SYMPOSIUM K

Engineering Biointerfaces via Cell-Interactive Materials

November 30 - December 1, 2005

Chairs

Prabhas Moghe
Dept. of Biomedical Engineering
Rutgers University
98 Brett Rd.
Piscataway, NJ 08854
732-445-4951

David Kaplan
Dept. of Chemical & Biological Engineering
Tufts University
4 Colby St.
Medford, MA 02155
617-627-3251

Susan Enders
Max-Planck-Institute & Process Development
Heisenbergstrasse 3
Stuttgart, D-70569 Germany
49-711-689-3424

Shrirang Ranade
325 Paramount Dr.
Raynham, MA 02767-0350
508-828-3203

* Invited paper
SESSION K1: Engineered Interfaces I

Chairs: David Kaplan and Prabhas Moghe

Wednesday Morning, November 30, 2005
Room 204 (Hynes)

8:00 AM K1.1

Multiphoton Excited Fabricated Nano and Micro Patterned Extracellular Matrix Proteins Direct Cellular Morphology.

Paul J. Campagnola1, Larry Cunningham1, George Pins2 and Katie Bush2; 1CCAM/Cell Biology, University of Connecticut Health Center, Farmington, Connecticut; 2Biomedical Engineering, Worcester Polytechnic Institute, Worcester, Massachusetts.

Designing highly functional scaffolds for tissue engineering requires a fundamental understanding of the mechanisms by which the three-dimensional architecture and the biochemical composition of the tissue scaffolds modulate cell adhesion, migration, and differentiation, as well as the regeneration of native tissue functions. Fabrication methods must thus be able to reproduce both the native topographic and biochemical composition of the extracellular matrix (ECM). To this end, we use multiphoton excited (MPE) photochemistry to create artificial ECMs directly from matrix proteins. The MPE process directly protein photocrosslinking with submicron precision by utilizing the inherent 3-D confinement of non-linear optical processes in analogy to intrinsic sectioning in a two-photon fluorescence microscope. Here, we investigate the morphology of human dermal fibroblasts adhered to photocrosslinked linear structures of fibronectin (FN), fibronogen (FG), and bone sialoprotein (BSP). These proteins were chosen in order to systematically investigate the roles of topography and ECM biochemistry on cell spreading, since fibroblasts bind directly to both FN and FG at RGD sites through integrins, whereas BSP provides no RGD sequences. Crosslinked patterns are created from parallel linear structures 700 nm in width, 200 microns in length, spaced by either 10 or 40 microns. Immunofluorescence staining of FN and FG was used to assay the functionally of crosslinked protein, the results indicate a high level of activity and specificity. The morphological metrics of orientation, elongation, and cell perimeter were then used to quantitate the resulting cellular behavior on the crosslinked protein patterns. These parameters reflect statistically similar differences for cells on RSA, relative to the statistically similar behavior on fibronectin and fibronogen. These analyses show that cells on the RSA patterns are constrained by mechanical guidance and orient in a parallel manner between linear substrates. By contrast, cells adhered to FN and FG both have a greater propensity to spread orthogonally across neighboring structures, suggesting the importance of biochemistry in governing the spreading behavior. Focal adhesion staining of cells on these surfaces also indicates a strong influence of ECM cues guiding the cell morphology. These findings are consistent with our hypothesis that the FN and FG protein structures are expected to direct cell adhesion and spreading, where the outcome results from a combination of cell-matrix and topographic interactions.

8:15 AM K1.2

Molecular Structure of Adsorbed Fibronectin at Bio-interfaces: Correlation with Integrin-Mediated Cellular Adhesion.


Materials in contact with biological media are instantly covered by proteins which adsorb from solution. This layer of adsorbed proteins, in turn, largely determines the interactions that occur at the cell-material interface. Fibronectin is an extracellular matrix (ECM) protein which preferentially adsorbs from serum and plays a major role in the adhesion and function of a wide variety of cells through its interactions with integrin receptors found on the cell surface. Recent studies using self-assembled monolayers (SAM) of different functional groups have elegantly demonstrated that the surface properties of bio-interfaces can be used to control fibronectin adsorption and thereby modulate cell adhesion and function. In the present study, we investigate the structure of adsorbed fibronectin and bovine serum albumin to various SAMs (X = OH, COOH, NH2 and CH3) and SiO2 using in-situ atomic force microscopy (AFM) and correlate it with quantitative measurements of a531 integrin-mediated cellular adhesion. The combination of the spinning disc apparatus and K562 erythroleukemia cells in the work represents a unique system which allows us to study the steady-state molecular interactions at the receptor-ligand level. Even with this simplified system, we demonstrate the complexity of the various interactions that exist at bio-interfaces and nanostructured materials. Understanding fundamentally the correlation between the nano-scale structure of functional proteins, such as fibronectin, and cell adhesion allow us to better optimize the design of material interfaces for biotechnological and tissue engineering applications. Supported by an Interdisciplinary Pilot Grant in Biomedical Research from the Institute of Medicine & Engineering (Univ. of Pennsylvania).

8:30 AM K1.3

Micropatterned Surfaces Directing α531 Integrin-Mediated Adhesion.

Timothy A. Petrie1, Nathan D. Gallant2, Jeffrey R. Capodona3 and Andres J. Garcia3; 1Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia; 2School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia; 3Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Integrin-mediated cell adhesion to extracellular matrix components triggers signals directing cell survival, growth, and differentiation. In particular, binding of α531 integrin to fibronectin controls bone and muscle cell differentiation. To enhance integrin-mediated cell adhesion, numerous groups have incorporated bioadhesive ligands, such as RGD, onto surfaces to promote integrin binding. However, binding of α531 to FN also requires the FN P14(15) energy site. Using micropatterned mixed self-assembled monolayers (SAM) of alkanethiols on gold, we have created model non-fouling surfaces with well-defined islands of selectively tethered FN recombinant fragments. An E. coli-based expression system was developed to produce wild-type and related derivatives of FN spanning the 9th-10th type III repeats which contain both RGD and PHRSN sites in the correct structural orientation (FNIII1-10). FNIII1-10 was selectively tethered to mixed SAMs of functionally terminated alkanethiols (ratio of HS-[CH2]11-COOH: tri(ethylene glycol)-terminated thiol, EGS3 presenting COOH anchors within a tri(ethylene glycol)-terminated thiol) via NHS/EDC chemistry. Tethered FNIII1-10 surface densities and weights were validated by IR, ellipsometry and antibody-based assays. The surface density of tethered FNIII1-10 increased with coating concentration until reaching saturation values. Confocalization-based cell adhesion assays revealed a significantly six-fold higher cell binding activity for the FNIII1-10 tethered surface compared to RGD-functionalized surfaces. More importantly, osteoblast adhesion to FNIII1-10 tethered surfaces was mediated by α531 integrin, while αv35 controlled adhesion to RGD. These results demonstrate enhanced adhesive capacity as well as integrin binding specificity of the FNIII1-10 mixed SAM surface compared to RGD tethered surfaces. These mixed SAM surfaces of tethered FNIII1-10 were micropatterned into circular adhesive regions using microcontact printing. Antibody staining revealed well-formed, equally-sized and spaced islands of FNIII1-10. Osteoblasts adhered and remained constrained to these distinct FNIII1-10 tethered islands, demonstrating the ability of this system to effectively control cell spread area, shape, and focal adhesion assembly. Finally, we are currently using these surfaces to evaluate the effects of cell shape, cell-cell contact, and integrin binding on downstream cell signaling pathways, adhesion strength, and expression of differentiated phenotypes.

8:45 AM K1.4

Large-Scale Carbon Nanotube Patterns as a New Substrate for Bioengineering Applications: Cell Growth and Protein Motor Assay.

Kyoung-Eun Byun1, Sungyou Park1, J. Y. Kim2, Yongdoo Park2, K. B. Lee3, Lori McFadden4, P. Bryant Chase4, Min-Gon Kim5 and Seunghun Hong6; 1Physics and Nano Systems Institute, Seoul National University, Seoul, South Korea; 2Department of Artificial Organ Center, Korea University, Seoul, South Korea; 3Department of Biological and Science and Molecular Biophysics, Florida State University, Tallahassee, Florida; 4Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology, Taejeon, South Korea.

Recently, carbon nanotubes (CNTs) have been drawing attention as a new biomimetic material due to their high surface-to-volume ratio and exotic mechanical properties. However, it is still extremely difficult to prepare large-scale patterned carbon nanotube substrates, which has been hindering their applications in tissue engineering, protein assays, etc. One promising method to prepare large-scale patterned CNT substrates can be “surface-programmed assembly” process (Nature 425, 36 (2003)), where surface molecular patterns guide the “substrate assembly” and ‘precision alignment’ of carbon nanotubes on a solid substrate. Using this method, we prepared micro- and nanoscale CNT patterns covering ~1 cm x 1 cm area of cover glass surfaces and successfully demonstrated the growth of mesenchymal stem cells and the in-vitro assay of protein motors on the prepared CNT patterns. Mesenchymal stem cells, which are the progenitors of new bone, cartilaginous and connective tissue cells in the body, show enhanced affinity to the carbon nanotubes on the substrate. The in-vitro motility assay has been performed using actin polymerizing myosin motor proteins on carbon nanotube patterns, where the motion of actin filaments is guided by the carbon nanotube patterns. The possible impacts and future implications of the CNT-patterned substrates in advanced tissue engineering also will be discussed.
9:00 AM K1.5
Cell-Interactive Materials Based on Thermotropic Liquid Crystals. Nathan A. Lockwood, Jeffrey C. Mohr, Lin Ji, Sean P. Palecek, Juan J. de Pablo and Nicholas L. Abbott; Chemical and Biological Engineering, University of Wisconsin, Madison, Wisconsin.

We have investigated the design of biomaterials to cells that are based on the use of low-molecular-weight thermotropic liquid crystals. The use of liquid crystalline interfaces forms the basis of new approaches to deliver physical cues to cells so as to control cell behavior and enable reporting of cell functions. We have developed a method that permits the culture of cells on interfaces of thermotropic liquid crystals and allows imaging of the reorganization of the extracellular matrix by cells. Our results indicate that the extracellular matrix couples to the orientations of the underlying liquid crystal in such a way that the appearance of the liquid crystal indicates the location and organization of the matrix. We also show that it is possible to use this coupling to observe the reorganization of the matrix by the cells. These results suggest new approaches to the culture of cells and measurements of cell-extracellular matrix interactions.

9:15 AM K1.6
Nanopatterned Surface Induced Endothelial Cell Behavior. John H. Slater1,2 and Wolfgang Frey1, 2; Biomedical Engineering, University of Texas at Austin, Austin, Texas; Center for Nano and Molecular Science and Technology, University of Texas at Austin, Austin, Texas.

Cellular attachment to surfaces mediated by focal adhesions is essential in development, differentiation, and survival of adherent-dependent cells. Focal adhesions, clusters of integrin receptors that recognize specific peptide sequences in extracellular matrix proteins, have shown to initiate intracellular signaling pathways that influence cellular proliferation, motility, adhesion strength, cytoskeletal arrangement, and phenotype. Using self-assembly methods and orthogonal chemical functionalization, we have fabricated nanostructured surfaces that provide well-defined nanoscale cellular attachment sites as small as 47 nm. These attachment areas then provide a well-defined number of integrin adhesions while the surrounding areas are non-adhesive. Using these surfaces, we have independently probed the effects of cellular attachment area, attachment spacing, and nanotopography on focal adhesion formation, cytoskeletal organization, cell spreading area, proliferation, and cell motility. We have demonstrated that endothelial cells (ECs) recognize and attach exclusively to the nanopatterns while ignoring the passive glass background. Even at 47 nm pattern size, proliferation is not significantly changed between a fibronectin control and patterns of different sizes. The varying surface nanopatterns, however, induce, over a seventy-two hour time period, a clear change in other cellular behavior based on pattern size, but only minimal changes are induced by topography and interadhesion site spacing. ECs seeded on patterns ranging from 68 to 372 nm in length display an order of magnitude increase in focal adhesion density when compared to non-patterned control surfaces. We suggest that certain surface patterns hinder the maturation process of focal adhesions and do not allow for the development of full-blown adhesions. Chemical Adhesion: Chemical Patterns. Patterns with 68 nm lengths induce increased fibroplasia formation and a strong decrease in actin stress fiber formation yet do not influence cell spreading area or proliferation changes. In contrast, ECs seeded on a slightly larger pattern, 93 nm, show few fibroplasia and an increase in cytoskeletal network formation. We have shown that EC behavior can be influenced via nanostructured cell-surface interactions without changing the proliferation rate. We suggest that the number of integrins allowed to aggregate into a focal adhesion site influences the attachment-dependent signaling and subsequent cellular phenotype.

10:00 AM K1.7
Engineering Biointerfaces to Control Cell Adhesion. Andres Jose Garcia; Mechanical Engineering, Georgia Inst. Technology, Atlanta, Georgia.

Cell adhesion to adsorbed extracellular matrix (ECM) proteins and adhesive sequences engineered on synthetic surfaces plays critical roles in biomaterial, tissue engineering, and biotechnological applications. Cell adhesion to these adhesive motifs is primarily mediated by integrin receptors that aggregate into focal adhesion plaques, integrin binding activates signaling pathways regulating cell survival, proliferation, and differentiation. While tethering short adhesive peptides derived from ECM ligands (e.g., RGD for fibronectin) promotes cell adhesion and function in several cell systems, these biomimetic strategies are limited by reduced biological activity compared to the native ligand, lack of specificity among integrins, and inability to bind non-RGD integrins. These limitations are of particular importance to tailoring specific cellular responses since different integrins trigger different signaling pathways. We have engineered biointerfaces that mimic the secondary and tertiary protein structure of fibronectin and type I collagen. These surfaces convey integrin binding specificity, focal adhesion assembly and signaling as well as both non-adhesive and adhesion, proliferation, and differentiation. A recombinant fragment of fibronectin spanning the 7th-10th type III repeats of fibronectin and encompassing the PHRSN and RGD motifs was tethered to polyelectrolyte supports to specifically bind alphabeta2 integrin and trigger focal adhesion assembly and signaling. Binding of this receptor is critical to osteoblast proliferation, differentiation, and matrix mineralization. To target alpha2beta1 integrin, a triple-helical collagen-mimetic peptide was tethered to GPECE polymers and this surface was tethered to model non-adhesive supports. These biomimetic surfaces supported alpha2beta1 integrin-mediated adhesion and focal adhesion assembly and directed osteoblast specific gene expression and matrix mineralization to higher culture media supports. Second-generation interfaces have been developed to display controlled fibronectin-/collagen-mimetic ligand mixed densities to independently target alpha3beta1 and alpha2beta1 integrins. These mixed ligand surfaces synergistically modulate cell adhesion activities. Finally, these approaches have been combined with micropatterning techniques to generate biointerfaces that control cell-substrate adhesive area and integrin binding. These biomolecular engineering strategies provide a basis for the rational design of robust biointerfaces that tailor adhesive interactions and elicit specific cellular responses for the development of bioactive implant surfaces, scaffolds for enhanced tissue reconstruction, and growth supports for enhanced cellular activities.

10:30 AM K1.8
Engineering Interfaces with Biomimetic Peptide-Amphiphiles for Controlled Cell Adhesion. Efrosini Kokkoli, Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, Minnesota.

Cell-matrix adhesion mediated by integrins regulates several aspects of cell behavior and is critical to cellular responses on biomaterial surfaces. While many integrins recognize a short Arg-Gly-Asp (RGD) motif present in several extracellular matrix proteins, integrin αβ3 requires a synergy sequence, Pro-His-Ser-Arg-Asn (PHRSN), present in the 9th type III domain of fibronectin (FN), in addition to its primary recognition sequence Gly-Arg-Gly-Asp-Ser-Pro (GRG DSP), present in the 10th type III domain of FN, for enhanced specificity and higher affinity binding. The focus of this work was to engineer αβ3 integrin-specific bioadhesive interfaces using supported bilayer membranes constructed from peptide-amphiphiles that mimic the adhesion domain of fibronectin. Novel peptide-amphiphiles were designed that contain both the GRG DSP and PHRSN sequences in a single peptide formulation, separated by a spacer. Cell adhesion, blocking assays, and confocal microscopy were used to evaluate human umbilical vein endothelial cell (HUVEC) response on biomimetic interfaces. The effect of the PHRSN, the spacer length between the GRG DSP and PHRSN in the new peptides, and incubation time on HUVEC adhesion to the new FN-mimetic peptide will be discussed.

11:00 AM K1.9
Biomimetic hydrogel materials from modular artificial proteins. Stephen Fischer, Lixin Mi, Sara Sundelaurz, Brian Chung and James L. Hardin; Chemical and Biomedical Engineering, Johns Hopkins University, Baltimore, Maryland.

De novo protein design and recombinant DNA methods have been used to develop a library of self-assembling proteins with useful biomaterials properties. These proteins are engineered to include molecular recognition elements that (1) direct their self-assembly into multi-component nanostructured hydrogels with tailored topology and (2) interact with the surface receptors of a number of different cell types in order to guide their growth and development. The overall protein design is based on a modular, multi-block architecture that includes independent inter-chain binding end domains and flexible biomimetic linker domains. The associated inter-block amphiphilic helices that serve as smart crosslinking agents of the hydrogel, whereas the central linker domain is a water soluble, disordered sequence that encodes specific binding and signaling functions of extracellular matrix (ECM) constituents. Production of these materials through recombinant DNA methods gives unmatched control over their specific structural and biofunctional attributes. Through the use of microscopic and cell proliferation assays, we show that these multi-functional hydrogels are capable of inducing appropriate cellular responses in a variety of human cell types. In doing so, we illustrate the utility of flexibility and modularity in a biomaterial as a means to induce desired cellular responses. Such a combinatorial approach to biomaterials for artificial ECM applications, in which the end user can choose from a library of bioactive modules, mixing and matching as needed, is a promising strategy for tissue engineering applications.
The ability to control the adsorption of proteins and the interaction of cells on a solid surface are important for the development of cellular biosensors, biomaterials, and high-throughput drug screening assays. The critical problem in spatially directing protein and cellular interactions at a surface is the rapid adsorption of a complex layer of proteins with high affinity for protein of contact with serum in cell culture or upon implantation in vivo. I will describe two different strategies for the synthesis of biologically-nonfouling polymer coatings that are applicable to diverse substrates. The first strategy involves coating a surface with an amphiphilic comb polymer that present short oligoethylene glycol side chains. A number of different fabrication methods to synthesize these nonfouling coatings will be described including spin-coating, dip-coating and surface-initiated ATRP. ATRP initiator immobilized on gold through the formation of an alkanethiol self-assembled monolayer (SAM) and on silicon oxide through a silane SAM. The second strategy involves the grafting of a stimuli-responsive biopolymer, derived from an oligomeric sequence found in mammalian elastin. This polypeptide, which we term an elastin-like polypeptide (ELP), is a biopolymer with the repeat unit Val-Pro-Gly-Ser-Pro, and exhibits a lower critical solution temperature (LCST) transition in aqueous solution. Surfaces grafted with these polypeptides exhibit a hydrophilic-hydrophobic transition in response to increased temperature or salt concentration. The change in the interfacial properties can be exploited to create dynamic substrates that enable reversible, triggered binding of proteins onto surfaces in response to an external stimulus. The implementation of both these nonfouling strategies in combination with nano- and micron-scale patterning methods is currently under investigation. This work is supported by grants from the National Science Foundation and the National Institutes of Health.

SESSION K2: Engineered Biointerfaces

11:30 AM #K1.10 Passive and Active Surfaces to Control Protein and Cell Interactions. Ashleigh Chilotti, Duke University, Durham, North Carolina.

1:30 PM #K2.1 Quantitative Modeling of Cell-Interactive Substrates Used in Biophysical Experiments. Camilla Mohriek1 and Eduard Arzt1,2; 1Institute for Metal Research, University of Stuttgart, Stuttgart, Germany; 2Max Planck Institute for Metal Research, Stuttgart, Germany.

Cell-interactive materials, such as dynamic and force-sensitive substrates, are increasingly used in biological experiments to measure cellular responses to mechanical stimuli. A prominent example of these sensors are elastic pillar substrates that can, for instance, be utilized to explore traction forces exerted by living cells or to determine the force distribution in actin networks of cytoskeletal fibers. Quantitative analysis of these experiments requires an analytic account of the interaction between the substrate and the biomaterials adhering to it. In this presentation, we will develop a theoretical description of the interaction between displacement and force in a pillar substrate and the forces acting in a fiber network adhering to the tops of the pillars. This description makes clear that pillar substrates are force gradient sensors rather than force sensors and shows that the entire displacement field of interconnected pillars must be known to evaluate the force distribution in the fiber network or the cell that adheres to these pillars. Furthermore, the theory is general and applies to networks of any geometry: regular and random networks, and also to any state of activation of the network including deformation of cells. Thus, the analysis of these experiments provides a basis for the quantitative understanding of the aforementioned experiments. We will show several examples of application of our theory and demonstrate that they can serve as guidelines for experiments that aim at evaluating forces in the cellular and sub-cellular regime.

2:00 PM K2.2 Chemistry and Nanophase Topography: Effects on Mesenchymal Stem Cell Functions. Aaron J Duglar-Tulloch1,3, Rena Bizios2,3 and Richard W. Siegel1,2; 1Materials Science and Engineering, Rensselaer Polytechnic Institute, Troy, New York; 2Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, New York; 3Rensselaer Nanotechnology Center, Rensselaer Polytechnic Institute, Troy, New York.

Modification of the chemistry and surface topography of nanophase ceramics can provide biomaterial formulations capable of directing the cellular functions of adherent cells. This effect is hypothesized to rely on the type, amount, and conformation of adsorbed proteins for the adhesion of mesenchymally-derived lineages (e.g., osteoblasts, fibroblasts, etc.). However, the mechanisms driving this response are not yet well-understood and have not been investigated for any of the relevant functions of mesenchymal stem cells (MSCs). Here, we demonstrate that nanophase lineages critical to the design of clinically efficacious biomaterials. The current study addresses these omissions by examining the in vitro adhesion, proliferation, and osteogenic differentiation of human MSCs as a function of chemistry and grain size, with particular attention to the protein-mediated mechanisms of cellular adhesion. To this end, alumina, titania, and hydroxyapatite substrates were prepared with 1500, 206, 50, and 24 nm grain sizes, characterized with respect to surface properties, porosity, composition, and phase, and seeded with MSCs. Adhesion was found to be dependent upon both chemistry and grain size. Specifically, adhesion on alumina and hydroxyapatite was significantly reduced on 50 nm substrates, while adhesion on titania substrates was independent of grain size. Investigation into the protein-mediated mechanism of this response identified vitronectin as the dominant adhesive protein, demonstrated random protein distribution across the substrate surface without aggregation or segregation, and confirmed the importance of the type, amount, and conformation of adsorbed proteins on cell adhesion. Minimal proliferation was observed on 50 and 24 nm substrates of any chemistry, which resulted in cellular densities orders of magnitude below those found on 1500 or 206 nm surfaces. Further, proliferation was up-regulated on 206 nm substrates, resulting in higher cellular densities than found on 1500 nm substrates after 14 days of culture. Osteogenic differentiation was not detected on 50 nm substrates throughout the 28 day culture period. In contrast, osteogenic differentiation was strongly enhanced on 206 nm substrates, occurring approximately 7 days earlier in greater amounts than observed on 1500 nm substrates. In summary, the current study elucidated the chemical and topographical cues necessary to optimize the vitronectin-mediated adhesion, proliferation, and differentiation of human mesenchymal stem cells on nanophase surfaces. These results further the fundamental understanding of surface-mediated cellular functions and provide information central to the design criteria for next-generation orthopedic and tissue engineering biomaterials. This work was supported by Philip Morris USA and the Nanoscale Science and Engineering Initiative of the National Science Foundation under NSF Award Number DMR-0117792.

2:15 PM K2.3 Endocytosis of SiO2 Nanowires and Interfacial Chemistry. Lidong Wang1, D. Zhang1, K. Dziwianowska2, G. A. Boah3, C. Berven1 and D. N. McIvor2; 1Physics, University of Idaho, Moscow, Idaho; 2Microbiology, Molecular Biology & Biochemistry, University of Idaho, Moscow, Idaho.

The controlled delivery of biologically-active molecules into mammalian cells has broad and significant applications, such as drug delivery and cell biology. For example, many drugs and compounds cannot permeate through cell membranes, i.e., endocytosis into the cell is prohibited. A method for sidestepping this obstacle is to use nanomaterials as carriers, or cargo holds for medicinal compounds. However, the endocytosis of nanomaterials is not well understood. In this study, we have developed a process for introducing SiO2 nanowires into the bovine epithelial cells to exploit receptor/ligand interactions employed by intercellular bacteria. Fibrocin, which is a normal protein produced by vertebrate animal, was applied as a molecular bridge to allow the penetration of the nanowires into the living cells. Scanning electron microscopy (SEM) images were taken to observe the nanowires in the cells after a certain duration time. After comparing the secondary electron SEM images to the back-scattered electron images, we can verify the amount of the nanowires in the cells quantitatively. X-ray mapping using energy dispersive spectroscopy was also performed to obtain the distribution of the individual elements from the cells. From these preliminary results, we believe that endocytosis of SiO2 nanowires into mammalian cells using fibrocin as a promoter is a promising process for introducing biological compounds into the living cells. Further studies on tailoring the interface of the nanowires to facilitate the desired chemical bonding with specific chemical structures is undergoing.


Directing the behavior of biological cells using nanoscale materials and devices has great promise for improved control and understanding of biological processes. In particular, it is envisioned that active cells such as stem cells and neurons would be a step towards
regenerative medicine by directing the body's own machinery to heal damaged tissues. However, biological signaling is largely mediated by chemical messengers, which have a finite reach and spatial control. With the advent of nanotechnology, it is now possible to create materials and structures with features much smaller than the size of a cell, and such an approach will be within the reach of scientists. The design and testing of a system of electrically addressable nano-scale reservoirs that can selectively release biological signaling molecules with spatial control of ~200 nanometers. This system is comprised of an array of hemispherical nano-scalars, each containing the signaling molecule, and the ability to precisely deliver the signal to a specific location in a living system. The reversible permeability of the lipid membrane under and applied electrical bias (electroporation) allows the cells held within the reservoir to be released into the surrounding medium for potential applications in tissue engineering. Due to the unique properties of lipid bilayers, the opening will then reseal. These devices are sufficiently small that such arrays of up to 1000 reservoirs may be located under a single cell, each of which may be loaded with a different biological signal. This chip represents a means to translate between electrophysiological and the chemical communication of cells, allowing direct investigation of the effects of signal delivery time, location, and composition upon cell behavior.

3:45 PM K2.5
Immunological Synapse Arrays: Control of T cell assembly and surface membrane patterns in multi-component patterned protein substrates. Junsang Do and Darrell Irvine

1Department of Chemical Engineering, MIT, Cambridge, Massachusetts; 2Biological Engineering Division, MIT, Cambridge, Massachusetts; 3Department of Material Science and Engineering, MIT, Cambridge, Massachusetts.

T cells are activated during the initiation of immune responses when they encounter protein receptor signals displayed on the surface of antigen-presenting cells (APCs). When a T cell interacts with its target APC, receptor pairs on the membranes of the two cells form an organized structure at the cell-cell interface termed the immunological synapse (IS). We sought to engineer surfaces that can 'replace' the APC to induce T cell receptors (TCR) of T cells to interact with targets in a manner to dissect the roles of the synapse's physical structure on its function in T cell activation. To this end, we developed a novel photoresist polymer that can be processed under mild conditions in order to pattern multiple proteins on surfaces without denaturation. A random terpolymer composed of photosensitive o-nitrobenzyl methacrylate (o-NBA), methyl methacrylate (MMA) and poly (ethylene glycol) methacrylate (PEGMA) was synthesized by free radical polymerization, and biotin was covalently attached to the hydroxyl end groups of the PEGMA repeat units. While the thin films of the copolymer were initially water-insoluble, on UV irradiation, carboxylates generated from the o-nitrobenzyl groups of the resist adhered the film to glass, through photo-induced hydrolysis of water at low pHs, but dissolved in aqueous solution having a pH > 6.6. This pH-dependent solubility enabled a 'lift-off' process that typically requires harsh processing conditions to be performed simply by increasing the pH. After lift-off, the film was dried in air. Utilizing the polyelectrolyte character of this resist, we developed a photolithographic process to enable segregated micro-scale patterning of two proteins on a surface without exposure of the biomolecules to conditions outside the narrow range of physiological pH, ionic strength, and temperature where their stability is greatest. This protein patterning technique was extended to patterning protein-conjugated nanoparticles to simultaneously control ligand presentation on multiple length scales. We applied this patterning approach to present two protein signals to T cells in defined surface patterns as 'immunological synapse arrays': the adhesion molecule ICAM-1 and a T cell receptor ligand anti-CD3. The structure of synapse surfaces was varied on micron and nanometer length scales and the effect of ligands spatial distribution on T cell activation was studied. T cells 'self-arranged' on surfaces presenting arrays of anti-CD3 surrounded by stop and go signals provided to T cells by these two ligands. In addition, receptors on individual cells accumulated in patterns defined by the pattern of ligands presented by the surfaces. The physical pattern of receptor presentation was found to allow for up-regulation and down-regulation of T cell activation responses, depending on the structure and composition of the synapse arrays.

4:00 PM K2.6
Surface-Patterned Poly(ethylene glycol) Nanohydrogels to Control Bacterial Adhesion. Peter Krako and Matthew R. Liber.

1CBME Dept., Stevens Institute of Technology, Hoboken, New Jersey; 2New Jersey Dental School, Newark, New Jersey.

We are interested in developing patterned poly(ethylene glycol) [PEG] hydrogels to mediate interactions between microorganisms and physiological systems. We used focused electron beam to crosslink PEG in order to create thin film hydrogels with diameters as small as 100 nm on glass and silicon. These can be patterned to modulate cellular/microorganism interactions by varying length scales ranging from subcellular to macroscopic. The degree of gel swelling can be controlled from unity to as much as fifteen times by varying the electron dose. We found that cells of the disease-associated pathogen Staphylococcus epidermidis are repelled by lightly crosslinked, high-swelling gels in a manner consistent with PEG's well-known anti-fouling properties. However, these bacteria adhere to heavily crosslinked, low swelling PEG hydrogels. Interestingly, we found that the bacterial cell colonies, which form on the low-swelling, adhesive PEG hydrogels, grow significantly more slowly than those on unmodified silicon or glass. Given the flexibility of e-beam processing, we created surface-patterned hydrogels with spatially modulated bioadhesiveness. These enabled us to localise Str. epidermidis adhesion further to the lateral extent to which bacterial colonies grow. Significantly, bacteria that adhered to these constrained regions failed to form biofilms. When bioadhesive regions in microns in lateral extent, comparable to the dimensions of individual S. epidermidis cells, were patterned into otherwise-repulsive surfaces, only one or two cells adhered to each adhesive region, and their proliferation stopped. In addition to providing a new experimental platform with which to study cell-material interactions, the ability to create precise nanoscale patterns of surface-associated bacteria should be useful to the development of novel biosensors, drug screening and delivery systems, and other nanofabricated analytical devices.

4:15 PM K2.7
Nanotubes as Cholesterol Nanotrap. Inder P. Batra, Bikash C. Gupta, Anthony J. Ciani and Purusottam Jenna

1Physics, UIC, Chicago, Illinois; 2Physics, Virginia Commonwealth University, Richmond, Virginia.

With the ultimate goal of removing excess cholesterol from human body without drug therapy, we present our speculative hypothesis and preliminary calculations for dealing with excess cholesterol through mechanical means based on nanoscience. The cause of high cholesterol is due to some defect in the receptors of the liver responsible for removing cholesterol from the blood. Frequently used statin drugs, work by deactivating an enzyme that is needed to produce mevalonic acid, the molecule from which cholesterol is made. Statin drugs operate by turning off the supply at the source. In as much as they are interfering with the natural processes of the cells, the side effect may be a chemical imbalance in the body. If one could devise a method of removing excess cholesterol from the body without interfering with its natural chemical processes, the chances of ill side effects may be minimized. Our calculations are directed towards such a discovery, a small step towards a long journey. Our direction is to take advantage of the advances in Nanotechnology (in particular, the properties of nanotubes) and explore the potential of the use of excess cholesterol through need for mechanical means. The aim is to study the feasibility of creating cholesterol nanotrap, which can be injected in the body as needed. They would trap excess cholesterol from the blood stream, liver and intestines and will be excreted. With this in mind, we have started rudimental studies on cholesterol and its interaction with metal atoms. We present the results for electronic structure and total energy calculations based on density-functional theory. We have optimized the geometric structure of an isolated cholesterol molecule, a medium-sized hydrocarbon-type entity (C27H46O). We found d(C=O) = 1.44Å and d(O-H) = 0.97Å. The HOMO and LUMO energy gap is calculated to be in the 5-6 eV range. It is thus clear that cholesterol molecule is insulating in character. We next investigated the interaction of cholesterol molecule with various metal atoms and found that P atom bonds with the O-H group with a binding energy of 3.2 eV. The O-H however continues to bind favorably with carbon nanotube. This suggests that functionalizing nanotube with P can be used to hook the cholesterol molecules. Once in the nanotube, the metal can be removed at a later stage by using another metal with a sufficient escape barrier to cholesterol molecules and thus keep the molecules trapped. The metal atom will draw them into the nanotube and the van der Waals interactions will prevent them from escaping. A nanotube will serve as a storage unit which can be excreted without interfering with any natural processes. We realize at this point there remain many unanswered questions. Our next step is to tackle them with combined forces from materials science community.

4:30 PM K2.8
Tunable Contact Guidance Using Feature Geometry of Biodegradable Substrates. Chris John Bettinger, Brian Orrick, Robert Langer and Jeffrey Borenstein

1Materials Science, MIT, Cambridge, Massachusetts.
Controlling cell orientation and morphology through sub-stratum mechanical cues is a phenomenon that is applicable to a wide variety of medical applications such as implants and tissue engineering scaffolds. Previous work in this field, termed contact guidance, has demonstrated the application of this cellular response on a wide variety of material substrates such as silicon, quartz, glass, and poly(dimethylsiloxane) typically using grid-groove geometries. One limitation of these studies in terms of biomedical applications is the choice of material. However, demonstrating contact guidance and topography in a biodegradable material platform is a promising strategy for control of cellular arrangements in tissue engineering scaffolds. Recent studies have investigated several approaches to advance contact guidance strategies and technology to more practical applications. Results include the improved response and control of the contact guidance of bovine aortic endothelial cells (bAECs) on microfabricated poly(glycerol-sebacate) substrates with minimum feature sizes of 500 nm. Cell alignment distributions were selectively controlled by simply adjusting the feature geometry of the microstructures. This new ability to control cell alignment, which we term directed contact guidance, showed no observable dependence on feature sizes across the interval of 1-5 microns. Directed contact guidance could lead to the development of engineered surfaces for implantable scaffolds to facilitate specific cell-cell interactions that promote new tissue formation.

4:45 PM K2.9 Engineering Polymeric Microstructures for Cell Alignment and Force Measurement. Yi Zhao and Xia Zhang; Department of Manufacturing Engineering, Boston University, Boston, Massachusetts.

The limited ability of regeneration and the scarcity of donors contribute to the need for a large pool of transplantable cardiac tissue. After being transplanted, the tissue needs to coordinate with the host both mechanically and electrically, for serving in whole or as part of the driving source for blood pumping. The contractility is thus a vital concern. Although dramatic progress have been made on contractility study of suspending and attaching cells, mechanical behavior of in vivo-like myocytes, as those in artificial tissues, still remains unknown. In this paper, we demonstrate an engineering polymeric microstructures array. This array can align the adhered cardiac myocytes, resulting in different morphology as the cells in vivo. Meanwhile, the force evolution of the cells can be achieved by observing the deflection of the embedded micro pillars. Such a substrate reflects the mechanical interaction in physiological situation, and is expected to give some insight in artificial cardiac tissue construction. Micrometer scale polymeric pillars with the elevated sidewalls were built by using a pressure-assisted micro molding process. PDMS (polydimethylsiloxane) was used in this study due to its transparency and biocompatibility. The prepolymer was spin-coated on a glass slide and degassed, followed by a thermal curing at 65°C for a partial crosslinking. Afterwards, a silicon mold with high aspect ratio holes was placed on top of the polymer, and pumped to a certain vacuum. After being held in vacuo for 5 minutes, the mold was brought back to atmosphere and experienced a complete thermal curing. Polymer pillars with the elevated sidewalls were formed on the substrate upon mold removal. The elevation of polymer into the mold is due to the internal pressure between the trapped air under the mold and the ambient air. The correlation between the resulting height and the operation parameters, material properties, and the lateral dimension is determined by theoretical and experimental approaches. Cardiac myocytes isolated from Wistar rats were cultured on the substrate. After 7 days culture, most cells accomplished the remodeling and had stretched out along the sidewalls, differing from the randomly oriented ones cultured on a plain substrate. This alignment is believed due to the extra area for cell attachment offered by the vertical surfaces, which avoid, to some extents, the artificial polarization of cells induced by conventional dishes, thus allowing a more in vivo-like cellular morphology. Meanwhile, the micro pillars were deflected dynamically upon cell contraction. The deflection map was achieved by comparing with a reference array and the mechanical forces distribution with a subcellular resolution was obtained by multiplication with a predetermined spring constant.

SESSION K3: Poster Session I. Engineered Biointerfaces

Chairs: Susan Enders, David Kaplan and Shrirang Ranade

Wednesday Evening, November 30, 2005

8:00 PM

Exhibition Hall D (Hyenas)

K3.1 Abstract Withdrawn

K3.2 Neuronal-diamond Interfacing. Oliver A. Williams1,3, Christian Specht1, Stephane Curat1, Ralf Schoepf2 and Richard B. Jackman1;
1 London Centre for Nanotechnology, University College London, London, United Kingdom; 2 Pharmacology, University College London, London, United Kingdom; 3 Institute for Materials Research, University of Hasselt, Diepenbeker, Belgium.

Diamond offers great promise for engineering bio-interfaces, since it is an inherently biocompatible material, with high chemical and physical stability, whose surface may be functionalized in a number of ways. In addition, it can be doped to reveal semiconducting properties, enabling the integration of electronic devices with cellular material. We have been investigating a range of methods for the attachment, and controlled growth, of neurons to diamond surfaces, as part of our programme aimed at the formation of diamond-based neural networks and their electronic interrogration. Proteins have been specifically patterned on diamond surfaces by micro-contact printing. Mouse cortical neurons were then cultured on these substrates. Neuron adhesion and outgrowth was specific for those areas of the diamond that had been stumped with laminin, resulting in ordered growth with high resolution over large areas. Neurons survived in culture for the duration of the experiment, and laminin patterns were stable for at least one week in culture. Other methods for patterning neural growth on diamond have also been explored; surface functionalization enables selective hydrophobic/hydrophilic regions to be formed, and focused ion-beam milling has been exploited for topographical etching. This paper will present results from each of these methods, and discuss the prospects for the use of diamond-based systems within cellular bio-sensing applications.

K3.3 Influence of nanotextured titanium surfaces on cellular behaviour. Ludovic Richet1, Hyun Y. Kwon1, Federico Rossell2, James Wuest1 and Antonio Nanci1;
1 Laboratory for the Study of Calcified Tissues and Biomaterials, Universite de Montreal, Montreal, Quebec, Canada; 2 Department of Chirurgie, Universite de Montreal, Montreal, Quebec, Canada; 3 Institut national de la recherche scientifique, Varannes, Quebec, Canada.

A long term problem associated with metal implants is their fibrosis enwrapment that decreases the tissue stability especially for bone implants, where the capsule limits bone integration with the implant. Past studies have developed a simple and versatile chemical treatment (reduction coupled with controlled reoxidation) to generate precisely sized and reproducible nanotopics. This technique enabled us to show the influence of nanotextured surfaces on cellular behavior [1]. Our objectives are to elucidated and optimized interactions of living cell pertinent to orthopedic and vascular prostheses with nanotextured surfaces. To obtain optimal nanotextured surface, we studied the influence of treatment variables (time, temperature...) on surface properties. Surfaces porosity and roughness were characterized by High Resolution Scanning Electron Microscopy (HR-SEM) and Atomic Force Microscopy (AFM). The growth of cell lines (muscular and bone cells) was measured on wide range of textured substrates of titanium. Nanotexture had little effect on cell adhesion but it altered markedly proliferation responses. Previous studies showed an early (6h) influence of nanotexture on cell proliferation, but these modifications are also specific to cell types. We observed that increasing the degree of nanotexturing inhibited fibroblast proliferation whereas osteogenic cells were stimulated. However, when the nanostructuring exceed a certain threshold level (with the appearance of microstructure), the growth of osteoblasts was inhibited. Studies by Quartz Crystal Microbalance (QCM-D) are showed influence of these topographies on serum proteins adsorption and can explain a part of cell behaviour modifications by nanotexture. This nanostructuring approach seems promising for limiting fibrosis and promoting hosts tissue regeneration and integration particularly for orthopedics devices. Bibliography [1] A. Nanci, J. D. Wuest, L. Peru, P. Gagnon, F. Rossell, V. Sharma, S.-Zalalutdini, and M. D. McKee, “Influence of titanium surfaces for covalent attachment of biological molecules,” Journal of Biomedical Materials Research, vol. 40, no. 2, pp. 324-35, 1998. [2] P. Tambasco de Oliveira and A. Nanci, “Nanostructuring of titanium-based surfaces upregulates expression of bone sialoprotein and osteopontin by cultured osteogenic cells,” Biomaterials, vol. 25, no. 3, pp. 403-413, 2004.

K3.4 Poly(ethylene oxide)-based Nanocollodids Made by Electrified Jetting for Biomedical Applications. Kyung-Ho Roh1, Sonsoles de Olayo2, David C. Martin2,3 and Joerg Lahann2,3;
1 Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan; 2 Macromolecular Science and Engineering, University of Michigan, Ann Arbor, Michigan; 3 Department of Materials Science and Engineering, University of Michigan, Ann Arbor, Michigan.

Electrofused jetting is a hydrodynamic process that uses electric fields to generate liquid jets. We employ this process to fabricate two-dimensional micro-fluidic channels to convey blood nanoparticles - notably, to particles of small length scales. Moreover, it can be used to manipulate materials distributed within the nanoobjects. Herein, we report the fabrication of water-stable poly(styrene oxide) nanocolloids using the jetting of polymer-precursor mixture solution followed by UV crosslinking.

Two jetting capillaries are employed in side-by-side configuration for parallel injection of two distinct polymer solutions under high electrical potentials. Moreover, by tuning the electrical conditions, the polymers in each solution are suppressed throughout the jetting process and results in binary Janus particles with sub-micrometer diameters. The anisotropic characteristic of the particles were confirmed with transmission electron microscopy (TEM), scanning electron microscopy (SEM), and scanning electron confocal microscopy. Moreover, this polymer nanocolloids can be modified with biological ligands. Our preliminary results show that particles with streptavidin-modified surface are selectively attached to patterned substrates under the use of the binary, nanocolloids for controlled interactions with living cells will be presented. Eventually, we foresee the binary nanocolloids to be of use for advanced drug delivery applications.

K.3.5 Nanoscale Mapping of Electrostatic and van der Waals Interactions on Silicon-Substituted Hydroxyapatite. Jennifer Vanvliet, Delphine Dean, Nelesse Patel, Claudia Botelho, M. A. Lopez, Serena Best, William Bonfield, and Christine Ortiz; 1Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; 2Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, Massachusetts; 3Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, United Kingdom; 4Laboratorio de Biomatérias, Instituto de Engenharia de Materiais, Porto, Portugal. Results of this study, the first time enhanced osteoblast (bone-forming cell) functions on nanophase compared to conventional gold size ZnO. Specifically, osteoblast adhesion was up to two times greater on nanophase compared to conventional ZnO. In this manner, the present study highlights the need to conduct further experiments on nanophase ZnO for orthopedic applications.

K.3.7 Increased Endothelial and Vascular Smooth Muscle Cell Adhesion on Nanostructured Titanium for Improved Stent Applications. Saba Choudhury, Karen Haberstroh and Thomas Jay Webster; Biomedical Engineering, Purdue University, Lafayette, Indiana.

Vascular tissue possesses numerous nanostructured surface features due to the presence of proteins such as collagen and elastin. Despite this fact, most vascular stents proposed to restore blood flow are smooth at the nanoscale. As the first step towards creating the next generation of vascular stent materials, the objective of this work was to investigate vascular cell (specifically, endothelial and vascular smooth muscle cell) adhesion on nanostructured compared to conventional commercially-pure (c.p.) Ti. Nanostructured c.p. Ti was created utilizing constituent c.p. Ti nanoparticles as opposed to conventional micron-sized particles. Results of this study showed that the first time increased endothelial and vascular smooth muscle cell adhesion on nanostructured compared to conventional c.p. Ti after 4 hours. In addition, compared to conventional c.p. Ti, endothelial and vascular smooth muscle cells had a more well spread morphology on nanostructured c.p. Ti. Results of surface characterization studies demonstrated similar chemistry but significantly greater root-mean-square (rms) surface roughness as measured by AFM for nanoparticles as compared to conventional c.p. Ti. For these reasons, results from the present study provided evidence that vascular stents composed of nanostructured compared to micron-sized particles may invoke cellular responses promising for improved vascular stent applications.

K.3.8 Modeling the Hydrodynamic Interactions Between Two Cells Rolling Along an Elastic Substrate. Alexander Alexey, Rolf Verberg and Anna C. Balas; Chemical Engineering Department, University of Pittsburgh, Pittsburgh, Pennsylvania.

Leukocytes adhesion and rolling within blood vessels are vital steps in the inflammatory response. Recent experiments have shown that hydrodynamic interactions occur between closely rolling leukocytes, significantly affecting their dynamic behavior. In particular, dynamic rolling slow down the rolling velocity and promote the recruitment of leukocytes from the free stream. We study the hydrodynamic interaction between two vesicles, which mimic blood cells. The vesicles roll along a compliant substrate due to an imposed flow. Each vesicle consists of an elastic shell that is filled with a viscous fluid. To model this multi-component system, we combine the lattice Boltzmann and lattice spring methods for the fluids and elastic solid, respectively. This hybrid technique allows for a dynamic interaction between moving elastic walls and the surrounding fluid. To mimic the adhesive interactions among the vesicles and compliant substrate, we consider vesicles that interact with each other and with the substrate through a depletion potential. We describe the effect by the properties of the vesicle-substrate and vesicle as the flow parameters, on the rolling dynamics of two closely placed cells. We modify the stiffness of the vesicles and substrate in order to determine how the mechanical properties can affect the interaction of the rolling vesicles. Our results show that depending on the vesicle’s substrate stiffness, the vesicles either attract each or separate. Furthermore, the decrease in rolling velocity greatly depends on the material properties of the vesicles and substrate, as well as on the channel width. In particular, in the narrow channel the rolling velocity increases as the vesicles approach each other; this is in contrast to the observations for the wide channels. Our results suggest that mechanical properties of the vesicle-substrate and cells play an important role in the hydrodynamic interaction and should be accounted for in an accurate description of leukocyte rolling. Moreover, the results can be applied to the design of micro-reactors that utilize elastic capsules to transport reagents and carry out the reactions.
K3.9
Molecular Imprinting of Polysialoxane Scaffolds for Selective Protein Binding. Jianxin Liu, M. S. Aderibigbe, Ryanui Lee and E. D. A. Pulcro; Bone Biomaterials Lab., Center for Biomedical Engineering, University of Kentucky, Lexington, Kentucky.

The increasing number of implants being used to restore or replace the functionality of tissues and organs necessitates designing implants to have better stability, performance, and longevity. Most events at the tissue-implant interface are uncontrolled and nonspecific in nature, eliciting a host response that may lead to tissue regeneration of the tissue. Hence a challenge for tissue engineering is the fabrication of materials that not only serve as scaffolds but also proactively stimulate tissue formation. In order to have specific protein interactions at the interface to stimulate specific cell responses, one potential approach is modification of the implant surface using molecular imprinting techniques. Molecular imprinting is a unique approach for fabricating synthetic materials with surfaces presenting sites for specific binding of molecules. The most common imprinting process is a non-covalent approach, in which functional monomers assemble with template biomolecule in solution and form a crosslinked network that locks in the shape and chemical functionality of the template molecule. Subsequent removal of this template molecule results in nanocavities with specific shape and defined arrangement of functional groups that make them selective for the template molecule. Methacrylate polymers are often used for imprinting, but inorganic materials, such as polysialoxanes, offer distinct advantages. The present study involved development and characterization sol-gel derived, foamed, molecularly imprinted polysialoxane scaffolds. Effects of materials processing parameters on the physical and mechanical properties of the scaffolds and properties of protein-imprinted scaffolds to selectively bind template molecules were investigated. Results indicate decreased porosity and surface area and increased compressive strength of the scaffolds with increasing amounts of acid, solvent, and surfactant. Examination of morphology using SEM revealed highly textured surfaces and cross-sections with meso- and macroporosity, which would enhance interaction of these materials with proteins and cells. In order to study the imprinting process, materials were loaded with 4-fluorescently-labeled lysozyme as a model template biomolecule. The amount of surface-imprinted protein and of rebound protein was proportional to the surface area available. Selectivity studies using a solution containing equal amounts of lysozyme and RNase, which has molecular weight similar to lysozyme, showed that the scaffolds still bound more than twice as much lysozyme as RNase. These findings indicate the potential of polysialoxanes for molecularly imprinting. Scaffolds properties and the imprinting process can be manipulated by varying composition of the materials. Thus these molecularly imprinted, foamed polysialoxanes are a novel biomaterial that could establish controlled protein and cell interactions at the tissue-implant interface.

K3.10
Engineering Biointerfaces and Bioactive Systems via Laser Direct Write Deposition of Biomolecules and Viable Cells. Anand Doraiswamy1, R. J. Narayan2, T. Lippert2, L. Urech2, A. Wokaun1, M. Nagel1, B. Hopp1, M. Dinescu1, R. Modl6 and D. O. Chrisey2; 1Bioengineering Program, School of Materials Science and Engineering, Georgia Institute of Technology, Atlanta, Georgia; 2Paul Scherrer Institut, Villigen PSI, Switzerland; 3Laboratory for Functional Polymers, EMPA Swiss Federal Laboratories for Materials Testing and Research, Dübendorf, Switzerland; 3Hungarian Academy of Sciences and University of Szeged, Dom ter 9, Hungary; 4Plasma and Radiation Physics, National Institute for Laser, Bucharest, Romania; 5US Naval Research Laboratory, Washington, District of Columbia.

We demonstrate a novel laser forward transfer approach termed matrix assisted pulsed laser evaporation direct write or MAPLE DW to develop two-dimensional patterns of various mammalian cells and biomolecules on plastic and polymers to assist in desorption. Paramount to the undamaged deposition of sensitive biomaterials by laser forward transfer is the complete absorption of UV, or other wavelength, laser energy in absorbing layers. In this paper, we show viable transfers with various mammalian cells (human osteoblasts, rat neuroblasts and mouse myoblasts) using the novel polymer coating. The triazene-polymers are ideally suited for this application as they are explosive polymers designed to absorb in the UV and decompose almost completely. These explosive fragments have the potential for forward transfer of sensitive biological materials, it can provide complete laser absorption with soft desorption. Our results showed that the triazene polymer was biocompatible and supported adherent cell growth. However, these polymers were not suitable for transferring directly the cells over the fluence and thickness range studied. Instead, an intermediate layer of extracellular matrix was required. In this paper, we demonstrate the potential of MAPLE DW technique for engineering CAD/CAM generated bioactive systems and biological interfaces for a wide range of biomedical applications.

K3.11
Effects of Hydroxyapatite Crystal Morphology on Osteoblast-like Cell Response in Two- and Three-Dimensional Substrates. Micah R. Rogel1, Joseph A. Van Naude2, Carmen J. Narvaez2, Jo Ellen Welsh3 and Ryan K. Roeder1; 1Department of Aerospace & Mechanical Engineering, University of Notre Dame, Notre Dame, Indiana; 2Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana.

Hydroxyapatite (HA) shows promise as a reinforcement phase in synthetic orthopedic biocomposites due to a close resemblance to bone mineral, as well as demonstrated biocompatibility and bioactivity. Polymers reinforced with HA whiskers have exhibited improved biomechanical properties compared to conventional HA powders. Preliminary studies have also indicated that HA whiskers are at least as biocompatible as conventional HA powders. Osteoblast-like cells (MC3T3-E1) were cultured on the two-dimensional (2D) surface of HA whisker and powder compacts. On both types of surfaces, cells appeared elongated, with increased spreading apparent on whisker substrates. Cell attachment was significantly greater on whisker substrates after 24 h in culture. Cell viability (MTT assay) was not statistically different between either type of substrate after 24 h in culture. Therefore, this study investigated cell attachment, proliferation and differentiation on 2D HA substrates over longer time periods. Osteoblast-like cells (MC3T3-E1) were cultured for 1, 3, 7 and 14 days on the surface of uniaxially pressed or traditionally sintered HA whisker and powder substrates. Scanning electron microscopy (SEM) was used to image the substrate surface topology, which reflected the HA whisker or powder morphology. HA whiskers were approximately 18 x 2 micrometers, while the powder comprised plate-like crystals approximately one micrometer wide. After each culture period, cells were fixed in glutaraldehyde, stained with 0.1% crystal violet, and counted using an optical microscope in order to examine cell morphology. MTT assay showed greater cell viability on HA whiskers by day 3; however, by day 14, cells had reached confluence on both substrates. Cells on HA whisker substrates demonstrated slightly higher alkaline phosphatase activity than cells cultured on the powder. In order to more closely mimic an in vivo physiological environment, osteoblasts were also cultured in novel three-dimensional (3D) scaffolds composed of a collagen hydrogel reinforced with either HA whiskers or powder. Cell differentiation and differentiation were characterized using the MTT assay and measuring alkaline phosphatase activity. Confocal microscopy and SEM were used to examine the morphology of 3D matrices with and without cells present.

K3.12
Probing Transepithelial Substrate Permeation with Sub-Cellular Lateral Resolution. Petra Göring4, Ralf Wehrspohn4, Christina Rommel4, Jan Endell4, Joachim Wegener5, Hans-Joachim Gall4, Claudia Steinem3 and Andreas Janushoff2; 2MPI of Microstructure Physics, Halle, Germany; 3Department of Physics, University of Paderborn, Paderborn, Germany; 4Institute of Analytical Chemistry, University of Regensburg, Regensburg, Germany; 5Institute of Biochemistry, University of Muenster, Muenster, Germany; 4Institute of Physical Chemistry, Johannes Gutenberg University Mainz, Mainz, Germany.

Measuring substrate permeation across epithelial cell layers in vitro is important for both, fundamental research addressing the molecular mechanisms of epithelial barrier function as well as for applied studies of drug absorption and drug targeting. In current in vitro approaches cell layers are grown to confluence on permeable filter membranes, the compound of interest is applied to one side of the cell layer and its concentration in the opposite compartment is determined as a function of time. To measure transepithelial transport with detailed lateral resolution, epithelial cells are grown on nano-porous silicon wafers. If needed, the substrates can be coated with adhesive proteins. These protocols are not only used to assist in desorption, but also on the substrate surface (depth 10 micrometres) serve as a lateral array of capillaries in which the permeation probe is collected after permeation across the cell layer. The lateral density of the pores is sufficient to distinguish paracellular from transcellular transport. The diameters of the pores are exactly defined and do not differ on one substrate. Pore diameters and density can be tailored to the cell size. We assume that the interactions between the cells and the paths between pores are similar to those on plane, so the spreading the diffusion under the cells is not totally free, but limited by increased viscosity boundary water layers. This reduces the problem of lateral diffusion between cell and substrate surface. Once a drug molecule enters the porous path of the substrate, lateral diffusion is impossible, the information is collected. As the lateral diffusion in the capillaries of the silicon chip is impossible, defect areas produce only local artifacts but do not interfere with readings at a different position in the cell layer. The method is open to other read
out techniques, like Confocal Laser-Scanning Microscopy (CLSM), Scanning Ion Conductance Microscopy (SICM) and Time of Flight Secondary Ion Mass Spectroscopy (ToF-SIMS), especially with gold, make electrical investigations available.

**K3.19**

Micropatterned cell co-cultures using layer-by-layer deposition of extracellular matrix components. Junji Fukuda1, Ali Khademhosseini2,3, Judy Yeh1, George Eng1, Jianjun Cheng1, Omid Farokhzad2 and Robert Langer1,2. 1Chemical Engineering, MIT, Cambridge, Massachusetts; 2Harvard-MIT Division of Health Science and Technology, MIT, Cambridge, Massachusetts; 3Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts.

Micropatterned cellular co-cultures can be used to control both the degree of homotypic and heterotypic cell-cell interactions in culture and the spatial organization of multiple cell types in relation to each other. Most approaches to fabricate patterned co-cultures have been conducted on micropatterned surfaces with different properties such as hydrophilic/hydrophobic. However, many synthetic polymers used in these systems are not optimized for interactions with cells and lack biological function associated with natural extracellular matrices. In this work, we present a novel method of fabricating biomimetic patterned co-cultures based on layer-by-layer deposition of three biocompatible extracellular components: hyaluronic acid (HA), fibronectin (FN) and collagen. In this scheme, cell-repellent HA was micropatterned on a glass hydrophilic substrate. The exposed substrate was then coated with FN to generate cell adhesive islands. Once the first cell type was immobilized on these adhesive regions, subsequent electrostatic adsorption of collagen to HA patterns switched the non-adherent HA surfaces to adhere, and thereby facilitated the adhesion of a second cell type. Patterned co-cultures of either hepatocytes or embryonic stem cells with fibroblasts were created. This technique utilizes non-cytotoxic, native extracellular matrix components of high biological affinity. This biocompatible co-culture system may be a useful tool to study cell-cell communication and cell-matrix interactions in culture, and for tissue engineering applications.

**K3.14**

Amphiphilic Block Copolymer Micelle Thin Films for Nanostructured Protein Delivery. Ryan D. Bennett1, Naomi T. Kohern2, Robert E. Cohen3, Darrell J. Irvine2,3 and Paula T. Hammond1. 1Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; 2Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; 3Biological Engineering Division, Massachusetts Institute of Technology, Cambridge, Massachusetts.

The ability to pattern proteins at the nanoscale has gained significant interest for the applications of biological sensors, protein arrays and as biomaterials to study cell function and behavior. We are investigating the self-assembly and amplification of collagen amphiphilic micelle thin films as a platform for nanoscale protein patterning for such biotechnological applications. Poly(styrene-block-acrylic acid) (PS-b-PAA) forms micelles in organic solvents that can be spin-coated onto a substrate to form arrays of spherical micellar domains within a PS matrix. On exposure to aqueous solutions, these substrate-assembled micelles reorganize to expose carboxylic acid groups of the PAA domains at the film surface, which serve as sites for subsequent covalent or noncovalent protein immobilization. In this strategy, the size, center-to-center spacing, and areal density of PAA domains are key features of interest for protein presentation from these functional group nanoclusters. To investigate these parameters, we determined the effect of PS-b-PAA molecular weight, loading of metal salts into the micelles, the addition of PS and PAA homopolymer into the micellar solution, and the mixing of different micellar solutions on surface microncale structure. Through these routes, we demonstrated the ability to tune the size and morphology of micellar thin films from 4.7 nm to 16 nm and spatial density from 6 x 10^{10} to 6.5 x 10^{10} micelles per cm^{2}. We also used PDMS based soft lithographic techniques to introduce micropatterned patterning of these nanostructured micelle films. The control of the areal arrangement of these carboxylic acid functionalities demonstrates the potential of this system as a biomaterial.

**K3.15**

Increased Osteoblast Functions on Undoped and Yttrium-doped Nanocrystalline Hydroxyapatite Coatings on Titanium. Michiko Sato1, Marisa A. Sanibio2, Arash Aslan3, Nader M. Kheshtkar1, Ellen B. Shumanovich2,1, and Thomas J. Webster1,1. 1School of Materials Engineering, Purdue University, West Lafayette, Indiana; 2Spire Biomedical Corp., Bedford, Massachusetts; 3Weldon School of Biomedical Engineering, Purdue University, West Lafayette, Indiana.

In order to improve orthopedic implant performance, the objective of this in vitro study was to analyze the relationship between nanocrystalline hydroxyapatite (HA) powder and using such powders to coat titanium. HA was synthesized through a wet chemical process. The precipitated powders were either sintered at 1100 °C for 1 h in order to produce UltraCap HA (200 °C or more) or were treated at 200 °C for 20 h to produce nanocrystalline HA size HA. Some of the UltraCap and nanocrystalline HA powders were doped with yttrium (Y) since previous studies demonstrated that Y-doped HA in bulk implant and osteoblast (or bone-forming cell) function. The original HA particles were characterized using XRD, ICP-AES, BET, a laser particle size analyzer, TEM, and SEM. These powders were then deposited onto titanium by a novel room-temperature process, called IonTite. The properties of the resulting HA-coatings on titanium were compared to respective properties of the original HA powders. The results showed that the chemical and physical properties of the original HA powders were retained when coated on titanium by IonTite, as determined by XRD, ICP, TEM, and SEM analysis. More importantly, results showed increased osteoblast adhesion on the nanocrystalline HA IonTite coatings compared to non-doped HA. These results encourage further studies on nanocrystalline HA IonTite coatings on titanium for improved orthopedic applications.

**K3.10**

Surface Immobilization of Anti-Inflammatory Agent on Chronic Neutral Implants for Enhanced Neuro-Integration. Wei He and Ravi V. Bellamkonda. Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Implanted silicon microelectrode arrays (Si-MEAs) are neural implants with great potential to enhance both fundamental knowledge of plasticity and physiology, as well as in the treatment of central nervous system (CNS) trauma by providing single cell recordings in the adult cortex. One of the greatest obstacles to such potential is the instability of the implant-host interface due to astroglial scarring response. The glial scarring adversely impacts the function of Si-MEAs by electrically and mechanically isolating them from the neurons. One strategy is to locally release anti-inflammatory agents at the site of injury. However, due to micromotion, it is likely that Si-MEAs in the brain cause a continuing chronic injury at the site of implantation, decreasing the likelihood that short-term anti-inflammatory therapy would have long-term functional consequences. To address this issue, we functionalized the surface of Si-MEAs by immobilizing an anti-inflammatory neuropeptide a-melanocyte stimulating hormone (a-MSH) and evaluated the performance of the modified implant both in vitro and in vivo. Chemical composition of the modified surface was verified by X-ray photoelectron spectroscopy (XPS). We observed that a-MSH grafted silicon surface inhibited the production of nitric oxide and lipopolysaccharide (LPS)-activated microglia in vitro, demonstrating that the immobilized neuropeptide remained biologically active. The gene expression of pro-inflammatory cytokines, tumor necrosis factor alpha (TNF-a) and interleukin-1 (IL-1), was measured by qPCR using real time quantitative reverse transcriptase polymerase chain reaction (RT-PCR). The results indicated that the immobilized a-MSH reduced the expression of both TNF-a and IL-1 in response to LPS activation. We implanted both bare and a-MSH coated microelectrodes into adult rat brains. Reactive tissue response was assessed by quantitative immunohistochemistry for GFAP (astrocytes) and ED-1 (microglia). The presence of a-MSH reduced microglial reaction 1 and 4 weeks following surgery. The astrocytic response was also weakened 4 weeks post surgery. These results suggest that surface immobilization of anti-inflammatory agents may provide a means to attenuate long-term brain tissue response to chronic neural implants, leading to a stable implant interaction.

**K3.8**

Artificial bone synthesis using genetically engineered viruses. Seong-Wuk Lee1,2,3, Soo Kwan Lee4, Je Song2,1, Angela M. Belcher5 and Carolyn R. Bertozzi1,2. 1Chemistry, University of California, Berkeley, Berkeley, California; 2The Molecular Foundry, Lawrence Berkeley National Lab, Berkeley, California; 3Biomedical Engineering, University of California, Berkeley, California; 4Department of Materials Science and Engineering, MIT, Cambridge, Massachusetts; 5Biological Engineering Division, MIT, Cambridge, Massachusetts.

Artificial bones were synthesized using genetically engineered viruses. Genetically engineered M13 bacteriophage (virus) was used as a template to nucleate hydroxyapatite crystals. Phage display was used to identify hydroxyapatite binding peptides. The cores containing one billion different amino acid sequences expressed either
on pHIII or on pHVIII coat proteins of M13 bacteriophage, was screened to find specific binding moieties against single crystalline hydroxyapatite surfaces. After four rounds of selection, a pseudorepetitive consensus amino acid sequences possessing periodic hydroxyl side chains in every two or three amino acid sequences were obtained. These sequences resembled the Gly-Pro-Hyp repeat of human I collagen, a major component of extracellular matrices of natural bone. In addition, a consistent presence of basic amino acid residues was also observed. These peptides were synthesized and then used to template the nucleation and growth of hydroxyapatite nanocrystalline matrix mineralization. XPS, FTIR, and SEM (and EDX) were used to characterize the shape and orientation of mineral growth of the peptide-mineral composites. These mineral binding peptides are expected to be further incorporated into 3-dimensional biomimetic bone-like materials.

K3.18
Characterization of a bioactive nanotextured surface created by controlled chemical oxidation of titanium. Ji-Hyun Yi1, Fabio Variola1, Sylvia F. Zalal1, Caroline Bernard1, James D. West3, Federico Roselli2 and Antonio Nanci1; 1Laboratory for the Study of Calcified Tissues and Biomaterials, Faculté de Médecine Dentaire, Université de Montréal, Montréal, Québec, Canada; INRS-EMT, Université du Québec, Varennes, Quebec, Canada; 3Department of Chirurgie, Université de Montréal, Montréal, Quebec, Canada.

Events at bone-implant interfaces are influenced by implant surface properties. Our previous work has revealed that a nanotextured titanium surface, obtained by controlled chemical oxidation, enhances osteogenic activity. To better understand the biological effect of the underlying surface properties, a more detailed characterization of the surface is essential. With this aim, the morphology, structure and chemical composition of the nanotextured titanium surface, derived from controlled chemical oxidation using a H2SO4/H2O2 mixture, were thoroughly investigated. X-ray photoelectron spectroscopy (XPS) combined with grazing angle Fourier transformed infrared (FTIR) spectroscopy revealed that the oxidized Ti surface consists of almost pure TiO2 with a Ti:O ratio of 1:2.08. X-ray diffraction (XRD) patterns indicated that the chemically treated Ti surface is composed of crystalline grains of anatase, with a mean size of ～25 nm. Scanning Electron Microscopy (SEM) observations clearly showed that the treated titanium substrate acquires a surface with a high porosity consisting of micrometer-sized pits, of which the average diameter and fractal dimension are 22.4 ± 7.0 nm and 1.2, respectively. Atomic Force Microscopy (AFM) revealed a three-fold increase in RMS roughness. The thickness of the oxide layer on the treated Ti surface is estimated to be ～40 nm. Finally, our results suggest a two-layered oxide structure on the treated Ti surface, an inner dense layer and an outer porous layer.

K3.19
Myelin-mimetic surfaces for cell adhesion. Amit Banu and Anastasia Murphy; Chemistry, Brown University, Providence, Rhode Island.

Carbohydrate coated surfaces have potential applications as substrates for cell adhesion and growth. A carbohydrate-carbohydrate interaction (CCI) between glycolipids mediates the compaction of the myelin sheets. We have developed methods for attaching myelin carbohydrates to glass slides via a mild and efficient copper catalyzed dipolar cycloaddition process. These slides can be thought of as minimally myelin-mimetic surfaces, and they provide a platform for examining the carbohydrate mediated adhesion of various neural cells. Details of the surface preparation, characterization, and evaluation of their cell adhesive properties will be described.

K3.20
Controlled Co-Cultures of Embryonic Stem Cells with Embryonic Feeder Cells. Judy Yeh1, Ali Khademhosseini2,3, Lino da Silva Ferreira1, Jeffrey Karp4, Junji Fukuda5, George Eng6, Jay Gantz4, Robert Langer5,1,2,3; 1Biological Engineering Division, Massachusetts Institute of Technology (MIT), Cambridge, Massachusetts; 2Division of Health Science and Technology, Harvard-MIT, Cambridge, Massachusetts; 3Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts; 4Department of Chemical Engineering, MIT, Cambridge, Massachusetts.

Embryonic stem (ES) cells have great potential in regenerative medicine because of their ability to differentiate into all cell types of the body. Currently, co-cultures of ES cells with mouse embryonic feeder (MEF) cells are widely used to propagate human ES cells in culture. MEF cells provide cues that induce self-renewal and maintenance of ES cells. Novel ways of controlling the degree of homotypic and heterotypic cell-cell interactions and the ease with which ES cells are retrieved from these cultures may be beneficial in ES cell studies. In this work, we present a novel method of fabricating co-cultures of ES cells with feeder cells with control over properties such as size and shape of ES cell colonies. The approach is based on using the topographical features of a substrate to physically control the formation of ES cell aggregates on monolayers of feeder cells. PDMS substrates were formed with topographical features in the shapes of wells or lanes ranging in diameter from ～20 μm to ～300 μm. Monolayers of feeder cells were formed on these substrates so that they could be subsequently seeded onto these cultures, and based on the dimensions of the microwells, their size, shape, and the degree of aggregation could be controlled. ES cells forming these cultures can thus be prepared in a homogenous manner. It is anticipated that these co-cultures may provide an advance over present systems of three-dimensional culture and also enhance cell function. Thus, this approach may be a useful tool for studying cell behavior and for tissue engineering applications.

K3.31
The Development of Tailored 3-D Microenvironments for the Study of Valvular Interstitial Cells. Darshita Shah1 and Kristi S. Anseth2,3; 1Chemical and Biological Engineering, University of Colorado, Boulder, Colorado; 2Howard Hughes Medical Institute, Boulder, Colorado.

While cellular response to various factors is frequently studied on tissue culture plastic, what is observed in vitro may not occur in vivo due to the disruption of cell contact with their natural extracellular matrix (ECM). Thus, to gain a better understanding of cellular processes such as differentiation, there is a need for the development of 3D cell microenvironments where cell function can not only be maintained and studied, but also can be controlled. We have developed a method for producing tailored microenvironments (VIMCs) that have the unique ability to differentiate into myofibroblasts, a phenotype present during valve development and repair and characterized by the expression of α-smooth muscle actin (αSMA) and the secretion of large amounts of extracellular matrix proteins. The objective of this study was to develop a hydrogel niche that would permit VIC viability and promote the activation of VICs to a myofibroblast phenotype. VICs have been photoencapsulated within poly(ethylene glycol) diisocyanurate (PEGDM) hydrogels; however, because PEGDM is a relatively non-adhesive substrate, additional components were incorporated into the hydrogel structure to promote the survivability of adherent dependent cells such as VICS. Methacrylated hyaluronic acid (HA), which is known to possess specific binding interactions with fibronectin, was incorporated into the networks at 1.1 wt%, 5.5 wt%, 10 wt%, and 20 wt% of the total macrocollamer (10 wt%). VIC survival increased with increasing HA content. Transforming growth factor-β (TGF-β), an activator of the VIC myofibroblast phenotype, was delivered to constructs at 1-100 ng/mL levels and VIC αSMA expression was assessed. Results related to our most recent efforts in this area will be presented. 1. Malliaris D, Shah DN, Walker G, Leimwand LA, Anseth KS. Designing scaffolds for valvular interstitial cell: cell adhesion and function on naturally derived materials. J Biomed Mater Res 2004; 71A(1): 172-80. 2. Walker GN, Masters KS, Shah DN, Leimwand LA, Anseth KS. Valvular myofibroblast activation by transforming growth factor-beta: implications for pathological extracellular matrix remodeling in heart valve disease. Circ Res 2004; 95(3): 253-60.

K3.22
Photocrosslinkable Hyaluronic Acid for Surface Micropatterning. George Eng1, Ali Khademhosseini2,3, Judy Yeh1, Junji Fukuda1, James Blumling1, Jeanne Morin-Leisk1, Jay Gantz4, Robert Langer1,2,3; 1Department of Chemical Engineering, Massachusetts Institute of Technology (MIT), Cambridge, Massachusetts; 2Division of Health Science and Technology, Harvard-MIT, Cambridge, Massachusetts; 3Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts; 4Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania.

Surface micropatterning is potentially useful for a variety of applications ranging from cell biology to high-throughput screening. Typically, synthetic materials such as poly(ethylene glycol) have been used for surface patterning. Natural polymers such as polysaccharides may have potential advantages for biological surface patterning because they are natural components of the cell microenvironment and are biologically degradable. In this study, we have used hyaluronic acid (HA) comprised with methacrylate side chains to enable the photocrosslinking of the material for micropatterning applications. HA micropatterns were generated either as two-dimensional patterns or as three-dimensional microstructures using a capillary molding process. To generate two-dimensional patterns, bioresistant HA was spun coated on methacrylated glass to form thin films. A patterned
PDMS mold was then placed in conformal contact with the HA film and crosslinked by exposure to UV light. Proteins such as fibronectin and albumin are known to enhance the generation of cell arrays through cellular adhesion on these protein-coated regions. To generate three-dimensional HA hydrogel microstructures, thicker films were molded with a PDMS stamp and then crosslinked by UV exposure. The HA microstructures enabled the formation of arrays of cells aggregated of embryonic stem (ES) cells or hepatocytes. Subsequently, ES cells or hepatocyte aggregates can be directly immobilized within molded HA microgels and subsequently exposed to over time. These soluble hydrogels may be a useful material for bioMEMS-related applications.

**K.3.23** Nano-featured Collagen and Nanohydroxyapatite Composite Scaffolds for Bone Tissue Engineering. Vinoy Thomas,1 Sunita Jagannath2,3, Kolaanda Johnson2, Moncy Jose2, Derrick Dean2, William Clem2,4, Susan L. Bellis2,5, Shane A. Catledge1 and Yogesh K. Vohra2,6,1 Department of Physics, University of Alabama at Birmingham, Birmingham, Alabama; 2 Department of Chemistry and BioScience, University of Alabama at Birmingham, Birmingham, Alabama; 3 Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, Alabama.

An ideal 3D-scaffold for tissue engineering requires resemblance to natural counter parts in terms of chemistry, physical nano-features and bio-performance. While most conventionally fabricated scaffolds are synthetic polymers, the cells do not necessarily recognize these surfaces. In contrast to synthetic polymers, collagen has an especially favorable surface for cellular attachment. Collagen is a major natural component, and is designed to give fibrous scaffolds with fiber bundles varying in diameter from 50-500 nm. The collagen fibrous structure is organized in a three-dimensional network with fibers that are formed hierarchically by nanometer-scale multi-fibrils. The physicochemical properties allow attachment and retention of osteogenic cells, to be presented to the cells in the microenvironment. Integrin binding is critical for the necessary cell-ECM communication that is lacking when synthetic polymers are used. A combination of nano-fibrous collagen and nanohydroxyapatite (nanoHA) that mimic the nano-scale features of ECM could be more promising system for next generation scaffolds for bone tissue engineering. Therefore, nano-featured composite hydrogels of type I collagen and nanoHA of varying compositions were prepared by electrospinning and used to resemble mineralized bone tissue. Electrospinning produced well interconnected-porous structure and nonwoven composite fibrous mesh of collagen-nanoHA having fiber diameters ranging from 70 to 1000 nm and a large surface to volume ratio. Determination of mechanical properties (tensile) of collagen mesh showed an average tensile strength of 2.3 MPa and average modulus of 28 MPa. Chemical crosslinking of collagen and presence of nanoHA influenced further the mechanical stability. The collagen and collagen-nanoHA fibrous materials exhibited improved tensile properties than PLA and PLGA-nanoHA. In vitro biodegradation/mineralization and in vitro cell culture studies of these nanofibrous scaffolds using mesenchymal stem cells and chondrocytes demonstrated that nano-structured scaffolds anticipate excellent cell proliferation, migration, and differentiation of the cells. We acknowledge the support by NSF-NIRT program under DMR-0402891 and UAB Centre for Metabolic Bone Disease (CMBD).

**K.3.24** Genetically Engineered Peptide-Based Molecular Constructs for Functional Immobilization. Cagdas Tamerler1,2, Turgay Kacak1,2, Mustafa Gungor1,2, Melvin Zim1, Xiaorong Xiong3, Parviz Amir Babak4, Alex Jen2 and Mehmet Sarikaya1,2,1 Molecular Biology & Genetics, Istanbul Technical University, Istanbul, Turkey; 2 Materials Science and Engineering, University of Washington, Seattle, Washington; 3 Electrical Engineering, University of Washington, Seattle, Washington.

The immobilization of biological catalysis and enzymes has so far been accomplished mostly as thiol inactivated enzymes on metals and glasses, respectively. Although considerable advances have been made in using these chemical linkers on solid substrates as well as in the use of on-microparticles, their utilization has not been fully realized due to the inherent problems associated with them. First and foremost is the fact that thiols and silanes are prepared in non-biological solutions. Secondly, their molecular architecture only allows limited space for functional molecular attachments. And, thirdly, their assemblies into two-dimensional ordered structures rely on intermolecular forces in addition to molecule-substrate interaction and, therefore, require dense coverage for useful applications. In this work, we demonstrate the functional utilization of inorganic-binding polypeptide amphiphiles and self-assemble of biological enzymes which selectively bind to inorganics, e.g., metals (gold, platinum, and palladium) and ceramics (silica, alumina, and hydroxyapatite). The genetically designed molecular constructs consisting of inorganic-binding polypeptide-express is used by E. coli or M13 phage as the host organisms. The inorganic-binding polypeptides are selected using combinatorial biology, either cell surface or phage display approach that we have developed for a variety of practical materials systems. The peptides are further engineered to have multiple repeats or their amino acid sequences changed and, hence, the binding domains are tailored depending on the nature of the applications. Both inorganic- and flat inorganic nanopatterned surfaces are used for self-directed immobilization of the molecular constructs. We demonstrate the bi-functionality of the constructs using gold, platinum, and silica, and hydroxyapatite as specific substrates and show simultaneous inorganic-binding and enzymatic or biorecognition functions. The molecular biomimetic approach opens up new avenues for the design and utilization of multifunctional nanomaterials systems in wide ranging applications from tissue engineering, drug delivery and biosensors, to nanotechnology and materials system integration. This work is supported by TR-SPO, US-ARO-DURINT and AFOSR Bioinspired Systems Programs.

**K.3.25** Cell Adhesion and Growth on Chemically Patterned Polymer Surfaces. Benjamin B. Katz, Benita M. Comeau and Clifford L. Henderson, School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Cell patterning of mammalian cells can be useful in many applications such as cell-based sensors and diagnostics, and the engineering of complex and functioning tissues. Conventional cell patterning techniques commonly rely on first patterning extracellular matrix (ECM) proteins on a bio-inert substrate, with subsequent cell adhesion to the ECM proteins. While these techniques are successful in two dimensional (2D) surfaces, they lack the flexibility to be used easily with three dimensional (3D) tissue scaffolds. Our recent work has focused on utilizing chemically amplified photoresist (CAR) microreactive layers to locally modify poly(ethylene glycol) and polyelectrolyte brushes and using this material property change to induce preferential cell adhesion on the surface. Carboxyl, in simplest form, consists of a polymer backbone that contains an alcohol moiety which has been converted to an acid-label and a protecting group and a reactive photoacid generator (PAG). Upon exposure to UV light, acid is produced from the PAG which catalyzes the conversion of the protected alcohol back to its alcohol form. In photoresistors, this change from a hydrophobic protecting group to a hydrophilic alcohol is used to switch the solubility of the polymer in a developing solvent to produce patterned relief structures. In our case, we are using the chemical change in the polymer itself to promote selective cell adhesion on the material surface. The use of an exposure-induced polymer property change, in combination with a photo-polarizable monomer, can allow for the patterning of complex 3D polymer scaffolds. We are pursuing such fabrication methods using dual wavelength stereolithography techniques. This paper will present results of our work on the interaction of cells with model chemically amplified polymer surfaces that can be chemically patterned via exposure to light. Contact angle and FTIR measurements are used to monitor the deprotection reaction in the polymer and characterize the resulting hydrophilicity of the polymer surface. Several cell seeding procedures were tested with fibroblasts and pre-osteoblasts, and cell adhesion to the patterned surfaces was examined for pattern density, morphological and functional assessments. Qualitative and quantitative data of cell patterning studies will be presented which show the differences in patterning that can be achieved using cell seeding in both serum-free and serum containing media followed by cell growth in serum containing media.

**K.3.26** Oriented and Vectorial Patterning of Cardiac Myocytes using microelectrodes? towards engineered cardiac tissue with controlled macroscopic anisotropy. Mo Yang and Xin Zhang: Manufacturing Engineering, Boston University, Brookline, Massachusetts.

Major initiatives are underway to create implantable engineered heart constructs for repair of damaged myocardium, but integration of such engineered patches of heart muscle with the injured heart with require deeper understanding of the interactions between cardiomyocytes in networks of cells. Furthermore, while many engineered cardiac constructs lack preferential cell orientation, the actual in vivo tissue is structurally and functionally anisotropic. Anisotropy is an essential feature of cardiac tissue. The unique architecture of cardiac tissue enables an orderly sequence of electrical and mechanical activity and efficient pumping of blood from the heart. Previous attempts to mimic it in vitro have employed 2-D micropatterning, extracellular matrix microarrays, and surface microabrasions. Here, we present a novel approach is to use AC electrical field to align the cardiomyocytes along the electric field direction, which could be well controlled in our system by the designed spatial feature of the microelectrode. Cardiac myocyte tissue-like structure is constructed using a combination of
dielectrophoresis and electro-orientation via a microfluidic chip. The rod shaped cardiac myocytes were patterned on the edge of microelectrodes fabricated along a 90° angle. Using different patterns of microelectrode, 90° and 45° orientation angles were achieved. Finite element Modeling (FEM) was used to evaluate the dependence of microfluidic geometry on patterning efficiency. The dependence of microfluidic channels on the magnitude and orientation of cell elongation were listed and evaluated in this study.

K3.27
Self-assembled monolayers (SAMs) presenting precisely defined functional groups modulate protein binding and direct cell attachment and spreading. Brett R. Downing 1,2,3
Ernesto Soto 4,5, Katie Bush 1,3, W. Grant McCimpsey 2 and George D. Pits 2
1Biomedical Engineering, Worcester Polytechnic Institute, Worcester, Massachusetts; 2Chemistry and Biochemistry, Bioengineering Institute, Worcester Polytechnic Institute, Worcester, Massachusetts; 3Department of Biomedical Sciences, University of Massachusetts Medical School, Worcester, Massachusetts.

Protein adsorption to biomaterials is important for regulating cell function through integrin-mediated cell matrix. Interactions of cells with ligands presented in the extracellular matrix have been shown to influence cell survival, proliferation, and differentiation. For example, keratinocytes removed from tissue culture polyostrene and cultured in suspension undergo irreversible inhibition of DNA synthesis and terminal differentiation. When fibronectin is added to the suspension culture and brought to the cell surface, terminal differentiation of the cells is inhibited. In this study, eleven different self-assembled monolayers (SAMs) consisting of a decanethiol linked covalently to a variety of chemical groups were tested. Cu(II)-[2-[(10-sulphhydroxy-decylxoy)-pyridine-2, 6-dicarboxylic acid, or 4-(10-sulphhydroxy-decylxoy)-pyridine] were used to investigate relationships between molecular surface determinants, protein adsorption, and cell spreading. The surfaces presented a range of bulk and molecular-level properties which had a systematic effect on the amount of fibronectin adsorption. Fibronectin was fluorescently labeled with Alexa/Fluor 594 and 0, 50 or 100 μg/ml of protein was adsorbed to the surface of the SAMs for 24 hours. Quantitative analysis of fibronectin adsorption revealed differences in the amount of the protein adsorbed on the surfaces with the least adsorption to dodecanethiol and the greatest adsorption to isothiocyanate. The fibronectin-coated surfaces presented different concentrations of protein were seeded with human keratinocytes for four hours, fixed and stained with Hoechst nuclear dye as well as fluorescein-5-maleimide, then analyzed for cell attachment and spreading. Most surfaces exhibited an increase in cell attachment and spreading consistent with a decrease in fibronectin concentration on the surface. Spreading values increased from 409 μm² to 1681 μm² on dodecanethiol surfaces treated with 0 μg/ml and 100 μg/ml of fibronectin respectively. Differences in cell adherence and spreading were also found on surfaces with different surface chemistry but with the same concentration of fibronectin adsorbed to the surface. Pyridine-decanethiol had the greatest adherence, 263 cells/mm², whereas dodecanethiol had the least adherence, 173 cells/mm², following surface adsorption with 100 μg/ml of fibronectin. These results establish an experimental framework for the further analysis of the molecular determinants for protein adsorption and conformation and subsequently cellular function on these surfaces. Further, results from this and future studies will help elucidate surface chemistry and adsorbed protein densities to elicit a desired response from cells in contact with biomaterials.

K3.28
Organic Template Mediated Self Assembly of Mineral as a Model System for Biomimetic and Bone Tissue Engineering. David H. Kohn 1,2, Kyungsup Shin 2, Sun Il Hong 2, Elena V. Leonova 1, Paul H. Kwek 1, A. Rossella 1,2
1Biologic & Materials Sciences, University of Michigan, Ann Arbor, Michigan; 2Biomedical Engineering, University of Michigan, Ann Arbor, Michigan; 3Metallurgical Engineering, Chungnam National University, Taejon, South Korea.

The formation of bone from progenitor cells is variable, and controlled by factors in the cellular microenvironment, including the supporting biomaterial. A balance between mineral and bone can be achieved if a bone-like mineral sheath is formed on a biomaterial surface in vivo. Therefore, a system in which an organic template can self-assemble a bone-like mineral extracellular matrix analogue in a controlled manner, regulates the rate and extent of cell differentiation and bone formation, as well as provide insight into biomimORIZATION. The purpose of this study was to parametrically assess the effect of ionic strength of mineralizing solutions on mineral composition and morphology, and relate these material characteristics to bone marrow stromal cell (BMSC) adhesion and bone formation. Three dimensional, porous organic (poly(2-lactide-co-glycolide)) scaffolds were functionalyzed by exposing to CaCl₂ solutions of varying ionic strength and pH to control mineralization kinetics, mineral composition and mineral morphology. Templates were analyzed for mineral composition and morphology via FTIR, XRD, SEM and TEM. Man-made bone marrow stromal cells were seeded onto the scaffolds via a micromass seeding technique. Cell proliferation was assessed by counting attached cells in 6 random fields under a light microscope following washing and fixing. Cytoplakolysis were assessed via indirect immunofluorescent localization of vinculin, using confocal microscopy. Scaffolds were implanted ectopically into nude mice for 6 weeks and bone regeneration was assessed qualitatively using undecalcified histology and quantitatively via microcomputed tomography. The structure of the mineral was controllably changed with incubation time and ionic activity product; at lower ionic strengths crystals grew within the plane of the substrate, whereas at higher strengths growth was out of plane, and Ca/P ratios varied inversely with ionic strength. Amorphous Ca-P was formed, with nucleation of crystalline apatite from the ACP, followed by growth of needles (100-1000 nm) along the a-axis. At all times up to 4 hrs, significantly more cells adhered to mineralized substrates (p<0.05). On non-mineralized substrates, focal adhesion contacts were localized at the periphery of the cell, whereas on mineralized substrates they were distributed throughout the cell surface. The difference in distribution was quantified and shown to be statistically significant (p<0.001); cell adhesion contacts on mineralized PLGA are significantly closer to the cell center than on PLGA. In vivo, a significantly higher bone volume fraction was achieved by transplanting cells in mineralized scaffolds, compared to controls (31 +/- 14 % vs. 15.8% +/- 0.01 %). Collectively, these data demonstrate the ability to controllably self-assemble 3D mineralized constructs, providing material-based control over cell function. NIH R01 DE 013980 and R01 DE 015411.

K3.29
Fabrication of Patterned Biomolecular and Bioconjugated PolymERIC Nanostructures Using ScannIng Probe Lithography. Woo-Kyung Lee1,2, Hongwei Ma1,2, Ashutosh Chikitori1,2 and Stefan Zauscher1,2
1Mechanical Engineering and Materials Science, Duke University, Durham, North Carolina; 2Center for Bioinspired Materials and Systems, Duke University, Durham, North Carolina; 3Biomedical Engineering, Duke University, Durham, North Carolina.

Patterning of polymeric and biomolecular nanostructures on surfaces and the control of their architecture are critically important for the fabrication of functional interfaces for single cell research, and for biomolecular devices and sensors. Here we present several methods that we have developed that allow for molecular-level control in the fabrication of polymeric and biomolecular nanostructures. We first describe the fabrication and characterization of stimulus-responsive elastin-like polypeptide (ELP) nanostructures grafted onto ω-terminus-thioester that patterned onto gold surfaces by dip-pen nanolithography (DPN). Here we demonstrate that a hydrophilic-hydrophobic phase transition of ELP in response to a change in ionic strength as a switch in order to reversibly immobilize a designed ELP fusion protein onto the ELP nanopattern above the lower critical solution temperature. Our work demonstrates the potential for ELP nanoarrays in reusable lab-on-chip devices for protein purification or nanoscale analysis. Furthermore, we show how we use AFM amodization lithography to chemically modify protein non-fouling polymer brushes directly to allow conjugation of biomolecules with nanometer resolution. We prepared surface-confined non-fouling and protein resistant polyl(ethylene glycol) methyl methacrylate) (P(EO)MA) brushes on silicon substrates by surface-initiated atom transfer radical polymerization (ATRP) in a "grafting-from" approach. These P(EO)MA brushes were then patterned directly by AFM anodization lithography, generating nanostructured features with carboxylic acid functionality. Amineterminated biotin derivative was covalently conjugated with the chemically activated carboxylic acid nanopattern. The surface was then incubated with streptavidin to form streptavidin nanostructures, mediated by molecular recognition between biotin and streptavidin. Finally, switchable "smart" protein nanopatterns were fabricated by molecular-recognition mediated immobilization of immobinotiolated protein from solution. Our research demonstrates that "smart," surface-confined biomolecular patches can be built on a nanoscale and raises the intriguing possibility that nanostructures of recombinant proteins and peptides could be created directly on a surface by affinity capture from cell lysate without the necessity of purification steps. The nanoscale miniaturization of the rate of separation and the presentation and triggered release of captured proteins made possible by our methodologies should be integrable into nanoscale bioanalytical devices and should have direct application in single cell research.

The present study aimed at understanding the hierarchical interactions of protein-mediated mammalian cell adhesion to nanoscale surface features. Spherical silica particles of 4, 20, or 100 nm diameters were used to fabricate substrates with surface coverage by particles in the range of 0 to 100%. The materials formulated and fabricated provided a set of model substrates with well-controlled and well-characterized nanoscale surface features. These model substrates were utilized to evaluate the adsorbed select proteins, and subsequent adhesion of cells that are critical to the clinical efficacy of biomaterials. The size of nanoscale silica particles on native oxo-coated silicon pieces affected the adhesion of rat calvarial osteoblasts and rat skin fibroblasts differently. Specifically, osteoblast adhesion was enhanced, while fibroblast adhesion was reduced, on surfaces decorated with 4 or 20 nm particles, in contrast to surfaces decorated with 100 nm particles. It was also observed that a threshold density of nanoscale surface features greater than 50% was necessary to elicit such size-selective adhesion from osteoblasts or fibroblasts. Adsorption of fibronectin and vitronectin onto native oxo-coated silica surfaces decorated with either 4, 20, or 100 nm diameter silica particles, or a control (no oxide) was assessed. Protein adsorption was observed to increase as the particle diameter increased on surfaces 80% covered by particles. Subsequent osteoblast adhesion on surfaces decorated with 4 or 20 nm particles and preadsorbed vitronectin was enhanced compared to surfaces decorated with 100 nm particles and preadsorbed with fibronectin. Alternatively, osteoblast adhesion was similar on surfaces decorated with each particle size tested and preadsorbed with vitronectin. Circular dichroism (CD) spectroscopy provided evidence that the secondary structures of fibronectin molecules adsorbed on either 4 or 20 nm diameter particles in buffer were similar, but fibronectin exhibited decreased beta sheet content and increased unordered structure on 100 nm particles. CD spectroscopy revealed that the secondary structure of vitronectin was similar for all particle sizes tested. The alteration of fibronectin and vitronectin secondary structure correlated well to adhesion of osteoblasts on nanoparticle-decorated surfaces with fibronectin or vitronectin preadsorbed. This study offers insights into a molecular mechanism that is linked to nanostructured material surface feature size through quantified changes in protein structure and cell adhesion behavior. These results provide an explanation of the molecular level events occurring on nanostructured material surfaces that contribute to protein-mediated and size-selective cell adhesion. This work was supported by the USA and the Nanoscale Science and Engineering Initiative of the National Science Foundation under NSF Award Number DMR-0111792.

SESSION K4: Engineered Biointerfaces III Chairs: Susan Enders and Shrikanth Ranade Thursday Morning, December 1, 2005 Room 204 (Hynes)

8:00 AM K4.1 Inverted Colloidal Crystal Hydrogel 3D Scaffolds Control Differentiation of Stem Cells: Toward Artificial Bone Marrow and Thymus, Nicholas A. Kotov, Jung Woo Lee, Jong Hwan Bahn, Megan Caddie, Shaoqeng Wang, Joaquin Cortiella and Joan Nichols, 4 Chemical Engineering, University of Michigan, Ann Arbor, Michigan; 2Biomedical Engineering, University of Michigan, Ann Arbor, Michigan; 3Nomadic Inc., Stillwater, Oklahoma; 5Infectious Deceases, University of Texas Medical Branch, Galveston, Texas.

Successful engineering of functional tissues requires the development of three-dimensional (3D) scaffolds that can provide optimum microenvironment for tissue growth and regeneration. A new class of 3D scaffolds with high degree of organization and unique topography was fabricated from polyacrylamide hydrogel. The hydrogel matrix was molded by inverted colloidal crystal. The process of stem cell differentiation can be additional enhanced by incorporation of chemokines in the hydrogel matrix of the scaffold. The walls of ICC hydrogel cell support can also be covered by Notch ligands, that allow to tune the process of stem cell differentiation and replication. Ease of production, unique 3D structure, biocompatibility, and optical transparency make this new type of hydrogel scaffold suitable for most challenging tasks in tissue engineering.

8:15 AM K4.2 Topological and Chemical Control of Cultured Rat Hippocampal Neurons, Jiayi Zhang, Somnaya Venkataramani and Arto V. Nurmikko, 1, 2Physics, Brown University, Providence, Rhode Island; 3Engineering Division, Brown University, Providence, Rhode Island.

In-vitro cultured neuronal networks are promises seeding bed for access and study of neuro-electrical interfaces and information processing between interconnected neurons. In standard cultures their random spatial distribution and the overlapping of neurites leads to a complex technical problem; hence many recent efforts at creating patterned cellular circuits. Here, we present a novel method in both topological and chemical control of cultured hippocampal neuron and electrical and optical measurements of their activity. In our approach, negative photoresist SU8 microstructures are designed to constraint the 2D geometry for the growth of neurites. The 5µm-high microstructure is composed of pits with diameter 50µm to 200µm and grooves with width 20µm to 50µm. A liquid phase surface adhesion polymer of amino acid Poly-L-lysine is patterned correspondingly into the pits and grooves. Both soma and neurites grown in these types of structures encounter not only geometrical but also selective neuronal signals for orientation and growth, which open the possibility of optically accessible study of development and physiology of few-cell neuron networks. The flexibility in pattern geometry and their dimensions further enables the experiment of the synaptic strength for optimizing the signaling capability within the neuron network. Research supported by NSF Biophotonics program and DARPA.

8:30 AM K4.3 Neuron Responses to Simultaneous and Competing Extracellular Cues, Natalia Gomez, Christine E. Schmidt and Shaochen Chen, 1Chemical Engineering, The University of Texas at Austin, Austin, Texas; 2Biomedical Engineering, The University of Texas at Austin, Austin, Texas; 3Mechanical Engineering, The University of Texas at Austin, Austin, Texas.

Neurons are cells particularly influenced by extracellular cues during several phases of their development [1-2]. Numerous environmental stimuli, including substrate topography [3], growth factors (e.g., nerve growth factor, NGF) [4], extracellular matrix components [5], electrical activity [6] and support of cells [7], have been investigated for interfaces with neurons. Although all these stimuli have shown to induce responses in neurons independently, in this investigation we have mostly focused on cell behavior when two or more cues are simultaneously presented to the cell. This includes both combinatorial and competitive factors. In particular, we have studied the immobilization of NGF on poly(ethylene glycol) (PEG) and poly(dimethylsiloxane) (PDMS) as a biochemical stimulus. This novel presentation of the protein has been combined with stimuli originated from the substrate, such as the electrical activity of PPF or contact guidance by microchannels patterned on PDMS, to influence neurite outgrowth. NGF has been fixed to the different substrates by using arylazide compounds able to create covalent bonds upon UV exposure. Different surface concentrations have been achieved (~0.2-1.5 ng/mm²) and the retained activity of the tethered protein has been corroborated. The immobilized NGF creates bioactive surfaces on both PPF and PDMS, which can be combined with other cues. Specifically, stimulation of cells on PPF with fixed NGF has revealed a significant increase in neurite length (~30%). We are currently studying cell responses to the immobilized NGF on microchannels in PDMS. Additionally, we are investigating the nature of cell response specifically between contact guidance (microchannels) and chemical guidance (tethered NGF). For this study, the two factors have been designed to be simultaneous but spatially independent; individual neurons have been precisely micropositioned in between the competing stimuli in order to sense both stimuli and initiate a decision-making process. Neurite length on both cues and neuron polarization (i.e., definition of an axon toward a particular side) are further analyzed on long-range with this system. The contribution of cell reactions to simultaneous cues will contribute to a more complete understanding of neurite outgrowth, and therefore, it will enable the development of better methods to enhance axon growth and nerve regeneration. References 1. T. Esch, V. Lemmon, G. Banker. J.
tiny probe for nanomedicine? Daniele Gerion, Lawrence Livermore National Laboratory, Livermore, California.

Silanized quantum dots are small fluorescent nanoparticles embedded in a thin silica shell. The silica shell offers multiple opportunities to functionalize or dope the nanocrystals. For instance silanized quantum dots can be functionalized with nuclear targeting peptides, transfecting agents and MRI contrast agents. These different functions can all be grafted onto these tiny nanoprobos, about 10 nm across. What is more interesting is that the probe itself is compatible with living tissues, therefore such probes might be used in noninvasive and intracellular fluorescent imaging. Our work seeks to establish this bridge. I will focus on our attempts to track and visualize live cells by MRI and on attempts to observe molecular kinase activity in real-time. But first of all, I will describe how such nanoprobos can be synthesized and what their properties are.

10:00 AM *K4.7 Molecular and Materials Design for Cellular Delivery of Oligonucleotides. Charles M. Roth, Chemical and Biochemical Engineering, Rutgers University, Piscataway, New Jersey.

The ability to modulate cell behavior through genetic modification has great potential as a therapeutic strategy and has applications in functional genomics and tissue engineering. For example, antisense oligonucleotides (AS ONS), which are most commonly single-stranded DNA molecules 15-25 nucleotides in length, modulate gene expression by binding to a complementary segment on the mRNA from the targeted gene, thus repressing the translation of the gene. While antisense technology is becoming a viable therapeutic entity and platform for functional genomics, intracellular delivery of the genetic material (polynucleic acid) remains a major barrier to more widespread use. The recent development of RNA interference (RNAi), an endogenous gene silencing mechanism based on 19-21 nucleotide double-stranded RNA called short interfering RNA (siRNA), is active in mammalian cells has created an exciting new avenue for oligonucleotide-based control of gene expression. Numerous recent reports attempt to establish the superiority of RNAi over antisense, while others refute these claims. We are investigating the molecular design variables that govern the effectiveness of these approaches using unmodified and stably GFP-transfected CHO cells. In particular, we use a molecular thermodynamic approach to select identical AS ON and siRNA sequences targeting susceptible regions of the RNA. In this system, antisense effects appear to occur more quickly and are short-lived compared to RNAi effects. Nonspecific effects occur more prevalently with the AS ODN approach. If these results prove general for other cell types, they will have a major impact on the selection of AS ONS and siRNAs for various applications. We are also developing a molecular approach to improving cellular delivery by controlling the electrostatic interactions and intracellular trafficking involved in cationic lipid and polymer-mediated delivery of AS ODNs. The complexes between cationic lipids or polymers and antisense oligonucleotides are understood at the component level, within the cationic lipid-DNA complex, or trimethylammonium protonated diethylene triamine (DOTAP) both in vitro and in vivo. We show that the addition of DOTAP improves the in vitro efficiency of DOTAP with regards to ON delivery and antisense activity. In characterization studies, DOTAP/ON complexes were formed even when substantial amounts of DOTAP were added. In both CHO and A127 cells, incorporation of DOTAP into DOTAP/ON complexes improves substantially the cellular uptake of fluorescently labeled oligonucleotides and the AS ON activity (using GTP as the target) over a range of DOTAP concentrations, particularly in serum-free media.

9:00 AM K4.5 Rate of adhesion between targeted drug or gene carriers and celluar membranes, as a measure of controlling binding and internalization. Nily Dan, Chemical and Biological Eng., Drexel University, Philadelphia, Pennsylvania.

Targeted drug delivery and gene therapy require cellular internalization of macromolecular complexes or carriers. Targeting, namely, binding to a specific cell receptor and does not take place instantaneously: Due to the dilute concentration of receptors, as well as binding kinetics, the targeting moiety must remain in the vicinity of the cellular membrane for a minimal length of time if attachment is to occur. Similarly, to trigger endocytosis the carrier must adhere to the membrane for a characteristic length of time. Adhesion between particles and cell membranes is composed of several contributions, which include electrostatic interactions (due to cellular charge), hydrophobic interactions (arising from the cellular glyocalyx layer and, when relevant, attached PEG on the particle), as well as van der Waals and solvation forces. In this study we calculate the effect of system parameters such as the cell-type, the particle charge, and the presence of PEG chains on the interactions between cells and carriers, and relate these to the characteristic adhesion time.

9:15 AM K4.6 Silanized quantum dots with MRI signatures: an integrated...
that are several orders of magnitude more potent than the corresponding monovalent inhibitors and can neutralize anthrax toxin in vivo. The inhibitors developed during this work may enable the successful treatment of anthrax during the later stages of the disease when antibiotic treatment is ineffective. We will also briefly discuss the application of polyanionic covalent drug delivery systems that also induce the formation of nanoparticles. We will then discuss the design of polyvalent inhibitors based on biomimetic scaffolds—liposomes. Investigating the interaction of biofunctionalized liposomes with target proteins (bound to the surfaces of cells) will allow us to test the predictions of theoretical models that have analyzed recognition events at interfaces, as well as to design inhibitors that function in vivo. We will also discuss the influence of the composition of lipid bilayers on the transport of adsorbed biomolecules (DNA) at interfaces.

11:00 AM **K4.9**

**Nanostructured Polymer Drug-Delivery Coatings for Microfabricated Neuroprosthetic Devices**

David Charles Martin, 1, 2, 3 Donghwan Kim, 2, 3, Mohammad Abidian, 4, Sarah Richardson-Burns, 5 Jeffrey Hendricks, 6, Cynthia Sequerah, 6 and Matt Meier, 5, Materials Science and Engineering, The University of Michigan, Ann Arbor, Michigan; 2Department of Biomedical Engineering, The University of Michigan, Ann Arbor, Michigan; 3Biomedical Engineering, The University of Michigan, Ann Arbor, Michigan.

We are investigating the use of polymers to make soft, bioactive coatings for microfabricated neural prosthetics intended for direct implantation into the Central Nervous System. There is interest in the local delivery of anti-inflammatory agents (such as dexamethasone) to reduce the reactive response around the implanted device, and for trophic factors (such as Nerve Growth Factor) for promoting interactions with neurons. The polymer coatings used for these biosensors must be able to accommodate the exchange in charge transport from electrons in the device to ions in the tissue. Pharmacological agents of interest can be incorporated into the coatings using several different procedures including electrochemical polymerization of conducting polymers. They can also be embedded in nanoparticles or nanofibers of biodegradable polymers. The morphology of the polymers can be precisely controlled by using templates that are later removed, and this provides for the ability to tailor the local delivery of the biological agents.

11:30 AM **K4.10**

**Non-Viral Gene Delivery Regulated by Mechanical Properties of Engineered Cell Adhesion Substrates**

Hyun Joong Kong, 1, Jodi Liu, 2, Kathryn Riddle, 2 and David J. Mooney, 1, 2, DEAS, Harvard University; 2Chemical Engineering, University of Michigan, Ann Arbor, Michigan.

Non-viral gene vectors are increasingly used in various gene therapy strategies due to safety concerns with viral vectors, but are plagued by low levels of gene transfection and cellular expression [1]. The current emphasis in efforts to increase the efficiency of non-viral gene delivery is designing a vector to control the gene vector. In contrast, the influence of the cellular environment in DNA uptake is often ignored. Recently, we found that the mechanical properties (e.g., rigidity) of the substrate to which a cell adheres mediate many aspects of cellular behavior, including proliferation, migration, and differentiation [2]. These results suggest that the physical properties of the adhesion substrate may regulate a cell's ability to transfer exogenous signaling molecules. In this talk, we will present a critical role for the rigidity of the cell adhesion substrate on the level of gene transfer and expression utilizing a fluorescent resonance energy transfer (FRET) technique [4]. We will also discuss the mechanism of this effect, which relates to material control over cell proliferation. We envisage that this study will provide a new material-based control point for non-viral gene therapy. References 1. Nidome, T., & Huang, L. Genetic engineering progress and prospects: Nonviral vectors. Gene Ther. 9, 1647-1652 (2002). 2. Brown, M.D., Gschetal, A., & Uchegb, I.F. Gene delivery to cardiomyocytes, Int. J. Pharmaceutics, 229, 1-21 (2001). 3. Kong, H., Kogut, T., Abberk, E., & Mooney, D.J. FRET measurement of cell-attachment forces and nano-scale clustering of adhesion ligands varied by substrate stiffness, Proc. Nat. Acad. Sci. (USA) 102, 4900 (2005). 4. Hyun Joong Kong, Jodi Liu, Kathryn Riddle, Takuya Matsumoto, Kent Leach, David J. Mooney. Non-Viral Gene Delivery Regulated by Stiffness of Cell Adhesion Substrates. Nature Mater. (In press, 2005).

11:45 AM **K4.11**

**Designed Peptides for Hydrogel Self-assembly Enabling 2-D and 3-D Osteogenic Cell Growth and Mineralization**

Haasna Rahman1, Darrin J. Peterson2, and Patrick M. Schneider2, 2Materials Science and Engineering and Delaware Biotechnology Institute, University of Delaware, Newark, Delaware; 2Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware.

Peptide hydrogels are potentially ideal scaffolds for tissue repair and regeneration due to their ability to mimic natural extra cellular matrix. The 20 amino acid peptide MAXI (H-N-VKVKVKVKVKVKVK-VPTKVKVKVKCONH2), has been shown to fold and self-assemble into a rigid hydrogel based on environmental cues such as pH, salt, and temperature. In addition, cell-material interactions, and design variations in the arms of the MAXI sequence allow for tunability of the self-assembly/hydrogel kinetics. Due to its environmental responsiveness, hydrogel assembly can be induced by cell culture media, allowing for 3D encapsulation of osteogenic cells. Initially, 2D cultures of MC3T3 cells proved that the hydrogel is nontoxic and sustains cellular attachment in the absence of serum proteins without altering the physical properties of the hydrogel. The cell-material structure relationship in normal and pathological conditions was further investigated by 3D encapsulation. As shown by optical and laser scanning confocal microscopy, cells were viable for 3 weeks and grew in clongenic spheroids. Characterization of the proliferation, differentiation and expression of mesenchymal markers was performed using spectrophotometric methods. The well-defined, fibrillar nanostructure of the hydrogel directs the attachment and growth of osteoblast cells and dictates the mineralization rate in a manner that is tuneable. This study will enable control over the interaction of cellular systems with the peptide hydrogel with designs for biomedical applications of bone repair.

SESSION K5: Poster Session II: Engineered Biointerfaces

Chairs: Susan Enders and Shrirang Ranade
Thursday Evening, December 1, 2005
8:00 PM
Exhibition Hall D (Hynes)

K5.1 Optimization of the organic-inorganic interface for the development of robust biotherapeutic microdevices

Jorge Eduardo Quijano and Shalini Prasad; Electrical and Computer Department, Portland State University, Portland, Oregon.

Microelectrode arrays have proved to be a convenient method for the recording of electrical activity in cells to be used in biosensors. Nevertheless, the actual techniques have several constraints such as attenuation of the recorded signal, deficient coupling between the cell and the electrode, problems of selectivity related to the size of the microelectrode of hydrolyzed to that of a neuron, and the lack of electrochemical reactions in the culture media when external voltages are applied for electro stimulation. The use of nanomaterials in the fabrication of electrodes readily improves the performance of biopotential transducers due to the properties available in the nano scale. We have explored two approaches in the design of nanoelectrode arrays: use of nanoparticles to modify the electrode surface and use of vertically aligned nanowires in the geometry and surface topography that optimize our design in order to decrease the electrode impedance and increase the signal resistance. It results in the acquisition of more reliable signals, in addition to promoting cell adhesion and growth [1]. The use of silicon array apt for cell culture. We have characterized the devices by determining its biocompatibility, signal to noise ratio, cyclic voltammetry and suitability to be used for arrangement of cells using electric fields.

K5.2 Purification of Single-Walled Carbon Nanotubes to Produce Nanotube Scaffolds for Cell and Tissue Engineering

Takuya Uchida, 1, Yoshinori Sato, 1, Kenichi Motomiya, 1, Balachandran Jeyadevan, 2, Rikozi Hatakeyama, 3 and Kazuyki Tojii, 1, 1Graduate School of Environmental Studies, Tohoku University, Sendai, Japan; 2Graduate School of Engineering, Tohoku University, Sendai, Japan.

Carbon Nanotubes (CNTs) have been actively studied by many researchers and expected to have great potential in many fields due to their extraordinary mechanical, electrical and thermal properties. However, these extraordinary properties can be utilized only if we succeed in the preparation of sintered bodies, reinforced composites, high strength fibers, and thin film electodes of CNTs. Moreover, considerable efforts have been made to explore new biological application in recent years. In this study, we pay attention to production of single-walled carbon nanotube (SWCNT) scaffolds in an effort to produce a material that possesses both flexibility and strength. Recently, we succeeded in the preparation of MWCNT composites using polymer as binder [1]. However, these composites cannot be used for cell growth due to the presence of polymer, which is harmful (not biocompatible). Therefore, it is necessary to produce binder-free SWCNT sheets for cell breeding. Furthermore, the SWCNTs, which have been proved to be biocompatible [2], have to be
highly pure and free of harmful residual metal catalysts used during the synthesis of the same. In this report, we focus on a simple purification method to obtain high purity SWCNTs to produce nanotube scaffold for cell and tissue engineering. We synthesized raw SWCNT soot by a direct current (dc) arc discharge method. The arc discharge is generated by a current of 65-70 A in a helium atmosphere at a pressure of 760 Torr. Packed graphite rod, containing a mixture of Fe and Ni powders of 99.9% purity are used as the anode of dc arc discharge. A pure graphite rod of 10 mm in diameter is used as cathode. Our purification method is simple and consists only of oxidation and reduction. Firstly, the sample is heated at 725 K for 30 min in the air to remove carbonaceous particles such as an amorphous carbon and fulleren-like nanocarbon. Secondly, the sample is heated again at 773 K for 30 min in the air. Then, the sample is immersed in 2M HCl at 333 K for 12 h to dissolve catalytic metal particles. The dispersion is filtered, washed with distilled water and dried at 333 K for 2 h. Finally, the sample is again heated at 773 K for 30 min and soaking in HCl, filtering and drying processes are repeated once. Characterization of SWCNTs are confirmed by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Raman spectroscopy. In the presentation, we will discuss the characterization of purified SWCNTs and production of pure SWCNT scaffolds for cell and tissue engineering.

K5.3 Characterization of reflection related proteins isolated from the squid Loligopeale, Michi Izumi1, Yoshiko Okamura1, James C. Weaver1, Roger T. Hamlin2 and Daniel E. Morse1; 1Institute for Catalysis, Pacific Northwest National Laboratory, Richland, WA; 2California NanoSystems Institute, University of California, Santa Barbara, Santa Barbara, California; 3Marine Biological Laboratory, Woods Hole, Massachusetts.

Iridophores are light reflecting cells that are distributed across the animal kingdom. Two types of iridescent patterns of reflectance from these cells have been reported. Passive iridescence refers to constant reflectance, while active or adaptive iridescence refers to behavior of reversibly changeable reflectance. Typically, iridophores have been found to contain multiple stacks of thin reflective plates; each stack is known as an iridosome. While the iridomes in fish and reptiles contain flat and highly reflective purine crystals that undergo dynamic changes in spacing and positioning in adaptive reflection, the mechanisms controlling adaptive changes in the cephalopod (octopus, squid, etc.) iridocome remain to be fully elucidated. Iridophores located in the skin of the mantle of the squid, Loligopeale, exhibit active iridescence. It previously was demonstrated that squid iridophores contain a novel family of proteins, named reflectins, that apparently mediate the dynamically adaptive changes in reflectance in these species. Using biochemical and molecular genetic techniques to analyze the reflectin proteins from the iridophores of L. peali, we are aiming to elucidate the mechanism underlying the adaptive changes in this polynucleotide synthetic reflector and this enable the development of a new class of biologically inspired adaptive reflector.

K5.5 Abstract Withdrawn.

Processing and Characterization of Biodegradable Polyurethane Fibers for an Application in Tissue Engineering. Danielle Nicole Rockwood1, Kimberly A. Woodhouse2, Joanna Fromstein3, D. Bruce Chase4 and John F. Rabolt; 1Materials Science and Engineering, University of Delaware; 2Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada; 3DuPont Company, Wilmington, Delaware.

The goal of tissue engineering is to create biomimetic materials for the reconstruction of failing tissue. Conventionally, biocompatible matrices are synthesized, seeded with cells, and then transplanted into the body. Traditional methods for scaffold fabrication include particulate leaching, solvent casting, membrane lamination, and thermally induced phase separation (TIPS). These processes have high porosity and surface area necessary for cell attachment but lack interconnected pores. This element is critical for cell transportation through the matrix and also for diffusion of nutrients and waste. An alternative to these methods is electrospinning. Electrospinning is a technique where a polymer solution can be electrically charged to create nano- or micro-fibrous membranes. These scaffolds can provide many desired matrix properties including a fully interconnected porous network. The polymer studied in this work is a biodegradable polyurethane that has been designed with a phase-segregated morphology in order to give it elastomeric properties. This matrix degradation is mediated through hydrolysis as well as enzymatic cleavage of the polymer backbone. It is expected that as cells begin to lay down matrix proteins that the synthetic scaffold will slowly erode leaving native tissue. We have used our polyurethane solution electrospun to create a scaffold for murine skeletal myoblasts. C2C12 cells have been grown on these biomimetic matrices and differentiation has been observed. The goal of this project is to develop a viable cardiac patch that can be implanted at the site of an infarction. It is hoped that this construct can either restore or augment heart functionality.

K5.7 Media Reaction of Resorbable Synthetic Bone Implant. Loughlin Tuck, Michael Sayer and Rovey Astala; Physics, Queen's University, Kingston, Ontario, Canada.

Silicon diffusing from nearby quartz into a precipitated calcium hydroxyapatite (HA) coating during firing at >1000°C gives rise to a mixed phase material (~80% Si-TPC) bearing unique bioactive properties. Electron microscopy and X-ray diffraction and fluorescent techniques have been used to provide information about key elements of the microstructure critical for osteoclast (OC) resorption and osteoblast (OB) bone deposition in bone remodeling. Elements such as growth factor, phase composition, and silicon distribution can have significant effects on bioactivity. Ultrasonically stimulated studies of silicon doped powders immersed in simulated body fluid (SBF) or H2O show that powder grains transform dramatically through the formation of a surface layer of calcium phosphate crystals. Transmission electron microscopy imaging shows that these needle-like crystals emanate from the surface of what was once a smooth silicon doped calcium phosphate particle after only 12 hours. XRD diffraction of the bulk material reveals a complete reversion to an apatite phase within 1 hour of contact with the media, with the morphology having the form of single crystal apatite grains elongated along the c-axis. Chemical analysis of the SBF media reveals a release of Ca and Si and a depletion of phosphorus which equilibrates after approximately two hours. These experimental results complement computer simulations of Si-doped calcium phosphate surfaces in H2O. Support from the Natural Sciences and Engineering Research Council and Millenium Biologix Corporation is acknowledged.

K5.8 Effect of surface roughness on cell culture. Chehung Wei and Chau-yu Lai; Mechanical Engineering, Tatung University, Taipei, Taiwan.

Three different cells (Klebsiella pneumoniae, staphylococcus aureus, pseudomonas aeruginosa) were cultured on four different roughness (0.022 micron, 0.571 micron, 1.024 micron, 1.858 micron) silicon surface. For each cell, the surface roughness was the only control variable. Cell viability was evaluated by minimum inhibitory concentration (MIC) and was examined every 12 hour. For Klebsiella pneumoniae and staphylococcus aureus, the viable time for different surface roughness was from 3 to 5 days and for pseudomonas aeruginosa was from 4 to 9 days. The shape of the cells in the former case was ball-like while the shape of pseudomonas aeruginosa was like...
a rod. Different cell shape seems to have different viable time. Among these four roughness surfaces, 0.571 micron surface was the most favorable culture condition while the smooth surface 0.022 micron was the worst one. Given the different shapes of these cells, the complex adhesion-dependent control of apoptosis might be the main reason for this discrepancy.

K5.9

The surface energy of a conjugated polymer film is a function of the chemistry (side chains, dopant) and the oxidation state of the polymer. This is a useful tool in numerous situations, but the dominance of surface effects on the interaction of small volumes of liquid with their environment makes the control of wettability key in e.g. microfluidic devices and self-assembled monolayers. Here, we describe an electronically controlled wettability switches on flexible substrates. The devices use electrochemical oxidation and reduction of conjugated polymer films on a solid polymer electrolyte to regulate water contact angles (in air) and guide liquid droplets on the surfaces. Polymers, like poly(3-thiophene) that are initially undoped are more hydrophilic when oxidized (doped) compared to the neutral (undoped) state. This is because the surface becomes more polar upon doping. Other polymeric systems, such as polyaniline doped with the surfactant dodecylsulfonic acid, work the opposite way and have higher water contact angles when oxidized and lower contact angles when reduced. Owing to the high mobility of the surfactant molecule to the polymer chain is believed to be the dominant effect of the surface energy change. Conducting polymers are currently being used in a wide range of applications. Electrochemical switching of switching electrodes is used to regulate the requirement of different device applications. Intermediate wettability levels have also been achieved by blending different polymers. The typical contact angle switching effect is around 25°-30° with driving voltage less than 5V. Since the conjugated polymers are used both as electrochemically active surfaces and conducting wires, the devices require only a few processing steps. All-organic surface energy switches can potentially be manufactured with reel-to-reel printing techniques and integrated into a vast array of analytical systems to control flow and movement of liquids, particularly in single-use analysis chips.

K5.10

This study combines (i) the design and fabrication of an integrated circuit platform (IC) and (ii) the synthesis ex situ of electrically insulated and conducting neural interfaces with nanodevice level for electrophysiological studies of neuronal cells. The IC platform, which features an array of electrically-insulated electrodes deposited on a silicon substrate, was fabricated using three different scales of resolution to enable recordings of field potentials, action potentials and ionic potentials, respectively, at the multicellular, intercellular and intracellular levels. A conducting nanotube / insulator composite was fabricated by polymer infiltration of carbon nanotube arrays to achieve electrical insulation between adjacent nanotube bundles (1). Alternative methods are currently being undertaken to fabricate conducting nanowires / insulator composite constructs using anodic alumina templates as the insulating material (2). Conducting nanowire arrays are grown within the pores of the alumina template by: (i) high temperature carbon decomposition in a chemical vapor deposition environment; (ii) electron beam evaporation of either carbon, deposition of gold or copper in an electrolytic cell; and (iii) electron beam evaporation of carbon, copper or nickel. The composites are then positioned in intimate contact with the previously manufactured multiple electrode arrays, thus forming a novel interface between an underlying IC platform and neuronal cells. The device will eventually be interfaced with external amplification and data acquisition instruments in order to either record bioelectric signals from both single and multiple neural cells or to electrically stimulate them with ultimate nanometric space resolution. This work was supported by Philip Morris USA and the Nanoscope Science and Engineering Initiative of the National Science Foundation under NSF Award No: DMR-0117702. References (1) L.M. Dell’Acqua-Bellavitis, J.D. Ballard, R. Bizios, R.W. Siegel (2004) Synthesis of nanoscale devices for neural electrophysiological imaging. Mater. Res. Soc. Symp. Proc. 872, 318.17.1. (2) G.W. Meng, T.J. Yang, A.Y. Cao, R. Vajtai, P.M. Ajayan (2003) Controlled fabrication of hierarchically branched nanotubes, nanotubes, and nanowires. Proc. Natl. Acad. Sci. USA 102, 7074-7078.

K5.11
Adsorption of Protein onto Ultra-Water-Repellent Films and Several Self-Assembled Monolayers. Nagahiro Saito 1, Naoki Mastuda 2 and Osamu Takai 2; 1 Department of Molecular Design and Engineering, Nagoya University, Nagoya, Japan; 2 On-Site Sensing and Diagnostics Research Laboratory, National Institute of Industrial Science and Technology, Tsu, Japan; 3 EcoTopia Science Research Institute, Nagoya University, Nagoya, Japan.

Cell Adhesion is a key factor in order to understand the biointerface between materials and cell. Proteins play a great important role on the adhesion at the biointerface. Many researchers have investigated physical and/or chemical aspect at the interface. Ultra-water-repellent (UWR) films has a water contact angle over 150°, the water penetration is generally very difficult. The hydrophilicity of the UWR films is necessary for subsequent functionality such as cell adhesion. In this study, the adsorption of adhesion proteins on UWR films was investigated by scanning probe microscopy, optical waveguide spectroscopy. Moreover, the adsorption on various self-assembled monolayers was also investigated for comparison. Fibronectin, fibrinogen, albumin and heparin were selected as adsorbed proteins. The proteins were adsorbed on the surface by immersing the substrates into the solution containing the proteins. The optical waveguides images of proteins on different substrates were acquired. The adsorption was also investigated by in-situ impedance QCM and optical spectroscopy.

K5.12
o-Nitrobenzyl-Protected Polymer Surfaces for Protein Photopatterning Under Mild Aqueous Conditions. Joshua S. Katz 1, Junsa Doh 2 and Darrell J. Irvine 1, 2; 1 Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts; 2 Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; 3 Biological Engineering Division, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Micron-scale patterns of proteins immobilized on surfaces are used in a broad range of applications, including proteomics arrays, biosensors, and studies of cell-substrate interactions. To enable protein patterning using photolithographic methods but under mild conditions where proteins remain intact, we synthesized o-nitrobenzyl-protected methacrylate co-polymers as ‘bio-friendly’ photoreactive resists and characterized their solubility behavior as a function of composition. O-Nitrobenzyl methacrylate (oNBA) was co-polymerized with free-radical photoreactive (4-vinylcyclohexene glycol) methacrylate (PEGMA) and methyl methacrylate (MMA). Upon UV irradiation, the oNBA moiety rearranges to yield methacrylic acid, significantly altering the solubility properties of the polymer. As the contents of PEGMA and oNBA in the resin terpolymer were increased, films of the irradiated resist exhibited water solubility at decreasing pH. This trend was found to be true for all ratios of oNBA:PEGMA:MMA, except near compositions of 35:65:0 wt:wt:wt, where the polymer’s solubility in water was seen to decrease at all pH’s after UV irradiation. Before exposure, the polymer film’s dissolution is facile, but it is significantly slowed after irradiation, potentially as a result of intramolecular hydrogen bonding. This phenomenon was further explored and we found that in the presence of water, induced by solvent evaporation, the polymer, due to its porosity, could adsorb water and was thereby solubilized. This phenomenon caused solubility to increase with decreasing pH. Further, we investigated the use of patterns to enable two-component patterning of proteins on a surface under mild aqueous conditions (pH 6.8, temperatures 4°C-37°C). With a single UV exposure performed prior to protein immobilization, the unexposed portions of the film can be removed with water within a few seconds at room temperature, while the exposed regions dissolve over a few hours. During the interleave time the protein can be immobilized on the now-exposed surface. Increasing the temperature to 37°C and incubation in PBS speeds the dissolution of the UV-exposed portion of the film, allowing for the adsorption of a second protein within a few hours. These resist multilayer platforms provide a facile way for patterning proteins and other fragile biomolecules under conditions that maintain their optimal functionality and avoid the use of toxic organic solvents.

K5.13
Patterned 3D hydrogel films for biological screening.
Rein Vincent Uljijn and Mohammed Zouroob; School of Materials, University of Manchester, Manchester, merseyside, United Kingdom.

With the advent of microarray technology, biological ligands immobilized on flat glass surfaces are becoming increasingly important for the rapid parallel screening of biological interactions of both whole cells and purified proteins. Microarrays are also becoming increasingly common in the highly successful DNA micro-arrays as bioocompatibility of the surface becomes important and amplification methods (such as PCR) are not available. The ideal micro array would be one that (i) prevents non-specific protein adsorption at the surface (ii) has a high binding capacity of ligands, (iii) highly hydrated (wet) to study interactions at biologically relevant conditions. Here we present a novel hydrogel array platform that has each of these properties. It was tested in single cell screening experiments involving enzymes and whole cells. The hydrogel array is based on patterned poly ethylene glycol-acrylamide co-polymer (PEGA) directly onto a functionalized glass surface. The gel patterns have the same chemical properties (inertness, swelling, accessibility, biocompatible) as the agarose-based bead-captured PEGA-beads that have found many uses in biological screening experiments. The advantage of using such as 3 D array is that it has a higher substrate loading, avoids protein denaturation at the solid-liquid interface, universal handling (like conventional micro titer plates), easy to read using CCD camera, very low substance consumption. These patterned surfaces were used in a simple screening experiment involving proteases and in a cell binding assay.

K.5.14 Abstract Withdrawn

K.5.15 Electrospin Nanofiber Tissue Scaffolds Based on Polycaprolactone and Hydroxyapatite, Derek Dean1, Sunita Jagun1, Kolanah Johnson1, Shaune Forbes1, Mony Jose1, Vinoy Thomas2,1, Vitor Rocha1, and Elijah Johnson1,2; 1 Materials Science and Engineering, University of Alabama at Birmingham, Birmingham, Alabama; 2 Physics, University of Alabama at Birmingham, Birmingham, Alabama; 3 Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, Alabama; 4 Physical Sciences, Alabama State University, Montgomery, Alabama.

With the continual aging of the population in the United States, bone fractures and diseases such as osteoporosis, osteomalacia and osteitis deformans (Paget’s disease of bone) present a need for the development and perfection of bone regeneration methods. While numerous bone fractures can be treated by bone grafting, success rates vary, and the treatment is limited in several ways. Biodegradable bone scaffolds may provide solutions that strike a balance between function and mechanical properties. In living systems, the extracellular matrix, ECM, plays a vital role in controlling cell behavior. In addition, it has been shown that nanometer size features influence cell behavior, and cells attach well to fibers with diameters smaller than the cell size. The emergence of electrostatic spinning of polymeric fibers offers a nanofabrication technique by which three-dimensional structures (i.e. fibers) with nanoscopic diameters, high surface area and porosity can be obtained. The application of synthetic nanofibers offers the potential to fabricate well-defined architectures that mimic the dimensions of the ECM, and promote cell growth. While a significant number of studies have investigated biodegradable polymers for bone tissue scaffolds, one problem which has not been solved is the mismatch between the mechanical properties of the bone and the scaffold materials. Thus, the objective of research proposed herein is the development of biodegradable tissue scaffolds with nanometer dimensions, an interconnected pore structure, and enhanced mechanical properties. We have recently electrostatically spun fibers of polycaprolactone (PCL) and hydroxyapatite (HA) with diameters ranging from 50 to 1000 nm. The strengths increase from 1.2 MPa for pure PCL to 2 and 2.5 MPa for 5 and 10 wt% hydroxyapatite, respectively. In vitro Cell growth studies using mouse osteoblast-like cells were undertaken. In addition to enhancing the mechanical integrity of the PCL, it is anticipated that the presence of the hydroxyapatite will help stimulate cell growth. We acknowledge support from the UAB Center for Metabolic Bone Disease; NSF-NIH under DMR-0402891 and NSF-REU program under DMR-0249040.

K.5.16 Electrical field stimulation enhanced excitation-contraction coupling in engineered muscle constructs: effects of voltage and frequency, HyoEunghun Park1, Rajiv Saigal1, Rajat Bhalla2, Milica Radisic1, Nicki Watson2, Robert Langer1 and Gordana Vunjak-Novakovic1; 1 Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts; 2Whitbread Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge, Massachusetts.

In our previous studies, electrical field stimulation designed to mimic signals present in the native heart was applied to neonatal rat cardiomyocytes cultured on collagen scaffolds. Electrical stimulation induced cell elongation and alignment, and enhanced the formation of gap junctions and sarcomeres. Over only eight days, cultured cells assembled into cardiac-like tissue that contracted synchronously in response to electrical pacing and had remarkably well developed ultrastructural properties (Radisic et al., 2004). We observed that the structure and function of engineered muscle constructs depended strongly on the initiation and duration of electrical stimulation. In further studies, we explored if the tissue outcomes also depended on the voltage gradient and frequency of electrical stimulation, and whether excitation-contraction coupling extends to other electrically excitable cells. In the present study, electrically excitable muscle cells (C2C12 cell line) were seeded onto collagen scaffolds (6 x 8 x 1.5 mm) and cultured for 3 days in myogenic medium without stimulation, to allow cell attachment to the substrate and synthesis of extracellular matrix proteins. Starting at day 4, tissue constructs were stimulated continuously for an additional 5 days, using rectangular biphasic signals (2 ms in duration) at a range of voltage gradients (2, 5 or 7 V/cm) and signal frequencies (1 or 2 Hz). Non-stimulated constructs were served as a control. The amount of DNA was comparable for all constructs, and only few apoptotic cells were detected. Constructs cultured with electrical stimulation were excitable, as evidenced by macroscopic contractions in response to electrical pacing, in contrast to control constructs that could not be induced to contract. The excitation threshold, a minimum voltage inducing synchronous contractions, was in the range of 3 to 7 V. The stimulation voltage gradient of 5 V/cm resulted in constructs with highest expression of ±-sarcomeric actin and troponin I (observed in immunostains), which correlated with elongated cells with parallelized and organized sarcomere structures (observed in transmission electron microscopy). Our data suggest that electrical stimulation enhances organization of contractile proteins and improves contractile behavior electrically excitable cells.

K.5.17 Patterned co-cultures of three-dimensional cell spheroids and support cells using microstructure patterned substrates, Ali Khademhosseini1, Yvonne Green2,3,4, Jeff J. Huang5,6,7, Robert Langer1,2, and Howard M. Chang1,2,3,4,7; 1 Massachusetts Institute of Technology, Cambridge, Massachusetts; 2 Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts; 3Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Patterned co-cultures can be used to control cell-cell interactions and to design biomimetic tissue engineering constructs by positioning multiple cell types relative to one another. These cultures are typically based on the selective adhesion of a specific cell type to particular regions of a micropatterned substrate and the subsequent adhesion of a second cell type to the surrounding regions. Previous developed patterned co-culture approaches have been based on cell monolayers and have therefore limited two-dimensional cultures. The development of patterned co-culture methods in which three-dimensional aggregates of cells (i.e. spheroids) are formed may be beneficial for cell types such as hepatocytes and fibroblasts, in which homotypic cell-cell interactions are important for cellular function. In this work, we present a method of using patterned arrays of microwells by fabricating patterned co-cultures that maintain the 3D structure of cellular aggregates. Cell spheroids are formed within poly(dimethyloxiane) microspheres coated with hyaluronic acid (HA) or used directly. The spheroid shape and size were controlled by the initial dimensions of the well. Once spheroids were formed, the subsequent adhesion of a second cell type in the regions in between the microwells could be controlled. Patterned co-cultures of hepatocytes or embryonic stem cells with fibroblasts were successfully generated using this approach. It was seen that the co-cultures produced in this scheme remained stable over time, presumably due to the biodegradable microwell structure which minimized cell migration out of the wells. It is anticipated that the addition of three-dimensional spheroids into patterned co-cultures could be used to enhance cell function, and thus this approach may be a useful tool for studying cell behavior and for tissue engineering applications.

K.5.18 Cell Growth on Polymer-Clay Surfaces, Aprajita Mattoo1, 2, Aattar Singh2, Ying Liu3, Lourdes Collazo4, and Mirmir Rasafa1,2,5; 1Columbia University, Jericho, New York; 2Cornell University, Ithaca, New York; 3Materials Science & Engineering, Stony Brook University, Stony Brook, New York.

In order to further the advancement of biomaterials science, it is necessary to study the interactions between polymer surfaces and cells. Previous studies indicate that most polymers are poor substrates for cell growth. Preliminary studies conducted within our
lab suggest that the addition of clay to PB (poly butadiene) increases the polymer's ability to support cells. In this study, PMMA (poly methyl methacrylate), a nanocomposite with a more defined structure and investigated as viable substrates for cell compatibility. Cell growth was studied as a function of clay concentration. Solutions of Cloisite 6A clay and PMMA were prepared at 5%, 10%, and 20% clay by weight and spun onto glass coverslips or Petri dishes to label cells grown on these substrates were used to examine cytoskeletal arrangement and cell morphology. A, atomic force microscopy was used to study the effects of clay on the mechanical properties of the polymer substrates. Human dermal epithelial fibroblasts were seeded onto the aforementioned surfaces and growth curves were generated over a 12-day span. The addition of clay to PMMA was found to facilitate improved cell proliferation, particularly at higher concentrations of Cloisite 6A. Substrates with PMMA alone supported less than 50% as many cells as those with clay, and samples with 20% clay exhibited the control. The similarity between polymer-clay nanocomposites and the control may be explained by the presence of silicates in the clay, which give the substrates glass-like properties. In the future, PANI-EB (poly aniline emodindole base) and PEO (poly ethylene oxide) surfaces will also be studied.

Kakoli Das1, Howard L. Hiosck2, Susmita Bose3 and Amit Bandopadhyay4; 1'MME, WSU, Pullman, Washington; 2'SBS, WSU, Pullman, Washington.

We have studied cell materials interaction of bioactive TiO2 layer grown on commercially pure titanium substrate using anodization process. Titanium is a bioinert material and, therefore, gets encapsulated after implantation into the living body by a fibrous tissue that isolates them from the surrounding tissues. Titanium surface could be made bioactive by several methods including growing a TiO2 layer on the Ti-surface. In this work, TiO2 layer was grown on Ti-surface by anodization process using H3PO4, HF and H2SO4 electrolyte solutions. Effects of anodization time and voltage on growth behavior of TiO2 films were studied. Mineralization study was done under simulated body fluid (SBF) with ion concentration nearly equal to human blood plasma to understand biomimetic apatite deposition behavior. Human osteoblast cell growth behavior was studied with as received and anodized Ti substrates using an osteocprecursor cell line (OPC-1) for 3, 5 and 11 days. Colonization of the cells was noticed with distinctive cell-to-cell attachment in HF anodized TiO2 films. Good cell adherence was noticed on TiO2 film made using H3PO4 electrolyte but the cells were confined to small regions. TiO2 layer made by H2SO4 electrolytes did not show cell growth on the surface and some cell death was also noticed. It was clear that electrolyte composition matter for cell materials interaction for TiO2 on Ti surfaces. The presentation will focus on microstructural evaluation and in vitro cell-materials study of anodized Ti substrates.

K5.20 Highly Parallel Fabrication of Nanopatterned Surfaces for Controlling Cell Response: Jonathan Gass1,2, and Wei Wang Frey1,2.1 Biomedical Engineering, University of Texas at Austin, Austin, Texas; 2Center for Nano and Molecular Science and Technology, University of Texas at Austin, Austin, Texas.

Cellular adhesion to artificial surfaces and extracellular matrix is mediated by integrins. Their organization serves as points of attachment and mediators in a variety of surface-initiated signaling events in the cell. Integrin clustering into focal adhesions is important for the stability of cellular attachments, for the formation of cytoplasmic protein assemblies at the clusters, the subsequent signaling through several pathways, and the build-up of stress along actin stress fibers. Knowledge of how specific signaling events and the resultant cell responses can be induced by engineering the nano-scale surface parameters is important for the development of advanced biomaterials. Experiments to elucidate this knowledge require the use of a functionally functionalized surface pattern down to the molecular scale. However, many processes that can achieve these resolutions are serial in nature and cannot yield the fabrication speed needed to produce the large quantity of samples needed to acquire statistically relevant data. We present a technique based on a parallel imprinting technique that allows the rapid fabrication of biologically functionalized nanopatterns with 60 nm resolution. The surface pattern is translated into a nanopatterned chemical functionality by orthogonal functionalization of the patterned nanopatterns with a small number of integers to bind specifically to each pattern island while the surrounding areas are inhibiting cell adhesion. Because the fabrication process is highly parallel, we can simultaneously produce a matrix of 96 different patterned surfaces each on a size of 1 x 1 mm each. This allows us to vary the attachment island size and spacing independently from 60 nm to 900 nm within one sample. We have seeded vascular endothelial cells on these surfaces and compared the results with a more densely self-assembled surface-functionalized pattern. This has allowed us to judge the influence of surface pattern defects on the behavior of cells and achieve size to spacing ratios that could not be achieved with the self-assembly technique. Additionally, the matrix of varying surfaces patterns has enabled us to systematically examine the dependence of focal adhesion maturation on the integrin cluster size and the cluster spacing.


We have studied cell-materials interactions on nanoporous titania films on Ti surfaces. Titanium is a bioinert material and, therefore, gets encapsulated after implantation into the living body by a fibrous tissue that isolates them from the surrounding tissues. Titanium surface could be made bioactive by several methods including growing a TiO2 layer on the Ti-surface. Nanoporous titanobas were grown on Ti-surface using aqueous solution mixture of sodium fluoride, citric acid and sulfuric acid as an electrolyte. The electrolyte pH was maintained at 4.5. Anodization runs were carried out potentiostatically at 20V for 2, 4 and 10h. It was found that increasing anodization time initially increased the height of the nanotubes while maintaining the tubular array structure, but beyond 10h, growth of nanotubes decreased with a collapsed array structure. After 4h anodization time, 1 micron long nanotube was obtained. To better understand and compare cell-materials interactions with and without TiO2 nanotubes, anodized sample surfaces were etched with different patterns with H2NO solution. As received, as grown and patterned surfaces were used for human osteoblast cell growth study for 3, 5 and 11 days. It was observed that nanotubes actually help the microextensions projecting from cells to grasp the substrate. Good cell attachment was observed in nanoporous area compared to the etched surface where Ti surface was exposed. Mineralization study was done under simulated body fluid (SBF) with ion concentration nearly equal to human blood plasma to understand biomimetic apatite deposition behavior. Though apatite layer formation was noticed, but it was non-uniform even after 21 days in SBF.

K5.22 Nanophase Calcite Rods - Fluorescent Multifunctional Materials by Controlled, Confined Crystallization. Richard Lloyd Carroll, Dmitry Spivak and Mike Falvo; Physics and Astronomy, University of North Carolina - Chapel Hill, Chapel Hill, North Carolina.

Two important strategies exploited in biomineralization are molecular scale templating (to control crystallization) and spatial confinement of the reaction process (to control overall morphology). Through the use of these strategies, among others, biological systems build exquisitely complex architectures across a range of size scales, from the single cell to the plankton to the skeleton of the blue whale. Here we have improvised on these themes to synthesize nanophase calcite structures. We have made single crystal calcite microrods and nanorods through controlled crystallization within the pores of polycarbonate track etched membranes (PCTE) via a diffusion-limited vapor-solution interaction. By engineering the interfacial interaction of the calcite components, we have expressed control over the formation of the crystal. We have modified the surface functionality of the templates and studied how this affects the crystal structure and habit of the resulting calcite nanorods. We will also present results which show that control over the crystal orientation of each nanorod is possible by modifying the surface chemistry present at the interface. We have observed an array of calcite nanorods extending from a face of the single rhombohed calcite crystal. The morphological features of the distal ends of these rods suggest they have consistent crystalline orientation (they are part of the same single crystal) as the base rhombohed. We will describe further efforts to characterize these structures and to control this behavior in the interests of understanding the phenomenon and in making use of such crystalline arrays in technical application. As a biorelevant material that can be made to fluoresce at various colors, nanophase calcite has potential of applications based on its optical properties. Through the careful introduction and control of ppm levels of some earth activators, we have been able to modify nanophase calcite material. Results of experiments to understand the effect of activator concentration on fluorescent intensity, emission spectra, and crystal form and habit will be presented. Of central interest to our group is the goal of using nanophase calcite in optical applications, including as a marker in biological contexts. We will describe efforts to characterize the biocompatibility and usefulness of nanorods in these types of experiments.
K5.23
Control of Neuron Adhesion and Neurite Outgrowth Using Micropatterns of an Electrodeposited Polymer Composite.
H.-K. Song$^{1,2}$, D. Hoffman-Kim$^{1,2}$ and G. T. Palmore$^{1,2}$
$^{1}$Engineering, Brown University, Providence, Rhode Island; $^{2}$Biology and Medicine, Brown University, Providence, Rhode Island.

We have developed an electroactive polymer composite to which biomolecules can be coupled. This polymer composite consists of a polycationic matrix of polypyrrole (pPy) and a polyanionic dopant of poly-L-glutamic acid (pGlu). Micropatterns of this polymer composite (pPy[pGlu]) can be fabricated by dipping an electroactive substrate into an aqueous solution containing pyrrole and pGlu and subsequent application of potential. The polyanionic dopant was chosen so as to create a high concentration of carboxylic acid groups protruding from the surface of the polymer composite. As such, the carboxylic acid groups can be activated for subsequent covalent attachment of any biomolecule, such as poly-L-lysine (pLys), a material known to promote neuron adhesion and neurite outgrowth. In this talk, the electrosynthesis and characterization of micropatterned substrates coated with pPy[pGlu]-pLys will be described. Spectroscopic analysis of the substrates during each stage in the fabrication will be shown. The dependence of neuron adhesion and neurite outgrowth on the concentration of pLys and other biomolecules covalently confined to the surface of the polymer composite will be demonstrated.

K5.24
Keratin Protein based Novel Biomaterials for Antibiotic Drug Delivery and Bone Regeneration. Grace Jeong Lim, Sang Jin Lee and Mark Van Dyke; Regenerative Medicine, Wake Forest University, Winston Salem, North Carolina.

Keratin biomaterials draw many of their novel properties from their source, human hair, a fiber that is readily available and one of the few reserves of human tissue that can be obtained without trauma or injury to the donor. We were able to efficiently extract various types of keratin proteins from human hair fibers by way of both oxidation and reduction, and investigated to utilize their novel properties for biomedical applications such as drug delivery system and simultaneously bone tissue regeneration. We incorporated bioceramic filler and antibiotics to the keratin proteins to create a keratin bioceramic antibiotic putty (KBAP) which is a prototype bone graft substitute. We have discovered that KBAP had a characteristic capability of osteoconductivity and osteoinductivity in vitro as well as controlled and sustained antibiotic release for extended period into an easy to use and inexpensive product. In addition, keratin biomaterials had a unique property of self-assembly into nanofiber structure that exhibited a distinct advantage over conventional biomaterial grafts that are difficult for cells to populate and remodel. These human keratin proteins were shown to be highly functional as biomaterials in enhancing bone cell proliferation and controlled release of biologically active substances.

K5.25
A simple method for fabrication of micropatterned cell arrays using photocrosslinkable chitosan. Milica Radić$^{1,2}$, Yoon Yeo$^{3}$ and Wenliang Geng$^{3}$; $^{1}$IBBME, University of Toronto, Toronto, Ontario, Canada; $^{2}$Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada; $^{3}$Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

We developed a simple and inexpensive method for patterning cell arrays on glass and tissue culture plastics using photocrosslinkable chitosan. Chitosan was modified with photoactive azidoenzenzic acid to make an in situ photocrosslinkable hydrogel. Subsequently, it was dissolved in normal saline at 20mg/ml and spin coated onto the glass or polystyrene surface at 2000 rpm for 10s. Lanes of crosslinked hydrogel that prevent cell adhesion were created directly by UV illumination for 5s to 4 min through the photomask applied to the surface opposite to the chitosan layer. By varying UV exposure time a single photomask was utilized to pattern the surfaces with a range of chitosan lane sizes. Atomic force microscopy indicated that the lane height varied from 401μm for 500μm lanes spaced 500 μm apart to 203μm for 100μm lanes spaced 100 μm apart. The patterned surfaces were supportive of primary cell adhesion and differentiation. Cardiac fibroblasts, isolated from neonatal rat hearts, were plated onto Petri Dishes with a pattern consisting of 700μm wide chitosan lanes spaced 300μm apart. The cells selectively adhered only in between chitosan lanes, remained stable for 24 days and expressed intermediate filament vimentin. Neonatal rat cardiomyocytes were plated on patterned glass surfaces with chitosan lane spacing varying from 70 to 100μm. Cardiomyocytes adhered in between the chitosan lanes with the degree of alignment dependent on the spacing size. After 8 days of culture the cells expressed cardiac troponin I and remained functional as evidence by pacing from 60bpm to 220 bpm using electrical field stimulation at 4.3 V/cm. Since chitosan can be degraded at