Tuesday Evening Poster Sessions, November 14, 2006

Biomaterial Interfaces

Room 3rd Floor Lobby - Session BI-TuP

Biomaterial Interfaces Poster Session

BI-TuP1 Effect of Cross-linking Ultra-high Molecular Weight Polyethylene on Surface Molecular Orientation and Wear Characteristics, *S. Sambasivan,* ***D.A. Fischer****, S.M. Hsu,* National Institute of Standards and Technology

Cross-linked ultra-high molecular weight polyethylene (UHMWPE) has become the dominant material used in human artificial joints due to substantial increase in wear resistance and ability to form conformal contacts. Wear debris resulting from wear has been identified as a major contributor to the eventual loosening of acetabular prostheses and failure of implants. Cross-linking of UHMWPE by gamma irradiation has been developed but the detailed mechanism of how cross-linking increases wear resistance is not understood. Wear of the UHMWPE surface layer changes the hardness and brittleness of the surface, and this, in turn, affects the wear resistance. This study uses a soft x-ray spectroscopic technique Near edge X-ray absorption fine structure (NEXAFS) to examine the degree of molecular orientation of the worn UHMWPE surface layer (up to 10nm). NEXAFS measurements at Carbon-K edge of worn UHMWPE samples which were subjected to gamma, ethylene-oxide (EtO), and electron beam sterilization techniques was conducted. Results conclusively suggest that cross-linking resists orientation when the samples were mechanically pulled or worn in a knee simulator. The molecular orientation in the C-C alkyl chains showed a monotonic decrease with increase in gamma radiation dosage levels suggesting highly crosslinked samples resists molecular orientation. EtO sterilized samples showed more C-C chain orientation compared hence less resistance to wear as compared to electron beam radiated samples. Direct comparison of surface molecular orientation of C-C alkyl chains of UHMWPE may offer explanation which of the cross-linking methods are more effective to produce a wear resistant artificial joints.

BI-TuP2 Biosurfaces Generated Using AFM-Based Nanolithography and Surface Activation Chemistry, ***J.N. Ngunjiri****, W. Serem, J.C. Garno,* Louisiana State University

The immobilization of biological ligands in precisely defined locations on surfaces is a critical technology for the integration of biological molecules into miniature bioelectronics and sensing devices. The selectivity of protein adsorption with designed surfaces is compared at the nanoscale using in situ atomic force microscopy (AFM). The high-resolution capability of AFM characterization is combined with nanografting for investigations of protein binding on chemically activated self-assembled monolayers (SAM). Using a computer program, the AFM tip is translated at designated speed, direction, and force to enable fabrication of arrays of SAM nanopatterns with well-defined shapes and sizes. Nanografting provides superb control of parameters of ligand density, pattern spacing and the size of array elements. Nanostructures ranging in size from 10-100 nm are inscribed within a resistive matrix SAM (such as methyl or hydroxyl) which imparts selectivity for protein adsorption. The terminal moieties of carboxylate-terminated SAMs can be reacted with coupling agents such as N-ethyl-N'(dimethylaminoporpyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to establish covalent coupling of proteins to arrays of SAM nanopatterns. The activity of the immobilized proteins for binding immunoglobulin G (IgG) and peptides can then be investigated by viewing successive changes in the height and morphology of nanostructures during in situ AFM experiments. The resistive matrix SAM surrounding the nanopatterned proteins selectively defines regions for viewing adsorption of proteins with exquisite detail. In situ AFM images of nanoengineered surfaces will be presented which provide direct views of the progression of biomolecular reactions on surfaces.

BI-TuP3 QCM-D and AFM Characterization of the Adsorption and Functionality of Laminin in Large Area Nanopatterns for the Study of Neural Stem Cells, ***J. Malmstrom****,* University of Aarhus, Denmark*; H. Agheli, E.M. Larsson,* Chalmers University of Technology, Sweden*; M. Textor,* ETH Zurich, Switzerland*; D.S. Sutherland,* University of Aarhus, Denmark

A number of reports have made use of chemical or topographic surface structures to pattern assemblies of neural cells in an effort to control their growth and development. Here a nanostructured surface is used to immobilize Laminin at discrete locations on a solid substrate in well-defined nanometer scale patterns in order to investigate the response of neural stem cells (AHP - adult hippocampal progenitor). Nanostructured quartz crystal oscillators and silicon wafer chips with ~120nm diameter regions of alkanethiol modified gold on a silicon oxide background are produced by colloidal lithography. Immobilization of protein resistant layers (PLL-g-PEG) specifically to the silicon oxide parts of the surface allows the generation of nanoscale patches of protein by the non-specific adsorption to the hydrophobically modified gold. The adsorption of Laminin onto homogenous and nanostructured surfaces is studied with parallel sets of samples using the Quartz Crystal Microbalance with dissipation (QCM-D) and Atomic Force Microscopy (AFM). Viscoelastic modeling of the QCM-D data, supported by surface plasmon resonance (SPR) data, on homogenous surfaces indicate a thick hydrated Laminin layer (71±2 nm, >95% water) with high antibody binding capacity (~3 polyclonal anti-Laminin IgG's binding per surface bound Laminin). QCM-D data indicates a higher surface density of Laminin binding for the structured surfaces than the homogeneous surfaces and a greater IgG binding capacity. A new approach to analyze AFM height histograms is used to quantify protein and monoclonal/polyclonal anti-Laminin antibody binding to the nanoscale patches showing

BI-TuP4 Investigating Model Peptides on Surfaces using XPS, SIMS and NEXAFS, ***J.S. Apte****, L.J. Gamble, D.G. Castner,* University of Washington

The purpose of this investigation is to study protein-surface interactions at a fundamental level using model peptides to examine individual adsorption phenomena that comprise the collective interactions of larger proteins with surfaces. The model peptides used were made up of leucine (L) and lysine (K) amino acids arranged in specific sequences to reliably create @alpha@-helix or @beta@-sheet secondary structures. These peptides were adsorbed onto self-assembled monolayer (SAM) surfaces that have well-defined, extensively studied characteristics. The terminal functional groups of the SAMs used in these studies are a methyl group, a carboxylic acid group, an alcohol group and an amine group. Studies were also done on a plasma-deposited fluoropolymer for comparison. Initial adsorption isotherm studies with the 14-mer LK @alpha@-helix peptide showed that the adsorption behavior varied greatly on the different SAM surfaces. Percent nitrogen measured with XPS was used to determine the amount of adsorbed peptide. It was found on methyl- and COOH-terminated SAMs that adsorption from PBS formed patches of peptide with bare spots. There was no adsorption on OH-terminated SAMs and uniform adsorption on fluoropolymer. It was also found that the concentration required to achieve a partial peptide layer with some bare spots on methyl SAMs was 100-fold higher that required for COOH SAMs. Adsorption was investigated for three different buffer salt concentrations to observe the influence of ionic strength on adsorption. On methyl SAMs, it was found that no peptide adsorption was detectable from deionized water, even at peptide concentrations of 1 mg/mL. In contrast a nitrogen concentration of about 5 atomic percent was detected when adsorbed from PBS. Angle-dependent XPS and NEXAFS studies were used to probe the organization of the adsorbed peptides.

BI-TuP5 Characterization of Nitrilotriacetic Acid-Terminated Self-Assembled Monolayers for Orientation-Controlled Immobilization of Proteins, ***F. Cheng****, L.J. Gamble, D. Graham, D.G. Castner,* University of Washington

In recent studies of orientation and conformation in adsorbed protein films, it is essential step to immobilize proteins onto surfaces in a controlled and well-defined manner. The oriented immobilization of genetically engineered histidine-tagged proteins onto nitrilotriacetic acid (NTA)-terminated self-assembled monolayers (SAMs) on gold has been a widely used model system. X-ray photoelectron spectroscopy (XPS), angle-resolved XPS (ARXPS) and surface plasmon resonance (SPR) have been used to characterize monolayers generated via self-assembly of the NTA thiol onto gold and backfill of methyl-terminated thiol (mercaptoundecane, MUD) into the loosely-packed NTA SAMs. In pure NTA SAMs, self-assembly conditions can significantly affect lateral packing density and nickel-activation efficiency. In mixed NTA SAMs, the correlation between the MUD backfill time and surface compositions indicates a two-step backfill process: 1) at short backfill times (@footnote 1@1h), MUD binds to unoccupied gold sites in NTA monolayer; 2) at long backfill times (@footnote 2@1h), MUD displaces NTA from the gold surface. ARXPS analysis shows that the MUD backfill method can optimize the vertical distribution of NTA endgroup and enrich NTA endgroup in the outmost layer of the films. The binding constants of oligo (histidine) onto the NTA surfaces were determined by SPR.

BI-TuP6 Interface Biology at Structured Surfaces of Titanium Dental Implants, ***H.P. Wiesmann***, *L. Lammers, F. Abusua, U. Joos,* University M@um u@nster, Germany

In the present investigation three comparable threaded dental titanium implants were evaluated for the bone cell reactions at the surface in cell culture and after insertion in the mandible of minipigs. Implants with comparable size and geometry but various surface structures were used; TBS surfaces, sandblasted - acid-etched (SLA) surfaces, and microgrooved surfaces. Osteoblast-like cells were seeded under defined culture conditions on the implants and cell reactions were investigated during a 14-day culture period. For the in vivo experiments implants were placed in the mandible of 10 minipigs. Light- and electron microscopy as well as energy dispersive x-ray analysis were used. Cultured osteoblasts attached to all tested surfaces. TBS and microgrooved surfaces showed significantly more attached cells after 1 day. Proliferation of cells was best on microgrooved surfaces followed by TBS- and SLA-surfaces. After 14 days a high expression of osteocalcin, osteonectin, fibronectin, collagen type I, and osteopontin of osteoblasts cultured on microgrooved surfaces was demonstrated, whereas a lower level was present on SLA surfaces. In vivo, the most prominent difference at the interface between the implant systems was the extent of titanium wear. Number and size of titanium particles in the vicinity of the implantation bed were high around TBS-implants and low around microgrooved implants. SLA-implants showed many but small titanium particles in the interface region. In conclusion, grooved surfaces offer under in vitro conditions a better cell attachment and proliferation as well as a higher expression of typical bone related matrix proteins than the other surfaces studied.

BI-TuP9 Proteins Patterning on Plasma PEG Surfaces by Microcontact Printing, ***A. Ruiz***, *L. Ceriotti, F. Brétagnol, D. Gilliland, H. Rauscher, P. Colpo, F. Rossi,* European Commission, Institute for Health and Consumer Protection, Italy

Arrays of surface-bound biomolecules are significantly needed in a variety of applications, such as diagnostic immunoassays, DNA microarrays, cell culturing or biosensing. Among all known techniques, soft lithography is a printing processes used for the fabrication of micro and nano structures based on a physical contact between a stamp and a substrate. The most representative technique of this technology is the microcontact printing. This technique is based on the use of PDMS stamps with micropatterned relief inked by biomolecules that are transferred to a substrate. Surfaces with biomolecules localised in a well arrangement can be used for cell growth studies and for improving the understanding of their interactions with different cell types. In this framework, we have used microcontact printing for patterning PEG surfaces with Poly-L-Lysine and BSA. We used the fact that PEG is known to be antifouling, i.e. protein repellent, in solution but accept protein adhesion when printed in dried conditions, as previously suggested by Delamarche.@footnote 1@ The proteins stamping is done in dried conditions and the stability of the protein patterns when put in solution has been proven. Characterisation by Fluorescence Microscopy, ToF-SIMS and Ellipsometry showed well-defined stable motifs even after 24h in water. The PLL microstamped PEG surfaces were then incubated with a solution of L929 fibroblasts. It was clearly observed how the cells adhered and grew in the regions patterned with PLL. Controlled patterning of biomolecules on an antifouling substrate like PEG has been achieved by microcontact printing technique, which also offers the advantage of being a simple, convenient, inexpensive and accessible method. @FootnoteText@ @footnote 1@ E. Delamarche, C. Donzel, F.S. Kamounah, H. Wolf, M. Geissler, R. Stutz, P. Schmidt-Winkel, B. Michel, H.J. Mathieu, K. Schaumburg. Langmuir 19 (2003) 8749 - 8758.

BI-TuP10 Optical Waveguide Spectroscopy of DNA Molecules Adsorbed on an Amino-Terminated Surface, ***H. Mori***, *M.A. Bratescu, N. Saito, O. Takai,* Nagoya University, Japan

Deoxyribonucleic acid (DNA) chip has recently emerged as a powerful tool for genetic research. The optical properties of DNA have received considerable attention in the past few years, motivated by the detection of the adsorption processes in a DNA chip. Our work is focused on the adsorption processes study of the DNA molecule, on an amino-terminated self assembling monolayer (SAM) surface. The investigation method is the absorption of the evanescent light by the DNA molecules lying on the surface, in a depth of hundred nanometers. DNA absorption of the evanescent light was performed by using a slab optical waveguide (SOWG), made from quartz, in the UV region, around 260 nm. The average number of total reflections on SOWG surfaces, controlled by the incident light angle was established in order to obtain a maximum absorbance signal. A comparative analysis of the DNA molecules adsorption was investigated on SOWG surface covered with an amino-terminated SAM and on the clean quartz surface of SOWG. The amino-terminated SAM was obtained by dipping the freshly clean SOWG into 1 wt % solution of (3-aminopropyl) trimethoxysilane in toluene for 3 hours at 60°C. Different DNA molecules are investigated: Oligo-DNA (5@super '@-AAAAAAAA-3@super '@), Oligo-DNA (5@super '@-AAATAAAA-3@super '@), Oligo-DNA (5@super '@-TTTTTTTT-3@super '@) and Oligo-DNA (5@super '@-carboxydecyl-TTTTTTTT-3@super '@). The concentration of DNA solution in distilled water was in the range from 25 @micro@M to 200 @micro@M. The time dependence of the absorption spectra shows an increased DNA UV absorbance on SOWG surface covered with amino-terminated SAM as compared with clean SOWG surface.

BI-TuP11 The Influence of Material Surface Properties on the Polymerase Activity in Microchip-Based PCR, ***S. Forti***, *R. Canteri, R. Dell'Anna,* ITC-irst, Italy; *C. Della Volpe,* University of Trento, Italy; *L. Lunelli, L. Pasquardini, L. Vanzetti, C. Pederzolli, M. Anderle,* ITC-irst, Italy

PCR (polymerase chain reaction) represents the most widely used method for amplification of defined DNA sequences for medical and biological applications. As a result of the increasing demand for high performance PCR devices with high sample throughput, but low reagent consumption, PCR has recently become the focus of investigation for miniaturization in order to enhance its efficiency. However, in order to perform PCR in microchips, because of the increased surface-to-volume ratio upon miniaturization, special attention must be paid to the internal surface, which comes into contact with the PCR-reaction mixture. Effects related to the non specific surface adsorption of PCR reagents (e.g. the replicating enzyme DNA polymerase) may become significant and reduce the efficiency of DNA amplification in microchip PCR. Therefore, effects of silicon (with different deposited oxide layer), glass, chromium and titanium nitride surfaces on Taq (Thermus aquaticus) DNA polymerase adsorption will be investigated by immunofluorescence, using anti-Taq DNA polymerase monoclonal antibody. Surface distribution as well as protein amount will be determined and a correlation with surface morphological and physico-chemical properties, as determined by atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), time of flight secondary ion mass spectrometry (ToF-SIMS) and contact angle (CA) analysis, will be presented.@footnote 1@ @FootnoteText@ @footnote 1@This work was accomplished in the framework of LaTEMAR (Laboratorio di Tecnologie Elettrobiochimiche Miniaturizzate per l'Analisi e la Ricerca - Laboratory of Miniaturized Electrobiochemical Technologies for Analysis and Research), Centre of Excellence funded by MIUR (Italian Ministry for Education, University and Research) grants - FIRB 2003-2004 - for public/private structures involved in research fields characterized by strategic value.

BI-TuP12 Gold Nanoparticle Arrays as a Template for Designing Biosurfaces, ***J Hyun***, *J. Jung, K. Na,* Seoul National University, Korea

We demonstrate a new, reliable and simple method for fabricating micropatterned nanoparticle arrays that can serve as templates for the surface-initiated polymerization of polymeric brushes, which can be used for the control of bilogical adsorption. As a proof of concept we micropatterned Au nanoparticles (Au-NPs, ~10 nm) onto glass, silicon, polystyrene, and gold surfaces by a simple three-step process: 1) ÂμCP of soluble polymer, 2) incubation with a solution of Au-NPs, and 3) lift-off of the template in a mixture of ethanol and deionized water. Forty-micrometer wide features were successfully fabricated without any significant defects or nonspecific adsorption on the background. To demonstrate the utility of these Au-NP templates, we subsequently polymerized N-isopropylacrylamide (NIPAAm) by surface-initiated polymerization, using a surface-bound initiator.

BI-TuP13 Surface Characterization of Plasma-Polymerized Cyclohexane Thin Film used as Non-Biofouling Surface for Proteins, *C. Choi, S. Yeo,* Sungkyunkwan University, Korea; *H.K. Shon, J.W. Kim,* Korea Research Institute of Standards and Science (KRISS); *Y. Park, D.W. Moon,* Korea Research Institute of Standards and Science (KRISS), Korea; *D. Jung,* Sungkyunkwan University, Korea; ***T.G. Lee***, Korea Research Institute of Standards and Science (KRISS), Korea

In this work, plasma-polymerized cyclohexane (PPCHex) thin film, which can be used as a non-biofouling surface for proteins, was characterized by using time-of-flight secondary ion mass spectrometry (TOF-SIMS) along with a principal component analysis (PCA), X-ray photoelectron spectroscopy and Fourier transform infrared spectroscopy. The PPCHex thin film was deposited on a glass surface by using the inductively coupled plasma chemical vapor deposition method and cyclohexane as a precursor.

The non-biofouling property of the PPCHex surface was controlled in a reproducible manner as a function of sample bias plasma power and was correlated with the surface chemical composition and hydrophobicity of each surface. In addition, a PCA of the TOF-SIMS data provided insight in a systematic manner into the surface chemical compositions and molecular cross-linking on plasma-polymerized thin films as a function of sample bias plasma power.

BI-TuP14 Organophosphonate Functionalized Silicon Nanowires for DNA Hybridization Studies, ***D. Pedone***, *A. Cattani-Scholz,* Technical University Munich (TUM), Germany*; M. Dubey, J. Schwartz,* Princeton University*; G. Abstreiter, M. Tornow,* Technical University Munich (TUM), Germany

Semiconductor nanowire field effect devices have great appeal for label-free sensing applications due to their sensitivity to surface potential changes that may originate from charged adsorbates. In addition to requiring high sensitivity, suitable passivation and functionalization of the semiconductor surface is obligatory. We have fabricated both individual and sets of parallel, eventually freely suspended, silicon nanowires from Silicon-on-Insulator substrates using standard nanopatterning (electron beam lithography, reactive ion etching) and surface micromachining techniques (sacrificial oxide etching). The wires of length 2 µm were typically 100 nm wide and high, and consisted of boron p-type 10@super 18@ cm@super -3@ implantation doped Si. Subsequent to nanofabrication, the devices were bio-functionalized in a 3-step sequence: First, a hydroxyalkylphosphonate monolayer was covalently attached to the native oxide of the Si wire, affording stable and dense passivation in aqueous solutions. Then, bifunctional linker groups were bound to this surface, and in the final step thiol-terminated DNA oligonucleotides were allowed to react with a maleimide moiety of the linker. In initial experiments we investigated DNA hybridization on such functionalized nanowires using a difference resistance setup, where subtracting the reference signal from a second wire could be used to exclude most unspecific effects. A net change in surface potential of the order of -2.5 mV could be detected upon addition of complementary DNA.

BI-TuP15 Dimensional Analysis of Living Cells in Liquid by Scanning Probe Microscopy, ***Y.-J. Kim***, Myongji University, Korea*; H.-D. Kim, K.-H. Lee,* Seoul National University, Korea*; J. Kim, Y.J. Choi, Y.-S. Kim, C.J. Kang,* Myongji University, Korea

Recent advances in atomic force microscopy (AFM) allow us to determine fine structures of biological materials even under physiological liquids. Divers cancer tissues are well established to have their own structural identity defined as fractal dimension when they are growing in vivo. The dimensionality of these tissues is thought to reflect their invasiveness and malignancy. However, the fractional dimension of living cells has not been elucidated and may be largely attributed to the technical limitations of conventional imaging tools such as optical microscopy and surface electron microscopy. In this work, we have identified fractional dimension of breast cancer cells (MCF7) and normal breast epithelial cells (MCF10A) derived from the same origin. Using AFM technique, high-resolution surface images of living cells were obtained from both MCF7 cells and MCF10A cells under physiological conditions. AFM images of cells showed finer structure of cell boundary compared with SEM images after fixation. Box-counting analysis of its boundary have defined fractal dimension of each cell line. These results suggest that AFM imaging is a feasible tool for analyzing surface structures of living cells with high resolution, and could provide new insights into cell surface structure.

BI-TuP17 Synthesis and Optical Properties of Shape-Controlled Europium-Doped Gadolinium Oxide Nanocrystals, ***S.Y. Seo***, University of Florida

Shape-controlled luminescent oxide nanocrystals have been synthesized by a high temperature solution phase growth method. Thermal decomposition of metal precursors in the presence of trioctylphosphine oxide (TOPO), hexadecanediol, oleic acid, and benzyl ether resulted in highly crystalline, monodisperse oxide nanocrystals . This route provides crystalline nanoparticles with shapes of square plates or spheres dependent upon the growth variables, such as the choice of metal precursor and capping molecules. Upon evaporation of the hexane solvent, the plate-type Gd@sub 2@O@sub 3@ nanocrystals self-assembled into stacked plates lying on their edge. Alternatively, the reaction of Gd(acac)@sub 3@. with hexadecanediol resulted in spherical Gd@sub 2@O@sub 3@ nanocrystals with a diameter of 2 nm, showing that the type of metal precursors plays an important role in control of the shape of nanocrystals. The effects on the crystal growth of other reaction parameters, including reaction temperature and different combinations of organic solvents, will be discussed. The effects of the shape and size of Gd@sub 2@O@sub 3@:Eu nanocrystals on luminescent properties will also be reported.

BI-TuP18 Gradient Functionalized Plasma Polymer Films for on Probe Affinity Capture (OPAC) MALDI MS, ***C.J. Mathai***, *M.Z. Segu, R.G. Kinsel,* Southern Illinois University

Surface modification of the sample targets used for Matrix Assisted Laser Desorption / Ionization (MALDI) Mass Spectrometry (MS) is receiving considerable attention because these devices allow for rapid, on-probe fractionation of complex protein mixtures. This on-MALDI-probe fractionation of proteins/peptides is now being explored as a novel approach for disease diagnosis. Our research has focused on the use of rf pulsed plasma technique for incorporation of the surface chemical functionality on the MALDI target. In the present study a new rf plasma reactor design is described which allows for rapid deposition of discreet, or continuous, gradients of chemical functionality directly on a MALDI target. The reactor system has a cylindrical chamber with two adjustable electrodes kept parallel to each other. One of the electrodes is powered by 13.56 MHz rf generated by a function generator and amplified by an rf power amplifier. A matching network is used to couple the rf power to the electrode. For pulsed operational mode, a signal generator is employed to pulse the function generator. By varying the pulsing parameters, plasma on and off times can be varied to achieve maximum control on the film chemistry. Patterned and gradient plasma polymer films are obtained by mounting MALDI probes on an automated XY stage with specially designed masks. The complete coating system including the XY stage is fully controlled by using LabView software. Using this system, plasma polymer thin films having gradient hydrophobicity or acidity are depositied directly on the MALDI probe as a series of discreet spots. All surface chemical modifications are fully characterized by FT-IR and contact angel measurement. Subsequently, these gradient surface modified MALDI probes are used to perform on-probe fractionation of various mixtures of peptides and proteins with a goal of identifying optimal conditions for fractionation and analysis of the peptide/protein mixture constituents.

BI-TuP19 Metal Ion Impregnated PMMA as On-Probe Affinity Capture MALDI (OPAC-MALDI) Probes for the Fractionation of Protein Mixtures, ***G.S. Fernando***, *G.R. Kinsel,* Southern Illinois University

MALDI Mass Spectrometry (MS) is currently broadly used in the structural characterization and identification of proteins and other biological molecules. Fractionation of proteins into groups according to their physical or chemical properties is usually an important first step before MALDI MS analysis. Conventional methods typically involve external sample manipulation which limits the overall throughput of the analysis. Incorporation of these fractionation steps directly on a MALDI target plate has given rise to various novel protein chip technologies. In the present studies, the use of a metal impregnated, commercially available, comparatively inexpensive and easily fabricated, poly (methyl methacrylate) (PMMA) surface for the separation of metal binding proteins is explored. Identification of metal binding proteins is expected to facilitate the evaluation of the function of metal ions in protein folding, assembly, stability, conformational change, and catalysis. To produce the chemically modified MALDI target, PMMA is first hydrolyzed with 3M NaOH overnight to obtain carboxylic acid functional groups on the surface. After thorough washing with MilliQ water, the carboxyl groups are further reacted with metal ions, such as Cu2+, Zn2+ or Ni2+, by incubating the PMMA probes in a metal salt solution overnight. All surface modification steps are characterized by XPS, FTIR, AFM, MALDI MS and contact angle measurements and compared with unmodified PMMA. These metal incorporated PMMA MALDI targets are shown to selectively bind phosphorylated proteins, histidine rich proteins and proteins with terminal arginine residues in a series of control studies. Subsequently, the PMMA targets are further evaluated for their ability to fractionate complex mixtures of proteins derived from bacterial sources.

BI-TuP20 Preparation of Biodegradable Double-layered Microshells using Surfactant-free Emulsion Method, ***F. Nagata***, *T. Miyajima,* National Institute of Advanced Industrial Science and Technology (AIST), Japan

Double-layered microshells comprised of poly(lactic acid) (PLA) internal wall and calcium phosphate external wall were synthesized by a improvement surfactant-free emulsion method. PLA is one of the most commonly used hydrophobic and biodegradable polyesters which can encapsulate various poorly water-soluble drugs. PLA-based microcapsules have been investigated for biomedical applications such as drug carriers for targeted delivery. In this study, we demonstrate a method for direct synthesis of double layered microshells composed of PLA and calcium

phosphate using organic/inorganic interaction on the interface between oil and water phases. The microshells were prepared as follows. The organic phase composed of PLA (Mw = 20,000) and dichloromethane was poured in calcium acetate aqueous solution. The mixture was stirred at 300 rpm to yield emulsion at room temperature, then the beaker filled with the emulsion was set in an ice bath and was allowed to cool down for 30 min with stirring. Diammonium hydrogenphosphate aqueous solution was slowly added into the emulsion to precipitate calcium phosphate. After adding diammonium hydrogenphosphate aqueous solution, the beaker was taken out from an ice bath and allowed to return room temperature. The obetained microshells had a wide distribution ranging from 50 to 300µm and their wall thickness was 1 to 10µm. The internal wall of the microshells was smooth, contrary to this, the external wall have rough morphology, which indicate that the wall would have multiple layers. The external wall was identified as Dicalcium Phosphate Dihydrate (DCPD) by XRD. On the interface of the oil-in-water emulsion, DCPD would be precipitated on the nucleation sites of dissolved PLA in the oil droplets, which formed calcium phosphate microshell structure. After the precipitated DCPD stabilized the oil droplets, dichloromethane was volatilized and PLA would be deposited on the internal wall of DCPD microshells.

BI-TuP21 Control of Cell Shape, Cytoskeleton Structure, and Cell Migration Activity by using a Micro 3D Patterned Film, ***H. Sunami***, Hokkaido University, Japan; *E. Ito,* CREST, Japan Science and Technology Corporation (JST); *M. Tanaka, S. Yamamoto, M. Shimomura,* Hokkaido University, Japan

Recently we found that endothelial cells can proliferate rapidly on a micro 3D patterned film (honeycomb film). The cell shape and cytoskeleton structure on the honeycomb films were clearly different from those on a flat film. In order to elucidate the effect of honeycomb films as a 3D scaffold for cell culture, it is needed that the 3D observation of cell behaviors such as the morphological change, expression of cytoskeleton, expression of contact points on extracellular adhesion molecules, and migration on the honeycomb films during cell culture. In this research, effects of 3D honeycomb pattern on above cell behaviors were observed. The honeycomb films were fabricated by applying a moist air to a spread polymer solution containing an biodegradable polymer (poly(@epsilon@-caprolactone)(PCL)) and an amphiphilic polymer. The regular honeycomb structures with pore diameter of 10 µm and a wall thickness of 10 - 12 µm were prepared. The porcine vascular endothelial cells were cultured on the honeycomb films for 24 h at 37 °C. The cells were slender on the flat film, while the cells were spread widely on the honeycomb film. The cell migration activity on the honeycomb films was much lower than that on the flat film. The results of confocal laser scanning microscopic observation of CFSE-stained cytoplasm, the 3D shape of the cells on the honeycomb film was different from that on the flat film. Although the cells top-surfaces on both the honeycomb films and the flat films were flat equally, the bottom surface of cell body on the honeycomb films flagged approximately 1 - 3 µm along the honeycomb pores. The focal contact points (vinculin clusters) and actin cytoskeletons expressed strongly along the pores, too. These results showed the 3D structure of the honeycomb film effects on the cell shape, migration, and cytoskeletons. We will also report the relation between the 3D structure of honeycomb film and the good cell proliferation on the honeycomb film.

BI-TuP22 Nano-Sphere Lithography Based Chemical Nano-Patterns for Biosensor Design, ***A. Valsesia***, *P. Colpo, P. Lisboa, F. Rossi,* European Commission-JRC-IHCP, Italy

Patterning bio-molecules on biosensor platform surfaces is a cornerstone fabrication step for many applications ranging from medical diagnostics, environmental monitoring, food safety, and security applications to more fundamental themes such as cell-surface interactions. Many works are performed worldwide to develop advanced platforms with controlled surface chemistry and well defined nano-patterns. The goal is to be able to immobilize the biomolecules in an active state, avoiding non specific adsorption, in this manner the sensitivity and the specificity of detection is enhanced by surface densification of the recognition agents. An important consequence is the reduction of analyte volume needed for the detection. Among the different nanopatterning techniques nano-sphere lithography is a very flexible technique to produce nano-structured and chemically nano-patterned surfaces. Moreover this technique presents the advantage to be inexpensive and enable to produce nano-topography over large area surfaces. In this work, we present the fabrication strategy and the surface characterization of different types of nano-structures. In particular Poly Acrylic acid (carboxylic functional) nano-domes in anti-fouling matrix have been fabricated by combining colloidal lithography and Plasma Enhanced Chemical Vapor Deposition and carboxylic terminated nano-spots in an anti-fouling matrix have been produced by combining nano-sphere lithography and Self Assembled Molecular Monolayers on gold. We show that these chemical nano-patterns are able to immobilize proteins selectively in the carboxylic functional nano-domains, leaving the anti-fouling matrix clear. Moreover immunoassay experiments were set-up showing that nano-patterned surface constrains the immobilization of the antibodies in a biological reactive configuration, thus significantly improving the device performances as compared to more conventional non-patterned surfaces.

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