Biomaterial Interfaces Division
Room: East Exhibit Hall - Session BI-ThP

Biomaterial Interfaces Poster Session

BI-ThP1 Physicochemical Characterization of Loop Polymer Brushes Prepared by Immobilization of End-Functionalized Poly(dimethylsiloxane), ***S. Sakurai***, *H. Watanabe,* Jst, Erato, Japan, *A. Takahara*, Kyushu University, Japan

A loop polymer brush is surface-tethered polymer chain of which both ends were anchored on the substrate surface to form a loop at a molecular level. The dynamics and physicochemical characterization of the loop brush has been a hot topic of several theoretical and experimental studies for polymer physics because the formation and interaction of loop brushes are key processes in many important biological and engineering processes. In this study, we synthesized loop polymer brushes by using end-functionalized poly(dimethylsiloxane) (PDMS) with various molecular weights as follows. A commercially available amino-terminated PDMS was fractionated by size exclusion chromatography (SEC) in the molecular weight from M_n = 7,000 to 100,000 with narrow molecular weight distributions (M_w/M_n~1.2), and transformed to a triethoxysilane (TES) end-functionalized PDMS on both ends. The silicon wafer was immersed in dichloromethane solution of the telechelic TES-terminated PDMS at 293 K for 24 h to immobilize both chain ends of the PDMS. The surface morphology of the telechelic PDMS-immobilized silicon wafer was observed by atomic force microscopy (AFM) under air at room temperature. The root mean square (rms) of the surface roughness was 0.6 nm in a 2 x 2 μm^2 scanning area. The smooth morphology indicated that loop structure was formed without cross-linking and gelation of TES. Mono-functionalized PDMS was also synthesized to fabricate a linear brush on the silicon wafer. The thickness of these brushes in a dried state determined by ellipsometry increased from 2 to 5 nm with an increase in the molecular weight of M_n = 5,000 to 100,000. The loop and linear polymer brushes with same molecular weight showed the almost same thickness in a dried state. Apparent difference derived from a topology effect was not observed in a dried state. AFM measurement of the swollen thickness of the loop and linear brushes in a solution is in progress.

BI-ThP2 Surface Modification of Polylactic Acid (PLA) Fibers for Use as Tissue Engineering Scaffolds, ***N.M. Tambe***, *M.W. King, A. El Shafei*, North Carolina State University

Organ failure is one of the major problems faced by patients around the world. In the USA, there are currently millions of people waiting for organ transplantation with the annual healthcare costs for the treatment of these patients exceeding $500 billion. The goal of this study is to develop an innovative, resorbable porous tissue engineering (TE) scaffold with controlled biodegradability, good cytocompatibility, suitable surface chemistry for cell growth and optimum mechanical properties. Most scaffold materials do not possess any bioactivity and do not have the ability to promote extracellular matrix (ECM) secretion and support cell growth. So, the surface chemistry is very important as the outermost functional group is responsible for binding cells to the material.

In order to achieve these goals, there are a series of specific objectives:

1) To identify and compare various polymer surface modification techniques

2) To determine and study the process and material variables for those techniques

3) To characterize these techniques for changes in surface chemistry

4) To attach bioactive coatings onto the surface successfully.

Polylactic acid (PLA) was chosen as the scaffold material due to the fact that it has most of the desired TE scaffold properties. A PLA nonwoven web from Ahlstrom Nonwovens was used as the material. Surface modification was performed by thermally initiated free radical polymerization using vinyl monomers such as maleic acid and maleic anhydride. Potassium persulfate was used as the initiator and Mohr's salt was added to reduce the extent of homopolymerization. The grafted surfaces were coated with a bioactive coating of collagen using a spacer molecule called genipin. The grafted surfaces were then evaluated via Fourier transform infrared spectroscopy (FTIR), contact angle measurements, X-ray photoelectron spectroscopy (XPS), and dyeing with a basic dyestuff and using visible spectrophotometry.

The FTIR spectra show the grafting of carboxylic acid groups of maleic acid onto the surface of the PLA. The effect of monomer concentration is seen in the contact angle measurements. With increase in the monomer concentration, the contact angle fell, indicating an increase in the hydrophilicity of the material. The collagen was also successfully coated onto the grafted surface which is confirmed by the FTIR spectra showing the characteristic amide bands. Further evaluation of the surfaces is continuing with dyeing, XPS, and measuring the biological performance. In addition, other surface modification methods like plasma, atom-transfer radical polymerization (ATRP), are being undertaken.

BI-ThP3 Application of Layer-By-Layer Coatings to Tissue Scaffolds – A Novel Approach for Developing a Pro-Angiogenic Biomaterial, ***C.D. Easton***, CSIRO Materials Science and Engineering, Australia, *S.L. McArthur,* Swinburne University of Technology, Australia, *A.J. Bullock, S. MacNeil*, University of Sheffield, UK

Development of flexible coating strategies for angiogenesis promotion is critical for the health care industry to effectively treat chronic non-healing wounds. This need will continue to intensify in light of the increasing number of patients diagnosed with diabetes and an ageing population. In addition, such strategies are required within the tissue engineering community to overcome issues associated with engineered materials failing to engraft as a result of delays in neovascularisation.

An important requirement for a pro-angiogenic biomaterial is the ability to maintain a regulated release of bioactive growth factors to the wound site through the use of heparin. A number of strategies for the use of natural and synthetic heparin-mimetics have been developed, however in general they fail as the heparin can be rapidly lost from the wound site. Therefore new strategies to effectively immobilise heparin for the release of bioactive growth factors are being developed.

This work describes a layer-by-layer (LBL) approach that provides a platform for immobilising a significant amount of heparin to the substrate of choice. The LBL coating prevents desorption of immobilised heparin as a result of 'covalent bond-like' interactions from the electrostatic attraction between the multiple layers of polyelectrolytes. Employing plasma polymerisation, a thin functional coating was applied to the substrate to provide the necessary surface charge in order to build the LBL architecture. In this particular case, plasma polymerised acrylic acid (ppAAc) was deposited onto Si wafer and two different tissue scaffolds, a commercially available polypropylene type, and an electrospun PLGA (75:25) type previously reported in the literature. The LBL structure was then created by physically absorbing alternative layers of polyethyleneimine (PEI) and poly(acrylic acid) (pAAc), and once the desired number of layers was achieved, heparin was immobilised to the structures. The kinetics of the fabrication procedure was examined using a quartz crystal microbalance with dissipation (QCM-D) system, while the resulting structures were probed using X-ray Photoelectron Spectroscopy (XPS) and Atomic Force Microscopy (AFM). The effect of pH of the LBL solutions (PEI and pAAc) on the resulting structure and ability to immobilise and retain heparin were examined. This technique allowed for the immobilisation of a significant amount of heparin to the test substrates explored, more so than that previously reported using similar approaches. This approach therefore provides an inexpensive and easily expandable coating methodology for applying a pro-angiogenic interface to tissue engineered materials

BI-ThP4 In Situ Surface Initiated Enzymatic DNA Polymerization: Potential for Multiplexed Molecular Detection, ***L. Tang***, *V. Tjong, A. Chilkoti, S. Zauscher*, Duke University

We present a new technique called surface initiated enzymatic polymerization (SIEP), which uses terminal deoxynucleotidyl transferase (TdT), a template-independent DNA polymerase that catalyzes the sequential addition of deoxynucleotides (dNTPs) at the 3'-OH group of an oligonucleotide primer. We utilized TdT's ability to polymerize a long DNA chain while incorporating non-natural chemically reactive dNTPs to create new functional materials and to generate signal amplification. Previously, we have shown that fluorescent-dNTP can be directly polymerized by TdT. In this work, we will show the incorporation of amine- and aldehyde-modified dNTPs to impart reactive moieties into the polymerized DNA chain. We quantified the number of reactive dNTPs and their effect on the polymerization efficiency. We then further investigated the reactivity of the functional group for subsequent reactions, which include fluorescent dye conjugation for signal amplification, selective DNA metallization, and oligonucleotide conjugation to create a branched structure. We found that multiple reactive groups can be incorporated and they are active for subsequent reactions. For characterization of SIEP, we utilized a quartz crystal microbalance with dissipation monitoring (QCM-D) to monitor increase in surface mass during DNA polymerization in real-

time. The mass increase versus concentrations of dNTP allows us to determine the reaction constant, which reflects the growth kinetics of DNA polymerization on the surface. In addition, we examined the effect of grafting density on the polymerization reaction and the conformation of DNA brushes.

BI-ThP5 Plasma-based Approaches for Biointerface Preparation, *E.H. Lock,* ***S.H. North****, S.G. Walton, C.R. Taitt*, Naval Research Laboratory (NRL)

The ability to predict and manipulate biomolecule behavior at the biointerface determines the success of biomaterials in applications ranging from biosensing to medical devices and therapeutic products. However, precise biointerface engineering will remain elusive until the roles of physical and chemical properties of surfaces on abiotic and biotic interfacial interactions are well understood. We have shown that plasma treatment of polymers generates chemically reactive surfaces for successful silanization and biomolecule immobilization [1, 2]. The focus of this work is to investigate the influences of surface chemistry and surface morphology on biomolecule attachment. We fine-tune our plasma system to favor the production of specific functional groups that promote subsequent biomolecule attachment. The effects of surface morphology on biomolecule immobilization are also assessed. Plasma diagnostics and modeling allows us to elucidate the effects of plasma parameters (plasma density, electron temperature and the resulting kinetic ion energy) on the polymer surface modifications. The work was supported by the Office of Naval Research.

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BI-ThP6 A Novel ALD Al_2O_3-Parylene Bi-Layer Encapsulation for Biomedical Implantable Devices, ***X. Xie****, L.W. Rieth, F. Solzbacher*, University of Utah

Atomic layer deposited (ALD) Al_2O_3 has been widely used as encapsulation material for organic LEDs and solar cells due to its low water vapor transmission rate (WVTR) (~5×10^{-6} g-H_2O/m^2-day). However, its coating performance for implantable devices still needs investigation. Parylene has been commonly applied as encapsulation for implantable devices, such as Utah Electrode Arrays (UEAs). The idea of combing Al_2O_3 and parylene is based on the concept that Al_2O_3 works as moisture barrier and parylene as ion barrier. In this paper, Al_2O_3 was deposited by both thermal and plasma-enhanced ALD on interdigitated electrodes (IDEs) for comparison. AFM micrographs (Fig. 1) show that Al_2O_3 films deposited on silica substrate (RMS surface roughness of 0.17 nm) by thermal and plasma-enhanced ALD have RMS surface roughness of 0.51 nm and 0.48 nm, respectively. XPS shows that ALD films had an oxygen to aluminum ratio of 1.2 while thermal ALD Al_2O_3 is 1.09, indicating that the former is closer to Al_2O_3. A 6-μm thick parylene-C layer was deposited by CVD using Gorman process on top of Al_2O_3 and saline A-174 (Momentive Performance Materials) was used used as adhesion promoter. The samples were soaked in 1× PBS at 37 °C and 57 °C for accelerated lifetime test. Electrochemical impedance spectroscopy (EIS) and chronoamperometry were used to evaluate the performance of the encapsulation. Preliminary data shows that the leakage current (Fig. 2) remained very small (~ 7 pA) and the electrochemical impedance (Fig. 3) was consistently high (~ 3 MΩ at 1 kHz) after 5 days of soaking test at 57 °C (equivalent to at least 20 days of soaking test at 37 °C). Comparing with parylene and Al_2O_3 control samples, the Al_2O_3-parylene coated sample showed lower leakage current (Fig. 2). The impedance for three different types of samples was almost the same. However, the phase of parylene-C coated sample slightly declined after 5 days of soaking test (Fig. 3), suggesting that sample coated with Al_2O_3 had lower WVTR. No obvious difference has been observed yet for samples soaked at different temperatures since the soaking period is relatively short. Parylene came off after one day of soaking test for one sample, which might be caused by the poor adhesion between Al_2O_3 and parylene. In conclusion, preliminary results shows that the Al_2O_3-parylene bi-layer encapsulation scheme is promising encapsulation in terms of leakage current and electrochemical impedance. Long-term soaking tests are being performed to further investigate the functionality of this novel encapsulation scheme.

BI-ThP7 Complementary Electronic and Vibrational Circular Dichroism Analysis of Bovine Serum Albumin Adsorbed on Hydroxyapatite Microspheres, ***K.P. Fears****,* Naval Research Laboratory*, D.E. Day,* Missouri University of Science and Technology*, D.Y. Petrovykh,* International Iberian Nanotechnology Laboratory*, T.D. Clark*, Naval Research Laboratory

Bovine serum albumin (BSA) is a widely studied globular protein that contains ca. 68% of alpha-helices and <2% of beta-sheets in its native conformation. The well characterized secondary structure of BSA is commonly used as a benchmark for electronic (ECD) and vibrational (VCD) studies of proteins in solution. Both ECD and VCD indicated a substantial loss of helical structure accompanied by an increase of beta-sheet character in BSA thermally denatured in solution. In surface adsorption experiments, hydroxyapatite microspheres were incubated in solutions with low (1.0 mg/mL) or high (50.0 mg/mL) concentrations of BSA for one hour, then triple rinsed and re-suspended in buffer for analysis. The ECD spectra were similar for BSA adsorbed from low and high concentration solutions, both showing a sizeable increase in beta-sheet character upon adsorption, while being dominated by alpha-helical features. The VCD spectra also exhibited stronger peaks in the beta-sheet region upon adsorption of BSA on hydroxyapatite. VCD signal enhancement, however, was observed upon adsorption from the high concentration BSA solution, indicating the formation of macroscopic chiral structures. The analysis of proteins adsorbed on surfaces thus can be enhanced by taking advantage of the complementary sensitivities of ECD and VCD spectroscopies to the secondary structures of biomolecules.

BI-ThP9 Binding Affinities of *wt* and H93R PTEN to Lipid Membranes Containing PS and $PI(4,5)P_2$, ***S. Shenoy****,* Carnegie Mellon University*, A. Gericke,* Kent State University*, A.H. Ross,* University of Massachusetts Medical School*, M. Lösche*, Carnegie Mellon University and National Institute of Standards and Technology

PTEN is a phosphatidylinositolphosphate (PIP) phosphatase frequently mutated in human cancer [1]. By lowering $PI(3,4,5)P_3$ levels in the plasma membrane, it functions as an antagonist to PI3-kinase in the regulatory circuit that controls cell proliferation and survival. *wt* PTEN has only weak affinity to zwitterionic phosphatidylcholine (PC) membranes but a strong interaction with anionic lipids. Its C2 domain was shown to bind in a Ca^{2+} independent manner to phosphatidylserine (PS) and phosphatidylglycerol (PG), whereas a short N-terminal domain binds specifically to $PI(4,5)P_2$ [2,3]. H93R PTEN is an autism related mutant which has decreased phosphatase activity [4].

Using Surface Plasmon Resonance (SPR), we characterized the affinity of *wt* and H93R PTEN to tethered bilayer lipid membranes (tBLMs) that contain PC and PS, PC and $PI(4,5)P_2$, and PC, PS and $PI(4,5)P_2$. As compared with *wt* PTEN, we find that the H93R mutation is sufficient to cause significant increases in the protein's association with lipid membranes containing PS. $PI(4,5)P_2$ enhances the apparent binding constant for both proteins and leads to intriguing binding kinetics of the protein to the membrane. The binding of either protein to membranes containing both PS and $PI(4,5)P_2$ shows a biphasic behavior, suggesting two independent binding sites. This supports the hypothesis of non-competitive binding of the protein to PS and $PI(4,5)P_2$ [5]. We also performed neutron reflectivity experiments to determine the structure and orientation of PTEN bound to the membrane.

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BI-ThP10 Membrane Binding and Structure for Cytoplasmic Domain of Zeta Subunit of T Cell Receptor, ***P. Shekhar****,* Carnegie Mellon University*, F. Heinrich,* Carnegie Mellon University and National Institute of Standards and Technology*, K. Zimmerman,* University of Massachusetts Medical School*, M. Lösche,* Carnegie Mellon University and National Institute of Standards and Technology*, L.J. Stern*, University of Massachusetts Medical School

The cytoplasmic domain of the T-cell receptor zeta subunit, ζ_{cyt}, a cell surface protein complex responsible for binding peptide fragments of foreign antigens bound to major histocompatibility complex (MHC) proteins, is sufficient to couple receptor ligation to intracellular signaling cascades [1]. These domains carry immunoreceptor tyrosine-based activation motifs (ITAMs), *i.e.* signaling motifs that are phosphorylated by tyrosine kinases following receptor crosslinking. The phosphorylation of ITAMs is a first and obligatory step in signal transduction. ζ_{cyt} has been shown to be unstructured in aqueous solution and to assume a helical

conformation in the presence of anionic lipid vesicles [2,3]. Membrane binding and membrane-induced conformational changes likely plays an important role in signal transduction, but no direct structural information on these functionally important lipid-bound states was available so far. Using a synthetic membrane model, *i.e.*, fluid lipid bilayers tethered to planar solid supports [4,5], we report surface plasmon resonance (SPR) results on the binding kinetics and neutron reflectivity investigations of the association of the disordered ζ_{cyt} with membranes. We determine the extent to which the protein penetrates into the bilayer and discuss structural details of the ζ_{cyt}–lipid interaction.

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BI-ThP11 Microfluidic Bacterial Adhesion Assay to Characterize the Easy to Clean Properties of Surfaces, ***M.P. Arpa Sancet***, *C. Christophis, S. Bauer, A. Rosenhahn, M. Grunze*, Universität Heidelberg, Germany

Biofouling, the undesired growth of marine organisms on submerged surfaces, is a global problem with both economic and environmental consequences. The attachment of bacteria to surfaces is an important step in the biofouling process, and the development of ways to attenuate microbial attachment or to achieve their easy removal is desirable. We built a microfuidic system and applied it to determine the adhesion strength of bacterial biofilms. Marine bacteria, e.g. *Cobetia marina* are cultivated in this microfluidic device on the surfaces of interest. The bacterial detachment is caused by a hydrodynamic shear flow which is continuously increased and the removal is recorded via video microscopy. The adhesion strength is determined as the shear stress needed to detach 50% of the bacteria. The parameters incubation time, medium and increase of shear stress were varied in order to find the optimal conditions to carry out the biological assays. The applicability of the technique is demonstrated using self assembled monolayers with a different ability to bind water. Well hydrated surfaces lead to decreased adhesion strength while bacteria stick stronger to less hydrated surfaces. Based on these findings, a range of polysaccharide hydrogels were tested towards their potential to reduce adhesion strength.

Authors Index

Bold page numbers indicate the presenter

— A —

Arpa Sancet, M.P.: BI-ThP11, **3**

— B —

Bauer, S.: BI-ThP11, 3
Bullock, A.J.: BI-ThP3, 1

— C —

Chilkoti, A.: BI-ThP4, 1
Christophis, C.: BI-ThP11, 3
Clark, T.D.: BI-ThP7, 2

— D —

Day, D.E.: BI-ThP7, 2

— E —

Easton, C.D.: BI-ThP3, **1**
El Shafei, A.: BI-ThP2, 1

— F —

Fears, K.P.: BI-ThP7, **2**

— G —

Gericke, A.: BI-ThP9, 2
Grunze, M.: BI-ThP11, 3

— H —

Heinrich, F.: BI-ThP10, 2

— K —

King, M.W.: BI-ThP2, 1

— L —

Lock, E.H.: BI-ThP5, 2
Lösche, M.: BI-ThP10, 2; BI-ThP9, 2

— M —

MacNeil, S.: BI-ThP3, 1
McArthur, S.L.: BI-ThP3, 1

— N —

North, S.H.: BI-ThP5, **2**

— P —

Petrovykh, D.Y.: BI-ThP7, 2

— R —

Rieth, L.W.: BI-ThP6, 2
Rosenhahn, A.: BI-ThP11, 3
Ross, A.H.: BI-ThP9, 2

— S —

Sakurai, S.: BI-ThP1, **1**
Shekhar, P.: BI-ThP10, **2**
Shenoy, S.: BI-ThP9, **2**
Solzbacher, F.: BI-ThP6, 2
Stern, L.J.: BI-ThP10, 2

— T —

Taitt, C.R.: BI-ThP5, 2
Takahara, A.: BI-ThP1, 1
Tambe, N.M.: BI-ThP2, **1**
Tang, L.: BI-ThP4, **1**
Tjong, V.: BI-ThP4, 1

— W —

Walton, S.G.: BI-ThP5, 2
Watanabe, H.: BI-ThP1, 1

— X —

Xie, X.: BI-ThP6, **2**

— Z —

Zauscher, S.: BI-ThP4, 1
Zimmerman, K.: BI-ThP10, 2