Biomaterials
Room: Exhibit Hall B2 - Session BI-WeP

Biointerfaces and Surfaces II

BI-WeP1 Bioactive Surfaces for Control of Stem Cell Differentiation, ***J. Kelly***, *D. Dahlborg, S. Svedhem, D. Sutherland,* Chalmers University of Technology, Sweden, *P. Eriksson,* Sahlgrenska University Hospital, Sweden, *J. Gold,* Chalmers University of Technology, Sweden

Tissue engineering of the peripheral and central nervous systems stands to make great progress if the regenerative potential of the recently isolated neural stem cells can be harnessed and directed by the use of synthetic materials and constructs. We have produced surfaces with bound stimulatory molecules (proteins and growth factors) to control neural progenitor cells from the hippocampus of adult rats (AHPs) which have the ability to regenerate the progenitor phenotype or differentiate down one of two possible lineages to become neuron- or glial-like cells. The stimulatory molecule of interest may be patterned on surfaces with high spatial resolution by microcontact printing with a stamp fabricated by casting polydimethyl siloxane on a master with a negative of the desired pattern. We have combined this initial biopatterning step with either adsorption of a second, co-active protein or with a supported lipid bilayer (SLB). SLBs are membrane-like thin films which form by fusion of lipid vesicles on SiO2 or glass surfaces. Unmodified SLBs are resistant to protein adsorption and cell adhesion thus offering the ability to pattern areas of adhesive and unadhesive character. Moreover by inclusion of modified lipids we functionalised a SLB with a neuroactive 19-mer IKVAV sequence derived from laminin, via a maleimide coupling. This surface promoted high levels of cell attachment and presented an otherwise non-perturbing background to cells and proteins. We have assessed the biological activity of several proteins for control of cell function and lineage, including laminin, ciliary neurotrophic factor (CNTF) and fibroblast growth factor (FGF-2) by adsorption or printing on glass surfaces. Laminin supported a mixed population of proliferative and differentiated cells. AHPs on CNTF differentiated to glial phenotype as shown by expression of glial fibrillary acidic protein while on FGF-2, cell proliferation was maintained without differentiation.

BI-WeP2 Oriented Immobilization of Anti-Human Chronic Gonadotropin for Biosensor Applications, ***L. Liu***, *S. Chen, S. Jiang*, University of Washington

The orientation of immobilized antibodies on solid surfaces, is important to performance of a biosensor. Our previous studies show that it is possible to control antibody orientation during physical adsorption via adjusting surface and solution properties. For biosensor applications, it is desirable to chemically immobilize antibody molecules on surfaces. Our previous studies further show that chemical linkers (e.g., NHS/EDC and GLU) used for chemical immobilization alter surface charges, thus antibody orientation. In this work, we present a new method, which combines the site-directed immobilization (via the conservative carbohydrate or histidine rich region located in the Fc portion of the IgG) and the charge-control methods, for the oriented immobilization of antibodies on self-assembled monolayers (SAMs)/Au(111). Monoclonal anti-hCG (human chronic gonadotropin) will be a model antibody studied in this work. Surface charge is adjusted by changing SAM terminal groups and solution pH values. Atomic force microscopy (AFM) is used to characterize adsorbed antibody molecules on surfaces. Low detection limit and saturated adsorbed amount in surface plasmon resonance (SPR) biosensors are determined in this work for various antibody immobilization methods. Results from this work show significant improvement over those based on conventional immobilization methods.

BI-WeP3 Protein Absorption in Engineered MEMS Test-beds, ***D. Henry***, *K. Lenghaus, U. Jalgaonkar, J. Dale, J.C. Henderson, J. Hickman,* Clemson University

We are studying the influence of surface modification, channel geometry, flow conditions, etc., on protein absorption. The information found from these experiments will help create new knowledge for developing biocompatible MEMS systems. To this end we have developed a test system that involves clamping two silicon wafers together that have half of a channel or device etched in them, which allows for separation and examination of the wafer surface after the enzyme flow experiments have been conducted. The enzyme absorption to the channels can thus be investigated by standard enzyme assays as well as surface analysis directly in the channel halves. The primary enzymes used in this study include alkaline phosphotase, glucose oxidase, and TAQ polymerase. We have engineered a working version of this system, however, during development of the clamping system a problem arose with fluid leaking over and out of the channels. We hypothesized this was because the surface of the silicon wafer is hydrophilic and the fluid was drawn between the wafers rather than just flowing through the channels. Our results will present the solution to this problem via hydrophobic surface modification on the interior face of the silicon wafers. We will also present results on the development of the system, our experiment to optimize it, its application to determine how the proteins bind in the channels, where they bind in the channels, and if there is a difference in binding between angled and straight channels.

BI-WeP4 Experimental and Modeling Results for Protein Interactions with MEMS Devices, ***F. Wang***, *J. Hickman, R.A. Latour,* Clemson University

Microelecromechanical systems (MEMS) for biomedical devices and applications generally are used to interact with simple and complex biological fluids. Undesirable biomolecular surface adsorption, which causes channel plugging, is a leading factor in the failure of such systems. We are attempting to characterize these interactions using a combination of experimental analysis and a computational fluid dynamics (CFD) model. The adsorption process includes transport of biomolecules onto the surface and the adsorptive reaction at the surface. We model the transport by Navier-Stokes equations, which accounts for the mass, momentum and energy conservation for the flow; and the adsorptive reaction is modeled by a modified Langmuir adsorption isotherm. These two parts are integrated by incorporating the surface adsorption kinetics into the transport equation as a boundary condition. A commercially available CFD code, CFD-ACE+, based on a finite volume method is employed as a starting point. BioOpter, a peripheral to this code, has been developed to extract kinetic parameters by minimizing the least-square difference between the simulation and experiment data. We will present preliminary optimization results for experiments in which a 60ng/mL Alkaline Phosphatase solution was passed through a polyethyletherketones(PEEK) modified capillary (65 microns diameter by 100 mm long) at flowrate of 0.1mL/hr which gave the adsorption/desorption parameters as: Ka=7.2 x 10^5/m-s and Kd=2.1^{-5}/s. An optimization error surface model was used to visualize the location of the optimal parameters.

BI-WeP5 Fabrication and Characterization of Regular Porous Polymer Films for Biomedical Devices, ***M. Tanaka***, *M. Takebayashi, K. Sato, M. Miyama, K. Nishikawa, J. Nishida, M. Shimomura*, Hokkaido University, Japan

Porous polymer matrices are widely used in biomedical applications such as tissue engineering and artificial organs. The present studies describe the fabrication and characterization of highly regular porous polymer films formed by simple casting technique. The micro porous films were fabricated from biodegradable polymer such as poly(ε-caprolactone), poly(lactic acid-co-glicolic acid), poly(3-hydroxybutylate) and poly(L-lactic acid). Various experimental factors affecting pore size and thickness of the film, solvent, cast volume and so on, were studied. The porous film shows a highly regular hexagonal arrangement of holes in a large area and can be easily peeled off from a glass substrate as a self-supported porous film. The pore size can be controlled in the range from 1 to 50μm by changing the evaporation time of the polymer solutions. The thickness of the film becomes thinner with decreasing the concentration. The pores were connected to each other in the porous film. The films with 1-5 g/L of concentration of the polymer had the penetrated structure. On the other hand, the un-penetrated structure was formed when the concentration of the polymer exceeded 5 g/L. The porous film with controlled pore size is used for cell separation and biomedical devices.

BI-WeP6 Patterned Construction of Three-dimensional Neuronal Networks Using Ink Jet Directed Layer-by-Layer Deposition, ***T. Xu***, *M. Das, J. Hickman, T. Boland*, Clemson University

The ability of building three-dimensional constructs for tissue engineering applications has many obvious advantages. In particular, the outgrowth and regeneration of neurons depend on a three dimensional matrix of growth factors. We present here the use of an inkjet-based system to generate three-dimensional patterns for directing neuron growth. Using a home built inkjet printing system, a mixture of collagen and poly-D-lysine was printed with a pre-designed pattern onto a glass surface that was pretreated with agarose. Primary rat E18 hippocampal neurons were cultured on the surface and allowed to attach to the pattern. After 24h incubation, a second layer of the mixture of collagen and poly-D-lysine was printed on the surface. The hippocampal neurons were again seeded on the surface and allowed to form

a second layer. This was achieved by a fixing and locking mechanism built into the printer. Communication between the different layers of the hippocampal neurons was tested and results of electrophysiological tests will be presented. The layer-by-layer approach proved successful in 3D network design and may have many other applications in tissue engineering applications.

BI-WeP7 A Novel Surface Chemistry Platform for Biochips and Bioanalytical Devices, ***M.J. Lochhead***, *S. Metzger*, Accelr8 Technology Corporation

Biochips, biosensors, and other advanced bioanalytical devices require exquisite control of biomolecular interactions with surfaces. Specificity, signal to noise ratios and detection limits of these systems are often limited by surface non-specific binding, particularly in protein-based applications. Inhibition of non-specific binding is thus a critical performance feature in the design of improved synthetic materials that contact and operate in biological fluids. While non-specific binding to surfaces is most often undesired, specific biomolecule, particle or cell binding at surfaces often is desired. The goal is to bind only one type of molecule, particle, or cell, and to do so in a manner that preserves its recognition activity and native structure. We have developed a suite of functionalized surface coatings - OptiChem - that demonstrate both low non-specific binding and robust specific biomolecule attachment. OptiChem coatings can be applied to virtually all materials commonly used in bio-analytical devices including glass, silicon, and several plastics. The coatings are organic films that combine a low binding matrix with functional groups that provide for covalent attachment or affinity binding. Reduction of non-specific binding and control of reactive group density translates into increased signal to noise ratios, thus improving upon conventional surface chemistries resulting in faster assay turnaround and lower consumption of valuable or rare samples. The capacity for coating various substrates, ready scale-up of convenient fabrication and low preparation costs make the surface chemistry ideal for many microarray applications.

BI-WeP8 New Diazonium-Functionalized Support for Fabrication of Protein Microarrays, ***Y. Wu***, *G.P. Lopez*, University of New Mexico

Microarray technologies have rapidly become a major trend in high-throughput functional genomic study since its birth at the early 1990s. Recent advances of this technology have been focused on high-throughput proteomic analysis. The difficulty in immobilizing proteins onto solid surfaces without denaturation has led to the search for new general methods for coupling proteins to solid substrates. To this end, a chemical process for covalently linking proteins onto an ordinary microscope slide in a manner that preserves the ability of the immobilized proteins to interact with other proteins has been studied. The method uses p-aminophenyl trimethoxysilane (ATMS) /diazotization chemistry that was previously developed for formation of DNA microarrays. Preliminary results showed that protein microarrays fabricated on ATMS/diazotized surfaces produced enhanced levels of protein-protein interaction, low background fluorescence and high selectivity. Orientation of the immobilized proteins on the surfaces was also studied. In addition, the antigen-antibody reaction data has been analyzed quantitatively and successfully correlated with solution concentrations. In general, this method allows binding of protein onto a solid substrate that can lead to considerable improvements in antibody-antigen interaction, stability of affixed biomolecules, and preferable protein orientation.

BI-WeP9 Multi-Electrode Arrays Surface Modification by Aligned Microcontact Printing, ***W. Wang***, *M. Poeta, J. Hickman, T. Boland*, Clemson University

Multi-electrode arrays are widely used for neuron stimulating and recording. However, such stimulating and recording efforts are most efficient when neurons are placed precisely on the electrode sites. Microcontact printing is a versatile method allowing spatially resolved surface modification and has hence been used frequently to provoke cell attachment and spreading onto engineered patterns. Microcontact printing may have applications in forming artificially designed neuronal networks in vitro. In the present study, we use microcontact printing to modify the surface of a multi-electrode array with the aim of attracting neurons to only grow on the electrode areas of the arrays. We will present several methods to fabricate stamp replicas of the electrode arrays, and to align the stamps and the multi-electrode arrays surface. We achieve this by using micromanipulators to adjust the stamp and multi-electrode arrays and patterning under a microscope. As a result, the deviation between the patterned position and the electrodes are less than a few micrometers. The usefulness of this method of creating cell arrays will be presented.

BI-WeP10 Temperature-Responsive Polymer Coatings by Plasma Polymerization and Applications for Protein and Cell Patterning, ***X. Cheng***, *Y. Wang, Y. Hanein, K. Bohringer, B.D. Ratner*, University of Washington

The thermo-responsive polymer, poly (N-isopropylacrylamide) (pNIPAM), is of great interest for research and industrial applications in separation, controlled release, tissue engineering, sensor technology, etc. In this study, RF-plasma deposition is used to create temperature responsive ppNIPAM (plasma polymerized NIPAM) coatings. Films with a good retention of the monomer side-chain functionality are produced using low power continuous plasma deposition. Protein adsorption on the coating is studied at below and above the lower critical solution temperature (31°C) using both 125I-proteins and SPR. Dramatic increases of fibrinogen, IgG and BSA adsorption (8 fold, 8 fold and 10 fold respectively) are demonstrated at 37°C on ppNIPAM films compared to the adsorption at room temperature. Proteins adsorbed at 37°C do not detach two hours after switching the incubation temperature to 23°C, in contrast to the reported reversible cell detachment from pNIPAM upon temperature drop. Antibodies adsorbed on ppNIPAM at 37°C remain functionally active, as demonstrated by SPR studies. The coating has been deposited on micro-heater arrays. The individual micro-heaters control the phase transition of the ppNIPAM directly on top of it. Spatially controlled protein adsorption on the array has been visualized through a fluorescent marker. Based on preferential adhesion of cells to certain proteins, cells can be patterned on the protein arrays and used for tissue engineering applications.

BI-WeP11 Silicone Transfer during Microcontact Printing, ***K. Glasmästar***, *J. Gold, A.-S. Andersson, D. Sutherland, B. Kasemo*, Chalmers University of Technology, Sweden

Microcontact printing, muCP, is a widely used technique for fast and low-cost micropatterning of large surface areas. Within the field of biointerfaces it is routinely used to directly pattern SAM's and proteins or indirectly control cell adhesion and growth. During microcontact printing a stamp of an elastomer, typically PDMS, inked with molecules of interest, is brought into contact with the substrate and then removed, leaving a pattern of the "ink" on the surface of the substrate. Several reports have indicated that PDMS can be transferred to the substrate under particular conditions. However, this issue has earned surprisingly little attention so far. We have systematically studied the transfer of PDMS to the substrate during muCP. XPS, ToF-SIMS and water condensation patterns were used to identify and measure the transfer. Stamps were cast from Sylgard 184 silicone elastomer (Dow Corning). Stamps were used without further treatment or after UV/ozone treatment and no external force was applied during stamping. Significant amounts of PDMS were transferred from non-treated stamps during muCP under the model conditions used. The XPS results showed that the transfer of PDMS onto both Ti and Au was significantly lowered by UV/ozone treatment of the stamp. ToF-SIMS of Au samples stamped with flat stamps confirmed the XPS results. However, the use of a patterned stamp (5 µm lines, 15 µm space) transferred more silicone to Au than a flat stamp, and UV/ozone treatment appeared to be less effective in reducing PDMS transfer. In this work we show that the UV/ozone treatment of PDMS stamps before printing lowers the amount of silicone transferred to the substrate. Oxygen plasma treatment of the stamp is likely to have the same effect. It is important to consider the potential for transfer of PDMS onto substrates when using muCP to pattern SAM's or biological molecules for biointerface applications.

BI-WeP12 X-Ray PhotoEmission Electron Microscopy of Micro-contact Printed Protein and Polymer Coated Surfaces, ***C. Morin***, *A.P. Hitchcock*, McMaster University, Canada, *D.G. Castner, B. Wickes*, University of Washington, *A. Scholl, A. Doran*, Lawrence Berkeley National Laboratory

Synthetic biomaterials are widely used in medical applications. However, their interaction with the body is mediated through passive adsorption of a disorganized adsorbed protein monolayer. Mis-recognition of this adsorbed disorganized protein layer by surrounding cells may lead to the classic foreign body reaction and device encapsulation.[1] Next generation biomaterials, or 'engineered biomaterials', are being designed in which the surface contains specific bio-recognition moieties which control the biological response of the host. Microcontact printing (µCP) is one such method which can deposit biological signalling agents with spatial resolution and fidelity.[2] It uses an elastomeric template to transfer protein molecules to a surface of interest. µCP is combined with thiol-Au self assembly to form patterns on surfaces. We are exploring the use of X-Ray photoemission electron microscopy[3] to monitor methods for preparing patterned functionalized biomaterial surfaces, and to investigate the specificity of the interaction of model surfaces with key proteins. To test the reliability of the surface patterning method, we use highly specific bio-recognition pairs, such as the biotinylated ferritin-streptavidin couple, to

probe the quality of the patterned surface. Such structures are then investigated with elemental (Fe 2p) and molecular (C1s and N1s) speciation using NEXAFS microscopy recorded at the ALS BL 7.3.1 X-PEEM. This work is supported by research and partnership grants from NSERC (Canada) and a Canada Research Chair (APH). NESAC/BIO (DGC) is supported by NIH grant RR-01296 from the National Center for Research Resources. ALS is supported by U.S. DoE under contract DE-FG02-89ER60858.

[1] Ratner, B.D., J. Molecular Recognition, 9 (1996) 617.
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[3] S. Anders, et al. Rev. Sci. Inst. 70 (1999) 3973.

BI-WeP13 X-ray PhotoEmission Electron Microscopy of Polymeric Thin Films, ***A.P. Hitchcock***, *C. Morin*, McMaster University, Canada, *S.G. Urquhart*, University of Saskatchewan, Canada

Patterned thin polymer films are of increasing importance in biomaterials, displays, electronic materials, etc. High spatial resolution, high chemical sensitivity analytical microscopy techniques are needed to optimize these systems. We are using X-ray Photoemission Electron Microscopy (X-PEEM) for chemical imaging of phase segregated polymer blends, patterned biomaterials for non-fouling and bio-passivatable applications, and interactions of test proteins with these surfaces. Optimization of X-PEEM has been optimized for insulating, radiation sensitive organic thin films. Polystyrene (PS) and poly(methyl methacrylate) (PMMA) are completely immiscible and thus PS/PMMA blends may be a suitable model for patterned biomaterial-protein interactions. Spun cast thin film blends of mono-disperse high (1 Mdalton) and low (100 Kdalton) PS and PMMA with bulk compositions from 66/33 w/w up to 10/90 w/w PS/PMMA have been studied by X-PEEM and atomic force microscopy (AFM).[1] C 1s X-PEEM shows that there is significant enrichment of PS at the surface relative to the bulk and that the PMMA-rich domains contain PS. AFM shows the latter is a consequence of incomplete phase segregation, which results in a bimodal distribution of PS domain sizes, with the PS signal in PMMA domains arising from very small PS domains at the surface. This contribution will report on alternative techniques to prepare fully surface segregated PS-PMMA blends, and the outcome of protein attachment studies to these surfaces. X-ray microscopy carried out at the Advanced Light Source (supported by DoE under contract DE-AC03-76SF00098) and the Synchrotron Radiation Centre (supported by NSF under award DMR-0084402). Research supported financially by NSERC (Canada) and the Canada Research Chair Program. We thank the PEEM-2 staff (A. Scholl, A. Doran) for assistance in these studies.

[1] C. Morin et al., J. Electron Spectroscopy 121 (2001) 203.

BI-WeP14 NEXAFS Characterization of Poly (Amino Acids) at the Carbon, Nitrogen and Oxygen Edges, ***N.T. Samuel***, University of Washington, *D.A. Fischer*, National Institute of Standards and Technology, *D.G. Castner*, University of Washington

Near edge X-ray absorption fine structure (NEXAFS) spectroscopy has established itself as a powerful tool to characterize small molecules at interfaces. Recent developments in instrumentation have made it possible to image polymers and other biological molecules at very high spatial resolution and chemical specificity. In addition, NEXAFS offers the possibility to probe orientation and order in biological molecules at interfaces. Poly(amino acids) represent an important system of model compounds, since amino acids are the building blocks of proteins and peptides. Previous NEXAFS studies were done at the carbon and oxygen edges of individual and di amino acids. However, nitrogen is present in the backbone and some side chains of amino acids. In the present study, sixteen poly-amino acids were spin-cast or deposited as thick films onto silicon substrates. The samples were characterized by x-ray photoelectron spectroscopy (XPS) to ensure that a uniform film was obtained and no contaminants were present. The carbon edge spectra of these samples agrees well with the earlier work on amino acids. The nitrogen edge spectra of the poly(amino acids) exhibits three characteristic peaks, one due to the amide π^* resonance and the other two due to C-N σ^* resonances. Also, a pre-edge feature was observed that was associated with x-ray beam induced sample degradation. Overall, these poly-amino acids captured the major resonances in peptide and protein NEXAFS spectra. The different information content of NEXAFS and XPS will also be highlighted. These results coupled with previous results from our group indicate that NEXAFS can be used extract information about the orientation and order of biological molecules at interfaces.

BI-WeP15 Nanoporous Aluminum Oxide as Support Material for Enzyme Biosensors, ***A. Heilmann***, *N. Teuscher*, Fraunhofer-Institute for Mechanics of Materials, Germany, *D. Janasek, U. Spohn*, Martin-Luther-University, Germany

Enzyme sensors are of growing interest as detection tool in various clinical and food analysis. Up to now, limited operational stability of enzyme is even yet the main hindrance to their wider application to solve analytical problems. In the paper, we describe a novel method to create biosensors with good long-time stability by using nanoporous alumina oxide with well-defined pore sturcture as host material for enzyme immobilisation. The nanoporous aluminum oxide was made by anodic oxidation od alumnium in polyprotic acids. Free-standig membranes were realized by lifting the membrane film from the metal substrate. In this free-standig membranes, different enzymes were immobilized. e.g. pyruvate oxidase (PyrOD) from lactobacillus plantarum was enclosed in poly(carbamoylsulphonate) hydrogel and sucked into the porous structure before polymerization. In the paper, calibration curves and long-time stability of varous enzyme sensors were disccused, also by consideration the nanostructure of the filled alumina pores studied by scanning electron microscopy.

BI-WeP16 Formation of Biotin-reactive Silane Surfaces on SiO_2 for Specific Immobilization of Biomolecules, ***H.H.J. Persson***, *L.A. Ruiz-Taylor, D.A. Quincy, S. Follonier, J.K.C. Huang, T.L. Martin, A. Acharya, G. Kilcher, K. Belghiti, H.B. Lu, R.L. Cicero, P. Kernen, P. Wagner*, Zyomyx, Inc.

To provide robust and versatile surfaces for protein biochip applications, we have synthesized triethoxy silanes with thiol-reactive groups and report here on the formation of thin films on silicon oxide surfaces. Several surface analytical techniques such as contact angle, XPS, and ellipsometer have been applied to characterize such silane layers. Emphasis was given to layer thickness, wettability, molecular organization, homogeneity and reactivity. Effects of substrate pre-treatment, silanization conditions as well as post-silanization procedures have been evaluated. We used thiol reactive silane layers as reactive intermediates to produce reactive surfaces presenting biotin by in-situ coupling of PEGylated thiol-biotin crosslinkers. Streptavidin has been shown to bind to the surface specifically. Surface coverages, homogeneity, and reactivity of the streptavidin layer were evaluated mainly by radiometry.

BI-WeP17 Alkylsiloxane Self-assembled Monolayers on Titanium, *R.A. Brizzolara*, ***R.M. Lennen***, Naval Surface Warfare Center

Preparations of self-assembled monolayers (SAMs) of alkyltrichlorosilanes on silicon wafers or glass are well documented in the literature. On the other hand, little work has been done regarding SAM formation on other hydroxylated metal surfaces and little is known about the nature of the SAM. We have investigated the adsorption of different chain lengths of alkyltrichlorosilanes with different terminal functional groups on hydroxylated titanium as a function of reaction conditions using x-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and contact angle measurements. Angle-resolved XPS data was used for qualitative comparison of the vertical composition of the coatings, and the C1s/CKVV peak intensity ratio[1] enabled the calculation of comparative average layer thicknesses of the coatings. Reaction conditions included relative humidities of less than 1% and greater than 30%, with and without oven cure, and varying immersion times of the substrate in the adsorbate solution. In the future, these self-assembled monolayers will be used to investigate biofilm adhesion as a function of critical surface tension. This will lead to the development of ultra-thin antifouling coatings for shipboard titanium heat exchanger tubes with seawater intake. This work was funded by the NSWC Carderock Division In-House Laboratory Independent Research program.

[1] Brizzolara, R. A.; Beard, B. C.; Surf. Interface Anal., 27, 716 (1999).

BI-WeP18 A Novel Approach for the Detection of Antibiotics using Mixed SAMs of Thiols on Gold, ***K. Bonroy***, *F. Frederix*, IMEC, Belgium, *P. Cliquet*, University of Ghent, Belgium, *W. Laureyn, A. Campitelli, G. Borghs*, IMEC, Belgium, *E. Cox*, University of Ghent, Belgium, *P. Declerck*, KULeuven, Belgium

In numerous applications in the clinical, environmental and toxicological field, there is an increasing need for the detection of low concentrations of different biochemical parameters with low molecular weight. For example, the detection of antibiotic residues in milk is important because of the potential toxic and allergic effect for humans. Immunosensors have a huge potential as a fast and reliable system for the detection of such low molecular weight analytes. Our research is not only focussing on the transducer part of a biosensor, but also on the biological recognition layer. This biological recognition layer mainly determines the specificity, stability, reproducibility and durability of the biosensor. For the detection of small molecules, different assay principles are possible. In the current study

an indirect competitive assay was used. Therefore antibiotics were immobilised on the sensor surface and the optimal concentration of a monoclonal antibody (directed against b-lactam antibiotics), allowing quantification of antibiotics, was established.[1] For this competitive method, a reproducible and tuneable immobilisation of the antibiotics on the sensor-surface is indispensable. In order to realise such an immunosensor we coupled the antibiotics covalently to mixed Self-Assembled Monolayers (SAMs) of thiols deposited on gold. These mixed SAMs consist of two different thiols: one to bind the antibiotics and another thiol component to avoid non-specific adsorption 2. The binding of the antibiotics on mixed SAMs was characterised with cyclic voltammetry and GA-FTIR and the competitive immunoassay was evaluated using Surface Plasmon Resonance. In conclusion, we have developed a competitive immunoassay for the detection of antibiotics using a biosensor interface based on mixed SAMs of thiols.

[1] Cliquet P. et al. (2001).Journal Agricultural Food Chemistry, 49, 3349-3355
[2] Frederix F. et al. (2001).European Conference Organised Films, P11.03.

BI-WeP19 Poly(ethylene oxide)-Terminated Monolayer Formed at Solid/Vapor Interface, ***A. Hozumi***, *Y. Yokogawa, T. Kameyama*, National Institute of Advanced Industrial Science and Technology, Japan

Poly(ethylene oxide) (PEO) has been widely applied to the fabrication of protein or cell repellent surfaces in biotechnical and biomedical applications. Although extensive research on PEO-coating techniques has been reported in the literature, there have been few reports on preparing PEO-terminated monolayers from the vapor phase. Here, we report on the formation of a PEO-terminated monolayer on SiO_2/Si sample substrates through a chemical vapor deposition (CVD) method. Si substrates covered with na tive oxide (SiO_2/Si) were first cleaned by UV/ozone treatment. The cleaned samples were then exposed to vapor of organosilane, that is, 2-[methoxy (polyethyleneoxy) propyl] trimethoxysilane (MPEOPS) for 1~7 hours at 150 °C. We have investigated in detail chemical and electokinetic properties of this PEO-terminated monolayer. The SiO_2/Si surface after CVD became relatively hydrophobic showing a water-contact angle of ca. 67±2. Thickness of the MPEOPS-monolayer was ca. 0.8±0.1 nm as estimated by ellipsomrtry. As confirmed by AFM, the surface was very smooth and homogeneous with almost identical to that of the SiO_2/Si substrate. Zeta-potentials of the MPEOPS-monolayer covered SiO_2/Si substrates were measu red as a fun ction of pH by means of an electrophoretic light scattering spectrophotometer. Isoelectric point of the MPEOPS-monolayer covered surface was observed at around pH 5 which was higher than that of SiO_2/Si (~pH 2.0). These results are att r ibutable to a reduction in the density of surface silanol (Si-OH) groups on the SiO_2/Si substrate. Si-OH groups were consumed due to the formation of siloxane bondings with the MPEOPS. Furthermore, we demonstrated micropatterning of this MPEOPS-mo no layer based o n the photolithography using an excimer lamp radiating vacuum ultra violet light of 172 nm in wavelength.

BI-WeP21 Study of Bone Repairing Employing a Ricinus Camunis-based Biopolymer Additioned with Ascorbic Acid and Epidermal Growth Factor in a Rat Tibia Model[1], *C. Mendoza-Barrera*, UPIITA-IPN and Cinvestav-IPN, Mexico, ***M. Melendez-Lira***, *V. Altuzar, S.A. Tomas*, Cinvestav-IPN, Mexico

There is a huge demand of graft material to accelerate the bone healing process of lesions experienced after surgical interventions related with bony tumors or traumatic experiences. Autografts, allografts and biomaterials are sources of graft materials but it would be highly desirable to have materials without the limitations of the first two aforementioned. We discuss the effect of a Ricinus Camunis-based biopolymer mixed with Ascorbic Acid (AA)and/or Epidermal Growth Factor (EGF) on the bone repairing employing a rat tibia model. The bone healing process was monitored through the interface bone-graft material. No implant rejection or inflammatory reaction was observed during a 8 weeks period in our in vivo studies. The evolution of the osteogenesis in the lesion area was followed employing scanning electron microscopy (SEM), energy dispersive x-ray analysis (EDX), Photoacoustic Spectroscopy (PAS) and X-ray diffraction (XRD). Our study clearly indicates that the combined use of the Ricinus Camunis-based biopolymer, AA and EGF improves the remodeling characteristics of the biomaterial. XRD allow us to identify the structural characteristics of the biopolymer and its evolution during the bone healing process. Bone resorption was monitored by EDX studying the Ca/P ratio as function of time. PAS presents features that could be correlated with cellular activity during the bone mineralization process. All the results showed a good correlation and allowed us to obtain information to improve the composition of the biomaterial.

[1]This work was partially supported by CONACYT-Mexico.

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