIPAM) undergoes a phase change in a physiologically relevant temperature range that leads to cell release. Above its lower critical solution temperature (LCST, ~32°C), pNIPAM is relatively hydrophobic, and when grafted to a surface, it takes on a packed conformation. There have been numerous studies on the conformation change of pNIPAM across its LCST. Although it is known how pNIPAM chains tethered to a substrate behave when the temperature is changed, no study has probed the influence of a temperature gradient on the behavior of cells attached to the polymer. In this work, we present the investigation of cell detachment from pNIPAM-grafted surfaces resting on a temperature-gradient device. The polymer was deposited on the surface using plasma polymerization. This deposition technique creates a conformal, sterile film that is compatible with any surface chemistry, including the transparent well plates required for these experiments. Prior to their use for cell culture, it is imperative to characterize the pNIPAM films for film thickness, surface chemistry, and thermoresponse, as the surface characteristics determine cell attachment and detachment. The characterization was performed via interferometry, X-ray photoelectron spectroscopy (XPS), and contact angle measurements. Using a device fabricated in our laboratory, we studied whether there is a gradual progression of cellular detachment from the polymer along the temperature gradient, or if there is an abrupt step-change in the detachment. This work will have valuable insights regarding the optimal temperature for cell detachment from pNIPAM.

BI-ThP2 Protein Resistant Oligo(ethylene glycol) Terminated Self-Assembled Monolayers of Thiols on Gold by Vapor Deposition in Vacuum, *L. Kankate,* University of Bielefeld, Germany*, H. Großmann,* Johann Wolfgang Goethe-University, Germany*, U. Werner,* University of Bielefeld, Germany*, R. Tampé,* Johann Wolfgang Goethe-University, Germany*, A. Turchanin,* ***A. Gölzhäuser***, University of Bielefeld, Germany

Protein resistant oligo(ethylene glycol) terminated (OEG) self-assembled monolayers (SAMs) of thiols on gold are commonly used for suppression of nonspecific protein adsorption in biology and biotechnology. The standard preparation for these SAMs is the solution method (SM) that involves immersion of the gold surface in an OEG solution. Here we present the preparation of 11-(mercaptoundecyl)-triethylene glycol ($HS(CH_2)_{11}(OCH_2CH_2)_3OH$) SAMs on gold surface by vapor deposition (VD) in vacuum. We compare the properties of SAMs prepared by VD and SM using X-ray photoelectron spectroscopy (XPS), polarisation modulation infrared reflection absorption spectroscopy (PM-IRRAS) and surface plasmon resonance (SPR) measurements. VD and SM SAMs exhibit similar packing density and show a similar resistance to the nonspecific adsorption of various proteins (bovine serum albumin, trypsin, and myoglobin) under physiological conditions. A very high sensitivity of the OEG SAMs to X-ray radiation is found, which allows tuning their protein resistance. These results show a new path to *in situ* engineering, analysis and patterning of protein resistant OEG SAMs by high vacuum and ultra high vacuum techniques.

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BI-ThP3 Spontaneous Micro-Fluidic Flow Driven by Marangoni Effect, ***Y.C. Hu**, Y.H. Lin, Y.C. Ou*, National Applied Research Laboratories, Taiwan, Republic of China

This article presents an innovative new type of micro-fluidic chips; the marangoni effect is used as the driving force of the fluidics, while the fluidics is confined in "Surface tension-confined micro-fluidics" chips for regularly transportation. "Surface tension-confined micro-fluidics" is an open structure micro-fluidic chip, there is no micro-channels, only hydrophobic and hydrophilic coating patterns on the chips to confined fluidic flow. Normally the fluidics is driven by surface tension force, since there is no micro-channels structure, there is no way to use conventional pumps. In this study we propose a initiative driving force source that can be applied in "Surface tension-confined micro-fluidics " chips；low surface energy surfactants (such as alcohol) after atomization is sprayed on local area of the fluidics so that the fluidics surface can render surface energy gradient, according to the marangoni effect liquids with higher surface energy will attract liquids from lower surface energy area and generate fluidic flow, while the fluidic flow is confined along the hydrophilic pattern channel, with carefully designed patterns one can control the fluidic flow to a designed location. The surfactants spraying nozzle can be moved to any position so that one can easily control the fluidic flow just by simply move the spraying nozzle along the pattern channel. If one want to separate the fluidic flow in to two parts, just move the spraying nozzle to the middle area of the fluidic flow, it will be cut into two parts. By reciprocating oscillate the spraying nozzle one can disturb the fluidics and enhance the mixing efficiency.

This innovative micro-fluidic chip has the following advantages: the process is simple, low cost, easy to integrate and it is easy to use.

BI-ThP4 Printable Biological Ink on Gelatin for Self Crosslinking Wound Dressings, ***M. Yanez**, T. Boland*, University of Texas at el Paso

The printable biological ink on gelatin is a biopolymer that could be use in a handheld, portable device, such as a ink jet of printer and become a substrate for advanced wound care. Skin is very affected by when burned, and also by diabetic venous ulcers. Currently, some skin substitutes exist that for treatment of diabetic foot ulcers, but these products are really expensive. Based on this we want to create a low cost wound care material that helps not only people with diabetic foot ulcers and venous leg ulcers, but also with burned skin. We selected sodium alginate because it is a FDA approved carbohydrate that has many applications for tissue regeneration and cell therapy, and it is very compatible with human body. Oxidation of sodium alginate has been investigated to obtained alginate dialdehyde (ADA). Alginate dialdehyde is a fast acting self-crosslinking degradable polymer, when applied over collagen or gelatin. We investigated, the degree of oxidation, the degree of cross-linking and mechanical properties of the materials. The degree of crosslinking was determined by trinitrobenzene sulphonic acid assay maintaining constant ADA concentration in borax or PBS buffer and varying concentration of gelatin in borax or PBS. An increase of the degree of oxidation of sodium alginate, an increase in the cross-linking and a decrease of the gelling time was observed with increasing ADA concentration. We investigated the effect of ADA concentration on viscosity and found that at concentrations of about 10% ADA, the solutions are viscous. We will present data on the use of ADA solution as an ink in a printing device and the effect of printed ADA to cross-link gelatin. In general, control over the concentrations of ADA as well as the spatial dispensing via printing should allow us to generate wound dressings of tunable properties. The use of a portable device makes this solution attractive to low resource settings. Future work will include testing the wound dressing in a small animal model and investigating the effect of adding keratinocytes as well as endothelial cells to the material on healing and wound contraction.

BI-ThP5 Carbohydrate-functionalized Surfaces: Analysis by a Multi-Method Approach, ***P.M. Dietrich**,* BAM Federal Institute for Materials Research and Testing, Germany*, T. Horlacher,* Max Planck Institute of Colloids and Interfaces, Germany*, T. Gross, T. Wirth,* BAM Federal Institute for Materials Research and Testing, Germany*, R. Castelli,* Max Planck Institute of Colloids and Interfaces, Germany*, A. Lippitz,* BAM Federal Institute for Materials Research and Testing, Germany*, P.H. Seeberger,* Max Planck Institute of Colloids and Interfaces, Germany*, W.E.S. Unger*, BAM Federal Institute for Materials Research and Testing, Germany

Carbohydrate microarrays formed by hundreds of different sugars, covalently or non-covalently bound, have enabled an emerging field of applications in the last decade, i.e. in diagnostics or high-throughput analysis.[1-5] Saccharide microarrays are valuable tools to investigate interactions with other molecules since many glycans are involved in fundamental biological processes.

Carbohydrate-based microarrays are commonly prepared by covalent attachment of chemically modified saccharides that bind selectively to a functionalized solid support such as gold, glass or polymers. Arrayed structures of spotted sugars can be printed with standard robotic microarray printers.

For future applications a reliable surface chemistry combined with an advanced surface analysis is required for improved microarray qualities and performances.

Herein, we report on the combined XPS, NEXAFS and ToF-SIMS surface analysis of carbohydrate functionalized gold and glass surfaces containing synthetic glycans. Advanced characterization of spot shape, size and chemical composition across a spot surface are provided by surface sensitive methods as ToF-SIMS and XPS, used in their imaging modes.

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BI-ThP6 Enzymeless Flow Injection Analysis of 2,4,6-Trichlorophenol Based on Preoxidation by Ammonium Cerium (IV) Nitrate, ***J.S. Wang***, National Applied Research Laboratories, Taiwan, Republic of China

Enzymeless flow injection analysis of 2,4,6-trichlorophenol (2,4,6-TCP) based on preoxidation by ammonium cerium (IV) nitrate is present in this work. A preoxidation scheme is applied for 2,4,6-TCP determination without any enzyme treatment. This preoxidation scheme can provide a determination method with low cot and non-conductive polymerization reaction of 2,4,6,-TCP. In proposed scheme, the 2,4,6-TCP is oxidized to 2,6-dichloro-1,4-benzoquinone by ammonium cerium (IV) nitrate and the 2,6-dichloro-1,4-benzoquinone can be detect at low reduction potential. The linear range of 2,4,6-TCP determination was 0.4 to 750 mM with correlation coefficient (R^2) 0.9999 and the estimated detection limit (S/N=3) was 40 nM which were demonstrated by flow injection analysis. Twenty consecutive successive detect of 100 mM 2,4,6-TCP showed the relative standard deviation was 1.56 %. Several 2,4,6-TCP structure-like compounds were studied as interferences including 2,4-dichlorophenol, 2-chlorophenol, phenol and 4-aminophenol. No obviously influences were observed. Two water samples which were collected from local farm and pool were adopted as analytical application. The recoveries of two water samples are 105.2 % and 107.5%, respectively. An easy operation and enzymeless treatment detection scheme of 2,4,6-TCP is illustrated at this work.

BI-ThP7 Evaluation of Electrochemical Impedance Spectroscopy with an ITO Culture Chip, ***S.-Y. Hsiao***, Instrument Technology Research Center, NARL, Taiwan, *Y.-S. Lin,* Hungkuang University, Taiwan, *Y.-P. Lu,* Instrument Technology Research Center, NARL, Taiwan, *C.-Y. Lin,* Taipei Medical University, Taiwan, *H.-S. Huang,* Instrument Technology Research Center, NARL, Taiwan, *H.-M. Huang,* Taipei Medical University, Taiwan, *D.-C. Chen,* National Yang-Ming University, Taiwan, *T.-S. Liao, J.-S. Kao*, Instrument Technology Research Center, NARL, Taiwan

An electrochemical impedance spectroscopic (EIS) instrument with indium-tin oxide (ITO) culture chip module and lock-in impedance readout module was developed. The lock-in impedance readout module achieves impedance measurements with a small, bio-harmless AC signal. Moreover, transparent ITO culture-chip module is experience friendly; other optical inspections can be applied on the chip. The impedance readout module is designed to perform in a range from 1 to 10 kHz; the phase measuring errors are within 5.1% in that range. Typical examples of PBS solutions, BSA proteins and cell culturing tests are discussed in experiments. Higher concentration levels of PBS produced lower impedance. Higher concentration level of BSA solution also produced lower impedance. Furthermore, experiments of 0.25% BSA dissolved in sDDW and 1X PBS show that the mediums influence system impedance. Moreover, the log phase (period of cell proliferation) of B16F10 cell culture tests ended at 9, 16, 23, and 65 h for seeded cell densities of 5×10^4, 1×10^5, 2×10^5, and 4×10^5/mL, respectively. Finally, cultures are incubated and doped to demonstrate the monitoring of cell proliferation.

An EIS using microelectrode arrays has gained much attention as a promising, label free, fast, and real-time method for cellular analysis. The electric cell substrate impedance sensing (ECIS; Applied BioPhysics) and real time cell electronic sensing (RT-CES; Roche Applied Sciences) systems are widely applied in measuring cell proliferation, attachment and spreading, motility, toxicology, barrier function, wounding, and migration. The sensing chips are opaque and the influence of solutions is generally disregarded. However, transparent chips for optically related detection are required in many applications. And, in fact, chemical additives for biochemical treatments induce impedance variations.

This study develops an EIS instrument with an ITO culture chip module and lock-in impedance readout module. The instrument can apply and extract bio-harmless small signals by lock-in technology. Experimental results prove impedance readout module performance. The ITO culture chip module electrodes are connected via the electrolyte medium and the route of cell/tissue. Typical examples of the effects of solutions and cell culturing tests are discussed. A higher level of PBS causes lower impedance. Further study of solution ingredients will improve the measurement accuracy and results analysis. The ESI instrument with an ITO culture chip module and lock-in impedance readout module can be applied in other conductive liquid sample investigations.

BI-ThP8 Molecular Interactions between Lubricin and Type II Collagen Surfaces: Adsorption, Adhesion, Steric Repulsion, and Boundary Lubrication, ***D.P. Chang***, *F. Guilak,* Duke University, *G.D. Jay,* Brown University / Rhode Island Hospital, *S. Zauscher*, Duke University

Although many studies have tried to elucidate the lubrication mechanisms that occur in articular cartilage, the molecular details of how constituents of the synovial fluid interact with cartilage surfaces and mediate cartilage to cartilage interaction still remain poorly understood. One of the major constituents of the synovial fluid that is thought to be responsible for boundary lubrication is the glycoprotein lubricin; however, details of the molecular mechanisms by which lubricin carries out its vital functions still remain largely unknown. Here, we examine i) the molecular details in which lubricin interacts with type II collagen, the main component of cartilage that provides structural integrity and tensile strength, ii) whether collagen structure can affect lubricin binding and change the adhesive interactions and boundary lubrication between collagen surfaces. We found that lubricin adsorbed strongly onto denatured, amorphous and fibrillar collagens surfaces. Furthermore, we found large repulsive interactions, between the collagen surfaces in presence of lubricin, increase with increasing lubricin concentration. Lubricin attenuated the large friction and also the long-ranged adhesion between the fibrillar collagen surfaces. Interestingly, lubricin mediated the frictional response between the denatured and native amorphous collagen surfaces equally, and showed no preference on the supramolecular architecture of collagen. We speculate that in mediating interactions at the cartilage surface, an important role of lubricin is to attach to the cartilage surface and provide a protective coating that maintains the contacting surfaces in a sterically repulsive state.

BI-ThP9 A Study of the Effect of Solvent-based Sterilization on the Reversible Adhesion of Biological Cells to a Thermoresponsive Surface, ***L.J. Pawlikowski***, *V.J. Eriacho, H.E. Canavan*, University of New Mexico

Poly(*N*-isopropyl acrylamide) (pNIPAM) is a thermoresponsive polymer that is widely used in bioengineering applications, including tissue culture engineering, single cell adhesion/detachment, and biofouling prevention. Although there are many ways to treat surfaces with pNIPAM, plasma polymerization is one of the more adaptable ways for coating surfaces for the use in tissue culture experiments. While plasma polymerization creates a sterile environment useful for tissue culture, occasionally, additional sterilization techniques must be used. Some sterilization methods include using UV light to sterilize the surfaces, and the use of different solvents, such as ethanol. To date, there have been no studies on the effect of these sterilization techniques on the reversible adhesion of biological cells on pNIPAM treated surfaces. In this work, we investigate the effect of different sterilization techniques (e.g., ethanol and UV light) on the thermoresponsive nature of pNIPAM. Substrates were coated using plasma polymerization (ppNIPAM), after which they were sterilized using solvents, and characterized to determine if the solvents changed the thermoresponsive nature of the polymer. X-ray photoelectron spectroscopy (XPS) and interferometry were used to determine the surface chemistry and thickness of our ppNIPAM surfaces. Goniometry was used to confirm the thermoresponsive nature of our surfaces. Finally, we tested the adhesion and detachment of cells on the surfaces using bovine aortic endothelial cells (BAECs). We found that the use solvents as sterilizing agents does have an effect on the thermoresponsive nature of pNIPAM (as demonstrated by the decreased detachment of cells from the surfaces), even when the pNIPAM film's chemistry appears unaffected.

BI-ThP10 Probing the Molecular Interactions at Bio-Inorganic and Bio-Organic Interfaces using X-ray Photoelectron Spectroscopy, ***R.A. Rincón***, *K. Artyushkova, D. Ivnitski,* University of New Mexico, *M. Eby,* Universal Technology Corporation, *H.R. Luckarift, G.R. Johnson,* Air Force Research Laboratory, *P. Atanassov*, University of New Mexico

Our recent work has made extensive use of X-ray photoelectron spectroscopy (XPS) in order to identify molecular interactions at a series of bio-inorganic and bio-organic interfaces.

In the first project, biologically-synthesized silica-enzyme and silica-peptide nanocomposite materials were analyzed by means of high resolution XPS. Biosilicification, i.e., rapid precipitation of silica mediated by a biological catalyst at ambient conditions, provides an efficient method for controlled synthesis of complex nanoscale structures. The process mimics reactions that organisms use to form rigid structures such as diatom exoskeletons and sponge spicules. The molecular interactions that allow these peptides to induce silica mineralization have not been elucidated. The focus of our study used the antimicrobial decapeptide KSL (KKVVFKVKFK), which induces rapid biosilica formation from pre-hydrolyzed tetramethyl orthosilicate in phosphate buffer. XPS was used to probe elemental composition and coordination chemistry of hybrid peptide-silica nanoparticle surfaces and ultimately identify the molecular interactions at the bio-inorganic interface.

In separate work, XPS was used to define interactions between biomolecules and various materials molecules used for bioelectrochemical architectures. One complex used the in vitro biosilicification process to help associate glucose oxidase (GOx) and carbon nanotubes (CNT) to yield a conductive composite matrix. Silica encapsulation of GOx provided an immobilization efficiency of ~25% in the presence of CNT. XPS analysis confirmed that the formation of a heterogeneous silica matrix had incorporated lysozyme, GOx and CNT. Another materials approach used layer-by layer assembly (LbL) to immobilize a redox enzyme. The LbL approach fixed bilirubin oxidase (BOD) on an electrode surface by using a glutaraldehyde as a "cross-linker" in combination with electrostatic interactions between the negatively charged enzyme and the positively charged polymer. The composite matrix was characterized using angle resolved XPS and multivariate analysis. The methods defined: the physical architecture of BOD layers immobilized on the electrode, the relative thickness of each assembled layer, along with their elemental and chemical composition.

In the third project we have studied poly-azines, which have been popular electrocatalysts of choice for NADH oxidation for sensors and biofuel cell applications using NAD-dependent enzymes. Little is known about their structure and mechanism of polymerization. Through detailed XPS analysis of monomers and polymers we were able to propose mechanism of electropolymerization and elucidate polyazine structure.

BI-ThP11 Polysomes Interaction with Self-Assembled Monolayers, *L. Marocchi, L. Lunelli,* Center for Materials and Microsystems - FBK, Italy, *G. Viero,* CiBio – University of Trento, Italy, *F. Piras,* Center for Materials and Microsystems - FBK, Italy, *N. Arseni, A. Provenzani,* CiBio – University of Trento, Italy, *C. Pederzolli,* Center for Materials and Microsystems - FBK, Italy, *A. Quattrone,* CiBio – University of Trento, Italy, ***M. Anderle***, Ict - Pat, Italy

The cellular machinery dedicated to the synthesis of the polypeptide chain translated from the mRNA template is the ribosome. Clusters of translating ribosomes held together by mRNA form the so-called polysome. The study of such supramolecular assemblies has interests both in structural and functional aspects as addressed in this study. As consequence the analyses of polysomal mRNA are emerging as good estimators in directly representing cell phenotypes. Since traditional methods of polysome purification are still a time consuming and laborious procedures, high throughput polysomal purifications are attracting growing interest. Recently, more modern approaches have been developed, based on the specific affinity between genetically modified ribosomes and functional surfaces. However, native ribosomes are required for several analytical applications. This work is a first step in developing a convenient and fast strategy to purify native polysomes thanks to their adhesion ability to appropriate substrates. We studied the interaction between functionalized gold surfaces and polysome fractions purified from human cultured cells or ribosomes derived from rabbit reticulocyte lysate. Surfaces endowed with different chemical properties were obtained using functional thiols able to form self assembled monolayers on gold substrates. Substrate surface properties were studied with XPS, ToF-SIMS and AFM. The interaction of rabbit ribosomes with the surfaces was assessed with AFM and confocal microscopy.

Despite different imaging approaches have been used to visualize bacterial and eukaryotic polysomes, human native polysomes have never been observed in physiological conditions. Moreover nothing is known about the formation of the mRNA-ribosomes macroassembly. Here we present the very first AFM images of native polysomes from mammalian cell lines adherent to surfaces. For the first time to our knowledge, we could observe ribosome aggregates associated to RNA, providing the imaging of polysome assemblies. Our imaging approach suggests the existence of a complex pattern of conformations and reveals novel levels of polysomal organization.

BI-ThP12 Adsorption of a Therapeutic Monoclonal Antibody on Surfaces Characterized by X-Ray Photoelectron Spectroscopy and Atomic Force Microscopy, ***K.L. Steffens***, *J.R. Wayment*, National Institute of Standards and Technology

As the number of FDA-approved monoclonal antibody therapeutics increases, the need to understand and control aggregation and surface adsorption of these therapeutics becomes more critical. Aggregation of protein therapeutics is a concern, because aggregates may lead to serious immunological responses in patients. Currently, the cause of protein aggregation is not clearly understood. Recent evidence from several studies has suggested that the interaction of proteins with surfaces may influence solution phase aggregation processes. Therefore, investigation of these effects is critical in order to reduce or eliminate aggregation in biopharmaceutical products. In this study, we investigate the interaction of Rituxan™, a therapeutic monoclonal antibody, with various surfaces including alkanethiol self-assembled monolayers on gold, polyethylene glycol on gold, gelatin on gold, and bare glass. Both as-prepared and heat-stressed (aggregated) protein solutions were investigated with size exclusion chromatography (SEC) before and after contact with the surfaces to measure the presence of small antibody aggregates (monomers to trimers). The presence of larger antibody aggregates formed in solution was assessed by performing AFM of protein solutions deposited and dried on mica. Protein adsorption on the various surfaces was measured using x-ray photoelectron spectroscopy (XPS) to probe the surfaces before and after exposure to the protein solutions. In addition, the standard overlayer model was used to estimate the thickness of adsorbed protein layers. Results show that adsorption of the protein is highly dependent on the surface character as well as on the presence of solution phase aggregates.

BI-ThP13 Univariable Synthetic Material for the Study of Cell Response to Substrate Rigidity, ***A.T. Leonard***, *J.R. Funston,* University of New Mexico, *K.N. Cicotte,* Sandia National Laboratories, *M.N. Rush, E.L. Hedberg-Dirk*, University of New Mexico

There is a strong indication that the mechanics of a substrate plays an important role in many cellular functions. Native human soft tissue has elastic modulus in the range of 0.01 to 1000 kPa. The synthetic materials commonly used to fabricate cell culture platforms with varied moduli, including polyacrylamide and polydimethylsiloxane, are limited in applicability due to a restricted range of achievable moduli and/or surface chemistry instabilities. The copolymer network of n-octyl methacrylate (nOM) and diethylene gycol dimethacrylate (DEGDMA) offers attractive material properties that overcome these limitations. In our laboratory, co-polymer networks were fabricated with 3 to 33 wt% DEGDMA. The compressive modulus was 25±2 kPA for the 3% DEGDMA network and increased with increasing DEGDMA fraction to 4700±300 kPa at 33 wt%. The networks demonstrated consistent surface wettability over the range of gel formulations examined as determined by goiniometry. Surface interrogation with x-ray photoelectric spectroscopy (XPS) at the two extremes of formulations, 3 and 33 wt.% DEGDMA, showed similar elemental and chemical bond compositions. The formulation 3% DEGDMA had elemental composition of 82.1±2.0 % Carbon 1s and 16.8±0.7 % Oxygen 1s. The higher DEGDMA composition of 33 wt% DEGDMA showed 83.2±0.8 % Carbon 1s, 15.4±0.2 % Oxygen 1s. High resolution carbon XPS indicated similar ratios of ether, ester and alkyl groups at the two extremes of DEGDMA compositions. The murine osteoblastic cell line MC-3T3 was used as a model for cell attachment and viability at six and 72 hours, respectfully. Scanning electron spectroscopy was used to visualize the long range nano and micro surface topology. Atomic force microscopy was used to map as well as quantify the surface roughness for each of the formulations. Our results indicate nOM/DEGDMA substrates can vary in modulus over three orders of magnitude while maintaining comparable chemical and topographical surface features. These networks are the first that allow for the study of the effects of material mechanics without the interference of other material properties.

BI-ThP14 In Vitro Cytotoxicity Studies of Antimicrobial Conjugated Polyelectrolytes, ***K.N. Wilde***, *L. Ding, Y. Wang, E. Ji, T.S. Corbitt, L.K. Ista, D.G. Whitten, H.E. Canavan*, University of New Mexico

An estimated 19,000 deaths and \$3-4 billion in health care costs per year in the U.S. are attributed to methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Infected individuals inevitably touch a wide variety of surfaces. Therefore, making these surfaces antimicrobial would reduce or prevent the spread of potentially untreatable strains of bacteria. Current sterilization and disinfection techniques tend to be temporary and surface-specific, and require constant vigilance on the part of medical and support staff. Certain conjugated polyelectrolytes (CPEs) with arylene ethynylene repeat unit structure have been demonstrated to exhibit dark and light-activated antimicrobial activity. Both in solution and anchored to a support, these polymers have been effective at killing Gram-negative bacteria, specifically *Pseudomonas aeruginosa* strain PAO1 and *Cobetia marina*. This light-activated antimicrobial activity enables their use in a wide range of potential applications. However, until recently, it was unknown if the CPEs would exhibit similar biocidal activity toward mammalian cells. In this study, bovine aortic endothelial cells were exposed to two different CPEs for increasing periods of time, from 10 minutes to 24 hours, in both light and dark conditions. The relative cytotixicity was then assessed using a live/dead fluorescence assay, and imaged via epi-fluorescence microscopy. While CPEs demonstrate biocidal activity toward *P. aeruginosa* strain PAO1 and *C. marina*, these polymers do not appear to be toxic toward mammalian cells when the cells are exposed to the polymers in both light and dark conditions. Further work is underway to evaluate cytotoxicity at concentrations above the micromolar concentrations tested to date, to correlate mammalian test conditions to bacterial test conditions, and to include an epithelial cell line. Because membrane disruption is a common mechanism of action for antimicrobial agents, molecular dynamic simulations will also be performed to model polymer insertion into a

hydrated zwitterionic phospholipid bilayer. These combined data are important to determine how to best incorporate CPEs into antibacterial household and health care products.

BI-ThP15 Aptamer-based Protein Recognition using CMOS Single-Photon Detector Arrays for Time-Resolved Analysis, *L. Pasquardini,* Center for Materials and Microsystems - FBK, Italy*, M. Benetti,* University of Trento, Italy*, L. Lunelli,* Center for Materials and Microsystems - FBK, Italy*, D. Iori,* University of Trento, Italy*, L. Pancheri, F. Borghetti, L. Gonzo,* Center for Materials and Microsystems - FBK, Italy*, G.F. Dalla Betta,* University of Trento, Italy*, D. Stoppa,* ***C. Pederzolli***, Center for Materials and Microsystems - FBK, Italy

There is a high demand for convenient methodologies for detecting and measuring the levels of specific proteins in biological samples because their detection, identification and quantification can be very complex, expensive and time consuming. Biosensors are interesting tools offering certain operational advantages over standard photometric methods, especially with respect to sensibility, duration, ease-of-use, cost, simplicity and portability. Regarding this research field we are developing a monolithic silicon biochip suitable for detecting traces of proteins in biological fluids that are not detected by conventional immunoassays. Particularly in this contribute we discuss the performances of SPAD (Single-Photon Avalanche Diode) detector arrays fabricated in CMOS technology utilized within a lab-on-chip system consisting of a micro-reactor (MR) array for bioaffinity assays based on fluorescence markers. In a typical fluorescence lifetime experiment a pulsed laser is used to excite the fluorescent dyes and the emitted light is revealed by means of high sensitivity detectors. The utilized SPAD detector module, having a total area of 600 x 900 µm2 per MR, can be arranged to build a small pixel array to be directly coupled to the MRs. No emission filters are needed, since the ultra-short laser pulse is cut off in the time domain. Every module consists of a 10x10-SPAD sub-array, where each SPAD cell is equipped with dedicated active quenching and recharging circuit. A memory has also been implemented in order to enable only low dark count rate (DCR) SPADs, so that a total DCR of about 100kHz can be achieved for the whole photosensitive area. Two time-windows have been implemented in this architecture, with a time width that can be set within the range 500ps-10ns with a resolution of 500ps.

The biofunctional layer is based on a dual-site binding strategy with aptamers, that are short single-stranded DNA folded into well-defined three-dimensional structures to form binding pockets and cleft for the specific recognition and tight binding of the molecular target. The biological model chosen for the present work is the protein thrombin (factor IIa). Two aptamer sequences able to bind to different sites of this protein are used. The first aptamer, anchored to substrate, specifically immobilizes the target protein to the sensor surface, while the second sequence, carrying a fluorescent molecule, allows target detection.

BI-ThP16 Adsorption of Beta-Helical Peptides, ***J.L. Kulp***, *K.P. Fears, T.D. Clark*, Naval Research Laboratory

This poster will describe surface adsorption of beta-helical scaffolds derived from peptides composed of alternating D- and L-amino acids. The monomeric and stable conformation of beta-helical peptides in solution provides a well-defined starting point for discriminating the changes in secondary structure of peptides induced by surface adsorption. NMR, CD, VCD, and XPS results will be presented.

BI-ThP17 Surface Characterization of Baked-on Siliconized Vials for Biopharmaceuticals, ***G. Torraca***, *A. Vance, P. Masatani, L. Wong, B. Eu, M. Pallitto, M. Ricci, Z.-Q. Wen*, Amgen, Inc.

Protein formulations have been found to interact with the glass container surface, resulting in protein adsorption, glass delamination, or glass dissolution. To mitigate surface interactions, alternative primary containers with chemically modified surfaces are actively investigated. One container coating technology that was studied utilized a method of coating the vial interior with a dilute emulsion of polydimethysiloxane (PDMS) and then baking the vials to form a durable layer. The baked-on layer is meant as an isolation layer that insulates the drug product from the glass surface to prevent glass degradation as well as product interactions. Baked-on siliconized vials from two primary container vendors were examined for changes in surface properties over a 6-mo stability timecourse. To better understand and compare representative samples, three analytical techniques were employed to study the interior surface of the vials. These were Polarized Light Microscopy, X-ray Photoelectron Spectroscopy (XPS) which is also referred to as Electron Spectroscopy for Chemical Analysis (ESCA) and Contact Angle Measurement. The baked-on PDMS layer of the vial container was determined to be incompatible with the formulations that were studied. The coating characteristics and the robustness of these coatings from each vendor are discussed.

BI-ThP18 Accurate Delivery and Detection of Single Biomolecules Using Sub-Attoliter Pipettes on a Confocal/AFM Microscope, ***Y. Hu***, *S. Tadayyon, R. Taylor, J. Dechene, P.R. Norton*, University of Western Ontario, Canada

The development of novel techniques to accurately deliver, manipulate, and detect single molecules is one of the most interesting but challenging subjects in modern biotechnology and molecular biology. Most of the recent studies are focused on electrochemical methods using glass micro/nano pipettes that are mechanically pulled and an electrical field used to move molecules through microchannels. However, "single-molecule delivery" has not yet been achieved.

We report on our recent studies of single molecule delivery using a sub-attoliter reservoir formed at the end of what is essentially an NSOM optical fiber, mounted on a combined AFM/confocal fluorescence microscope. The reservoir is formed by chemical etching and focused ion beam (FIB) milling and is then coated with Al to confine the light for fluorescence detection in an NSOM geometry, and for the expulsion of the solution containing the molecule of interest. The volume of the reservoir can be in the range of attoliters by control of the etching and/or milling conditions. Initial experimental results using fluorescein dye indicates that when the solution concentration is low enough, single molecule delivery is achievable. Further study on cell labeling is discussed.

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BI-ThP19 Exploring Critical Parameters in Biointerface Design using Plasma-Modified Polymers, ***S.H. North***, NUARI-Institute for Advanced Sciences Convergence*, E.H. Lock, J. Wojciechowski, C.R. Taitt, S.G. Walton*, U.S. Naval Research Laboratory

The development of new transducing substrate materials, such as polymers, has spurred a growing interest in assessing the influences of surface topography and chemistry of the abiotic layer on the bio-functionality of the biotic layer. However, it is a challenge to differentiate the contributions of surface roughness and surface chemistry to biointerface functionality, as most surface modification methods tend to alter *both* at the same time. In this work, we discuss a dry and wet chemistry approach to biointerface development on polymeric substrates aimed at minimizing changes in the surface morphology. Specifically, an NRL developed electron beam-generated plasma processing system was used to functionalize the surface of polystyrene microtitre plates, which were then silanized to covalently immobilize biomolecules. Electron beam plasmas are unique in that they deliver high flux of low energy (< 5 eV) ions to substrate surfaces enabling selective, chemical alteration of the top few nanometers of any material *without* significant morphological modification. Thus, reactive hydroxyl groups, that make the characteristically inert material amenable to silane chemistry, can be introduced while maintaining substrate morphology. Silanization is an easy, controllable, and conformal covalent immobilization method that supports a wide variety of bio-immobilization schemes. Biomolecules covalently immobilized using this combined dry-wet chemistry approach retained functionality and demonstrated attachment efficiency comparable (and in some cases superior) to specialized commercial microtitre plates. We have used a combination of complementary surface analytical techniques to evaluate the relationships between the physical and chemical properties of the treated polymer surfaces, with particular emphasis on attachment of a functional silane layer, which is a critical parameter for efficient covalent bio-immobilization. Based on our results, we conclude that the development of novel interface materials with superior transducing capabilities is contingent on the deeper understanding of the complex physicochemical interactions between substrate polymer and the chemical/biological components attached to it.

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