SYMPOSIUM AA

Applications of Micron and Nanoscale Materials in Biology and Medicine

November 28 - December 2, 2004

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* Invited paper

TUTORIAL

FT AA: Micro- and Nanotechnology in Regenerative Medicine
Sunday November 28, 2004
1:30 PM - 5:00 PM
Room 206 (Hynes)

This tutorial will provide an overview of recent advances in the design and utilization of micron and nanoscale biomaterials in regenerative medicine.

Topics will include:
(1) the use of bio-electromechanical systems (bioMEMS) to perform bioanalytical processes and to create templates for cell and tissue regeneration,
(2) micro- and nanofabrication methods for the design of tissue engineering scaffolds based on synthetic and natural polymeric materials, and
(3) characterization of micro- and nanofabricated materials with emphasis on governing relationships of material structure and biological function.

The tutorial is primarily aimed at students and researchers, new to micro- and nanotechnology, who would like to learn more about how these technologies may impact their current research and the next generation of implantable biomaterials.

Instructors:
Edward A. Botchwey
University of Virginia

Tejal Desai
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SESSION AA1: Nano and Micron Scale Materials for Cell and Tissue Engineering - I
Chairs: Craig Halberstadt and Eleni Kousvelari
Monday Morning, November 29, 2004
Room 306 (Hynes)

8:30 AM *AA1.1
Design and Analysis of Adhesion Ligand-Cell Interactions in Tissue Regeneration. David J. Mooney, Division of Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts.

Hydrogels are attractive materials for tissue engineering and regeneration applications as they are structurally similar to the extracellular matrix of many tissues, can immobilize cells using mild conditions, and may be delivered into the body in a minimally invasive manner. We have covalently coupled cell adhesion peptides to alginate chains used to form gels, in order to infer specific means of cell adhesion. The ability of these materials to regulate cell function in vitro is dependent on the specific peptide, degree of peptide substitution on the polymer, mechanical background of the gel presenting the peptides, and the nanoscale organization of the peptides. Appropriate combinations of peptide presentation and gel physical properties allows one to regenerate new tissues (e.g., bone and cartilage) in vivo from transplanted cells. We have now begun to probe the relationship between peptide presentation and actual receptor binding using a combination of rheological, mechanical and FRET measurements in an effort to determine the relation between the number of cell receptor-ligand interactions in these systems and the cell response. This information may provide a new design criteria for cell-interactive biomaterials.

9:00 AM AA1.2
Two-Dimensional Differential Adherence and Myoblast Alignment Driven Fabrication of Engineered C2C12 Muscle Organoids. Timothy M. Patz[1,2], Rohit Modi[2], Roger Jagdish Narayan[1] and Douglas B. Chrisey[2]; [1]Material Science & Engineering, Georgia Institute of Technology, Atlanta, Georgia; [2]US Naval Research Laboratory, Washington, District of Columbia.

We have demonstrated two-dimensional differential adherence and controlled alignment of C2C12 myoblasts in extracellular matrix-enriched channels that have been created using a novel laser micromachining process. An ArF excimer laser (lambda=193 nm) was used to micromachine 60-400 um wide by 60-500 um deep and 1 cm long channels into a 2% agarose gel matrix in a 3-inch petri dish. Extracellular matrix (ECM) was pipetted into these channels. A C2C12 myoblast cell suspension was added to media in the petri dish and the cells preferentially adhered to the ECM-lined channels. These seeded patterns were incubated for a period of several days and monitored at critical time points to observe the cell growth, alignment, and three-dimensional organization. Live/dead assays at all time points tested (up to 3 days) demonstrated that the cells within the channel remained viable and multiplied. The cell size and nuclei number both increased over time, indicating that some of the myoblasts differentiated into multinucleated myotubes. The myoblasts and myotubes appear to remain aligned along the length of the channel for channel widths between 60 and 150 um and channel width/cell size ratios between 5 and 12.5. The 400 um channel (channel-width/cell-size ratio of 33) was not narrow enough to induce cellular alignment. The implications of this work on the development of porous tissue engineered materials are discussed.

9:15 AM AA1.3
Helical Rosette Nanotubes: A Potentially More Effective Orthopaedic Tissue Engineering Material. Ai Lin Chun[1], Hicham Fenniri[2] and Thomas Jay Webster[1]; [1]Biomedical Engineering, Purdue University, Lafayette, Indiana; [2]University of Alberta, Edmonton, Alberta, Canada.

Due to the nanometric properties of some physiological components of bone, nanomaterials have been proposed as the next generation of improved orthopaedic implant material. Yet, current efforts in the design of orthopaedic materials such as titanium (Ti) are not aimed at tailoring their nanoscale features, which is now believed to be one reason why Ti sometimes fails clinically as a bone implant material. Much effort is thus, being dedicated to developing improved bioactive nanometric surfaces and nanomaterials for biospecificity. Helical rosette nanotubes (HRN) are a new class of self-assembled organic nanotubes possessing biologically-inspired nanoscale dimensions. Because of their chemical and structural similarity with naturally-occurring nanostructured constituent components in bone such as collagen and hydroxyapatite, we anticipated that a HRN-coated surface may simulate an environment that bone cells are accustomed to interacting with. The objective of the present in vitro study was therefore to determine the efficacy of HRN as a bone prosthetic material. Results of this study show that both HRN functionalized with Lys and with Arg coated Ti displayed enhanced osteoblast (or bone forming cell) adhesion when compared to uncoated Ti. Enhanced cell adhesion was observed even at concentrations as low as 0.005 mg/ml. Moreover, studies into the mechanisms of increased osteoblast adhesion on HRN coated Ti revealed that these materials emulate certain properties of proteins that mediate cell attachment. These results point towards new possibilities in bone tissue engineering as they serve as a starting point for future manipulation of the outer chemistries of HRN to improve the results beyond those presented here. In this manner, this study represents one of the first to develop a novel organic nanomaterial that can be simply coated onto Ti for bone tissue engineering applications.

9:30 AM AA1.4
Fabricating Functional 3D Cardiac Patches by Rapid Prototyping Technology. Tao Xu[1], Catalin F. Baicu[2], Michae R. Zile[2] and Thomas Boland[1]; [1]Department of Bioengineering, Clemson University, Clemson, South Carolina; [2]Department of Medicine, Medical University of South Carolina, Charleston, South Carolina.

Rapid fabrication of 3D tissue or organ analogs with well-defined structures and functions is a challenge in regenerative medicine. We have developed a novel rapid prototyping technology, cell inkjet printing, to readily assemble and process living cells and biomaterials, including mammalian cells, engineered bacteria, and extra-cellular matrix proteins. Although printing anatomical structures for engineered tissues has been envisioned to lead to the creation of artificial organs, much development of the printing technique is needed. Here we focus on the latest application of this technology towards building 3-dimensional cardiac constructs. The technology allows rapid assembly of healthy functional cardiac tissue-like constructs possessing specific structures and forms. 3D cardiac patches were printed layer-by-layer using adult mammalian cardiomyocytes combined with biodegradable alginate scaffolds, crosslinked on demand with calcium chloride. Adult feline and murine isolated cardiomyocytes within the 3D printed patches exhibited their normal morphologies with clear sarcomere appearances after 1, 4, 8, and 13 days of culture. The mechano-electrical evaluations of the printed patches were performed using computer assisted videomicroscopy and IonOptix® video edge-detection protocols. Under electrical stimulations, not only were the individual adult cardiomyocytes observed to contract rhythmically within the printed patches, but also the whole patches were beating periodically. Electrically stimulated at 1Hz, the shortening extent of the total lengths of the patches was up to 3.5%, and the shortening velocity was about 113.4 ± 9.0 μm/s (n=3). Moreover, in response to increased stimulating rates, the printed patches beat more promptly and strongly. Additionally, other 3D cardiac constructs with complex structures, like tubes and half heart-like constructs were successfully printed. The passive

mechanical properties of the printed 3D constructs will be tested and the vascularization of the printed patches will be evaluated in vivo. The pulsatile ability of the printed patches and the flexibility to fabricate complex forms and structures of the cell printer open the possibility to construct functional 3D myocardial tissue on demand to meet the need of replacing lost or damaged tissues of failing myocardium. Finally, with its obvious advantages of high-throughput and flexibility, the bottom-up technology is offering the researchers a cost-effective tool to rapidly fabricate cell patterns and tissue-like structures for clinical tissue engineering.

10:15 AM *AA1.5
Mammalian Cell Interactions with Nanophase Materials.
Rena Bizios, Department of Biomedical Engineering and Nanotechnology Center, Rensselaer Polytechnic Institute, Troy, New York.

Current research on implantable biomaterials is motivated by the realization that, for the clinical success of implants it is not enough to minimize undesirable tissue-biomaterial interactions; it is also necessary to promote specific functions of (as well as induce select, desired and timely responses from) surrounding cells and tissues. In this respect, nanostructured, that is, materials with grain sizes less than 100 nm in at least one dimension, are extremely promising. Compared to conventional ceramics (such as alumina, titania and hydroxylapatite) nanophase formulations of these materials, as well as their composites with either poly(L-lactic acid) or poly(methyl methacrylate), promote enhanced functions (such as adhesion and proliferation) of mammalian cells (such as osteoblasts). Most importantly, nanophase materials promote interactions selectively, for example, those of osteroblasts but not those of fibroblasts. The type, amount and conformation of adsorbed proteins (such as fibronectin, collagen and vitronectin) are key aspects of the underlying mechanism(s) of subsequent cell interactions with nanophase materials. To date, most of the studies used differentiated mammalian cells from tissues pertinent to the intended biomedical application, for example, osteoblasts for orthopaedic applications. Recent endeavors expanded the scope of this research to include cells from various soft and hard tissues as well as adult mesenchymal stem cells. These cellular/molecular results provided evidence that nanophase materials have the potential for improving the efficacy of implants and for promoting neotissue formation pertinent to tissue engineering applications.

10:45 AM AA1.6
Poly(glycerol-sebacate) Microfluidic Scaffolds for Vascular Tissue Engineering.
Chris John Bettinger[1,4], Christina W. Fidkowski[2], Yadong Wang[3], Jeffrey T. Borenstein[4] and Robert S. Langer[5]; [1]Materials Science, MIT, Cambridge, Massachusetts; [2]Health Science and Technology, Harvard Medical School, Cambridge, Massachusetts; [3]Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia; [4]Charles Stark Draper Laboratories, Cambridge, Massachusetts; [5]Deparment of Chemical Engineeing, MIT, Cambridge, Massachusetts.

This work describes the integration of novel microfabrication techniques for vascular tissue engineering applications in the context of a novel biodegradable elastomer. The field of tissue engineering and organ regeneration has been born out of the high demand for organ transplants. However, one of the critical limitations in regeneration of vital organs is the lack of an intrinsic blood supply. This work expands on the development of microfluidic scaffolds for vascularized tissue engineering applications by employing microfabrication techniques. Building on previous advances in processing of PLGA (poly(L-lactic-co-glycolic) acid) constructs, this work focuses on fabricating scaffolds from poly(glycerol-sebacate) (PGS), a novel biodegradable elastomer with superior mechanical properties and biocompatibility. The transport properties of oxygen and carbon dioxide in PGS were measured through a series of time-lag diffusion experiments. The results of these measurements were used to calculate a characteristic length scale for oxygen and carbon dioxide diffusion limits in PGS scaffolds. Microfluidic scaffolds were then produced using fabrication techniques specific for PGS. Initial efforts have resulted in solid PGS-based scaffolds with physiologic fluid flow properties and capillary channels on the order of 10 microns in width. These scaffolds were pre-treated with protein solutions and seeded with human umbilical vascular endothelial cells (HUVECs) and perfused continuously in culture for up to 14 days resulting in partially confluent channels. Additional fabrication techniques using PGS were also demonstrated in the development of fully biodegradable, implantable microfluidic scaffolds with improved microvascular geometries.

11:00 AM AA1.7
Functional Fibrous Scaffolds for Liver Tissue Engineering.
Kian Ngiap Chua[1,2], Weiseng Lim[1], Hongfang Lu[1], Seeram Ramakrishna[2], Kam W. Leong[3,1] and Hai-Quan Mao[4,1]; [1]Division of Biomedical Sciences, Johns Hopkins in Singapore, Singapore; [2]Division of Bioengineering and NUSNNI, National University of Singapore, Singapore, Singapore; [3]Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, Maryland; [4]Department of Materials Science and Engineering, Whiting School of Engineering, Johns Hopkins University, Baltimore, Maryland.

Primary rat hepatocytes self-assemble into multi-cellular spheroids when cultured on a polymer membrane or hollow fiber surface conjugated with galactose ligand.[1,2] As high cell density packed multi-cellular aggregates, hepatocyte spheroids prolong differentiated hepatic functions as compared with a monolayer culture. For this reason, hepatocyte spheroids are attractive in the design of bioartificial liver assist device.[3] We have previously shown that galactosylated non-woven PET microfiber scaffolds could stimulate the spheroid formation. In this study, we investigated the influence of a nanofiber mesh on hepatocyte attachment, migration, spheroid formation and maintenance of the differentiated functions in comparison with the 2-D substrates. Nanofiber meshes comprising of fibers with an average diameter of 800 nm were prepared by electrospinning of a novel biodegradable polyphosphoester, poly(ϵ-caprolactone-co-ethylethylene phosphate) [PCL-co-EEP].[4] The hepatocyte specific nanofiber scaffold was synthesized by conjugating galactose ligand to poly(acrylic acid) grafted PCL-co-EEP nanofiber mesh, which was prepared by UV initiated acrylic acid polymerization on the fiber mesh surface.[1] The galactose ligand density on this functional nanofiber scaffold was 66 nmol/cm^2. Primary rat hepatocytes cultured on galactosylated PCL-co-EEP nanofiber scaffolds and thin film substrates exhibited similarly high hepatocyte attachment (80-90%) 3 hours after cell seeding. Both cultures showed similar functional maintenance profiles in terms of albumin secretion and cytochrome P450 enzymatic activity. Interestingly, spheroids formed on the functional nanofiber substrate exhibited a distinct morphology. Hepatocytes cultured on galactosylated PCL-co-EEP film formed 50-300 μm spheroids that were easily detached from surface upon agitation; whereas hepatocytes cultured on galactosylated nanofiber scaffold formed smaller aggregates of 20-100 μm that engulfed the functional nanofibers. It appears that galactose-ASGPR ligand-receptor interaction allowed hepatocytes to aggregate around galactosylated nanofibers. This unique interaction resulted in an integrated spheroid-nanofiber construct. In summary, galactosylated PCL-co-EEP nanofibers have shown the unique property of promoting hepatocyte aggregates within nanofiber mesh, forming an integrated spheroid-nanofiber construct. It suggests the potential application of this galactosylated PCL-co-EEP nanofiber scaffold in liver tissue engineering. Acknowledgement: The authors thank Dr. Chou Chai and Dr. Pengchi Zhang at JHS for their technical assistance and helpful discussions. PCL-co-EEP was provided by Dr. Jie Wen at JHU. References: [1] Yin C, et al. J. Biomed. Mater. Res. 2003; 67A:1093-1104. [2] Lu HF, et al. Biomaterials. 2003; 24:4893-4903. [3] Funatsu K, et al. Artificial Organs 2001; 25:194-200. [4] Wen J. and Leong K. 29th Intl. Symp. Contr. Rel. Bioact. Mater. 2002; 29:470.

11:15 AM AA1.8
Competitive Guidance Cues Affect Fibroblast Cell Alignment: Electric Fields vs. Contact Guidance.
Iain R. Gibson and Colin D. McCaig; School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom.

When bovine ligament fibroblast cells were cultured on parallel micro-grooved surfaces, they aligned their long axes parallel (approaching 0 degrees) to the groove direction. This alignment was dependent on the groove depth, with increasing groove depth (62, 347, 547 and 1024nm) resulting in increased guided cell alignment. When fibroblast cells were cultured in a physiological dc electric field (EF) on non-grooved, flat surfaces, the cells aligned in response to the electric field, with their long axes aligning perpendicular (approaching 90 degrees) to the electric field vector. This response was field strength dependent, with increasing field strength (20, 50, 100 and 200mV/mm) resulting in increased guided cell alignment, perpendicular to the EF vector. These two guidance cues were applied simultaneously, so that the EF vector was parallel to the groove direction. For high field strengths (200mV/mm) cells ignored the topography and were guided by the EF alone, with similar alignment, perpendicular to the EF vector, to cells on non-grooved surfaces. Low field strengths (20mV/mm) resulted in cells responding only to the topography as a guidance cue, with cells aligning parallel to the groove direction and the EF vector. Intermediate field strengths (50 to 100mV/mm) produced a mixed response, with cells appearing to be responding to both guidance cues, with cell alignment relative to the field vector ranging from approximately 30-55 degrees. The effect of removing serum from the culture medium on the EF and topographical guidance of fibroblast cells was studied and the results were compared to cells on non-grooved surfaces. Removal of serum produced a small decrease in the angle of cell alignment for cells on non-grooved surfaces, from 78 to 63 degrees, relative to the EF vector,

but did not completely suppress the EF guidance cue. In contrast, the EF guidance of cells on both grooved substrates was almost completely suppressed by the absence of serum, with cells responding only to the grooved topography, aligning their long axis parallel to the groove direction/EF vector. These results imply that alignment of fibroblasts by topography is serum-independent, but alignment by EFs is serum-dependent. An initial attempt has been made to try to identify which component of the serum is critical in EF-guided cell alignment by adding different concentrations of basic fibroblast growth factor (b-FGF) to serum-free culture medium. These additions did not restore the EF-guided alignment of cells on grooved surfaces observed in experiments containing serum. These results demonstrate that the alignment of fibroblast cells can be tailored by the dual guidance cues of topography and electric fields.

11:30 AM *AA1.9
Simulations of Chemotaxis and Random Motility in Finite Domains. Cameron Abrams and Ehsan Jabbarzadeh; Chemical Engineering, Drexel University, Philadelphia, Pennsylvania.

Rational design and selection of candidate porous biomaterials to serve as tissue engineering constructs rests on our ability to understand the influence of porous microarchitecture on the transport of chemical species (nutrients, signaling compounds), fluid flow, and cellular motion. We have begun to study the behavior of chemotactically active cells in response to time-varying signaling molecule concentration profiles using a simulation model that integrates finite-difference solution of reaction-diffusion equations and stochastic multicellular simulation, with an eye toward building a quantitative, pore-level model of mass and cell transport in porous tissue-engineered constructs. This talk will focus on our findings regarding the influence of no-flux boundaries on both the concentration profiles of cell-to-cell signaling molecules and the subsequent responses of cells in displaying chemotactic motility in model 2D domains. In particular, we find that the dominant role no-flux boundaries play in determining concentration profiles strongly influences chemotacitic response of cells moving in a domain contained by these boundaries. From our work we infer the importance of a proper treatment of boundary conditions in pore-level quantitative modeling of mass transport and cellular response in porous media.

SESSION AA2: Nano and Micron Scale Materials for Cell and Tissue Engineering - II
Chairs: Ken Gonsalves and Cato Laurencin
Monday Afternoon, November 29, 2004
Room 306 (Hynes)

1:30 PM *AA2.1
Topographic cues that model the native basement membrane differentially impact corneal epithelial cell behaviors. Paul F. Nealey[1] and Christopher J. Murphy[2]; [1]Chemical and Biological Engineering, University of Wisconsin, Madison, Wisconsin; [2]School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin.

Topographic cues that model the native basement membrane differentially impact corneal epithelial cell behaviors A fundamental question in the design of corneal prosthetics is how surface topography regulates cell behavior. Our previous and ongoing work has focused on defining the topography of native basement membranes and determining the phenotypic impact of biologically relevant length scales on cell behaviors. We have found that the native corneal basement membrane contains features from 20 to 400 nanometers in size. We fabricated silicon surfaces that contain feature sizes ranging from 400 to 4000 nm in pitch (groove width + ridge width) as well as planar regions. The smallest feature sizes on these chips were 70 nm ridges on a 400 nm pitch. Thus, we are able to replicate on a single chip features of biologically relevant size, as well as larger, micron sized features that provide a link to the bulk of the literature. We find that nanometer length features affect the behavior of epithelial and neuronal cell lines differently than do micron scale features. Specifically, primary human corneal epithelial cells (hCECs) cultured in Epilife medium (Cascade Biologics) align and elongate parallel to micron scale features, but perpendicular to nanometer length features. Adhesion of SV40-transformed hCECs under shear flow was dramatically enhanced when cells were cultured on nanometer length features over cells cultured on micron length features. hCEC proliferation and migration was decreased on surfaces with nanometer length scale features. PC12 cell neuritogenesis was dramatically enhanced on nanometer length features under sub-optimal conditions. When cultured in 5 ng/ml NGF for 3 days, cells plated on 400 and 800 nm pitch extended 3 times as many neurites as cells on flat and micrometer length features. Remarkably, for each of these findings a transition in the cellular response to topography occurs at approximately 1200 to 1600 nm pitch (ridge widths of 400 to 900 nm). Cells plated onto features smaller than this transition zone demonstrate differences in behavior from cells plated onto features larger than this zone. Thus, biologically relevant nanometer length features may be important regulators of cellular behavior. These studies have relevance to our fundamental understanding the role that topographic cues play in the normal development and maintenance of the corneal epithelium. Furthermore, this knowledge may contribute to the genesis of novel strategies in tissue engineering and advance the development of ocular prosthetics.

2:00 PM AA2.2
Real time study of neuro-synaptic contact formation on supported bilayer. Sophie Pautot[1], Hanson Lee[3], Arnd Pralle[3], Jay T. Groves[2] and Ehud Isacoff[3]; [1]Material Science Division, LBNL, Berkeley, California; [2]Chemistry, UC Berkeley, Berkeley, California; [3]Molecular and Cell Biology, UC Berkeley, Berkeley, California.

We present the first real time visualization of the neuro-synaptic contact formation between neurexin expressing cell and a supported bilayer containing neuroligin. Heterophilic adhesion molecules, such as postsynaptic neuroligin (Nlg) and presynaptic beta-neurexin (Nrx) are thought to mediate the formation of synapses between neurons by stabilizing cell-cell contact and nucleating the assembly of the synaptic transmission machinery. These molecules can function in non-neuronal cells, and we have shown that beads coated with an artificial lipid bilayer containing Nlg can trigger presynaptic differentiation in neuron. Among artificial substrate for cell adhesion, supported bilayer can the most closely mimic the live cell surface and thus increase the likelihood of recapturing functional aspects of protein behavior in vitro. The supported bilayer is formed on a flat optically clear surface that enable us to use confocal and Total Internal Reflection (TIR) microscopy to elucidate the details of the interaction between Nrx expressed in cell and Nlg reconstituted in the artificial bilayer. We show that supported bilayer containing Nlg can be used to direct the binding of Nrx-expressing cells. We were able to follow, Nlg in the bilayer and Nrx in the cell as they concentrate in small patches. Finally, we relate the degree of clustering of Nlg and Nrx at the bilayer-cell contact site using two measures of cell adhesion: gravity dissociation and cell immobilization. This approach makes possible single molecule level determination of the binding and nucleation dynamics of the proteins that mediate adhesion, signaling and transmitter secretion at the neural synapse.

2:15 PM AA2.3
Directed Membrane Compartmentalization by Surface Patterned Antigen. Min Wu[1], David Holowka[1], Harold Craighead[2] and Barbara Baird[1]; [1]Chemistry and Chemical Biology, Cornell University, Ithaca, New York; [2]Applied and Engineering Physics, Cornell University, Ithaca, New York.

Spatial localization is a key aspect of many cellular signaling processes. We are interested in the possible role of lipid-mediated membrane compartmentalization, commonly called 'lipid rafts', in directing such localization. The concept of membrane heterogeneity has been supported by accumulating data from various biophysical and biochemical approaches, such as detergent resistant assay, cholesterol depletion, imaging co localization. However, those methods either suffer from looking at localization after cell fractionation, or have to deal with the non-physiological stimulation conditions. As a result, the molecular basis and formation mechanism of such membrane compartmentalization still remains largely controversial. Understanding the compartments can be facilitated by our ability to control the formation of such compartments, followed by live cell dynamic imaging and to decouple the different regulation mechanisms by additional biochemical manipulation. In our research, spatially controlled stimuli on a 2D surface are utilized for systematic examination of localized signaling. Patterning supported lipid bilayers with liganded lipid on a silicon oxide surface using the parylene lift-off process provides the mobility of ligands for the cellular receptors as well as the localization at the interface. Uniform arrays of haptenated lipids with feature sizes down to 1μm width are revealed after the polymer is peeled away in one contiguous piece under water, ready for controlled engagement of cellular receptors and monitoring of subsequent cellular responses. We have demonstrated the effectiveness of such surfaces for specific stimulation of mast cells as indicated by specific antibody binding and redistribution, morphological changes and degranulation in the previous research. Here we show that these patterned surfaces provide a tool for visualizing localized intracellular signaling events, as well as providing new insight in the structural basis and functional relevance of membrane compartmentalization. Lyn kinase accumulates over the patterned DNP-haptens, pointing to the functional relevance of these spatially restricted membrane domains. Lipidated GFP constructs that associate with the cytosolic side of the plasma membrane also concentrate over the patterns, suggesting reorganization of this inner leaflet upon clustering of the transmembrane IgE-FcϵRI. Our results demonstrate that combination of patterned surface and high resolution live cell imaging can serve as

a powerful tool to study cellular signaling events where localization is critical and lay the groundwork for more general studies of cellular receptors and responses at interfaces.

2:30 PM AA2.4
Biocompatibility of CAD/CAM Ormocer Polymer Scaffold Structures. Anand Doraiswamy[1], Timothy Patz[1], Roger Jagdish Narayan[1], Boris Chichkov[2], Ruth Houbertz[3], Rohit Modi[4] and Douglas B. Chrisey[4]; [1]Georgia Institute of Technology, School of Material Science & Engineering, Atlanta, Georgia; [2]Laser Zentrum Hannover, Hannover, D-30419, Germany; [3]Fraunhofer Institute for Silicate Research, Wurzburg, ISC, Neunerpl 2, D-97082 , Germany; [4]Code 6360, Naval Research Laboratory, Washington, District of Columbia.

We have fabricated novel scaffolding structures using femtosecond two-photon polymerization of Ormocer polymers and tested their biocompatibility with several classes of mammalian cells. The novelty of using these inorganic-organic hybrid polymers for cell scaffolding is found in their physical dimensions and structure. These materials are fabricated based on a CAD/CAM laser processing technique, which uses two-photon polymerization initiated by femtosecond laser pulses and yields a controllably porous structure with nanometer feature size. Additionally, they can be functionalized to tailor their physical and chemical properties. Initial experiments have shown that the hybrid polymer materials used to achieve these nanoscale dimensions have also shown some qualitative biocompatibility, but they need to be quantitatively tested. We have tested the adhesion and biocompatibility of simple Ormocer planar films with several classes of adherent mammalian cells including: myoblasts, osteoblasts, pluripotent cells, endothelial, and epithelial cells, which represent a wide cross-section of cell properties, function, and cell adhesion. Cellular growth and morphology on these Ormocer films has been measured at several time points in the course of one week and compared to similar control results for polystyrene culture flasks and extracellular matrix coatings. Two-photon polymerization of hybrid polymers is demonstrated as a promising femtosecond laser-based nanofabrication technology for novel scaffolding structures.

3:15 PM *AA2.5
Drug and Cell Delivery from Injectable, Biodegradable Polymeric Scaffolds for Tissue Engineering. Antonios G. Mikos, Department of Bioengineering, Rice University, Houston, Texas.

Although the presence of a scaffolding material is necessary for the repair of many tissue defects, numerous studies have demonstrated that, often, scaffolds alone fail to provide a sufficient template to guide tissue regeneration. Consequently, our laboratory has developed a class of fumarate-based polymers for the controlled delivery of cells and bioactive agents to improve tissue repair. The repeating double bond in the backbone structure of these polymers allows solutions to be injected to a defect site and crosslinked into scaffolds *in* situ via either thermal or photo-initiation. Hydrolytic cleavage of the backbone's repeating ester bond then permits biodegradation of these scaffolds. Furthermore, cells and/or microparticles loaded with bioactive agents can be incorporated into these scaffolds prior to crosslinking to encourage neotissue formation as the scaffold degrades. For the repair of load-bearing bone defects, we have used this technology to fabricate porous poly(propylene fumarate) scaffolds. Loaded microparticles can be embedded within the scaffold network to allow sustained release of the desired drug. Scaffolds loaded in this manner with an osteogenic peptide exhibited enhanced bone regeneration in rabbits. Alternatively, for non-load-bearing applications, a more hydrophilic fumarate-based polymer, oligo(poly(ethylene glycol) fumarate), can be used to fabricate synthetic hydrogel scaffolds. Such scaffolds have been explored as injectable carriers for marrow stromal progenitor cells and have been shown to promote cellular differentiation and calcified matrix production *invitro*. For the repair of cartilage tissue, gelatin microparticles were loaded with growth factors and embedded within the hydrogel network. As these microparticles undergo enzymatic degradation, the growth factors are released to the surrounding tissue, and newly created pores allow for cell infiltration. As these examples demonstrate, fumarate-based materials can be easily tailored for specific applications and hold great promise as injectable carriers for delivery of cells and bioactive factors to a variety of complex defect sites.

3:45 PM AA2.6
Nanopatterned Adhesion Sites for the Control of Cellular Focal Contacts. Wolfgang Frey[1,2] and John H. Slater[1,2]; [1]Biomedical Engineering, University of Texas at Austin, Austin, Texas; [2]Center for Nano and Molecular Science and Technology, University of Texas at Austin, Austin, Texas.

The interaction of cells with the extracellular matrix determines cell adhesion and is mediated via integrin receptors. These transmembrane proteins are linked to a number of cytoplasmic proteins and act as an anchor for the cytoskeleton. When cells establish stable adhesion sites the integrins aggregate and form small nanometer scale clusters known as focal adhesions. The formation of focal adhesions is crucial in stress fiber development, cell motility, and proliferation. Providing clustered anchoring sites and controlling the focal adhesion of cells to biomaterials has recently been shown to impact the overall cell morphology and adhesion strength. We have developed a technique to fabricate large areas with well-defined, nanometer-sized adhesion sites of controlled area and spacing. Nanoclusters were fabricated via a self-assembly process. The nanopatterned surface consists of two materials, each of which can be functionalized independently. This orthogonal functionalization of the two materials leads to well-defined cellular attachment sites on a non-adhesive background. The binding sites presented either fibronectin or laminin proteins, or RGD or YIGSR peptides against a non-adhesive poly(ethylene glycol) background. Human umbilical vein endothelial cells (HUVECs) were seeded on nanopatterned surfaces, varying systematically adhesion area, site spacing, pattern height, and surface functionality. We monitored the cellular morphology, focal adhesion arrangement, and cytoskeletal arrangement over twenty four hours. Atomic force microscopy, confocal microscopy, fluorescence microscopy, and total internal reflection fluorescence microscopy were utilized to monitor cellular reactions. We show that the HUVECs recognize and bind specifically to the nanopatterns over a wide range of nanometer binding sizes. Pattern size and distance, however, influence the cytoskeletal and stress fiber development. Spreading area and proliferation are strongly reduced if the ratio of attachment site area to sites spacing becomes too small HUVECs on smaller patterns with larger adhesion site spacing extend lamellapodia in search of more accommodating patterns, and appear elongated This study of surface-induced focal adhesion formation will provide insight into the design of the next generation of biomimetic materials.

4:00 PM AA2.7
Tissue Engineering Applications of Multiphoton Nanofabrication. Paul Joseph Campagnola[1], Swarna Basu[1], Larry Cunningham[1] and George Pins[2]; [1]Cell Biology/CBIT, University of Connecticut Health Center, Farmington, Connecticut; [2]Biomedical Engineering, Worcester Polytechnic Institute, Worcester, Massachusetts.

We describe tissue engineering applications of multiphoton excited (MPE) nano and microfabrication, whereby essentially any protein or other biomolecule or protein/polymer composites can be optically crosslinked. MPE is used to precisely direct photocrosslinking with submicron precision by utilizing the inherent 3-D confinement of nonlinear optical processes in analogy to intrinsic sectioning in a two-photon fluorescence microscope. Using a custom laser -scanning microscope we have fabricated model scaffolds from a broad range of proteins including serum and ECM proteins, enzymes, and growth factors. Excellent biocompatibility is possible since the fabrication proceeds in aqueous solution and is demonstrated in several ways. Types I, II, and IV collagen matrices are fabricated using traditional photochemistries as well as our synthesized reagents, and we have characterized the minimum feature sizes using both 2 and 3 photon activation and find much smaller features for the latter due to the increased spatial confinement. The enzymatic degradations of MPE crosslinked collagens are probed using pepsin, collagenase, and MMP digestions in order to characterize the crosslinking process and crosslink density. The results are compared for different fabrication photochemistries and the findings are consistent with known enzymatic digestions. Directed cell adhesion on micron-scale crosslinked matrixes and line-like structures of sub-micron widths of fabricated collagen, fibronectin, fibrinogen, FGF2 and PDGF is also studied. When the adhesion on these crosslinked proteins is compared to BSA backgrounds, excellent specificity is observed for all the bioactive molecules. We observed that fibroblasts preferentially align along linear structures, as well as spanning linear regions. To further characterize MPE crosslinked protein matrixes, the lateral mobilities of several fluorescent dyes were examined and diffusion coefficients were found to decrease asymptotically with the crosslinking density of the matrix, and we find the measured values in proteins gels are approximately 3-4 orders of magnitude slower than those reported in solution. The diffusion coefficients were also measured at different levels through the 3D structures and were found to be highly location independent, demonstrating the uniformity of the fabrication process. Finally, both immunofluorescence labeling as well as quantitative fluorescence imaging of alkaline phosphatase using Michaelis-Menten kinetic analysis further indicates that the proteins retain the appropriate activity post-fabrication The MPE methodology provides improved 3-D capability and biocompatibility in comparison to photolithography, micro-contact printing, and current free-form fabrication methods and is thus a viable approach to the fabrication of 3-D biological devices to be used in applications such as tissue engineering and controlled release.

4:15 PM AA2.8
Ink Jet Printing of Mammalian Primary Cells for Tissue Engineering Applications. Rachel Saunders, Lucy Bosworth, Julie Gough and Brian Derby; Manchester Materials Science Centre, UMIST, Manchester, United Kingdom.

Free-form fabrication techniques have enabled the creation of complex detailed structures for use as tissue engineering scaffolds. The availability of precise scaffold structures does not however address the issues of cell seeding and placement. Current cell seeding techniques mainly rely on cell infiltration, which produce non-uniform cell distribution with little control. The ability to control both the placement and organisation or different cell types into an internal structure during the scaffold fabrication is an important step in the development of tissue-engineered organs. Ink jet printing has the potential to fulfil this role, however the high strain rates generated by droplet formation and impact exposes the cells to large stresses and deformation. A piezoelectric drop on demand printer has been used to print primary human osteoblast and bovine chondrocyte cells. After deposition the cells were incubated at 37°C and characterised using optical microscopy, SEM and cell viability assays. Cells showed a robust response to printing. Increasing the drop velocity results in a reduced cell survival and proliferation rates but both cell lines grew to confluence after printing under all conditions studied Chondrocyte matrix formation was stained with anti collagen I and anti-collagen II antibodies and analysed using a confocal microscope to assess the impact of printing on chondrocyte phenotype. Poly(ethylene glycol) diacrylate hydrogel, photo polymerised with UV light in the presence of 2,2-Dimethoxy-2-phenylacetophenone, has been investigated as a possible scaffold material. This hydrogel shows potential as a material for printed hybrid scaffolds.

4:30 PM AA2.9
Novel Polymer/Ceramic Composite Microsphere-Based Scaffolds For Bone Tissue Engineering: Osteoblast Growth on Scaffolds with Varied Calcium Phosphate Content.
Yusuf M. Khan[1], Dhirendra S. Katti[2] and Cato T. Laurencin[2]; [1]School of Biomedical Engineering, Science, and Health Systems, Drexel University, Philadelphia, Pennsylvania; [2]Department of Orthopaedic Surgery, University of Virginia School of Medicine, Charlottesville, Virginia.

Currently available bone grafts present certain limitations such as donor-site morbidity for autografts and risk of disease transmission for allografts, and suggest a need for alternative strategies. Polymer/ceramic composite scaffolds for trabecular bone tissue engineering capitalize on the benefits of both materials. Polymers are easily formed and shaped and add structural rigidity while calcium phosphates impart osteoconductivity and osteointegration. We have developed a biodegradable, microsphere-based scaffold for bone tissue engineering based on poly(lactide-co-glycolide)(PLAGA)/calcium phosphate composite microspheres in which a nanocrystalline hydroxyapatite (HA) is synthesized within forming microspheres. Briefly, PLAGA was mixed with methylene chloride and separate solutions of calcium nitrate tetrahydrate and ammonium hydrogenphosphate. Initial molar ratios of Ca/P were 1.67. The mixture was added dropwise to a stirring solution of polyvinyl alcohol that was maintained at 4°C, pH 10. The final composition of microspheres was manipulated by varying the pre-mix ratio of polymer:hydroxyapatite precursors resulting in two groups; low PLAGA/HA ratio (27% HA content post mix) and high PLAGA/HA ratio (17% HA content post mix). Scaffolds were formed from each group of microspheres by heating the microspheres above the Tg of the polymer (52°C). Calcium release studies over 24 hours showed elevated calcium ion release from scaffolds formed from the low PLAGA/HA ratio microspheres over scaffolds formed from the high PLAGA/HA ratio microspheres. Scaffolds formed from both the low and high PLAGA/HA ratio microspheres were seeded with mouse pre-osteoblast cells (MC3T3-E1) and evaluated for cell proliferation and alkaline phosphatase expression after 3, 7, 14, and 21 days. Cell culture was maintained with minimum essential medium supplemented with β-glycerophosphate and ascorbic acid and was changed every other day. Results indicated statistically significant proliferation between each time point over the 21 day incubation for cells seeded on both low and high PLAGA/HA ratio scaffolds, while alkaline phosphatase expression per cell was enhanced at the earliest time points with both the low and high PLAGA/HA ratio as compared to cells grown on tissue culture plastic (TCP). The formation of a nanocrystalline HA within the microspheres permitted dissolution of calcium ions more readily than would a more crystalline form of hydroxyapatite, and may enhance the *invivo* osteointegration of the scaffold to pre-existing bone by providing a site-specific delivery of calcium ions. The addition of nanocrystalline HA also appeared to have no inhibitory effect on cell proliferation while it enhanced alkaline phosphatase expression over cells on TCP. Given these restults, the composite microsphere scaffold formed from PLAGA and nanocrystalline hydroxyapatite shows great promise as a new composite scaffold system for bone repair.

4:45 PM AA2.10
Nanoscale RGD Peptide Organization Regulates Cell Proliferation and Differentiation. Susan X. Hsiong[1], Kuen Yong Lee[2] and David J. Mooney[1,2,3]; [1]Chemical Engineering, University of Michigan, Ann Arbor, Michigan; [2]Biologic and Materials Science, University of Michigan, Ann Arbor, Michigan; [3]Biomedical Engineering, University of Michigan, Ann Arbor, Michigan.

RGD (arginine-glycine-aspartic acid) containing peptide sequences, common cell attachment sites present in many extracellular matrix (ECM) proteins, mediate many important cellular processes. The role of nanoscale organization of RGD peptides in the regulation of the adhesion, proliferation and differentiation of both preosteoblasts (MC3T3-E1) and multipotential (D1) cell lines in vitro was investigated in this study. Alginate polymer chains with varying RGD peptide degree of substitution were mixed with unmodified polymer chains at different ratios to allow variation of RGD peptide spacing in the nanometer scale, independently of the overall bulk density of peptides presented from the material. Proliferation of both cell types was observed to be closely correlated to RGD island spacing, independently of overall bulk ligand density, following cell adhesion to alginate hydrogels. Increased RGD peptide spacing was observed to promote spreading of MC3T3-E1 cells while simultaneously suppressing their proliferation. However, increased RGD peptide spacing decreased spreading of D1 cells while also decreasing proliferation. Moreover, differentiation of preosteoblasts was significantly upregulated in response to decreased RGD spacing, whereas differentiation of multipotential cells was modestly regulated by this variable. These results demonstrate that the nanoscale organization of adhesion ligands may be an important variable in controlling cell phenotype and function. In addition, cellular responses to nanoscale ligand organization differ depending on the cell type, and this may be related to the differentiation stage of the cells.

SESSION AA3: Nanostructured Drug Delivery and Biosensor Systems
Chairs: Edward Botchwey and Lakshmi Nair
Tuesday Morning, November 30, 2004
Room 306 (Hynes)

8:30 AM *AA3.1
Micro- and Nano-scale Materials for Drug delivery.
Mark Saltzman, Yale University, New Haven, Connecticut.

The potential intersection between nanotechnology and the biological sciences is vast. Biological function depends heavily on units that have nanoscale dimensions, such as viruses, ribosomes, and molecular motors. In addition, engineered devices at the nanoscale are small enough to interact directly with subcellular compartments and to probe intracellular events. The ability to assemble and study materials with nanoscale precision leads to opportunities in both the basic biology (e.g. testing of biological hypotheses that require nanoscale manipulations) and development of new biological technologies (e.g. drug delivery systems, imaging probes, or nanodevices). This presentation will focus on the potential applications of nanotechnology in controlled release and drug delivery.

9:00 AM AA3.2
Interfacing Neurons with Microelectronics. Gunter Wrobel, Sven Ingebrandt and Andreas Offenhausser; Inst. Thin Films&Interfaces (ISG-2), Forschungszentrum Juelich, Juelich, Germany.

Future information systems will be inspired by the neuronal information process. A major challenge is the development of characterization methods and techniques to obtain the neuronal information needed. The interface between functional biological systems and inorganic materials is of central importance. The goal is the successful merge of micro- and nano-electronic systems with biological signal processing units. Our efforts aim at developing neuroelectronic systems as well as complex sensors for biological and chemical diagnostics. The interface between microelectronic devices, on the one hand, and the cells on the other, requires a tight contact of the solid surface and the cell membrane as well as suitable signals on the solid and on the cell side. In order to investigate the principles of the cell-transistor coupling genetically modified cells overexpressing voltage-gated ion channels are used. In our experiments we have focused on the time-dependence of the cell-transistor contact. We examined the electrical coupling of HEK293 cells, which were stably transfected with the voltage-gated ether-à-go-go potassium channel (beag1). The kinetics of the beag1-channels can be modulated by voltage-clamp protocols and different divalent cations in the extracellular medium. Cells were cultured on n- and on p-channel field-effect transistor (FET) devices to compare the influence of the

applied bias voltage on the signal shapes. The signals of the active ion channels of the whole cell and those in the contact region on the transistor gate are evaluated using electrophysiological techniques. We find clear differences in amplitude and time-dependence within the recordings made with p- and n-channel transistors, which strongly suggests that the bias voltage applied over the seal resistor in the cell-sensor junction influence the extracellular recorded signal. In all recordings on both chip types fast potassium currents (in the range of few ms) cannot be recorded. Our results strongly suggest that ion-sensitivity of the gate oxide and electrodiffusion of potassium ions in the small cleft are responsible for the observed effects. We introduce first attempts to model these effects in a Monte Carlo Simulation to explain signal shapes. This allows the development of theoretical models, which will lead to a better understanding of the cell-transistor interface and will be used to improve the recording devices.

9:15 AM AA3.3
Ultrasensitive Electric DNA recognition using Nanowells Array. Ho-Sup Jung[1,2], Hea-Yeon Lee[1,2] and Tomoji Kawai[1,2]; [1]ISIR-SANKEN, Osaka Univ., Osaka, Japan; [2]CREST, JST, Saitama, Japan.

Ultrasensitive biomaterials sensing systems have significantly advanced the areas of biomedical diagnostics, genetic disease, drug discovery, and biotechnology. The essential condition for producing a reliable analytic biochip with a planar substrate is to prepare immobilized probing biomaterials with a good coverage in geometry to minimize nonspecific bindings, which will interfere with wanted detecting reactions. Such highsensitive bioassays have been necessary for the precise measurement of highly specific DNA and amino acid sequences. We will describe an highsensitive electric sensing system for oligonucleotides, based on a 50 nm nanowells (NWs) array electrode, leading to good specificity or selectivity by eliminating nonspecific binding between DNA/DNA and DNA/protein.

9:30 AM AA3.4
Biological Applications of Porous Polyelectrolyte Multilayers. Michael C. Berg[1], Lei Zhai[2], Fevzi Cebeci[3], Robert E. Cohen[1] and Michael F. Rubner[2]; [1]Chemical Engineering, MIT, Cambridge, Massachusetts; [2]Materials Science and Engineering, MIT, Cambridge, Massachusetts; [3]Chemistry, Istanbul Technical University, Istanbul, Turkey.

Polyelectrolyte multilayers are ultra-thin polymer films that are constructed with molecular level control over structure and chemistry. These films have many interesting properties and potential applications as biomaterials due to their ease of processing, conformal coating of any geometry, and used with a wide range of applicable materials. This work focuses on biological applications in the areas of drug delivery and antibacterial coatings. Specifically, we have developed porous multilayers made from poly(allylamine hydrochloride) (PAH) and polyacrylic acid (PAA). The pores range in size from the nanoscale to microscale depending on the processing conditions, and can be loaded with drugs for controlled release. We have been successful in loading a variety of non-ionic small molecule drugs into the porous films, and have monitored the release into a buffer solution using UV-VIS spectroscopy. The films show a linear release profile that can be tuned through a variety of processing parameters. In the area of antibacterial coatings, we have developed porous multilayers that can be treated to become stable superhydrophobic films that resist bacterial adhesion. In addition, these films can be engineered to kill bacteria by releasing silver ions from nanoparticles grown in the films. We have found these films to be effective against both Gram-negative and Gram-positive bacteria in both airborne and waterborne models.

9:45 AM AA3.5
MAPLE Deposition of Polyanhydride Thin Films. Timothy M. Patz[1,2], Douglas B. Chrisey[2] and Roger Jagdish Narayan[1]; [1]Material Science & Engineering, Georgia Institute of Technology, Atlanta, Georgia; [2]US Naval Research Laboratory, Washington, District of Columbia.

In many medical conditions, it is important to maintain the release of a pharmacologic agent under strict control. Several polymers have been developed that provide controlled release of a pharmacologic agent. Unfortunately, many commonly used biodegradable polymers (e.g., polyorthoesters) exhibit a combination of bulk and surface erosion. Pulsatile release of a pharmacologic agent is not possible using these materials. Polyanhydrides, including copolymers of sebacic acid (SA) and 1,3 bis(p-carboxyphenoxy) propane (CPP), demonstrate only surface erosion. We propose the creation of a novel layered drug delivery system, in which pharmacologic agents are layered within a surface-eroding polymer. These devices would allow controlled, even pulsatile release of one or more pharmacologic agents over an extended period of time. We deposited thin films and multilayers of an 80:20 CPP/SA copolymer on Si and ZnSe substrates using matrix assisted pulsed laser evaporation. These films were characterized using Fourier transform infrared spectroscopy (FTIR), atomic force microscopy, dynamical light scattering, X-ray photoelectron spectroscopy, and scanning electron microscopy. FTIR revealed that the deposited polyanhydride film maintained the main functional groups as compared to a drop-cast sample. These novel materials will allow the development of sophisticated drug delivery devices for a variety of medical conditions.

10:30 AM *AA3.6
Micro and Nanofabricated Platforms for Biotemplating. Tejal Ashwin Desai, Biomedical Engineering, Boston University, Boston, Massachusetts.

A major goal in tissue engineering is to more closely replicate complex tissue architecture and arrangement by: a) designing physiologically relevant scaffold configurations and materials, b) understanding cellular differentiation and adhesion processes in response to substrates, and c) incorporating non-uniform chemical/physical forces. To achieve these goals, there is a significant need to develop and refine fabrication strategies that incorporate micro- and nanoscale features in a well-controlled and engineered manner, as these micro and nanoscale features have been shown to significantly affect cellular and subcellular function. In this talk, the fabrication of porous polymeric and metal-oxide films with controlled nano to micro architecture for biotemplating and drug delivery applications, will be discussed. The incorporation of hierarchical structure into tissue constructs may allow for more physiogically functional tissues in vitro and in vivo.

11:00 AM AA3.7
Targeted Stimuli-Responsive Dextran Conjugates for Doxorubicin Delivery to Hepatocytes. Noreen T. Zaman[1], Fred Tan[1,2] and Jackie Y. Ying[1,3]; [1]Chemical Engineering, MIT, Cambridge, Massachusetts; [2]Department of Biology, MIT, Cambridge, Massachusetts; [3]Institute of Bioengineering and Nanotehcnology, Singapore, Singapore.

A targeted, stimuli-responsive, polymeric drug delivery vehicle is being developed in our lab to help alleviate severe side-effects caused by narrow therapeutic window drugs. Targeting specific cell types or organs via proteins, specifically, lectin-mediated targeting holds potential due to the high specificity and affinity of receptor-ligand interactions, rapid internalization, and relative ease of processing. Dextran, a commercially available, biodegradable polymer has been conjugated to doxorubicin and galactosamine to target hepatocytes in a three-step, one-pot synthesis. The loading of doxorubicin and galactose on the conjugates was determined by absorbance at 485 nm and elemental analysis respectively. Conjugation efficiency based on the amount loaded of each reactant varies from 20% to 50% for doxorubicin and from 2% to 20% for galactosamine. Doxorubicin has also been attached to dextran through an acid-labile hydrazide bond. Doxorubicin acts by intercalating with DNA in the nuclei of cells. The fluorescence of doxorubicin is quenched when it binds to DNA. This allows a fluorescence-based cell-free assay to evaluate the efficacy of the polymer conjugates where we measure the fluorescence of doxorubicin and the conjugates in increasing concentrations of calf thymus DNA. Fluorescence quenching indicates that our conjugates can bind to DNA. The degree of binding increases with polymer molecular weight and substitution of doxorubicin. In cell culture experiments with hepatocytes, the relative uptake of polymer conjugates was evaluated using flow cytometry, and the killing efficiency was determined using the MTT cell proliferation assay. We have found that conjugate uptake is much lower than that of free doxorubicin. Lower uptake of conjugates may increase the maximum dose of drug tolerated by the body. Also, non-galactosylated conjugate uptake is lower than that of the galactosylated conjugate. Microscopy indicates that doxorubicin localizes almost exclusively at the nucleus, whereas the conjugates are present throughout the cell. Doxorubicin linked to dextran through a hydrazide bond was used to achieve improved killing efficiency. Following uptake, the doxorubicin dissociates from the polymer in an endosomal compartment and diffuses to the nucleus. The LC_{50} of covalently linked doxorubicin is 7.4 μg/mL, whereas that of hydrazide linked doxorubicin is 4.4 μg/mL.

11:15 AM AA3.8
In Situ Biological Agent Detection Using PZT/Ti Microcantilevers. John-Paul McGovern[2], Wan Y. Shih[2] and Wei-Heng Shih[2]; [1]Drexel University, Philadelphia, Pennsylvania; [2]Department of Materials Science and Engineering, Drexel University, Philadelphia, Pennsylvania.

With bioterrorism looming large in matters of national security and with the skyrocketing costs of medical diagnostic tests, the need for rapid, precise, specific, and in situ detection of biological agents is paramount. To this end, a composite, resonating microcantilever

constructed of a lead zirconia titanate (PZT) layer bonded to a titanium substrate is being used to accomplish specific detection and quantification of antigen cells and proteins in solution. Previous studies have shown that resonance frequency shifts of piezoelectric cantilevers can be used to detect a change in mass attached to the cantilever tip. In this study, we examine various surface functionalization techniques to optimize the immobilization of detection proteins on the surface of the titanium microcantilever tip. These detection proteins include cellular antibodies, e.g., those specific to Salmonella T. as well as antibodies specific to particular proteins such as Prostate Specific Antigen (PSA). The PZT/Ti cantilever has proven an effective detection device for cells and proteins in solution with a detection sensitivity better than 5 x 10^{-10} g/Hz. With the weight of a single salmonella cell approximately 3 x 10^{-12} g/cell, such sensitivities have the possibility of detecting just a relative few cells or a few nanograms of protein adhered to the surface whereas present biodetection and medical diagnostic techniques involve cell culture and protein amplification which often require lengthy periods of lab culture time.

11:30 AM AA3.9
Linear-Dendritic Diblock Copolymers for Receptor-Mediated Gene Delivery. Kris C. Wood, Robert S. Langer and Paula T. Hammond; Chemical Engineering, MIT, Cambridge, Massachusetts.

The application of nucleotide-based therapeutics such as DNA in clinical medicine represents a technology that may potentially revolutionize the treatment of human disease. Successful delivery systems for DNA must be nontoxic, nonimmunogenic, and mechanistically must bind and condense DNA, target it to specific cells for endocytosis, protect it from lysosomal or cytoplasmic degradation, and target it to the cell nucleus for expression. In this study, we present the design and synthesis of a highly functional delivery system consisting of linear-dendritic, poly (ethylene glycol) (PEG)-polyamidoamine (PAMAM) diblock copolymers that addresses many of these challenges. The linear, hydrophilic PEG block exhibits good aqueous solubility, chain mobility, and low cytotoxicity. Further, PEG has been used extensively in drug delivery applications to enhance biocompatibility and circulation time in vivo. The dendritc PAMAM block has been shown to ionically condense DNA, bind non-specifically to the cell membrane, and protect DNA from lysosomal degradation by acting as a reservoir for endosomal buffering. PEG-PAMAM systems form a protective delivery system by condensing and encapsulating negatively charged DNA in the micellar phase in solution. Further, shell-forming segments may be functionalized with ligands for selective, receptor-mediated delivery of encapsulated genes. Here, we detail the synthesis of ligand-functionalized PEG-PAMAM copolymers, as well DNA complexation and selective cell targeting mediated by these systems.

11:45 AM AA3.10
Functionalized Magnetic Nanoparticles for an Optimized Breast Cancer Drug Delivery System. Challa Kumar[1], Carola Leuschner[2], William Hansel[2] and Josef Hormes[1]; [1]Center for Advanced Microstructures and Devices, Louisiana State University, Baton Rouge, Louisiana; [2]Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana.

For an optimized cancer drug delivery system three properties are crucial: 1. Site-specificity so that only tumor cells (+ metastatic cells) are attacked and no other cells are damaged 2. Controlled delivery so that the drug can be released over a longer period of times and hence eliminates the need for multiple injections 3. Effective attack and killing of cancer cells. Based on a recently developed new drug-system for the treatment of breast cancer fulfilling basically requirements 1 and 3 (a combination of active lytic peptide conjugates and LHRH (Luteinizing Hormone Releasing Hormone), we are in the process of developing a system for a controlled delivery based on magnetic nanoparticles covered with a polymeric shell containing the active substances. For such a system, external magnetic field gradients can be used to guide the nanoparticles to the tumor site and an oscillating magnetic field can be used for a controlled release of the drug. A crucial part of this system are the nanoparticles and their geometric, electronic and magnetic properties, their bio-compatibility and their stability over reasonable times. For example, it is important for the here discussed application to have particles with the highest possible magnetic moments so that is possible to control these particles with "conventional" magnetic fields even when the targeted area is deep in a human body. It is also crucial to have nanoparticles that can be functionalized in a suitable way so that for example the active drug components can be attached to the particle without loosing their efficiency. We are using various wet-chemical methods - standard batch processes but also a newly developed micro-reactor system - for synthesizing various magnetic nanoparticles such as Co and Fe3O4. For magnetide nanoparticles the amine groups on their surfaces can be used to bind the bio-molecules of interest through carbodiimide activation and several experiments have been carried out to functionalize these particles with various drugs and ligands. This approach was also used to attach HRH and the active lytic peptide conjugate to the nanoparticles in various arrangements and first in vitro and in vivo experiments were carried with these functionalized particles to determine their . To stabilize and to optimize the magnetic and "chemical" properties of Co-particles, we are using various techniques to prepare suitable core-shell-systems. The electronic and geometric properties of those nanoparticles are investigated by X-ray absorption spectroscopy (XAS) (EXAFS = Extended X-ray absorption fine structure and XANES = X-ray absorption near edge structure) using synchrotron radiation from the CAMD storage ring; the magnetic properties by standard squid techniques.

SESSION AA4: Three Dimensional Scaffolds and Biomems
Chairs: Ken Gonsalves and Helen McNally
Tuesday Afternoon, November 30, 2004
Room 306 (Hynes)

1:30 PM *AA4.1
Control of Cell Adhesion, Shape, and Function by Material Biophysical Chemistry. Kevin E. Healy, Univ. of California at Berkeley, Berkeley, California.

The design and synthesis of polymers that circumvent their passive behavior in complex biological environments and actively regulate the response of mammalian cells has been a focus for my laboratory for over 15 years. Introduction of bioactive signals into a polymer requires that the base material prevents non-specific interactions with constituents of the biologic environment (e.g., proteins, lipids, cells). We have developed such polymers by surface-mediated photoinitiated polymerization to create ultra thin polymer coatings (20-200 nm thick) that control the spatial distribution of protein adsorption and subsequently cell interaction. Novel interpenetrating polymer networks (IPNs) based on polyacrylamide and poly(ethylene glycol) [p(AAm-co-EG)] have been grafted to both metal oxides (e.g., SiO2, TiO2) and polymers (e.g., PET, polystyrene). Characterization of the IPNs by contact angle goniometry, spectroscopic ellipsometry, XPS, static ToF-SIMS, and QCM-D has confirmed the formation of an interfacial IPN that resists protein adsorption and cell adhesion based on a combination of presentation of pEG chains and extremely low shear moduli of the full-hydrated IPNs. To exploit bimolecular engagement between ligands of the extracellular matrix grafted to the IPN and cell-surface receptors, a wide spectrum of physical and chemical characterization techniques are required during synthesis and performance evaluation. The ligand type (e.g., peptide), surface density, and binding affinity (e.g., cell adhesion) are critical determinants that must be quantified to understand the interactions between biomimetic polymers and mammalian cells. A number of techniques, including microwell and microfluidic patterning, can be used to create IPNs with ligands spatially distributed on the surface. Applications of these ligand-patterned IPNs as novel culture systems, implant surfaces, and cell-based biosensors will be addressed. The implications of this work are that the biophysical chemistry of a polymer can be modified to alter the kinetics of differentiation of mammalian cells, and may ultimately be used in cell-based diagnostic assays, and to control in situ tissue formation and regeneration within or surrounding medical implants and devices.

2:00 PM AA4.2
Polyphosphazene Nanohydroxyapatite Composite Nanofibers As Scaffolds For Bone Tissue Engineering.
Subhabrata Bhattacharyya[1], Lakshmi S. Nair[2], Jared D. Bender[3], Anurima Singh[3], Greish E. Yaser[4], P. W. Brown[4], Harry R. Allcock[3] and Cato Thomas Laurencin[2]; [1]Department of Chemistry, University of Virginia, Charlottesville, Virginia; [2]Department of Orthopaedic Surgery, University of Virginia, Charlottesville, Virginia; [3]Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania; [4]Materials Research Institute, Pennsylvania State University, University Park, Pennsylvania.

Polymeric nanofibers due to their extremely high surface area, high aspect ratio and similarity in structure to the extracellular matrix are generating a lot of interest as scaffolds for tissue engineering and drug delivery1, 2. Biodegradable polyphosphazenes due to their synthetic flexibility, wide range of physico-chemical properties, non-toxic and neutral degradation products and excellent biocompatibility form excellent candidates for biomedical applications 3. The objective of the present study is to develop composite nanofibers of a biodegradable polyphosphazene poly[bis(ethyl alanato)phosphazene] (PNEA) and nanocrystals of hydroxyapatite (NHAp) via electrospinning and evaluate the structural and morphological properties of the fibers by varying the process and solution parameters that affect the electrospinning process. These composite nanofibers which combine the biocompatibility of polyphosphaznes

with the bioactivity of NHAp can form potential candidates as novel porous scaffolds for bone tissue engineering. The synthesis and characterization of PNEA was carried out according to a reported procedure. The suspension of NHAp in ethanol or dimethyl formamide (DMF) was sonicated with 9% (w/v) polymer solution in tetrahydrofuran (THF) and used to electrospin composite nanofiber matrices at ambient conditions. In the present study the theoretical loading of NHAp was varied from 50%-90 %(w/w). The actual loading of the NHAp was determined by calcination of the composite nanofibers. The morphology, diameter and composition of the composite nanofibers was determined using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDS) and X-ray mapping. The formation of polyphosphazene-NHAp composite nanofibers with diameters in the range of 200-400 nm was revealed by SEM. SEM clearly showed the NHAp crystals embedded within the polyphosphazene nanofibers. The calcination experiment showed that the actual NHAp loading does not have a linear relationship with the experimental loading. It has been found that a theoretical loading of 50% (w/w) NHAp yields the maximum incorporation of NHAp within the fibers using both DMF and ethanol. However, higher percentages in theoretical loading did not show appreciable increment in the actual loading of NHAP in the fibers. Apparently, ethanol was found to be a better suspension medium than DMF in the present study. The distribution of NHAp crystals within the fibers was determined by TEM, EDS and X-ray mapping. The X-ray mapping clearly showed the presence of the element calcium along the fibers indicating the incorporation of NHAp on and within the nanofibers. Further TEM showed evidence of well dispersed NHAp nanocrystals in and along the fibers. The study demonstrated the feasibility of developing novel composite nanofibers of biodegradable polyphosphazenes and NHAp with more than 50% w/w loading of NHAp within/on the nanofibers.

2:15 PM AA4.3
Microfabricated 3D Scaffolds for Tissue Engineering Applications. Alvaro Mata, Aaron Fleischman and Shuvo Roy; The Cleveland Clinic Foundation, Cleveland, Ohio.

An effective tissue-engineered scaffold provides a three-dimensional (3D) space where cells can proliferate, migrate, and differentiate in a desirable manner. In addition, precise surface micro-textures are well known to stimulate cell behavior. Therefore, we have used microfabrication and soft lithographic techniques to combine SU-8 photoresist and Polydimethylsiloxane (PDMS) to develop 3D scaffolds with precise micro-architecture and surface micro-textures for tissue engineering applications. The present paper describes the processing steps used to develop these scaffolds. A multilevel SU-8 (MicroChem Corp., Newton, MA) process was developed to produce SU-8 molds including holes and posts. Three layers of SU-8 were processed on a standard 100 mm-diameter, (100)-oriented silicon wafer as follows. First, a 200 μm thick film of SU-8 2100 was spin coated, soft baked (95° C, 55 minutes), exposed (365 nm, 375 mJ/cm^2), and post exposure baked (95° C, 25 minutes). A 10 μm thick film of SU-8 2010 was then spin coated, soft baked (95° C, 5 minutes), exposed (100 mJ/cm^2), and post exposure baked (95° C, 5 minutes). Next, a 100 μm thick film of SU-8 2100 was spin coated, soft baked, exposed, and post exposure baked using the same process parameters as the first film. Finally, all three SU-8 layers were simultaneously developed to realize a multilevel SU-8 mold with 200, 10, and 100 μm-high features. Multilevel PDMS films comprising through-holes and micro-posts were molded using a dual sided soft lithography technique, where the SU-8 molds were aligned using a custom mechanical jig. This approach enabled the production of PDMS films with features patterned and positioned on specific locations on both sides of the films. Five multilevel patterned PDMS films were aligned with the mechanical jig and assembled into a 1.5 mm-high 3D scaffold using 10 μm thick films of uncured PDMS as adhesive between the PDMS layers. The top and bottom surface of each PDMS layer was textured with 10 μm diameter and 10 μm high micro-posts separated by 10 μm. The 3D scaffold exhibited a 40% porosity, which resulted from rectangular 200 x 400 μm through-holes between the PDMS layers and 300 μm diameter, 100 μm deep holes through the PDMS layers. The 300 μm diameter through-holes from one PDMS layer were aligned to offset those on the adjacent PDMS layers. This arrangement resulted in meandering pore geometry throughout the 3D scaffold.

2:30 PM AA4.4
New Materials and Methods for Hierarchically Structured Tissue Scaffolds. Clifford L. Henderson[1], Benita Comeau[1], Kenneth E. Gonsalves[2] and Yusif Umar[2]; [1]School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, Georgia; [2]Department of Chemistry, University of North Carolina-Charlotte, Charlotte, North Carolina.

The overall goal of our work is to develop new materials and methods for the fabrication of hierarchically structures 3-D tissue scaffolds. Most primary organ cells are believed to display anchorage dependent behavior and require specific environments for growth and proliferation. Such environments often must include a supporting material and structure that act as a template for growth in order to produce a viable tissue. Existing man-made templates, mainly in the form of simple polymeric tissue engineering scaffolds, possess many limitations such as a lack of mechanical strength, lack of interconnected channels and controlled porosity, and limited control over the pore distribution. As a general result of the crude methods that are currently prevalent for making such scaffolds, only the equivalent of porous sponges are currently utilized in any widespread manner. In addition, due to the simple homogeneous structure and lack of well defined internal order, these materials lack the ability to establish guided tissue regeneration in defined patterns. Such guided cell growth and proliferation can be critical for establishing the desired cell differentiation and function. To overcome these limitations in current scaffold design and fabrication methods, and to provide a highly flexible and revolutionary approach for obtaining complex tissue scaffolds, we are developing new materials and fabrication technologies for fabricating arbitrarily complex 3D scaffolds. This goal is being achieved by developing materials, tools, and methods for 3D microfabrication of hierarchically ordered polymeric scaffolds via microstereolithography (μSL). New monomers and polymers are being developed that can be used in conjunction with μSL techniques to produce 3D scaffolds of arbitrary size and complexity, and which provide the ability to further selectively and locally tailor the internal surface properties of the scaffolds. Such internal surface control is possible using monomers which can subsequently undergo photo-induced reactions that can modify their properties such as the surface hydrophobicity. This control over internal physical shape and size of the structure in conjunction with the local internal surface physical and chemical control offers the promise of highly functional engineered tissue constructs. This paper will present an update on results related to the production of hierarchically ordered tissue scaffolds using μSL methods including: the basic chemistry of the monomers and polymers we are using, the chemistry of the polymerization initiators we are using and the influence of initiator selection on cell and tissue viability, demonstration of the fabrication of 3-D hierarchically structured scaffolds, demonstration of 3-D scaffolds with local interior surface hydrophobicity control using dual wavelength photopolymerization, and results of early cell culture studies on these materials and scaffold constructs.

3:15 PM *AA4.5
Layer-by-Layer Printing of Cells and its Application to Tissue Engineering. Priya Kesari[1], Tao Xu[1], Catalin Baicu[2], Michael Zile[2] and Thomas Boland[1,2]; [1]Bioengineering, Clemson University, Clemson, South Carolina; [2]Medicine, Medical University of South Carolina, Charleston, South Carolina.

Tissues and organs exhibit distinct shapes and functions nurtured by vascular connectivity. In order to mimic and examine these intricate structure-function relationships, it is necessary to develop efficient strategies for assembling tissue-like constructs. Many of the top-down fabrication techniques used to build microelectromechanical systems, including photolithography, are attractive due to the similar feature sizes, but are not suitable for delicate biological systems or aqueous environments. A layer-by layer approach has been proposed by us to pattern functional cell structures in three dimensions. Freeform cell structures are created by the inkjet method, in which cells are entrapped within hydrogels and crosslinked on demand. The cells are viable, functional and show potential for cell maturation as exemplified by the diversion of hematopoietic stem cells into multiple cell types. These results show promise for many tissue engineering applications.

3:45 PM AA4.6
CAD/CAM Laser Fabrication of Heterogeneous Tissue Constructs Cell-by-Cell. Douglas B. Chrisey[1], Rohit Modi[1], Timothy Patz[2], Anand Doraiswamy[2] and Roger Jagdish Narayan[2]; [1]Code 6360, Naval Research Laboratory, Washington, District of Columbia; [2]Georgia Institute of Technology, School of Material Science & Engineering, Atlanta, Georgia.

We have fabricated heterogeneous tissue constructs by a novel CAD/CAM laser transfer process. This digital printing technique, termed MAPLE DW (matrix assisted pulsed laser evaporation direct write), allows the laser forward transfer of viable mammalian cells from a planar quartz disk, or ribbon, to a receiving substrate serving as the mechanical and adhesion support for the deposited cellular material. Depending on the laser spot size (10um diameter or greater) and areal cellular density on the ribbon surface, single cells or small groups of cells can be deposited. Using two different ribbons, we deposited adjacent and overlapping CAD/CAM patterns of C212 myoblasts and B35 neuroblasts. Their different morphology is easily observed using an inverted microscope and the cell patterns were nearly 100% viable as determined by a conventional live/dead assay.The cells coexist and proliferate in the same culture medium,

but depending on the local cellular, topographical and biochemical environment, the two types of cells will self-assemble and organize into different arrangements of living tissue . The success demonstrated by these results is an important step towards fabricating a macroscopic three-dimensional tissue construct.

4:00 PM AA4.7
Recognition of Angiotensin II Using Configurational Biomimetic Imprinted Polymers. Ellizabeth Hunter Lauten[1] and Nicholas A. Peppas[1,2,3]; [1]Biomedical Engineering, University of Texas at Austin, Austin, Texas; [2]Chemical Engineering, University of Texas at Austin, Austin, Texas; [3]Pharmaceutics, University of Texas at Austin, Austin, Texas.

Creating systems that use synthetic biomaterials to mimic natural biological recognition processes has been a major focus of current research. This is due to the fact that these systems hold promise of becoming the next generation of materials for therapeutic and diagnostic devices by overcoming difficulties posed by natural biomolecules and ligands which are relatively unstable and expensive. The first step in coordinating and duplicating the complex and physiological processes is to engineer the molecular design of biomaterials by controlling recognition and specificity. This can be achieved using a type of molecular imprinting termed configurational biomimesis, which produces surfaces and polymeric recognitive networks that have stereo specific three-dimensional binding cavities based on a given molecule. These imprinted polymers can then be used *invivo* to bind undesirable biomolecules. One biomolecule of interest is angiotensin II. Angiotensin II is an octapeptide hormone which is critical in vasomotor function and has been implicated in the development of atrial fibrosis when present in increased levels. Ultimately it would be advantageous to design a system that would allow for the reduction of circulating angiotensin II levels. Our research has therefore focused on sysnthesizing molecularly imprinted polymers using acrylamide as the functional monomer, poly (ethylene glycol) dimethacrylate as the crosslinking agent and angiotensin II as the template. The polymers were prepared by UV-initiated free radical polymerization. In order to optimize the imprinting process, the ratio of template to functional monomer was investigated. Studies were also performed to determine the structural and conformational integrity of angiotensin II under various pH and temperature changes. Binding and swelling studies were then conducted to determine the effectiveness of the imprinting process. Future work will include incorporating bioerodible components in order to improve the polymer's overall biomimesis.

4:15 PM AA4.8
Multifunctional Nanoparticles and Their Hierarchies for Biomedical Applications. I-W. Chen[1], H. Choi[1], A. Lipski[1], W. Znidarsic[1], P. Shastri[4,1], H. Kung[2], R. Zhou[2] and Y. Zhang[3]; [1]Materials Science and Engineering, University of Pennsylvania, Philaelphia, Pennsylvania; [2]Radiology, University of Pennsylvania, Philaelphia, Pennsylvania; [3]Hematology and Oncology, University of Pennsylvania, Philaelphia, Pennsylvania; [4]Children's Hospital of Philadelphia, Philadelphia, Pennsylvania.

Nanoparticles have unique attributes which make them suitable for biomedical applications. These include a very high surface-to-volume ratio and a size scale that is commensurate with receptors. Therefore, they can be used to efficiently express either specific information at the receptor level or a multitude of information at the cellular level. In addition, the mobility of nanoparticles is an advantage in applications such as targeted drug delivery. Our research seeks to design and synthesize engineered organic/inorganic nanoparticles for specific applications in tissue engineering and diagnostic imaging. These nanoparticles are multifunctional; i.e., they are electrically, magnetically, optically and biomedically active. This is achieved through the compositional/structural tailoring of the nanoparticle cores and the particle-tethered molecular moieties. The multifunctional nanoparticles have also been assembled into fibers and (metal and polymer) surface coatings, and used to direct further assemblies of three-dimensional hierarchies such as gels and fibrous composites, which are likewise endowed with multifunctionality. Examples of our material research and the in vitro/in vivo testing of the products, in MRI imaging and tissue engineering, are provided.

4:30 PM AA4.9
The Movement of Flagellated Escherichia coli Cells Near Porous Surfaces. Willow R. DiLuzio[1], Linda Turner[2], Michael Mayer[1], Byron Gates[1], Piotr Garstecki[1], Weibel Douglas[1], Howard C. Berg[2] and George M. Whitesides[1]; [1]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts; [2]The Rowland Institute at Harvard, Cambridge, Massachusetts.

Understanding how flagellated bacteria interact with surfaces is important in the early stages of biofilm formation and pathogenic infection. The movement of flagellated cells near surfaces is quite different from the three-dimensional, random-walk trajectories that cells execute in free solution. For example, individual E. coli cells are known to swim in clockwise, circular trajectories near planar surfaces for much longer times than are predicted by a random-walk model for their motions. We have developed a new technique for studying the behavior of flagellated bacterial cells near surfaces by confining individual E. coli cells in shallow poly(dimethylsiloxane) (PDMS) microchannels. Agar comprises the floor of these microchannels and a PDMS stamp comprises the sidewalls and ceiling of the microchannels. We found that cells preferentially "drive on the right" in these composite microchannels; cells swim along the channel wall to their right and follow traffic rules. When cells are confined between agar and a PDMS stamp, cells likely minimize the resistance to their movement by swimming closer to the porous agar surface than to the top PDMS surface, leading to their preferential movement to the right. We have also examined the response of individual E. coli cells to microfabricated, porous materials. The possibility that porous materials offer less resistance to bacterial movement than solid surfaces has important implications in environmental and medical microbiology. Affecting cell movement by controlling the porosity of one channel wall provides a new strategy for directing the transport of cells in microchannels. We envision new, cell-based microdevices, which require no external pumping or valving for the manipulation of cells. Instead, cells would navigate through complex, microfluidic networks autonomously.

4:45 PM AA4.10
Bone Tissue Engineering using Polycaprolactone Scaffolds Fabricated via Selective Laser Sintering. Suman Das[1], Jessica Williams[2], Adebisi Adewunmi[1], Rachel Schek[2,3], Colleen L. Flanagan[2], Paul Krebsbach[3,2], Stephen Feinberg[5] and Scott Hollister[2,4]; [1]Mechanical Engineering, University of Michigan, Ann Arbor, Michigan; [2]Biomedical Engineering, University of Michigan, Ann Arbor, Michigan; [3]Oral Medicine, Pathology and Oncology, University of Michigan, Ann Arbor, Michigan; [4]Surgery, University of Michigan, Ann Arbor, Michigan; [5]Oral and Maxillofacial Surgery, University of Michigan, Ann Arbor, Michigan.

Polycaprolactone (PCL) is a biocompatible, bioresorbable polymer with potential applications for bone and cartilage repair. In this work, porous polycaprolactone scaffolds were computationally designed and then fabricated via selective laser sintering (SLS), a rapid prototyping technique. The microstructure and mechanical properties of the fabricated scaffolds were assessed and compared to the designed porous architectures and computationally predicted properties. Scaffolds were then seeded with BMP-7 transduced fibroblasts and implanted subcutaneously to evaluate biological properties and demonstrate tissue ingrowth. The work done illustrates the ability to design and fabricate PCL scaffolds with porous architecture that have sufficient mechanical properties for bone tissue engineering applications using SLS. Compressive modulus and yield strength values ranged from 52-67 MPa and 2.0-3.2 MPa respectively, and lay within the lower range of properties reported for human trabecular bone. Finite element analysis (FEA) results show that mechanical properties of scaffold designs and of fabricated scaffolds can be computationally predicted. Histological evaluation and micro-computed tomography analysis of in vivo scaffolds showed that bone can be generated in vivo. Finally, to demonstrate the clinical application of this technology, we designed and fabricated a prototype mandibular condyle scaffold. The integration of scaffold computational design and freeform fabrication techniques presented here could prove highly useful for the construction of scaffolds that have anatomy specific exterior architecture derived from patient CT or MRI data and an interior porous architecture derived from computational design optimization.

SESSION AA5: Poster Session
Chairs: Craig Halberstadt and M. Thiyagarajan
Tuesday Evening, November 30, 2004
8:00 PM
Exhibition Hall D (Hynes)

AA5.1
Self-Assembly of Magnetic Biofunctional Nanoparticles. Xiangcheng Sun[1], David E. Nikles[2] and J. W. Harrrell[2]; [1]Chemistry Depart., Rutgers, The State University of New Jersey, Piscataway, New Jersey; [2]Center for Materials for Information Technology, The University of Alabama, Tuscaloosa, Alabama.

Nanobiotechnolgy, in which nanoparticles are applied to the analysis of biomolecules. Smaller and smarter magnetic nanoparticles have been explored for many biological applications such as targeted drug delivery, biomedical sensing, and magnetic resonance imaging contrast enhancement etc. Many challenges clearly remain before magnetic nanoparticles achieve their full potential in the biological arena [1, 2].

However highly functionalized magnetic particles have shown very promising progress [3, 4]. In this work, an alternative approach have been used to prepare 4 nm FePt nanoparticles by simultaneous chemically reduction of $Pt(acac)_2$ and $Fe(acac)_3$ by 1, 2-hexadecanediol at high temperature in the presence of stabilizers oleic acid and oleyl amine. As-prepared particles have chemically disordered face-centered cubic structure and exhibited superparamagnetic. As-prepared FePt particles are surrounded by a coat of long chained hydrocarbons with oleic acid and amine functionalized ends. These surfactants are used to disperse the particles in hydrocarbon solutions such as hexane, and self-assembled into particles arrays with a variety of close-packing arrangements. It has been shown that these surfactants, oleic acid, and olyel amine, can be replaced by organic compounds containing a thiol functional group. The thiol binds to the surface and creates an anchor for other molecules such as proteins. Both TEM images and HAADF images of 2D self-assembly have given a clear evidence of the success of surface exchanging of FePt particles. These highly functionalized FePt particles can then be applied to many biological applications, most specifically to magnetic biosensor. References [1] P. Gold, Materials Toady, 2 (2004) 36. [2] Q. A. Pankhurt et al., J. Phys. D: Appl. Phys., 36 (2003) R167. [3] H. Gu et al., Chem. Comm., 15 (2003) 1966. [4] L. E. Euliss et al., Nano Letters, 3 (2003) 1489.

AA5.2

Functionalised Nanostructured TiO_2 Layers for Sensor and Biosensor Applications. Antonella Curulli[1], Antonio Cusma[1], Saulius Kaciulis[1], Stella Nunziante[1], Luca Pandolfi[1], Giuseppina Padeletti[1], Federica Valentini[2] and Marco Viticoli[1]; [1]Istituto per lo Studio dei Materiali Nanostrutturati-CNR, Rome, Italy; [2]Scienze e Tecnologie Chimiche, University of Rome Tor Vergata, Rome, Italy.

The immobilization of biomolecules on solid surfaces is a hot topic in modern bioelectrochemistry, since it is of great interest for several applications such as biological fuel cells, chemical and biological sensing. Furthermore, the development of biosensors on miniaturized platforms appears fundamental to overcome the extensive packaging, the complex electronic interfacing and the regular maintenance of conventional biosensors. The crucial aspect involved in the realization of a bioelectronic system is the immobilization of biological components on electrodes surfaces, able to ensure an effective electronic communication between the biomaterials and the electronic transducers. In latest years, the functionalisation of solid electrodes with thin films of biocompatible materials reveals very attractive, since these configurations could provide a rapid translation of the biological processes occurring on the surface to electronic outputs. In this study we report the realization of functionalised TiO_2 thin films onto silicon substrates for the immobilization of several enzymes and biological molecules. TiO_2 films with different characteristics were realized by MOCVD and Sol-Gel techniques. Deposition processes revealed to be the key parameters for the determination of chemical and microstructural features of the films, which influenced the immobilization. Surface roughness, chemical composition and microstructural features of TiO_2 films were investigated by Atomic Force Microscopy (AFM), X-ray Photoelectron Spectroscopy (XPS) and FT-IR Spectrometry. Nanostructured TiO_2 films deposited at different conditions exhibited a homogeneous microstructure, characterised by low roughness values. The presence of residual carbonylic and carboxylic groups was detected on TiO_2 surfaces deposited at lowest temperatures. Glucose Oxidase and Horseradish Peroxidase immobilized onto TiO_2 nanostructured surfaces exhibited a pair of well-defined and quasi-reversible cyclic voltammetric peaks. The electron exchange between the enzyme and TiO_2 electrodes was greatly enhanced in the TiO_2 nanostructured environment. In addition, several biological molecules, as neurotransmitters were also investigated by Cyclic Voltammetry (CV) and detected with the Differential Pulse Voltammetry (DPV) technique. For dopamine, epinephrine and norepinephrine very high current values, a wide range of potential, and a shift of their oxidation potential (very important to minimize the interference effects) were observed by CV. The study of the interactions between TiO_2 film surfaces and biologic components performed by XPS and FT-IR revealed that the presence of residual carbonylic and carboxylic groups promotes their immobilization TiO_2 surfaces. The electrocatalytic activity of HRP and GOD embedded in TiO_2 electrodes toward H_2O_2 and glucose, respectively, may have a potential perspective in fabricating the third-generation biosensors based on direct electrochemistry of enzymes.

AA5.3

Synthesis of Nanoscale Materials for Neural Electrophysiological Imaging. Ludovico M. Dell'Acqua-Bellavitis, Jake D. Ballard, Pulickel M. Ajayan, Rena Bizios and Richard W. Siegel; Nanotechnology Center, Rensselaer Polytechnic Institute, Troy, New York.

Arrays of aligned, multi-walled, electrically-conducting carbon nanotubes were grown on silicon dioxide wafers using a chemical vapour deposition method and subsequently infiltrated with in situ-polymerized polymethylmethacrylate to achieve electrical insulation between adjacent nanotube bundles. This composite construct was then positioned on an array of electrically-insulated electrodes previously deposited on thermally-grown silicon dioxide substrates. The design aimed at achieving electrical conductivity in the range (2.5 E2 , 5.0 E2) $1/(\Omega*m)$ (1), which will eventually allow interfacing this novel construct with external amplification and data acquisition instruments in order to either record bioelectric signal from both single and multiple neural cells or to electrically stimulate them with nanometric space resolution. This work was supported by Philip Morris USA and the Nanoscale Science and Engineering Initiative of the National Science Foundation under NSF Award No. DMR-0117702. References (1) A. Cao, G. Meng, P.M.Ajayan (2004) Nanobelt-Templated Growth of Carbon Nanotube Rows. Adv. Mater. 16, 40-44

AA5.4

Functionalized Nanoparticles for Cellular Tracking and Delivery. Wei Fu[2,4], Dinesh Shenoy[1,4], Curtis Crasto[3,4], Sanjeev Mukerjee[3,4], Mansoor Mustafa Amiji[1,4] and Srinivas Sridhar[2,4]; [1]Pharmaceutical Sciences, Northeastern University, Boston, Massachusetts; [2]Physics, Northeastern University, Boston, Massachusetts; [3]Chemistry, Northeastern University, Boston, Massachusetts; [4]Nanomedicine Consortium, Northeastern University, Boston, Massachusetts.

In order to improve the delivery efficiency of DNA using non-viral vectors in gene therapy, it is necessary to characterize the cellular transport pathways and study the barriers to efficient gene transfection. We have prepared functionalized gold (Au) nanoparticles for intracellular tracking and delivery of DNA. Au nanoparticles, with an average diameter of 10-15 nm and a narrow size distribution, were prepared by reduction of chloroauric acid (HAuCl4) with freshly-prepared sodium citrate under reflux conditions. Coumarin was attached to the Au nanoparticles surface using a PEG spacer (Mol. wt 1,500 daltons). A hetero-bifunctional PEG derivative containing end-groups thiol and alcohol derivatized as the coumarin carbamate was efficiently prepared. Using scanning fluorescence confocal microscopy, fast data acquisition, and custom particle tracking software, we have tracked these nanoparticles inside BT-20 human breast cancer cells. The results show that the functionalized Au nanoparticles were rapidly internalized in BT-20 cells and within the first 5 minutes of incubation, majority of the particles were in the endosomes of the cells. Within 30 minutes of incubation, the nanoparticles had traversed to the nuclear membrane and were predominantly localized in the peri-nuclear region. Acknowledgements: This study was supported by a National Institutes of Health grant RO1-CA095522 and by the Electronic Materials Research Institute of Northeastern University.

AA5.5

Biodistribution and Targeting Potential of Poly(Ethylene Glycol) Modified Gelatin Nanoparticles in Tumor-bearing Mice. Goldi Kaul and Mansoor M. Amiji; Northeastern University, Boston, Massachusetts.

Purpose: In order to develop a safe and effective systemically-administered delivery system for solid tumors, the biodistribution of control gelatin and poly(ethylene-glycol) modified (PEGylated) gelatin nanoparticles was examined in Lewis lung carcinoma (LLC)-bearing female C57BL6 mice. Methods: Type B gelatin and PEGylated gelatin nanoparticles were radiolabeled (125I) for the in vivo biodistribution studies after intravenous (i.v.) administration through the tail vein in LLC-bearing mice. At various time intervals, the tumor-bearing mice were sacrificed and tumor, blood, and major organs were harvested for analysis of radioactivity corresponding to the localization of the nanoparticles. Percent recovered dose was determined and normalized to the weight of the tissue or fluid sample. Non-compartmental pharmacokinetic analysis was performed to determine the long-circulating property and preferential tumor targeting potential of PEGylated gelatin nanoparticles in vivo. Results: From the radioactivity in plasma and various organs collected, it was evident that the majority of PEGylated nanoparticles were present either in the blood pool or taken up by the tumor mass and liver. For instance after 3 hours, the PEGylated gelatin nanoparticles were almost 6 times higher in the blood pool than the unmodified nanoparticles. PEGylated gelatin nanoparticles remained in the blood pool for a longer period of time due to the steric repulsion effect of the PEG chains as compared to the control gelatin nanoparticles. In addition, approximately 4-5% of the recovered dose of PEGylated gelatin nanoparticles was present in the tumor mass for up to 12 hours. The plasma and the tumor half-lives, area-under-the-curve, and the mean residence time of the PEGylated gelatin nanoparticles were significantly greater than those of the control gelatin nanoparticles. Conclusions: The results of the

study confirmed long-circulating property and preferential tumor targeting potential of PEGylated gelatin nanoparticles in a murine tumor model.

AA5.6
Copolymers of N-isopropylacrylamide, HEMA-lactate, and Acrylic Acid with Time-dependent LCST for a Bioresorbable Carrier. Bae Hoon Lee and Brent Vernon; the Harrington Department of Bioengineering, Arizona State University, Tempe, Arizona.

Polymeric materials with biodegradable thermosensitive properties are useful for biomedical applications such as injectable drug delivery and tissue engineering. Poly(ethylene glycol)/poly(lactic acid-co-glycolic acid) triblock and graft copolymers, triblock copolymers of methoxy poly(ethylene glycol) and poly(propylene fumarate) and poly(organophosphazenes) with poly(ethylene glycol) and hydrophobic amino acids are typical polymers. Injectable and in situ-forming devices using these biodegradable thermosensitive polymers have some advantages to avoid the use of organic solvents for delivery, harmful chemical crosslinking agents and sonication.(1) Recently, copolymers of N-isopropylacrlyamide (NIPAAm) and some hydrolysable comonomers such as 2-hydroxyethyl methacryl lactate (HEMA-lactate) and N-(2-hydroxypropyl)methacrylamide lactate (HPMAm-lactate) have been synthesized by Nevadovic et al. for degradable micelles. After hydrolysis, the copolymers exhibited lower critical solution temperature (LCST) below body temperature except copolymers of NIPAAm and HPMAm-lactate with more than 35 mol % HPMAM-lactate.(2) Generally, the LCST of NIPAAm-based copolymers can be controlled by the nature of comonomers: Hydrophobic comonomers decrease LCST of copolymers; hydrophilic comonomers increase that of copolymers. Especially, anionic comonomers increase largely in LCST of NIPAAm-based copolymers, depending the content of anionic comonomers. Hence, we designed copolymers showing LCST above 37 C after hydrolysis by using acrylic acid (AAc) as a comonomer for a degradable in situ gelling material. Copolymers of NIPAAm, HEMA-lactate, and AAc were characterized by NMR spectroscopy, Differential Scanning Calorimetry (DSC), Gel Permeation Chromatography (GPC), and rheometry. The copolymers showed LCST and gelation properties below body temperature in aqueous solution before hydrolysis. The LCST and gelation temperature of the copolymers decreased as the content of hydrophobic HEMA-lactate was increased. Also, the copolymers showed time-dependent LCST properties in 0.1 N PBS solution of pH 7.4 owing to hydrolysis of HEMA-lactate. Hydrolysis of HEMA-lactate caused the polymers to be more hydrophilic, resulting in the increase in LCST. The polymer solutions at above 20 wt % were soluble below room temperature and formed gels at body temperature. The formed gel dissolved during the degradation. It took 1 to 10 days for the polymer gels to dissolve. The gelation properties and degradation time depended on the content of HEMA-lactide. After hydrolysis, all the polymers exhibited the LCST values above body temperature and remained in sol state at body temperature. These polymers with time-dependent LCST and gelation properties can be expected to be useful for an injectable bioresorbable drug carrier. 1 Jeong B, Bae YH, Lee DS, Kim SW, Nature 388:860 (1997) 2 Neradovic D, Vannostrum CF, Hennink WE, Macromolecules 34:7589 (2001)

AA5.7
Preparation of Biodegradable Drug Delivery PLGA Nanocomposites by Rapid Expansion from Supercritical Solutions in Air (RESS) and Solvents (RESOLV). Alexandru D. Asandei[1,2], Gobinda Saha[1], Can Erkey[3], Carl Saquing[3], Diane Burgess[4] and Banu Sizanli-Zolnik[4]; [1]Institute of Materials Science, University of Connecticut, Storrs, Connecticut; [2]Chemistry, University of Connecticut, Storrs, Connecticut; [3]Chemical Engineering, University of Connecticut, Storrs, Connecticut; [4]School of Pharmacy, University of Connecticut, Storrs, Connecticut.

Biodegradable drug delivery nanocomposites based on poly(lactic acid) (PLA) and poly(lactide-co-glycolide) (PLGA) and dexamethasone (a drug for endocrine or rheumatic disorders) were prepared by the rapid expansion of the corresponding supercritical CHClF2 solutions (110 øC, 200-300 bar) in air (RESS) and in toluene (RESOLV). The RESS process leads to a broad particle size distribution (100-500 nm) while the RESOLV generates a narrower distribution centered around 100 nm and is accompanied by the formation of a few large particles, most likely due to aggregation.

AA5.8
MAPLE Deposition of PGLA Thin Films and Multilayers. Timothy M. Patz[1,2], Roger Jagdish Narayan[1] and Douglas B. Chrisey[2]; [1]Material Science & Engineering, Georgia Institute of Technology, Atlanta, Georgia; [2]US Naval Research Laboratory, Washington, District of Columbia.

Controlled release of pharmacological agents from a resorbable polymer matrix is a growing area of biomaterials research. Drug delivery devices provide several advantages over conventional oral or intravenous administration. These advantages include the ability to maintain the drug concentration within a therapeutic range, the ability to tailor dosing to drug and patient requirements, and increased patient compliance. Aliphatic polyesters (e.g., polyglycolides and polylactides) are most commonly used in drug delivery devices. We propose the creation of a novel layered drug delivery system, in which pharmacologic agents are layered within these polymers, in order to provide controlled release of one or more pharmacologic agents over an extended period of time. We deposited thin films and multilayers of a poly(glycolic-co-lactic) acid copolymer (m.w. 50-75,000) on Si and ZnSe substrates using matrix assisted pulsed laser evaporation. These films were characterized using Fourier transform infrared spectroscopy (FTIR), atomic force microscopy, dynamical light scattering, X-ray photoelectron spectroscopy, and scanning electron microscopy. FTIR revealed that the deposited PGLA film maintained the main vibrational groups as compared to a drop-cast sample. These novel materials will allow the development of sophisticated drug delivery devices.

AA5.9
Synchrotron X-ray Induced Gold Nanoparticle as Drug Delivery Carrier. Yung Chin Yang, Physics, Academia Sinica, Taipei, Taiwan.

In this study we introduced a new solution precipitation process of gold nanoparticles using synchrotron radiation. We demonstrated that by synchrotron x-ray irradiation Au particles were able to nucleate homogeneously in the aqueous solution without reducing agent, eventually growing to nanoparticles at room temperature (Fig. 1). Without stabilizer, e.g. surfactants and polymers, the Au nanoparticles were prepared by using synchrotron irradiation methodology and adjusting the pH value of reactant solution. Our data reveals that the pH value plays an important role in the synthesis of gold nanoparticles. The size of the nanoparticles precipitated was uniform around 10 nm, and it could be well dispersed in solution with sufficient OH ion (Fig. 2). However, the exceeding OH ion would promote nanoparticle growth (Fig. 1). In this study, keep the HAuCl4 solution in a neutral state revealed the smaller particle size and excellent particle dispersion. Otherwise, the concentration of HAuCl4 solution in the synthesis reaction was independent to the size of nanoparticle, it only influenced the fabrication rate of gold nanoparticle during synchrotron irradiation. Our results suggest that synchrotron x-ray can be used to induce solution precipitation of gold nanoparticles and therefore lead to a new application on drug delivery and release system. Au nanoparticle solution prepared from this simple and clean, especially fast fabrication process without toxic reductant is biocompatible and allows the subsequent surface modification and drug attachment could be performed simply.

AA5.10
A Novel Pressure Indicator for Continuous Flow PCR Chip Using Micro Molded PDMS Pillar Arrays. Yi Zhao and Xin Zhang; Department of Manufacturing Engineering, Boston University, Brookline, Massachusetts.

Continuous flow PCR chip has received considerable attention in recent years thanks to easy temperature control, simple fabrication and short thermal cycle. This approach requires precise flow rate control of PCR reagent in order to adjust residency time in each of three temperature regions. As such, even subtle change in fluid pressure can disturb the thermal cycle of PCR process, which would not cause any noticeable consequence in other microfluidic systems. In this paper, we demonstrates a novel pressure indicator integrated within a continuous flow PCR chip, which can *insitu* monitor fluid pressure using micro molded PDMS pillar arrays as the indicator. The PCR chip comprises two layers: a glass substrate with three heaters operated at three different temperature regions; and a polydimethylsiloxane (PDMS) film with the reagent inlet, outlet and serpentine channel. A micro pillar array fabricated on the top surface of the PDMS film serves as the pressure indicator, with a square chamber right underneath it. The chamber is connected with the reagent inlet and the serpentine channel at its two ends. Both the pressure indicator and the underlying chamber were fabricated in the same step together with other PCR channels using the double-sided micromolding process. In the process, the lower mold (SU-8) and the upper mold (silicon template) were placed face to face and aligned to each other, with PDMS prepolymer sandwiched in between. After curing for chemistry crosslinking, the molds were removed carefully. The polymerized PDMS was immediately put into contact with the glass slide, which has heaters deposited on. The continuous flow PCR chip with pressure indicator was thus formed with additional connections to fluid and electric supplies. In the pressure measurement, the fabricated PCR chip was put on the stage of an upright microscope with a video imaging system. The fluid pressure was tuned by changing the flow rate. The pillar spacing without

applied pressure was first measured as the reference (L_0). The PCR reagent was then perfused in. As indicated from the out-of-focus image observed under the microscope, the PDMS thin membrane suffered an out-of-plane bowing in response to the differential pressure change. This change in turn induced an enlarged spacing (L). With the smaller deflection assumption, the value of $(L-L_0)/L$ has a linear dependence with the differential pressure, thereby the fluid pressure can be derived giving the elastic modulus and geometry of the thin membrane. By using this method, the fluid pressure variation within the PCR channel was successfully *insitu* monitored, providing an alternative evaluation for the reliability and repeatability of the DNA amplification cycle.

AA5.11
DNA Electrophoresis on Micro-Patterned Surfaces.
Eric Petersen[1], Bingquan Li[2], Vladimir Samuilov[2], Miriam Rafailovich[2] and Jonathan Sokolov[2]; [1]Physics, Harvard University, Cambridge, Massachusetts; [2]Materials Science and Engineering, SUNY Stony Brook, Stony Brook, New York.

Conventional techniques of topographically confining DNA into channels create a myriad of problems due to the hydrodynamic confinement of the fluid. We created chemically micropatterned surfaces, in an attempt to confine DNA on the surface, without interfering with the hydrodynamics of the fluid. Micro-patterns of alternating gold and silicon strips were stamped onto silicon wafers by the Whitesides microcontact printing method [1].In order to understand the mechanism by which a chemical micropattern confines DNA on a surface, the mobility of DNA across patterned surfaces of varying period size with an electric field oriented normal to the strips was measured via laser induced fluoresence detection. Results suggest that the measured mobility of DNA depends on the period size of the gold striped pattern relative to the natural chain length of the DNA molecule. Similar measurements performed on striped patterns oriented parallel to an electric field indicate that DNA moves approximatley twice as fast as it does on patterns oriented normal to an electric field. Work is currently underway to image the motion of the DNA chains that are moving across the pattern. In this manner, we can determine if we have matched the pattern size to the chain length in order to obtain confinement in the channels. [1] A. Kumar and G.W. Whitesides, Appl. Phys. lett. 63, 2002 (1993). Work supported in part by the NSF-MRSEC program and the Department of Energy

AA5.12
Microfluidic Cell Volume Biosensor for High Throughput Drug Screening.
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Cell volume and its physiological functions are intertwined. Changes in cell volume accompany various functions, such as metabolism, excitation, hormone release, cell proliferation, and apoptosis. Therefore a real time monitor of cell volume provides a sensitive tool for studying numerous factors that affect cell volume. These include not only normal metabolism, but also factors leading to apoptosis, neurotransmitter activity, and environmental toxicity, such as the presence of agents from natural, industrial or chemical/biological warfare sources. We have constructed a microfluidic lab-chip biosensor that utilizes cell volume changes to monitor how cells respond to drugs or toxic chemicals. The biosensor is based on an electrical impedance method to measure changes in cell volume. In the present study, adherent cells on a solid substrate were placed in a shallow microfabricated chamber (15 microns deep). As the volume of the cells change, they displace the extra-cellular fluid in the chamber, thereby changing the electrical resistance of the chamber. A single microfluidic channel prototype sensor was built on silicon wafer. Cultured primary astrocyte cells were tested by varying the osmotic stimuli transmitted through the fluidic channel. We observe regulatory volume decrease (RVD) when the cells were exposed to hypotonic solution of 188, 220 and 273 mOsm. The regulatory volume increase (RVI) in astrocyte was also obtained due to hypertonic stimuli of 345, 399, and 417 mOsm. Results are in good agreements with previous reports using other methods to measure cell volume. Through the use of microfabrication technology to make precise chamber dimensions, our results further show that cell volume change even in response to osmotic gradient of less than 1 mOsm can be detected. To demonstrate the lab-chip as a potentially powerful screening tool for pharmacologic agents, we have also measured the effect of peptides on astrocyte volume regulation. We tested volume regulation in astrocytes using various amounts of GsMTx1, a peptides isolated from spider venom. The results shows that RVD of astrocyte was inhibited in the presence of GsMTx1 as low as 100 pico-moles. This work was supported by the National Science Foundation Grants NSF-CMS-0201293 and this support is gratefully acknowledged.

AA5.13
Imaging Therapeutic Proteins using Synchrotron X-Ray Tomography.
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In response to an event disrupting bone function, such as disease or trauma, specific proteins are naturally secreted to initiate healing. Disease and other factors can diminish the ability of bone to self-repair. Therefore, augmentation through the use of artificial bone may be required. The addition of therapeutic proteins, such as growth factors, to engineered bone constructs is believed to elucidate a more natural response at the defect site and to decrease healing time. Drug delivery vehicles must be carefully engineered in order to optimize the dose and dose rate, and these parameters must be monitored over time and space. One technique used to measure the pharmacokinetics of growth factors is radioactive labeling. However, this only yields an average value over the sample; it does not provide a three dimensional measure of the distribution of the drug within the delivery vehicle or of its release profile. The radioactivity of such a label adds further complications. Our results show that model proteins with nanoscale labels can be imaged using x-ray microcomputed tomography, and a three dimensional representation of the drug distribution can be determined. Gelatin microspheres, engineered to mimic the natural release of growth factor, were loaded with labeled protein and incorporated into either gelatin or chitosan matrices. The protein was labeled with non-radioactive iodine or gold nanoparticles, both of which provide x-ray absorption contrast. Using synchrotron radiation at the Advanced Photon Source at Argonne National Laboratory, we imaged these protein-loaded polymeric samples for the first time. Preliminary results demonstrate the feasibility of imaging the proteins in this manner and that for the same concentration the gold label provides better contrast as compared to the non-radioactive iodine. This technique will allow us to create a three dimensional representation of the drug distribution within the delivery vehicle and its pharmacokinetics, rapidly, and without radioactive tracers. Results will have significant impact on clinical design of bone implants.

AA5.14
A Quantum Dot Based TNT Nanosensor.
Igor Langier Medintz[1], Ellen R. Goldman[1], Andrew Hayhurst[2], Jessica L. Whitley[1] and Hedi Mattoussi[3]; [1]Center for Bio/Molecular Science and Engineering, Code 6900, U.S. Naval Research Laboratory, Washington, District of Columbia; [2]Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas; [3]Division of Optical Sciences, Code 5611, U.S. Naval Research Laboratory, Washington, District of Columbia.

Colloidal luminescent semiconductor nanocrystals or quantum dots (QD) possess unique attributes that make them superior to commercially available organic dyes when used for optical-based biological sensing assays. We have previously demonstrated a prototype fluorescence resonance energy transfer- (FRET) based QD nanosensor that targeted the nutrient maltose and utilized E. coli maltose binding protein as the bio-recognition element(1). We now extend this design to target the explosive TNT. The sensor consists of antibody fragments, that specifically recognize TNT, self-assembled onto the surface of the QD via metal-affinity coordination. These antibody fragments are pre-labeled with a TNT analog that is covalently attached to a quencher dye. The close proximity of the antibody fragment labeled with dye to the QD energy emitting core results in FRET based quenching of QD photoluminescence (PL). Addition of solution containing TNT results in the specific displacement of the dye analog and a concentration dependent increase in QD PL. This solution phase TNT sensor was tested in a number of different QD configurations and against a number of structurally different explosives. The sensor was also used to test TNT samples originating from a contaminated waste site. The results correlated well with other analytical methods. This sensor configuration may be uniquely suited for challenging environments such as the ocean. (1)Medintz, I.L., Clapp, A.R., Mattoussi, H., Goldman, E.R., Fisher, B., Mauro, J.M. Self-assembled nanoscale biosensors based on quantum dot FRET donors. Nat. Mat. Vol 2 (9) 630-638 2003.

AA5.15
Laser Fabrication of Micro-Joints for Encapsulation of Biomedical Devices.
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McAllister[4] and Simon Ng[5]; [1]Mechanical Engineering, Wayne State University, Detroit, Michigan; [2]Center for Smart Sensors and Integrated Microsystems (SSIM), Wayne State University, Detroit, Michigan; [3]Center for Laser Technology, Fraunhofer USA, Plymouth, Michigan; [4]Department of Neurosurgery, Wayne State University, Detroit, Michigan; [5]Chemical Engineering and Materials Science Department, Wayne State University, Detroit, Michigan.

The outermost shell of an implant device is the encapsulation, which is the physical interface between the device and the biological medium. The design of the encapsulation of advanced medical implant devices, such as devices that electrically stimulate and/or record neural activity, poses a number of serious requirements to the materials that are used. Among these requirements, biocompatibility and long-term stability are crucial. Often, the functionality of such devices requires the employment of dissimilar materials for which there is no reliable ways of joining that would also satisfy the need for biocompatibility and stability. Laser joining is a new emerging alternative to the conventional techniques of adhesive joining or soldering. As part of a larger project that aims at developing a functionally active biological encapsulation technology, we have studied the problem of laser joining of dissimilar biocompatible materials. We have identified laser conditions for successful joining of polymers, such as polyimide, PVDF, and polyurethane, to bulk Ti, Ti-coated borosilicate glass. These joints are of sub-millimeter widths and are suitable for encapsulation of miniature devices. In this work, we present the laser joining methods and discuss the ranges of parameters where joining is possible. We also present and discuss results from characterization by means of mechanical failure and He-leak hermeticity tests of joined samples that have been exposed for short and longer-term periods to physiological solutions such as cerebrospinal fluid (CSF). The laser joint regions and interfaces were studied by X-ray photoelectron spectroscopy (XPS), optical and electron microscopy and micro-Raman spectroscopy in order to understand the nature of the laser-fabricated materials interfaces. Our results suggest the formation of strong chemical bonds between Ti-containing species and certain polymeric functional groups. We have also found that laser-generated defects in the glass regions that surround the joints are responsible for the observed failure in some of the cases and we discuss the origin of such defects and approaches to minimize their concentration.

AA5.16
Ultrananocrystalline diamond as hermetic coating for BioMEMS. Xingcheng Xiao[1], Jian Wang[1], John A. Carlisle[2], Orlando H. Auciello[2], Mark Humayun[3], James Weiland[3], Brian Mech[4] and Honggang Jiang[4]; [1]Materials Science Division, Argonne National Laboratory, Argonne, Illinois; [2]Materials Science Division and Center for Nanoscale Materials, Argonne National Laboratory, Argonne, Illinois; [3]Doheny Eye Institute and Keck School of Medicine, University of Southern California, Los Angeles, California; [4]Second Sight Inc., Sylmar, California.

The biomedical application of the microelectromechanical systems, or BioMEMS, is a rapidly growing field that physiologically integrates the microfabrication technology into the in vivo treatment and in vitro diagnosis to improve human health. One example of this application is the artificial retina implant for restoring and substituting impaired or lost vision in human eyes. A passivation layer on the artificial retina is indispensable to prevent direct contact of the electronic components of the device with surrounding tissues, as well as to protect the device against erosion by the biological medium. CVD diamond has been considered as an ideal protective coating for bioimplants due to its excellent chemical inertness and biocompatibility. However, the high deposition temperature (> 700 oC) required for fabricating CVD diamond coatings greatly limits its practical application on devices with metallic components. In our presentation, a novel bioinert material, Ultrananocrystalline Diamond (UNCD) which can be deposited at low temperature (< 500 oC), will be introduced as a protective coating for the artificial retina microchips. UNCD was prepared in a microwave plasma enhanced chemical vapor deposition (MPECVD) system with 99% Ar/ 1% CH4 as precursors. Electrochemical tests were performed to evaluate the hermeticity of the deposited thin UNCD coatings (around 1 micron) on highly conductive Si slices in different chemical environment (5% HF solution and PBS at pH7.4). UNCD coated silicon chips were implanted in rabbit eyes for six months to study the biocompatibility and biostability of UNCD in the physiological environment. Different surface characterization methods were employed to compare the chip surface condition before and after implantation. The in vitro electrochemical experiment showed that UNCD coatings were dense and possess good hermeticity in harsh chemical environment. The in vivo implantation study indicated good bio-inertness of the UNCD coatings in the physiological environment with no interaction between UNCD and rabbit eyes. Results from this study suggest that UNCD is a promising candidate as the hermetic coating for the artificial retina and other BioMEMS devices.

AA5.17
Increased Capture of Bacteria by Using Nanophase Ceramics. Zhong-Hua Tong[2], Kathy M. Banks[2] and Thomas Jay Webster[1]; [1]Biomedical Engineering, Purdue University, Lafayette, Indiana; [2]Civil Engineering, Purdue UNiversity, West Lafayette, Indiana.

Nanotechnology is defined as the use of materials (such as particles, grains, fibers, etc.) with fundamental length scales less than 100 nm in at least one direction which demonstrate novel properties when compared to materials with conventional or micron dimensions. To date, numerous special properties of nanophase materials have been demonstrated including enhanced catalytic, mechanical, optical, electrical, and processing properties. However, except for tissue engineering applications, interactions of living cells with nanophase materials remains largely uninvestigated. This is despite their unique surface properties of increased surface area, greater numbers of atoms at the surface, increased electron delocalization, and a larger proportion of atoms at the surface compared to bulk; such properties would most certainly provide an intriguing surface for interactions with charged species like bacteria. For these reasons, this study is one of the first to determine the capture of a model bacteria (Pseudomonas fluorescens) on nanophase alumina. Results provided the first evidence of increased capture of Pseudomonas fluorescens on alumina with nanometer compared to conventional grain sizes. Specifically, capture after 30 minutes was up to twice as great on nanophase compared to conventional alumina. Since the nanophase and conventional alumina prepared in this study only altered in grain size (that is, chemistry (Al2O3), crystallinity, and crystal phase (gamma) were similar), this study elucidated a promising size-dependent relationship between bacteria capture and alumina nanometer grain size for the design of better environmental filters. In doing so, it provides the first evidence that nanophase materials should be further investigated for the purification of waste water and other fluids.

AA5.18
Fabrication of Novel Types of Colloidosome Microcapsules for Drug Delivery Applications. Vesselin N. Paunov[1], Olivier J. Cayre[1], Rossitza Alargova[2], Paul F. Noble[1] and Orlin D. Velev[2]; [1]Department of Chemistry, University of Hull, Hull, United Kingdom; [2]Dept. of Chemical Engineering, North Carolina State University, Raleigh, North Carolina.

Colloidosomes are core-shell microcapsules that consist of an aqueous core and a shell formed by fused colloidal particles. Recently, it has been recognised that such microcapsules offer a great potential in controlling the permeability of entrapped species in pharmaceutical, cosmetic and food products. Here we report a versatile fabrication method of novel colloidosomes microcapsules which is based on the following 3 stages: (i) Hot aqueous solution of gelling hydrocolloid is emulsified in a suitable oil in the presence of solid polymer particles dispersed in the aqueous phase to produce a water-in-oil emulsion stabilised by the solid particles and the system is cooled off to set the gel. (ii) The produced suspension of aqueous gel microcapsules coated with a particle monolayer is separated by filtration to remove the oil phase. (iii) The microcapsules are washed and collected into water. This methodology allows us to produce colloidosome microcapsules of diameters varying between several tens of micrometers to several hundreds of micrometers. The function of the gel cores was to support the particle shell around them and to give the microcapsules enough stiffness to be separated from the oil phase by filtration. Following this technique we have been able produce three different types of colloidosome microcapsules. (a) By combining monodisperse amino-latex microparticles and an oil which swells the latex we have fabricated integral colloidosomes of porous membrane where the pore size is controlled by the degree of swelling. (b) By using monodisperse amino-latex particles and cross-linking agent we were successful in producing colloidosomes of spherical particle monolayers, where the membrane pores are defined by the particle size. (c) By using polymer micro-rod particles as emulsifiers we have synthesized for the first time 'hairy' colloidosomes which shells consists of randomly assembled rod-like particles.

AA5.19
Abstract Withdrawn

AA5.20
Selective Labeling of Single Pathogenic Bacterial Cells with Semiconductor Nanocrystals. Megan A. Hahn[1], Joel S. Tabb[2] and Todd D. Krauss[1]; [1]Chemistry, University of Rochester, Rochester, New York; [2]Agave BioSystems, Ithaca, New York.

Water contamination and food poisoning caused by bacterial pathogens are urgent issues plaguing today's world. One possible route to detect these microorganisms is based on the photophysical properties of CdSe nanocrystals (NCs), which exhibit size-tunable fluorescence that spans the visible spectrum. Their narrow emission

spectra, broad absorption spectra, and high photostability versus traditional organic fluorophores make NCs attractive for biological applications: the simultaneous detection of multi-color labels using a single excitation source is possible, and certain long-lived biological processes can be followed in real time. We will present our efforts toward the development of a selective nanocrystal-based fluorimetric label for pathogenic *Escherichiacoli* O157:H7. Using fluorescence microscopy of individual bacterial cells, we have demonstrated that CdSe/ZnS core/shell NCs functionalized with streptavidin bind specifically to *E.coli* O157:H7 cells functionalized with biotinylated antibodies. We will also present results comparing *E.coli* O157:H7 cells labeled with NCs to cells similarly labeled with fluorescein isothiocyanate (FITC): under continuous excitation, the higher photostability of NCs over FITC is easily observed. The particular biochemical interactions incorporated in these methods can easily be generalized to allow for the rapid and selective detection of common pathogens.

AA5.21

Novel Electroporation System for Both Gram-Negative and Gram-Positive Bacteria Assisted by Multi-Walled Carbon Nanotubes (MWCNTs). Giersig Michael, Jose Rojas-Chapana and Miguel Correa-Duarte; Nanoparticle Technology, Center of Advanced European Studies and Research (CAESAR), Bonn, Germany.

Recent trends involving the convergence of research in cell biology and nanotechnology promote the development of biocompatible materials for the design of highly selective nanodevices for biomedical applications. Nanodevices with superior thermal, electrical and mechanical properties are ideal for biomedical applications because they can interact directly with the target both at the cellular and molecular level. From a biological perspective, cellular systems are fragile and require a controlled environment, which limits the kinds of forces and manipulators that can be used. For the manipulation of bacterial cells, however, the difficulty of targeting the cellwall and membranes without destroying the cells poses a real problem. Preparation of electrocompetent bacterial cells and mediators capable of disrupting the cell wall are frequently used to assist the transformation of cells. Usually an alternating electric field induces a dipole moment in the cell and a potential difference across the plasma membrane. This induced voltage leads inevitably to a breakdown of the membrane. Though this method allows a manipulation of living cells due to its unspecific features, it is also probable that the surviving cells have severely reduced efficiency rates as well as an altered physiological state. It is in this vein that we have recently initiated a program to develop selective methods for reversible nanomanipulation of bacteria (both gram-positive and negative) and eukaryotic cells via microwave (MW)-induced excitation of carbon nanotubes. When exposed to MW irradiation, the number of viable cells decreased according to the exposure time and the power used. In the presence of MWCNTs a short duration MW exposure (approx. 5 sec.) causes induced dipoles at their tips, converting them to single electroporation devices. Exposure to MW field was achieved in a MW cavity, powered by a magnetron source of 1200 W at a frequency of 2,45 GHz. The time of exposure was controlled. In this report we describe the development of a novel type of electroporation via water-soluble MWCNTs as vectors, which can be introduced across the bacterial cell envelope via controlled microwave (MW) irradiation. The bacteria used were a gram-negative Acidothiobacillus ferrooxidans, and a gram-positive Lactococcus lactis. This MWCNT-mediated electroporation process is not detrimental for cells, and after treatment, the same MWCNTs can serve as devices for the nanoinjection of macromolecules and nanoparticles into cells. The electroporative approach presented in this report provides several benefits over conventional electroporation modalities. Membrane disruptions can be minimized in nanoscale spacing and the procedure is able to manipulate cells still growing. The time consuming physical and chemical procedures are by-passed leaving the cells in their prime condition resulting in greater cell survival and increased transformation rates.

AA5.22

Novel Amino Acid Ester Polyphosphazene-Hydroxyapatite Composites for Bone Tissue Engineering. Cato T. Laurencin[1,2], Lakshmi S. Nair[3], J. R. Bender[4], A. Singh[4], H. R. Allcock[4], Y. Greish[5], P. W. Brown[5] and C. T. Laurencin[3,1,6]; [1]Department of Chemical Engineering, University of Virginia, Charlottesville, Virginia; [2]Department of Chemical Engineering, Drexel University, Philadelphia, Pennsylvania; [3]Department of Orthpaedic Surgery, University of Virginia, Charlottesville, Virginia; [4]Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania; [5]Intercollege Materials Research Laboratory, Pennsylvania State University, University Park, Pennsylvania; [6]Department of Biomedical Engineering, University of Virginia, Charlottesville, Virginia.

Approximately 1.3 million bone graft procedures take place per year in the US alone. Due to the inherent disadvantages of both autografts and allografts currently used for these procedures, tissue engineering has emerged as an alternative therapeutic strategy. Hydroxyapatite (HAp) being a major component of natural bone is the most extensively investigated biomaterial for skeletal tissue regeneration. Recently, low temperature apatites (LTA) have emerged as attractive candidates due to their moldability, resorbability, in situ self-hardening ability and osteoconductivity [1]. However, LTAs have poor mechanical properties and hence attempts are currently underway to improve their properties by developing composites of LTA with synthetic or natural polymers. Biodegradable polyphosphazenes have been developed as potential candidates for various biomedical applications due to their synthetic flexibility, non-toxic degradation products and excellent biocompatibility [2]. The objective of the present study was to develop novel composites of two calcium deficient low temperature apatites having calcium/ phosphate (Ca/P) ratios of 1.5 (CDHAp) and 1.6 (CDSHAp) with biodegradable polyphosphazenes and to evaluate the osteocompatibility of the matrices as candidate materials for bone tissue engineering. Biodegradable polyphosphazenes poly[bis (ethyl alanato) phosphazene] (PNEA) and poly[(ethyl alanato)(p-methyl phenoxy)phosphazene] (PNEA/mPh) were synthesized and characterized according to reported procedures [2]. The polyphosphazene HAp composites were prepared by mixing HAp precursors with corresponding polyphosphazenes in the ratio of 9:1 in 0.5% phosphoric acid (liquid to powder ratio 1:1) at 37?C for 24 h. The formation of HAp in the composite was followed by XRD. MC3T3-E1 cells were used for osteocompatibility evaluation. Cytotoxicity of the composite degradation products was followed by MTS assay. Adhesion and proliferation of cells on the matrices were followed by SEM and DNA assay. The phenotype expression of the cells on the matrices was followed by determining various phenotype markers such as alkaline phosphatase and osteocalcin. Poorly crystalline HAp was formed in the present study. The degradation products of the four composites were found to be non-toxic to MC3T3 cells. All matrices supported the adhesion and proliferation of osteoblast cells however the number of cells on CDSHAp composites was found to be higher than that of CDHAP. Furthermore, the cells on the matrices maintained their phenotype as evidenced from the expression of phenotype markers. We have demonstrated the feasibility of developing moldable, self setting, highly osteocompatible and biodegradable composites as candidates for bone tissue engineering. NIH Grant# 46560 1. Greish YE et al. Biomaterials 2005; 26: 1-9. 2. Allcock. Chemistry & Applications of Polyphosphazene, Wiley Interscience, 2002.

AA5.23

Fabrication of Gradient Hydrogels by a Microfluidics/ Photopolymerization Process. Ali Khademhosseini[1], Jason Burdick[2] and Robert Langer[1,2]; [1]Biological Engineering Division, Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Many biological processes such as cell migration and morphogenesis are controlled by gradients of soluble (chemotaxis) and immobilized (haptotaxis) molecules and substrate mechanics (durotaxis). Thus, it is of interest to develop techniques to fabricate model substrates that can be used to investigate these complex cell behaviors in vitro to provide information that can potentially be used for the development of therapies, such as scaffolding for tissue engineering. We introduce a method of fabricating photocrosslinked hydrogels with gradients of immobilized molecules and crosslinking densities. Two (or more) macromer/initiator solutions are injected into a unique PDMS channel system that produces a prepolymer gradient that is subsequently polymerized into a water swollen hydrogel with ultraviolet light exposure. The gradient is controlled by the injection flow rate (optimized to 0.3 microliter/min per inlet to produce a linear gradient). In addition, through simple modifications to the gradient generator, complex gradients can be generated and immobilized within hydrogels. The technique is investigated both through fabrication of adhesive ligand gradients that modulate spatial distribution of attached endothelial cells and gradients of crosslinking densities that lead to unique hydrogel architectures and spatially dependent swelling.

AA5.24

Molded Polyethylene Glycol Microstructures for Capturing Cells within Microfluidic Channels. Ali Khademhosseini[1], Judy Yeh[2], Sangyong Jon[2], George Eng[2], Kahp Y. Suh[3], Jason Burdick[2] and Robert Langer[1,2]; [1]Biological Engineering Division, Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [3]School of Mechanical and Aerospace Engineering, Seoul National University, Seoul, South Korea.

The ability to control the deposition and location of adherent and

non-adherent cells within microfluidic devices is beneficial for the development of micro-scale bioanalytical tools and high-throughput screening systems. Here, we introduce a simple technique to fabricate poly(ethylene glycol) (PEG) microstructures within microfluidic channels that can be used to dock cells within pre-defined locations. Microstructures of various shapes were used to capture and shear-protect cells despite medium flow in the channel. The size and shape of the microstructures could be easily controlled through the use of soft lithographic molding techniques. In addition, PEG microwells were fabricated either with exposed or non-exposed substrates. Proteins and cells adhered within microwells with exposed substrates, while non-exposed substrates prevented protein and cell adhesion (although the cells were captured inside the features). Furthermore, immobilized cells remained viable and were stained for cell surface receptors by sequential flow of antibodies and secondary fluorescent probes. With its unique strengths in utility and control, this approach is potentially beneficial for the development of cell-based analytical devices and microreactors that enable the capture and real-time analysis of cells within microchannels, irrespective of cell anchorage properties.

AA5.25
Microfabricated Arrays of Thermal Coolers for Cryobiological Applications. Aparna Prabhakar and Ram V. Devireddy; Mechanical Engineering, Louisiana State University, Baton Rouge, Louisiana.

There is an ever expanding interaction between the fields of micro electromechanical systems (MEMS) and biology to develop devices to monitor, control and act on living systems. Particularly in the field of cryobiology, there is a need to monitor and control temperature at the cellular level. An important step towards achieving this aim would be to fabricate a thermoelectric micro device or an array of Peltier coolers. The thermoelectric phenomena was chosen as a suitable technique because 1) it does not include any moving parts and 2) it can be micro electronically integrated to have the dual capability of controlling and monitoring temperature at the microscale. This study presents a method of fabricating an array of high aspect ratio Peltier coolers with a modified multi-step LIGA technique. Bismuth telluride microposts, to be used as thermo electric leg elements of the Peltier cooler, will be electrochemically deposited from solutions of $Bi_2O_3$and TeO_2 in diluted HNO_3onto the ceramic/gold-working electrode. A conventional three-electrode cell with platinum wire electrode and saturated calomel (SCE) reference electrode will be used. By controlling the cathodic potential either p- or n- type Bi-Te alloy micro posts will be produced. A patterned photoresist mask will be used to define the regions for deposition. Gold will be used for bottom interconnects and titanium for the top to ensure biocompatibility. A PMMA sheet will act as an interface between the cooler and the cells embedded in our experimental artificial tissue (AT) system. Within the array, the coolers will be interspersed to enable each one to be individually addressable. The thermoelectric micro device thus developed will result in the unique capability of temperature manipulation and control on cellular scales (micrometers). Acknowledgement: This work was supported in part by a Biomedical Engineering Research grant from the Whitaker Foundation to RD.

AA5.26
Presentation and Recognition of Epitopes on Nanofibers Formed by Branched Peptide Amphiphiles. Mustafa O. Guler[1] and Samuel I. Stupp[1,2,3]; [1]Department of Chemistry, Northwestern University, Evanston, Illinois; [2]Department of Materials Science and Engineering, Northwestern University, Evanston, Illinois; [3]Feinberg School of Medicine, Northwestern University, Chicago, Illinois.

Molecular recognition among ligands and receptors in biology requires appropriate presentation of epitopes. Recently, there has been great interest in design of cell scaffolds with artificial epitopes in order to trigger biological events important in regenerative medicine. The use of self-assembly in scaffold formation is particularly attractive because it can lead to noninvasive tissue delivery or in situ encapsulation of cells by liquids containing the molecules that build the scaffold. We report here on self-assembling peptide amphiphiles with branched covalent architecture that form nanofiber networks in physiological media. These molecules were designed for enhanced recognition of a bioactive epitope on the surfaces of nanofiber formed by these molecules. Branching at a lysine residue was also used to synthesize that present more than one epitope per molecule. We found that biotinylated branched structures form nanofibers with enhanced recognition by the avidin receptor relative to similar nanostructures formed by linear analogues. Experiments involved the measurement of fluorescence when nanofibers were incubated with fluorescein conjugated avidin.

AA5.27
Breaking the Immune Synapse Down, and Building it Back Up. Bryan Lawrence Jackson[1], Kaspar D. Mossman[1], Michael L. Dustin[2] and Jay T. Groves[1]; [1]Chemistry, University of California - Berkeley, Berkeley, California; [2]Molecular Pathology, NYU Medical Center, New York, New York.

Your immune system is responsible for defending your body against invading pathogens. When it works properly you recover from ailments such as the common cold. When it malfunctions the consequences may range from arthritis to multiple sclerosis. The immune system is of crucial importance to human health, but our understanding of it is far from complete. In 1999 it was discovered that information is transferred from infected cells to T cells via a bull's-eye pattern named the "immune synapse." We use microchips coated with supported lipid bilayers to query the response of live T cells as they form synapses. To impose nanostructure on a bilayer, we use electron-beam lithography to generate chromium patterns on glass, of linewidth 100 nm. The chromium acts as a barrier to lipid and protein mobility, thus frustrating a T cell as it attempts to organize a synapse. We are also developing a method to nanopartition lipid bilayers into pixelated synapses. To produce an artificial synapse, we use scanning probe lithography (SPL) to remove the lipid bilayer from selected pixels, and then add lipid vesicles of differing biological functionality. New vesicles fuse only over the exposed substrate. We have achieved resolution as fine as 1 m with this method. Artificial synapses will be invaluable to researchers who wish to determine whether patients will suffer immune reactions to newly developed drugs.

AA5.28
Incorporation of Luminescent Nanocrystals into Monodisperse Silica Microspheres. YinThai Chan[1], John P. Zimmer[1], Mark Stroh[2], Jonathan S. Steckel[1], Rakesh K. Jain[2] and Moungi G. Bawendi[1]; [1]Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]Steele Lab, Massachusetts General Hospital, Boston, Massachusetts.

Microspheres containing chromophores have been exploited in an extensive variety of applications, including photonic crystals, biological labeling, and flow visualization in microfluidic channels. In many of these applications, both the photostability of the chromophores as well as the monodispersity of the microspheres are of primary importance. This has led to recent efforts in incorporating semiconductor nanocrystals (NCs) into microspheres. We now report a facile procedure of incorporating core-shell CdSe/ZnS and CdS/ZnS NCs into monodisperse silica microspheres. By independently synthesizing the NCs and microsphere cores, we obtain high flexibility in incorporating different color-emitting nanocrystals into a variety of microsphere sizes, where the NCs are localized within a silica or titania shell. We illustrate the efficacy of our method in an experiment in which overcoated microspheres containing different color-emitting NCs are imaged simultaneously in vivo.

AA5.29
Patterning of Cells on Bioresist for Tissue Engineering Applications. Yusif Umar[1], Muthiah Thiyagarajan[1], Jae H. Choi[1], Catherine E. Austin[1], Craig R. Halberstadt[2] and Kenneth E. Gonsalves[1]; [1]Chemistry, University of North Carolina@Charlotte, Charlotte, North Carolina; [2]General Surgery Research, Carolina Medical Center, Charlotte, North Carolina.

Engineering functional tissues and organs successfully depends on the ability to control cell orientation and distribution. Materials used for such purposes therefore have to be designed to facilitate cell distribution and eventually guide tissue regeneration in 3D. In order to address this, we have developed a biocompatible, biostable chemically amplified bioresist, on which patterns are generated without involving harsh chemical treatment. Surface properties of different types of polymers affect cell adhesion, spreading and proliferation via their adsorptive properties with respect to serum proteins . In order to determine whether surface chemistry of the polymers has any direct influence on cell attachment, the copolymers, 3-(tert-Butoxycarbonyl)-N-Vinyl-2-Pyrrolidone-co-Methyl Methacrylate (MMA-co- t-BOC-NVP) and tert-butyl methacrylate-co-N-vinyl-2-pyrrolidone (t-BMA-co-NVP) and a terpolymer of tert-butyl methacrylate-co-N-vinyl-2-pyrrolidone-co-methyl methacrylate (MMA-co-t-BMA-co-NVP) with various compositions were synthesized by free radical polymerization. Due to its hydrophilic and good biocompatibility character, N-Vinyl-2-pyrrolidone was used in the above polymer systems. Photoresist solutions were prepared and patterned after exposure to UV light (365nm). Fibroblast cells were seeded on patterned surfaces. As another approach, patterned polymer surfaces were modified by linking them with RGD peptides, and fibroblast cells were then seeded on them. Details of cell orientation adhesion and proliferation will be presented. * Corresponding Author, E-mail : kegonsal@email.uncc.edu

AA5.30
Fabrication of Highly Ordered Nanopattern of Biological Molecules Using Anodic Porous Alumina. Futoshi Matsumoto[1], Masahiro Harada[2], Kazuyuki Nishio[1,2] and Hideki Masuda[1,2];

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Recently, high-density arrays of functional biomolecules such as proteins, antibodies, and DNA, which have molecular recognition ability, on two-dimensional substrates, have attracted considerable interest because of their tremendous potential in biomolecular nanodevices. In particular, the ideal ordering of biological molecular patterns with uniform size is expected to result in the high performance of the array in terms of increase in pattern density, decrease in analysis time, and improved precision of signal from each pattern. Ideal ordering and uniformity of pattern area are key factors in the development of the nanopattered array of biological molecules. Highly ordered anodic porous alumina, fabricated by anodizing in acid solutions, has a porous structure with nanometer-scale uniform pore diameter and interval. The use of the highly ordered porous structure for the ordering of biological molecules leads to the establishment of a nanometer-scale ideally ordered pattern of biological molecules with uniform size. In the present study, the highly ordered nanopatterns of biological molecules such as DNA and protein were fabricated by employing Au disk array fabricated using highly ordered anodic porous alumina by filling the pores of anodic porous alumina with Au. The observation of each pattern was examined using a scanning electron microscope and a fluorescence microscope based on the signals from a fluorescent dye fixed on each Au disk. [1] H. Masuda et al., Chemistry Letters, 33, 812 (2004). [2] F. Matsumoto et al., Jpn. J. Appl. Phys., 43, L640 (2004).

AA5.31

Synthesis, Sintering and Micro structural Characterization of Nanocrystalline Hydroxyapatite Composites. B. Viswanath[1], Suprabha Nayar[2], Sinha Arvind[2] and N. Ravishankar[1]; [1]Materials Research Centre, Indian Institute of Science, Bangalore, India; [2]National Metallurgical Laboratory, Jamshedpur, India.

Nanocrystalline hydroxyapatite exhibits better bioactivity and also biocompatibility with enhanced mechanical properties. In the present work, nanocrystalline hydroxyapatite is synthesized by wet chemical method. Sintering is carried out with suitable polymer additives to make it composite and enhance the strength. To get the details of the particle size distribution and structure of the hydroxyapatite nanocomposite, micro structural characterization is carried out using Transmission electron microscope (TEM). The ultimate aim is to achieve the microstructure similar to human bone which is nothing but the composite of hydroxyapatite and collagen fiber.

AA5.32

Functionalized Electrospun Polymer Fibers for Capture of Biological Agents. Chris Snively[1,2], John Rabolt[1] and Kristi Kiick[1]; [1]Department of Materials Science and Engineering, University of Delaware, Newark, Delaware; [2]Department of Chemical Engineering, University of Delaware, Newark, Delaware.

One of the challenges involved in the spectroscopic detection of small quantities of biological agents is interference from other sources, be they biological or chemical. One solution to this problem is to use a high surface area substrate, such as a mat, filter, or swab that is functionalized to selectively collect the agent of interest. The substrate is subsequently analyzed using a suitable analytical technique in order to detect the presence of the agent. Here, we describe an approach that combines functionalized polymers in conjunction with electrospinning as a potential solution to this problem. Formation of polymer fibers via electrospinning has the advantage of being able to rapidly produce fibers that possess diameters on the order of tens of nanometers to tens of microns. These fibers can be formed into self-supporting nonmoven mats by spinning them onto a flat substrate. By functionalizing the polymer with a capture agent, these mats can be used as a method of selectively capturing biological agents. The mats can subsequently be analyzed by a variety of analytical techniques, including fluorescence or vibrational spectroscopy in order to detect the presence of the biological agent of interest. The high surface area of the mats serves to increase the amount of agent that can be captured, which can in turn lower the concentration detection limit of this approach. Here, we use the biotin-avidin binding system as a proof-of-principle example due to the ready availability of functionalized biotin derivatives that can be used to biotinylate polymers with a variety of side chain functional groups. We will present results of the functionalization of poly(methyl acrylate), polylysine, and poly(acrylic acid) in terms of extent of functionalization and the effect of this functionalization on the ability to electrospin these polymers. The ability to capture and detect avidin will also be explored.

AA5.33

Improving Bone Formation Through Highly Dispersed Nanoceramics in Polymer Composites. Huinan Liu[2], Elliot Slamovich[2] and Thomas Jay Webster[1,2]; [1]Biomedical Engineering, Purdue University, Lafayette, Indiana; [2]Materials Engineering, Purdue UNiversity, West Lafayette, Indiana.

Much work is needed in the design of more effective bone tissue engineering materials since the average lifetime of an orthopedic implant is less than 15 years. Frequently, orthopedic implants fail due to insufficient integration into juxtaposed bone. Nanotechnology offers exciting alternatives to traditional bone implants since bone itself is a nanostructured material composed of nanofibered hydroxyapatite well-dispersed in a mostly collagen matrix. For this reason, osteoblast (bone-forming cell) adhesion on polylactic/glycolic acid (PLGA) and nanophase titania composites were investigated in vitro. PLGA was dissolved in chloroform and 30 wt.% nanometer grain size titania was dispersed in the solution by sonication. The surface characteristics, particle size and distribution of three composites fabricated with three different sonication output powers were studied. The dispersion of titania in PLGA was significantly enhanced by increasing the intensity of sonication. Moreover, results indicated for the first time that greater osteoblast adhesion with increased titania dispersion in PLGA. In this manner, the present study demonstrates that PLGA composites with well-dispersed nanophase titania can improve osteoblast functions necessary for increased orthopedic implant efficacy.

AA5.34

Protein binding properties of surface-modified porous polyethylene membranes. George Green[1], Harish Radakrishna[2] and Rina Tannenbaum[1]; [1]Materials Science and Engineering, Georgia Institute of Technology, Atlanta, Georgia; [2]Biology, Geirgia Institute of Technology, Atlanta, Georgia.

In this study we investigated methods of altering the surface chemistry of sintered porous polyethylene membranes to yield the properties necessary in lateral flow immunoassay applications. The surface of sintered porous polyethylene membranes was modified by adsorbing self-assembled polyelectrolyte monolayers, bilayer or multilayer nanostructures, in order to render intrinsically hydrophobic surfaces capable of driving the transport of fluids across the membrane without the aid of additional wetting agents, thus improving the protein binding capacity of the membranes through electrostatic interactions. We describe the characterization of the capacity of various modified membrane surfaces to bind IgG protein from and assortment of solutions with different pH and ionic strengths. Two analytical techniques were employed in order to gain insight into the mechanism, stength and nature of the interfacial forces between the adsorbing protein and the modified surfaces.

AA5.35

A Hybrid Laser/Aerosol Method for the Synthesis of Porous Nanostructured Calcium Phosphate Materials for Bone Tissue Engineering Applications. Shatoya Brown, Hyunbin Kim and Renato Camata; University of Alabama - Birmingham, Birmingham, Alabama.

Engineering and regeneration of bone tissue remains an outstanding problem in medicine and dentistry. Over the past decade numerous inorganic and polymeric materials have been explored for bone growth stimulation and scaffolding. Investigations have shown that porous biphasic calcium phosphate materials comprising mixtures of nonresorbable and bioresorbable calcium phosphate phases are among the most promising substrates for bone tissue engineering. Research on such mixtures has focused primarily on the combination of nonresorbable hydroxyapatite (HA) and resorbable tricalcium phosphate (TCP). It has been demonstrated, however, that pulsed laser deposition (PLD) allows the creation of biphasic calcium phosphate materials composed of nonresorbable HA and other bioresorbable phases such as tetracalcium phosphate (TTCP) that also have potential as bioceramic substrates to meet the stringent bioactivity and mechanical stability requirements for bone tissue engineering. In this study we present a new synthesis method based on PLD that can produce similar biphasic calcium phosphate coatings in a porous nanostructured configuration. This process uses laser ablation of crystalline HA targets to generate a calcium phosphate nanoparticle aerosol that is processed and deposited on a metallic substrate under well-controlled temperature and ambient. Because of the nature of this aerosol process, gas-phase aggregation of nanoparticles leads to highly networked calcium phosphate structures with controlled porosity. The resulting material comprises nanoscale calcium phosphate building blocks. A broad range of pore sizes suitable for growth of biogenic calcium phosphate materials can be achieved making these substrates interesting coatings and bone tissue engineering. We have explored the use of this method for the deposition of calcium phosphate layers with thickness ranging from a few microns to hundreds of microns on metallic surfaces. Laser ablation is carried out using a KrF excimer laser at fluences between 0.5 J/cm2 and 4 J/cm2 and temperatures ranging from 500C to 760C. A systematic study of calcium phosphate crystalline phase on as

deposited and annealed samples was performed with x-ray diffraction (XRD). Although this hybrid process leads to unstable calcium phosphate networks at temperatures below 600C, samples deposited above 750C exhibit good mechanical stability as a result of partial sintering undergone by the nanoscale building blocks. Our system features on-line, in-situ measurements of the size distribution of the gas-suspended calcium phosphate nanoparticles en route to the deposition surface. This is accomplished through the use of an aerosol spectrometer known as differential mobility analyzer. The use of this on-line monitoring technique associated with post-deposition XRD allows creation of calcium phosphate deposits with tailored crystalline phase, size of the nanoscale building blocks, and pore dimension. Support: NSF-REU (DMR-0243640).

AA5.36

Synthesis and Application of Molecularly Imprinted Polymers for Phenolic Steroid Hormones. Shuting Wei[1], Alexandra Molinelli[1], Edward Archibald McCallum[2], Ching-hua Huang[2] and Boris Mizaikoff[1]; [1]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia; [2]Environmental Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Phenolic steroid hormones including 17β-estradiol, 17α-estradiol, 17α-ethynylestradiol and estrone are suspected of adverse effects on the endocrine system in wildlife and humans. The existence of these compounds in aquatic environments has recently been reported. Therefore, considerable interest is focused on developing cost-effective analytical methods for quantitative determination of these compounds in environmental samples at low levels of concentration. Nowadays, non-covalently prepared molecular imprinted polymers (MIPs) are increasingly adopted as mimics of natural molecular receptors. The principle of this synthetic technique involves the formation of non-covalent complexes between template molecules and functional monomers in solution. In the presence of a cross-linker, a polymer is grafted by radical co-polymerization of functional monomer and cross-linker. The template molecule is then removed by extraction with an organic solvent and, ideally, specifically rebinds to the created synthetic cavity upon re-exposure. For chromatographic evaluation (e.g. HPLC) or solid phase extraction (SPE), imprinted polymer particles with sizes in the range of 10 to 25 μm are usually applied. For binding assays or polymeric biomimetic recognition layers used as chemical sensing surface, smaller particles (<1 μm) are desired. In the present study, MIPs were successfully prepared for 17β-estradiol and 17α-ethynylestradiol as template molecule based on a non-covalent imprinting approach. MIPs were prepared by bulk polymerization and by an innovative one-step precipitation polymerization, with the latter approach yielding micro- and nanospheres with controlled diameters ranging from 400 nm to 3 μm. HPLC results demonstrate that separation of several phenolic steroid hormones including enantiomers (17β- and 17α-estradiol) can be achieved by using the developed imprinted bulk polymer, as well as imprinted microspheres (approx. 3 μm) as stationary phase in HPLC. The developed MIPs were also used as sorbent material in SPE indicating great potential for selective sample clean-up and enrichment in environmental analysis. Imprinted nanospheres with diameters <400 nm were evaluated by radioligand binding assays revealing appreciable binding specificity for the template molecule. Consequently, these imprinted microspheres are useful as recognition element replacing antibodies in binding assays for 17β-estradiol. In summary, the developed MIPs offer a viable alternative to traditional methods for environment clean-up, enrichment or binding assays due to their unique recognition ability for template molecules.

AA5.37

Labeling Cells with Silver-Dendrimer Nanocomposites. Wojciech Lesniak[2], Anna Bielinska[2], Xiangyang Shi[2], Katarzyna Janczak[2], Chris Edwards[3] and Lajos P. Balogh[1,2,4]; [1]Dept. of Internal Medicine & Dept. of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan; [2]Center for Biologic Nanotechnology, University of Michigan, Ann Arbor, Michigan; [3]Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan; [4]MACRO, University of Michigan, Ann Arbor, Michigan.

The goal of this study was to determine the biocompatibility of silver dendrimer nanocomposites (DNC) at biological pH (pH=7.4) and the feasibility of their use as fluorescent biomarkers to monitor cellular processes. To achieve this goal, generation five PAMAM dendrimers with glycidyl and succinamic acid termini were synthesized from primary amine terminated dendrimer materials using standard techniques. The polymers were characterized by NMR, CE, PAGE, HPLC, SEC and potentiometric titration. Optimal silver/dendrimer ratios at pH=7.4 were determined by potentiometric titration of the dendrimers in the presence of AgNO3. At the experimentally determined Ag/D=25:1 ratios (close to optimal values at pH=7.4), three DNC solutions were prepared: {$(Ag(0))_{25}$-PAMAM_E5.NH2}, {$(Ag(0))_{25}$-PAMAM_E5.SAH}, and {$(Ag(0)_{25}$-PAMAM_E5.Gly}). These materials were characterized using UV-VIS spectroscopy, transmission electron microscopy, energy dispersive X-ray spectroscopy, and selected area electron diffraction. Size distribution and zeta-potential of the DNC materials were determined by dynamic light scattering. To identify optimum excitation and emission wavelengths, fluorescence of the silver nanocomposites was also measured. Stability studies were carried out by monitoring the change of the size-dependent plasmon absorptions at two different concentrations before and after dilution in the tissue culture medium used in the cytotoxicity experiments. The toxicity of DNCs and their host polymers were examined in vitro using cell lines. The DNC was found to be biocompatible and their effect on cells was comparable with that of host dendrimers. Detection of {Ag} DNCs internalized into the cells was achieved using confocal microscopy.

AA5.38

Bioreactor Based Bone Tissue Engineering: Influence of Wall Collision on Osteoblast Cultured on Polymeric Microcarrier Scaffolds in Rotating Bioreactors. Xiaojun Yu[1], Edward A. Botchwey[2], Levine M. Elliot[3], Solomon R. Pollack[4] and Laurencin T. Cato[1,2,5]; [1]Orthopaedic Surgery, University of Virginia, Charlottesville, Virginia; [2]Biomedical Engineering, University of Virginia, Charlottesville, Virginia; [3]The Wistar Institute, Philadelphia, Pennsylvania; [4]Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania; [5]Chemical Engineering, University of Virginia, Charlottesville, Virginia.

Introduction: To overcome the limitations associated with static culturing systems, considerable interest has been generated in tissue engineering by using the high aspect ratio vessel (HARV) rotating bioreactor. It is hypothesized that the conventional scaffolds undergo repeated wall collisions in the rotating bioreactors, which may disrupt bone tissue formation. In previous studies, we have described the development of novel poly(lactide-co-glycolide) (PLAGA) microsphere based mixed scaffolds that can avoid wall collision in rotating bioreactors [1]. In this study, we seek to test the effects of wall collision on human Saos-2 osteoblastic cells cultured on conventional and mixed PLAGA microcarrier scaffolds with equal motion velocities in rotating bioreactors. Materials and Methods: The lighter-than-water (LTW, density < 1 g/cm3) and heavier-than-water (HTW, density > 1 g/cm3) microspheres sized from 425-500 mm were fabricated using PLAGA in a 85:15 ratio [1]. The HTW or mixed scaffolds were fabricated into 4 mm x 2.5 mm by sintering either the HTW microspheres or HTW and LTW microspheres in a ratio of 1.5:1 at 80C for 3 hours [1]. The motion trajectories of scaffolds in rotating bioreactors were recorded using a real time microcapsule visualization unit [2], and the instantaneous velocity values of the scaffolds were calculated. Human Saos-2 osteoblastic cells were seeded onto scaffolds at a density of 5 x 104 cells/scaffold for 24 hours, and the scaffolds were separated into HARV vessels either maintained statically as controls or rotated at 54 rpm as rotating bioreactors. At days 4, 8, and 16, cell proliferation was analyzed by MTS assay and cell differentiation was analyzed by measuring Alkaline phosphatase activity. Results and Discussion: HTW scaffolds had frequent wall collision, while the mixed scaffolds can avoid wall collision. The motion velocities of the conventional HTW scaffolds and mixed scaffolds were equal. Osteoblastic cell proliferation and differentiation on HTW scaffolds in rotating bioreactors were significantly decreased as compared to those of static controls, but osteoblastic cell proliferation and differentiation on the mixed scaffolds in rotating bioreactors were significantly increased as compared to those of HTW scaffolds. Conclusions: As the velocities of the two types of scaffolds are equal, the difference in osteoblastic cell proliferation and differentiation between HTW and mixed scaffolds in rotating bioreactors are due to wall collision. Osteoblastic cell proliferation and differentiation in rotating bioreactors could be improved by adjusting motion trajectories to avoid wall collision. Acknowledgements: This work was supported by NSF grant 0343620, NASA grant NAG9-832, and Commonwealth Universal Research Enhancement Program, Pennsylvania Department of Health. Reference: [1] Botchwey et al., J. Biomed. Mat. Res. 55, 242-253 (2001). [2] Pollack et al., Tissue Eng. 6, 519-530 (2000).

AA5.39

Apatite-Polymer Composites for the Controlled Dual Delivery of BMP-2 and BMP-6 for Bone Tissue Engineering. Tseh-Hwan Yong[1,3], Shujun Gao[3], Anita Kris[1,3] and Jackie Y. Ying[2,3]; [1]Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [3]Institute of Bioengineering and Nanotechnology, Biopolis, Singapore.

The release of growth factors from tissue engineering scaffolds provides signals that influence the migration, differentiation, and proliferation of cells. The incorporation of a drug delivery platform that is capable of tunable release will give tissue engineers greater versatility in the direction of tissue regeneration. We have prepared a

novel composite of two biomaterials with proven track records - apatite and poly(lactic-co-glycolic acid) (PLGA) - as a drug delivery platform with promising controlled release properties. These composites have been tested in the delivery of a model protein, bovine serum albumin (BSA), as well as therapeutic proteins, recombinant human bone morphogenetic protein-2 (rhBMP-2) and rhBMP-6. The controlled release strategy is based on the use of a polymer with acidic degradation products to control the dissolution of the basic apatitic component, resulting in protein release. Therefore, any parameter that affects either polymer degradation or apatite dissolution can be used to control protein release. We have modified the protein release profile systematically by varying the polymer molecular weight, polymer hydrophobicity, apatite loading, apatite particle size, and other material and processing parameters. Biologically active rhBMP-2 was released from these composite microparticles over 100 days, in contrast to conventional collagen sponge carriers, which were depleted in approximately 2 weeks. The released rhBMP-2 was able to induce elevated alkaline phosphatase and osteocalcin expression in pluripotent murine embryonic fibroblasts, suggesting the commitment of these cells to the osteoblast lineage. To augment tissue engineering scaffolds with tunable and sustained protein release capabilities, these composite microparticles can be dispersed in the scaffolds in different combinations to obtain a superposition of the release profiles. We have loaded rhBMP-2 into composite microparticles with a fast release profile, and rhBMP-6 into slow-releasing composite microparticles. An equi-mixture of these two sets of composite particles was then injected into a collagen sponge, allowing for dual release of the proteins from the collagenous scaffold. The ability of these BMP-loaded scaffolds to induce osteoblastic differentiation in vitro and ectopic bone formation in a rat model is being investigated. We anticipate that these apatite-polymer composite microparticles can be extended to the delivery of other signalling molecules, and can be incorporated into other types of tissue engineering scaffolds.

AA5.40

Materials with Nanoparticle-Decorated Surfaces for Biomedical Applications. Jake D. Ballard[1,4], Ludovico M. Dell'Acqua-Bellavitis[2,4], Rena Bizios[3,4] and Richard W. Siegel[1,4]; [1]Materials Science & Engineering, Rensselaer Polytechnic Institute, Troy, New York; [2]Engineering Science, Rensselaer Polytechnic Institute, Troy, New York; [3]Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, New York; [4]Rensselaer Nanotechnology Center, Rensselaer Polytechnic Institute, Troy, New York.

Control of the events at the interface between living cells and materials is an important aspect for the realization of implantable devices with enhanced functionality and biocompatibility. Previous research demonstrated that nanoscale surface features modulate select responses of cells, but has not yet provide definitive information regarding the underlying mechanism(s). Our current research focuses on (1) the design, fabrication and characterization of silica nanoparticle-decorated silicon surfaces, (2) elucidation of the underlying cellular mechanism(s), specifically, the mediating role of adsorbed proteins on subsequent cell interactions, and (3) examination of select functions (such as adhesion of neurons and astrocytes) on surfaces decorated with nanoparticles of several specific sizes. These well characterized surfaces could provide novel alternatives to existing materials and optimize the performance of prostheses, for example, devices developed for the central nervous system.

AA5.41

Tissue Engineering of Myocardium Based on Channeled Elastomeric Scaffold and Oxygen Carriers. Milica Radisic[1], Hyoungshin Park[1], Yadong Wang[2], Robert Dennis[3], Lisa E. Freed[1], Robert Langer[1] and Gordana Vunjak-Novakovic[1]; [1]MIT, Cambridge, Massachusetts; [2]Georgia Institute of Technology, Atlanta, Georgia; [3]University of North Carolina, Chapel Hill, North Carolina.

In vascularized tissues, such as myocardium, oxygen is supplied by convection of blood through a capillary network and diffusion into the tissue space surrounding each capillary, with total oxygen content of blood increased by the presence of a natural oxygen carrier, hemoglobin. Our objective was to create an in vitro tissue culture system with in vivo-like oxygen supply to the cells within engineered cardiac constructs. A biodegradable elastomeric polymer poly(glycerol sebacate), was synthesized by polycondensation of glycerol and sebacic acid and processed into a 2.5mm thick highly porous scaffold using slat leaching technique. To mimic the capillary network, a cubically packed parallel channel array with diameter of 377 $\pm$ 52 μm was laser bored in the scaffold. The scaffold was seeded with neonatal rat heart cells using Matrigel and cultivated for total of 7 days. To mimic oxygen supply by hemoglobin, the channel array was perfused with culture medium supplemented with a 10%v/v synthetic oxygen carrier (OxygentTM, perfluorocarbon (PFC) emulsion). Constructs cultivated in the unsupplemented culture medium served as controls. The resulting constructs were assessed at the end of cultivation with respect to protein and DNA content, glucose consumption, tissue morphology, expression of cardiac markers and contractile function. Due to the settling of heavier PFC particles in the lower portions of the perfusion loop, the circulating PFC emulsion concentration was 17%. Supplementation by PFC emulsion did not have effect on medium pCO2 and pH with the values in both groups maintained within the physiological range. Decrease in the partial pressure of oxygen in the aqueous phase was 2 times smaller in the group supplemented with PFC emulsion compared to the culture medium alone (28mmHg vs. 45mmHg). At the flow rate of 0.1 ml/min, total oxygen concentration extracted from the culture medium was estimated to be 62 μM and total oxygen extracted from the PFC supplemented medium was higher, 82 μM (aqueous phase +PFC phase). Consistently, constructs cultivated in the presence of PFC emulsion had significantly higher DNA content and significantly lower excitation threshold compared to the constructs cultivated with pure culture medium. As a result, Western blotting revealed higher relative presence of cardiac markers troponin I and connexin-43 in the PFC group compared to the culture medium alone. Scanning electron microscopy revealed the presence of cells at the surface and cross section of constructs and a number of open channels with cell coated walls. In summary, the cultivation of cardiac fibroblasts and myocytes on scaffolds with an array of channels perfused with culture medium supplemented with the PFC enhanced the cellularity, presence of cardiac markers and contractile properties of engineered cardiac constructs.

AA5.42

Pulsed Laser Deposition of Bioactive Ceramic Nanocomposites. Roger Jagdish Narayan, Materials Science and Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Bioactive coatings mimic the mineral composition of natural bone. Unfortunately, problems with adhesion, poor chemical and mechanical integrity, and incomplete bone ingrowth limit the use of current bioactive ceramic coatings. By inserting the multiple layers of diamondlike carbon, silver, titanium nitride and titanium carbide, we achieved a considerable enhancement in mechanical properties and functionality of these composite layers. The underlying Ti-Al-V and Si substrates are first coated with titanium nitride, titanium carbide, and diamondlike carbon using pulsed laser deposition. The diamondlike carbon surface is then coated with hydroxyapatite to form the final multilayered material. The films were characterized using scanning electron microscopy, high-resolution electron microscopy, X-ray diffraction, Raman spectroscopy, and mechanical testing. These composite hydroxyapatite/diamondlike carbon bilayers have potential uses in several different orthopaedic and dental implant designs.

AA5.43

Microstructure Determination of Bioresists and Application for 3D Scaffold Fabrication. Muthiah Thiyagarajan[1], Kenneth E. Gonsalves[1], Yusif Umar[1] and Craig R. Halberstadt[2]; [1]Chemistry, University of North Carolina@Charlotte, Charlotte, North Carolina; [2]General Surgery Research, Carolina Medical Center, Charlotte, North Carolina.

We have recently developed polymers that are biocompatible, as well as imageable and termed as bioresists. For one such system N-Vinyl-2-pyrrolidone | tert-butyl methacrylate (V/B), copolymers were synthesized by free radical polymerization using AIBN as initiator. The copolymer compositions were determined from 1H NMR spectra. The comonomer reactivity ratios estimated using the Kelen −Tudos method are rV = 0.32 and rB = 1.16 Using these reactivity ratios, the sequence distribution of V- and B-centered triads determined from 13C NMR spectra of copolymer were in good agreement with triad concentration calculated from the Harwood Statistical model. The 2-D heteronuclear single-quantum correlation and correlated spectroscopy (TOCSY) were used to determine the microstructure. Similar microstructure determinations of TBOCNVP and MMA copolymers were conducted. The correlation of microstructure and bioactivity with respect to fibroblast cell adhesion and patterning will be presented. These copolymers are also essentially chemically amplified bioresists and can be imaged at 365 nm. Therefore these materials can be used for 3D scaffold fabrication in tissue engineering applications. In general, open pore scaffold structure and customized pore sizes promote greater cell attachment, proliferation and tissue development. 3D scaffolds of these polymers prepared by a solvent-casting / particulate leaching and μstereolithography methods, will be presented. Procedures on imaging these materials to develop aligned cell-to-cell structures will also be discussed. * Corresponding Author, E-mail : kegonsal@email.uncc.edu

AA5.44

A Single-Step Process for Fabricating Magnetic Nanoparticle Fluids Using Inert-Gas Condensation. Nguyen H. Hai[1,5], Raymond Lemoine[1], Shaina Rembolt[1], Michelle Strand[3,1], Steve Wignall[2,1], Jeffrey E. Shield[4,5] and Diandra Leslie-Pelecky[1,5]; [1]Department of Physics, University of Nebraska, Lincoln, Nebraska;

[2]Seward High School, Seward, Nebraska; [3]Pius X High School, Lincoln, Nebraska; [4]Mechanical Engineering, University of Nebraska, Lincoln, Nebraska; [5]Center for Materials Research & Analysis, University of Nebraska, Lincoln, Nebraska.

Fluids with magnetic nanoparticles have many biological applications, including magnetic imaging, drug delivery, and hyperthermic treatment for cancer. Challenges in fabricating these fluids include a) producing magnetic nanoparticles of different materials, b) producing nanoparticles with a narrow size distribution, and c) forming a stable, non-aggregating colloid. Inert-gas condensation directly into a surfactant/fluid mixture produces a magnetic nanoparticle fluid in a single fabrication step, and allows variation of the material and size of the nanoparticles while maintaining a narrow size distribution. Inert-gas condensation forms clusters via thermalizing collisions of an atomic or molecular vapor with an inert gas. The clusters land on the surface of a rotating drum coated with a thin film of a surfactant/low-vapor-pressure oil mixture that is held in a cooled reservoir. The surfactant prevents cluster growth and agglomeration, and adds to the stability of the resulting colloid. Particle size is controlled by the sputtering pressure, power and inert gas flow, as well as the surfactant type and concentration. This UHV-based technique can be used to make magnetic nanoparticle fluids from any material that can be sputtered or evaporated. A vacuum load lock allows removal of the fluid from the deposition chamber without exposure to air, thus allowing us to make fluids from air-sensitive materials. The surfactant Brij-92 in combination with oils of different vapor pressures produces iron and cobalt nanoparticle fluids with mean particle sizes of 15 to 20 nm and narrow particle-size distributions. The oil-based fluids are stable colloids; however, the nanoparticles can be extracted by high-speed centrifugation and then re-suspended in other carrier liquids. Fe-based fluids are superparamagnetic at room temperature and offer promise for magnetically targeted drug delivery, while Co-based fluids are ferromagnetic at room temperature and serve as prototypes for hyperthermic applications. In addition to advances in biological applications, the flexibility of the technique offers the opportunity for enhanced understanding of the fundamental properties of magnetic nanoparticles as a function of interaction strength and material characteristics. This project was supported by the Nebraska Research Initiative, the MRSEC Program of the National Science Foundation under Award No DMR-0213808 and the Center for Materials Research & Analysis at the University of Nebraska

AA5.45

Pre- and Post-Processing Functionalization of Electrospun Fibers for the Inclusion of Biologically Active Molecules in the Matrix. Cheryl Lynn Casper[1,2], Nori Yamaguchi[1,2], Weidong Yang[3,2], Mary C. Farach-Carson[3,2], Kristi L. Kiick[1,2] and John F. Rabolt[1,2]; [1]Materials Science and Engineering, University of Delaware, Newark, Delaware; [2]Delaware Biotechnology Institute, Newark, Delaware; [3]Department of Biological Sciences, University of Delaware, Newark, Delaware.

Due to small fiber diameters, high surface area, and interconnected porous network, electrospun fibers are finding increased utility in a variety of biomaterial applications. The characteristics of electrospun membranes structurally mimic the three-dimensional complexity of the extracellular matrix. However, additional modifications are needed to render these matrices biologically active for applications such as drug delivery, tissue engineering, and wound repair. In this work, two different systems were investigated to impart bioactivity to the electrospun matrices. The first system investigated includes the incorporation of heparin functionalized poly(ethylene glycol) (PEG-heparin), functionalized before electrospinning, into electrospun membranes. Heparin both binds and sequesters certain growth factors. Both free heparin and PEG-heparin were studied for their ability to be processed into nanometer diameter fibers via electrospinning. It was determined that heparin is incorporated into the electrospun membranes at concentrations of 3.5 micrograms (PEG-heparin) to 85 micrograms (free heparin). The other approach focuses on post-electrospinning functionalization of collagen and gelatin electrospun membranes with recombinant heparan sulfate modified perlecan domain I in order to increase the bioactivity and growth factor binding ability of the electrospun matrix. Perlecan, a heparan sulfate proteoglycan, is found in all basement membranes in cartilage and other tissues. Perlecan binds growth factors, such as basic fibroblastic growth factor, and is associated with formation of growth factor morphogen gradients in vivo. Attachment of perlecan to the electrospun matrix may facilitate cell attachment and growth factor binding. Functionalized electrospun fibers were characterized using energy dispersive x-ray analysis (EDX), field emission scanning electron microscopy (FESEM), and laser scanning confocal microscopy (LSCM).

AA5.46

Purification of nano-crystalline ultra-dispersed diamond to be employed as nanoprobes for cellular imaging. Joel De Jesus[1], Roberto Acosta Torres[1], Aleannette Lopez Cubero[1], Fabrice Piazza[1] and Gerardo Morell[2]; [1]Dep of Physics, University of Puerto Rico, San Juan, PR, Puerto Rico; [2]Dept of Physical Sciences, University of Puerto Rico, San Juan, PR, Puerto Rico.

The development of novel nanoparticles for imaging living cells in vivo without harming or disrupting their intracellular molecular dynamics is an important area of nanotechnology that intersects Materials Science, Physics, Chemistry, and Biology. Nano-crystalline ultra-dispersed diamond (UDD) particles are photostable, biocompatible and can be tailored for a range of excitation and emission wavelengths. Purification of the powder is necessary in order to remove the non-diamond carbon components and other impurities surrounding the nano-diamond crystals. The effects of UV exposure in air and of heat treatment in H2 and H atmosphere on the structure and properties of detonation UDD are investigated. The variation in the powder structure and properties after the exposure at different temperatures for variable periods is studied by using scanning electron microscopy (SEM), X-ray diffraction analysis (XRD), X-ray photoelectron spectroscopy (XPS), Electron Energy Loss spectroscopy (EELS), visible and UV Raman spectroscopy, and Fourier transform infrared spectroscopy (FTIR). The results show that it is possible to significantly increase the purity of the UDD powder by UV irradiation in air and heat treatment in H2 and H atmosphere. The role of oxygen and hydrogen radicals is discussed. Preliminary spectroscopic luminescence studies of the UDD particles are performed in vitro. We aim to learn how to tailor the nanoparticle size, composition, and surface termination, particularly targeting for long-lived yellow-green-blue luminescence states, in preparation for subsequent in vivo studies.

AA5.47

Microsphere-Based Scaffolds for Bone Tissue Engineering: Novel Angiogenic Compounds for Targeted Drug Delivery. Kristen A. Wieghaus[1], Scott M. Capitosti[2], Milton L. Brown[2] and Edward A. Botchwey[1]; [1]Biomedical Engineering, University of Virginia, Charlottesville, Virginia; [2]Chemistry, University of Virginia, Charlottesville, Virginia.

Microvascular remodeling and angiogenesis play a pivotal role in the regeneration of bone tissues, and have received increasing attention among researchers in the search for new strategies for therapeutic angiogenesis in tissue engineering. [1,2,3] In previous studies, our laboratory has investigated a unique microsphere-based scaffold fabrication method for bone tissue engineering. [4] In this study, we present a new multidisciplinary approach for the development of novel thalidomide-based compounds that may be delivered within microsphere based scaffolds to promote local angiogenesis at the site of bony repair. Others have released growth factors, including vascular endothelial growth factor (VEGF), from polymer scaffolds [5,6]; we are developing synthetic compounds that might exert similar effects on seeded endothelial cells while withstanding polymer processing procedures. Thalidomide has long been used as a sedative, and has traditionally been thought to inhibit angiogenesis in vivo. [7] We report here on the synthesis and characterization of SC-3-149, a novel thalidomide analogue possessing angiogenic potential. Our results show that that SC-3-149 had a significant effect on microvascular endothelial cell (HMVEC) proliferation after one week. After two weeks, SC-3-149 facilitated a nearly 1800% increase in endothelial cell proliferation. The compound also significantly reduced serum-deprived cell necrosis in HMVEC cells after 24 hours, maintaining the same cell viability level as serum-deprived endothelial cells supplemented with 0.1% VEGF. In addition, SC-3-149 significantly increased the number of vascular endothelial cords formed after 24 hours in 3-D Matrigel cultures. The effect of SC-3-149 stimulation of human osteoblast-like cells was also assessed in order to determine if its effects were selective for endothelial cells. Significant effects on proliferation were not seen on SaOS-2 after 3 days of subculture. 1. Smith MK, et al. Tissue Eng 2004;10:63-71. 2. Barou O, et al. Bone 2002;30(4):604-612. 3. Carrano RAD, et al. DDT 2003;8(21):980-989. 4. Botchwey EA, et al. JBMR 2001;55:242-253. 5. Peters MC, et al. JBMR 2002;60:668-678. 6. Murphy JL, et al. J Dent Res 2004;83(3):204-210. 7. D'Amato RJ, et al. Proc Natl Acad Sci 1994;91(9):4082-4085.

AA5.48

A Novel Nanofiber Scaffold by Electrospinning and its Utility in Cardiac Tissue Engineering. Dong Han and Pelagia-Irene Gouma; Materials Science and Engineering, Stony Brook University, Stony Brook, New York.

Non-woven cellulose acetate (CA) thin, porous membranes were produced by electrospinning polymer solutions (in acetone) at room temperature. During this process, polymer nanofibers were produced when a high electric field of 12 kV was applied to a precursor solution. The diameters of fibers obtained varied widely and the average diameter was approximately 50 nm. The electrospinning parameters used to control the morphology of the fibers and their membranes

were flow rate and distance between the syringe needle that ejects fluid and the collector (aluminum plate on which the membranes were deposited). These membranes are used as scaffolds for cardiac cells growth. In order to help cells grow effectively on these scaffolds, the morphology of the membranes had to mimic the structure of the top layer of natural extracellular matrix materials such as UBM, which are known to help cells proliferate. The non-woven fiber mats were examined by means of optical and electron microscopy and the nanofibers were seen to be oriented randomly and the structure was uniform. The problems of effectively biodegrading CA has been addressed by adding cellulase to the electrospun structures. The issue of strengthening the CA scaffold is currently studied by adding secondary ceramic nanoparticles in the polymer membranes.

AA5.49
Biodegradable Polymeric Nanoparticles for Tumor-Selective Tamoxifen Delivery: In-Vitro and In-Vivo Investigations. Dinesh Shenoy, Jugminder Chawla and Mansoor Amiji; Pharmaceutical Sciences, Northeastern University, Boston, Massachusetts.

This study was performed to evaluate the in-vitro and in-vivo tumor-cellular uptake and biodistribution pattern of tamoxifen (TAM) when administered intravenously as a simple solution and upon encapsulation into biodegradable, surface-modified poly(-caprolactone) (PCL) nanoparticles. PCL (MW 15,000) nanoparticles were prepared by the solvent displacement method and characterized for particle size/charge and surface morphology (by scanning electron microscopy). We investigated the nanoparticle-surface modification potential of the hydrophilic stabilizer (Pluronic F-68 and F-108) employed during the preparation by electron spectroscopy for chemical analysis (ESCA). Quantitative in-vitro cellular uptake of tritiated (3H) TAM in solution form and as nanoparticulate formulation was assessed in MCF-7 breast cancer cells. In-vivo biodistribution studies for the same formulations were carried out in Nu/Nu mice bearing MDA-MB-231 human breast carcinoma xenograft. Spherical nanoparticles having positive zeta potential (25 mV) were obtained in the size range of 200-300 nm. Pluronics (both F-68 and F-108), the triblock copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide), induced surface hydrophilization of the nanoparticles via adsorption as evident by ESCA. Nanoparticulate formulations of TAM achieved higher intracellular concentrations when exposed at therapeutic concentrations to tumor cells in-vitro compared to solutions. The in-vivo biodistribution studies carried out in nude mice bearing experimental breast tumor suggested increased tumor concentrations for the drug administered as nanoparticulate formulations besides longer retention times within tumor mass. This type of delivery system is expected to provide better therapeutic benefit by dual means: preferential concentration within the tumor mass via enhanced permeation and retention (EPR) pathway, and; subsequent controlled release, thus maintaining the local drug concentration for longer periods of time to achieve maximal cell-kill. Acknowledgements: This research project is funded by National Cancer Institute grant # 1R01CA095522-01A2.

AA5.50
Advanced Photocatalysis with Anatase Nanocoated Multi-walled Carbon Nanotubes. Sung-Hwan Lee, Georgios Pyrgiotakis and Wolfgang Michael Sigmund; University of FLorida, Gainesville, Florida.

Since 1972 when Fujishima and Honda discovered the photocatalytic split of water on titania electrodes under UV radiation, titania has been used in various photochemical applications ranging from photovoltaic cells to biological disinfection/purification. After UV irradiation and the electron hole pair generation a portion of the generated holes in the system are migrating to the surface where they create OH radicals to oxidize organic materials. It has been shown that the coupling of TiO2 with metals enhances the photocatalytic efficiency because the electrons flow to the metals and thus increases the amount of free holes by retarding electron-hole recombination. In this research a different approach is used to synthesize a photocatalytic nanocomposite, which utilizes the high aspect ratio of carbon nanotube (CNT) and the unique electrical properties to achieve higher photocatalytic efficiency. The particles were synthesized with sol-gel nanocoating on multi-walled carbon nanotubes (MWNTs). The nanostructure was characterized with the use of SEM, TEM, XRD, Raman, FTIR and UV-VIS spectroscopies. To determine and compare the photocatalytic efficiencies of commercial photocatalysts (Degussa P25) and TiO2 nanocoated MWNTs, the organic Azo dye degradation tests were performed and the time required for 50% present reduction of the concentration was measured and used. Destruction was observed with UV-A and visible light irradiations. Also biocidal tests have been performed with bacteria (E. Coli) and spores (B. Cereus). The values used for comparison at those experiments were the D value and the LD-90 value. Results from dye degradation and biocidal tests signify TiO2 nanocoated MWNTs have higher photocatalytic efficiency than the best photocatalysts in the market.

AA5.51
A New Class of Self-Assembling Peptide Detergents Significantly Stabilized Bovine Rhodopsin. Yusuke Nagai[1,2], Xiaojun Zhao[1], Philip Reeves[3], Gobind Khorana[3] and Shuguang Zhang[1]; [1]Center for biomedical engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]R&D, Menicon Co., Ltd., Kasugai, Aichi, Japan; [3]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts.

The integral membrane protein rhodopsin from the visual photoreceptor in the retina belongs to a typical G-protein coupled receptor (GPCR). A number of detergents including octyl-D-glucoside (OG) and n-dodecyl-D-maltoside (DM) are commonly used to solubilize rhodopsin from rod outer segments (ROS). These detergents stabilize rhodopsin by attaching their hydrophobic site with the transmembrane domain of rhodopsin. A great deal has been learned about rhodopsin using the simple detergents. However, in order to further gain detailed structural information of rhodopsin, it is necessary to use other detergents that can facilitate the high-resolution structural analyses. We designed a new class of peptide detergents that consists of a hydrophilic head of aspartic acid or lysine with a hydrophobic tail of consecutive Gly, Ala, Val, Ile and Leu. The individual peptide consists of 7 residues and numerous peptides self-assemble to form nanotubes and nanovesicles in the absence of proteins. We here demonstrate that one of the peptide detergents A6D (Ac-AAAAAAD) significantly stabilized bovine rhodopsin for extended time at 50 degrees C with DM. In the control experiment of the same concentration, DM did not significantly stabilize rhodopsin under identical condition. Moreover, when OG was replaced with A6D in the exchange reaction, bovine rhodopsin was further stabilized even at 55 degrees C. These results demonstrate that peptide detergents are useful materials for stabilizing and studying membrane proteins.

SESSION AA6: Nano and Micron Scale Materials in Biology and Medicine
Chairs: Cliff Henderson and Cato Laurencin
Wednesday Morning, December 1, 2004
Room 306 (Hynes)

8:30 AM *AA6.1
Nanoparticle Bioconjugates. A. Paul Alivisatos, Chemistry, University of California, Berkeley, Berkeley, California.

This talk will describe work related to the application of inorganic nanoparticles in biological detection. Work on the use of colloidal quantum dots is presently being extended to encompass new types of nanparticle groupings, which, as a unit, act collectively to provide a probe. In addition, new shapes and topologies of nanoparticles are being produced, which may lead to further types of biological probes.

9:00 AM AA6.2
Functionalized Luminescent Oxide Nanoparticles: New Tool for the Individual Detection of Sodium Channels on Cell Membranes. Domitille Giaume[1], Valerie Buissette[1], Khalid Lahlil[1], Thierry Gacoin[1], Jean-Pierre Boilot[1], Emmanuel Beaurepaire[2], Didier Casanova[2], Martin-Pierre Sauviat[2] and Antigoni Alexandrou[2]; [1]Solid State Chemistry Group, Laboratoire de Physique de la Matiere Condensee, CNRS - Ecole Polytechnique, Palaiseau, France; [2]Laboratoire d'Optique et Biosciences, CNRS - INSERM - Ecole Polytechnique, palaiseau, France.

Organic compounds including fluorescent proteins are extensively used for studying molecular and cellular processes. Nevertheless, rapid photobleaching is an important limitation for many studies such as individual tracking of biological species. Inorganic luminescent nanoparticles are expected to provide an interesting alternative to organic dyes, due to their high stability associated to relatively high emission yields. In this field, results obtained these last years using II-VI semiconductor quantum dots have indeed shown that these systems are very promising. The aim of the work presented here is to study similar applications in the case when the inorganic nanoparticles are oxide nanocrystals doped with luminescent rare earth ions. The biological issue we addressed is to study the localization of Na^+ channels in live cardiac myocytes. Specific interactions are expected through the functionnalization of the particles with guanidinium groups, which are the active part of complex toxins (tetrodotoxin and saxitoxin) that are well known for specifically plugging the Na^+ channels mouth. The luminescent oxide particles used in this work are YVO_4:Eu nanocrystals prepared following a simple aqueous colloidal route. Their emission spectra consist in narrow lines with a main contribution at 617 nm. Their emission yield is about 25% with an emission lifetime of 0.7 ms.

Surface functionnalization of the particles was achieved trough the covalent grafting of guanidinium groups on a thin shell of polymerized silane bearing epoxy groups. Our first studies concerned the optical detection of particles deposited on glass slides. Using wide field fluorescence microscopy, we showed that the detection of individual nanoparticles can be achieved quite easily. We then studied the biological activity of the guanidinium-functionalized nanoparticles compared to that of saxitoxin. Action potentials were recorded by means of intracellular microelectrode techniques. These experiments show that functionalized nanoparticles do indeed block the Na^+ channels similarly to saxitoxin, whereas no effect was measured using nanoparticles without guanidinium groups or free guanidinium groups alone. Individual imaging of the nanoparticles on cardiac cell membranes confirmed the interactions measured previously by the electrophysiology experiments. Moreover, the absence of particles bound to the membrane when the Na^+ channels are previously blocked by the saxitoxin demonstrates the high specificity of the interactions between the particles and the channels. It may then be concluded that functionalized YVO_4:Eu nanoparticles mimic the blocking effect of the saxitoxin and behave as artificial toxins. Such particles then provide a versatile tool for long-term single-molecule tracking, allowing further work on the dynamic and the aggregation behaviour of Na^+ channels on excitable cell membranes.

9:15 AM AA6.3
A Study of the Effect of Nanoscale Particles on Cells.
Nadine Pernodet[1], Xiaohua Fang[1], Assia Bakhtina[2], Abraham Ulman[2], Aditi Ramakrishnan[3], Nikhilesh Ray-Mazumder[3], Miriam Rafailovich[1] and Jonathan Sokolov[1]; [1]Stony Brook University, Stony Brook, New York; [2]Polytechnic University, Brooklyn, New York; [3]Ward Melville High School, Long Island, New York.

We have studied the effect of different nanoscale particles on the morphology and proliferation of human dermal fibroblast cells of different ages using confocal, atomic force, and transmission electron microscopy. We have found that nanoparticles can have a profound effect on the structure of the actin scaffold and the cell growth rates. Furthermore, the presence of nanoparticles can also alter the mechanical properties of the cells, stimulate the formation of vacuoles within the cell, and impair the cell ability to adhere to different substrates. These effects varied in intensity depending on the age of the cells, with older cells being most susceptible to damage. (Work supported in part by the NSF-MRSEC and the Department of Energy)

9:30 AM AA6.4
Properties and Applications of Novel DNA-based Surfactants.
Chun Xu, Pietro Taylor, Mustafa Ersoz, Paul D. I. Fletcher and Vesselin N. Paunov; Hull University, Hull, United Kingdom.

We have designed novel DNA-surfactants prepared by covalent attachment of a hydrophobic anchoring group to the (3'- or 5'-) end of short DNA oligonucleotides. This anchoring group turns these DNA-strands into amphiphilic molecules. Such DNA-surfactants can adsorb at air-water and oil-water surfaces which orients them with respect to the liquid surface and can promote programmable interaction based on Watson-Crick pairing. We studied the adsorption of DNA surfactants at the oil-water interface by Drop Shape Analysis and demonstrated that the interfacial tension isotherm at the oil-water interface depends strongly on the number of bases as well as the base sequence in the DNA surfactant. DNA hybridization at the oil/water interface was studied by measuring the interfacial tension of DNA surfactant during temperature jump across the melting point of complementary DNA-surfactants. Complementary sequences and non complementary sequence of DNA surfactant show clear difference during the temperature jump process. We also found that DNA surfactants can be immobilised on hydrophobic solid surfaces by hydrophobic interactions which allowed us to design a novel method for fabrication of DNA arrays based on microcontact printing of aqueous "inks" containing DNA surfactants on solid substrates. Special attntion was paid to the wetting properties of the ink with respect to the stamp and the solid substrates. The method allows for efficient attachment of DNA strands to solid surfaces and hybridisation with complementary fluorescently-tagged oligonucleotides. This new technology could be utilised for rapid preparation of DNA-assays and genetic biochips.

10:15 AM AA6.5
Synthesis and Application of Gold Nanoparticles Functionalized with Collagen Mimetic Peptides.
Xiao Mo and Seungju Michael Yu; Materials Science & Engineering, The Johns Hopkins University, Baltimore, Maryland.

Collagen is the principal tensile element of the extra cellular matrix in animals and is the basic scaffold for cells and tissues. Abnormalities in its structure are known to result in a number of debilitating human diseases. Collagen mimetic peptides (CMPs) with sequences of (Pro-Hyp-Gly)n n = (6, 7, 10) are capable of forming right-handed triple-helical structures similar to those of collagen triple helices. Recently, our group has shown that CMPs exhibit specific binding affinity to natural collagen under controlled thermal conditions. Using solid phase peptide synthesis method, we have prepared Cys-CMP conjugates that were used to modify gold nanoparticles with different sizes ranging from 3 nm to 15 nm. Transmission electron microscopy (TEM) observation shows that the Cys-CMP functionalized gold nanoparticles have affinity to reconstructed collagen fibers. We are investigating the interactions between Cys-CMP functionalized gold nanoparticles and reconstructed collagen fibers under various thermal conditions. The Cys-CMP conjugated nanoparticles can potentially be used as a tool to visualize and understand unstable domains of collagen fibers which are related to a number of pathological conditions of extra cellular matrices.

10:30 AM AA6.6
Radioactive Dendrimer Nanocomposites To Treat Tumor Microvasculature.
Lajos P. Balogh[1,4,5], Shraddha S. Nigavekar[6], Xiangyang Shi[4], Brian Bolton[2], Leah Minc[3], Fatema Mamou[6] and Mohamed K. Khan[6]; [1]Dept. of Internal Medicine & Dept. of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan; [2]Radiation Oncology, University of Michigan, Ann Arbor, Michigan; [3]Michigan Memorial Phoenix Project, University of Michigan, Ann Arbor, Michigan; [4]Center for Biologic Nanotechnology, University of Michigan, Ann Arbor, Michigan; [5]MACRO, University of Michigan, Ann Arbor, Michigan; [6]Radiation Medicine, Roswell Park Cancer Insitute, Buffalo, New York.

We describe the ongoing development of dendrimer based radioactive nanocomposite devices (NCDs) to deliver radiation selectively and efficiently to tumors. Our general approach is based on differences between the normal and tumor microvasculature permitting the delivery of NCDs specifically to the tumor, using size, charge and surface recognition characteristics of the composite nanoparticles. Dendrimer templated nanocomposites are an exciting new class of materials with many potential applications [1]. Their use for imaging and treatment of cancer angiogenesis is especially intriguing, as all tumors must maintain a microvasculature in order to exist. NCDs are synthesized as monodisperse hybrid nanoparticles composed of radioactive guests immobilized by functionalized dendritic polymer hosts of well-defined size and charge [2]. First, we have established detailed chemical characterization and studied in vivo biodistribution of several of these nanoscopic host particles [3], then we characterized the biodistribution of a folate targeted 5 nm host in tumor bearing mice [4]. We have synthesized {Au} nanocomposites by encapsulating Au(0) into poly(amidoamine) (PAMAM) dendrimers. These NCDs are nearly spherical, have a monodisperse distribution as well as well-defined and variable sizes (5 nm and 22 nm) in addition to a specifically charged (positively/ negatively charged and/or neutral) surface. First, we synthesize and characterize the gold containing cold device, then we activate the gold in neutron beam. Delivery of radiation is achieved by the {198Au} NCDs. Biodistribution of NCDs of various size and surface was investigated in mice models (B16 melanoma and DU145 human prostate cancer) before entering the therapy phase. Non-radioactive Au isotopes and H-3 labeled dendrimer hosts were used for in vivo experiments to study biodistribution. Isotope neutron activation analysis (INAA) was able to measure the presence of 10 ng Au per tissue sample. Various imaging techniques were applied to demonstrate the feasibility of the general nano-device concept in actual cancer cells in vivo [5]. REFERENCES [1] L. Balogh, D.R. Swanson, R. Spindler, D.A. Tomalia, Proc. ACS PMSE, 1997, Vol. 77, pp. 118-119; D.A. Tomalia, L.P. Balogh: US 6,664,315B2 [2] L.P. Balogh, S.S. Nigavekar, A.C. Cook, L. Minc, M.K. Khan, PharmaChem, 2003, Vol. 2(4), pp. 94-99 [3] S.S. Nigavekar, L.Y. Sung, C.W. Becker, T.S. Lawrence, L. Balogh, M.K. Khan, Pharm. Res., 2004 Vol. 21(3), pp. 476-483 [4] K.A. Candido, S.S. Nigavekar, I. Majoros, B. Keszler1, L.Y. Sung, C.W. Becker, L.P. Balogh, M.K. Khan, J.R. Baker, Jr.., (Submitted to Pharm. Research) [5] A. Bielinska, J.D. Eichman, I. Lee, J.R Baker, Jr. L. Balogh, J. Nanopart. Res., 2002, Vol 4, pp. 395-403,

10:45 AM AA6.7
Hybridization-Based Unquenching of DNA Hairpins on Au Surfaces: Prototypical "Molecular Beacon" Biosensors.
Hui Karen Du[1], Christopher M. Strohsahl[2], Benjamin L. Miller[3] and Todd D. Krauss[1]; [1]Chemistry, University of Rochester, Rochester, New York; [2]Department of Biochemistry and Biophysics, University of Rochester, Rochester, New York; [3]Department of Dermatology, University of Rochester, Rochester, New York.

The strong need for novel arrayable-based bio-analytical tools has been driven by intense interest in the use of rapid genetic analysis for understanding biological processes, for unlocking the underlying molecular causes of disease, and in the development of biosensors. Techniques that do not require labeling of the target sample are highly sought after, since they save time, money, and limit potential errors inherent in the analysis. We will present a novel, "label-free"

surface-based molecular-beacon biosensor based on unquenching of DNA hairpins on Au. Traditional molecular beacons consist of a DNA hairpin functionalized at one end with a fluorophore, and at the other with a quencher. In the absence of target DNA, the proximity of the quencher to the fluorophore inhibits fluorescence. Addition of the target leads to hairpin unfolding and signal generation. Using confocal-epi-fluorescence microscopy we examined the fluorescence of DNA hairpin-functionalized Au films in the presence and absence of complementary targets, whereby the Au surface serves the role of the quencher. Hairpins studied include portions of Bacillus anthracis Pag gene, Staphylococcus aureus genomic DNA, Staphlococcus aureus FemA and mecR methicillin-resistance genes, and are "naturally occurring" because they were identified via a novel "partial gene folding" technique. This technique is advantageous in that it provides probes containing complete specificity for their designed target. We found fluorescence from the probes could be enhanced by up to 100-fold in the presence of the DNA complement. Furthermore, we found our technique could distinguish single-base mismatched DNA targets with high sensitivity under identical hybridization conditions, indicating that the surface immobilized DNA hairpins retain their ability to bind complementary sequences selectively. Measurements of hybridization kinetics and device performance as a function of input target concentration will also be presented.

11:00 AM AA6.8
Yttrium/Gadolinium/Fibrin Composites For Localized Radiation Therapy. Anna Gutowska, Novella Bridges, Darrell Fisher, Kevin Minard, Robert Wind, James Morris and Lyle Sasser; Pacific Northwest National laboratory, Richland, Washington.

The purpose of this project is to develop a new localized radiation therapy method based on fibrin surgical adhesive serving as an effective delivery vehicle for therapeutic radionuclides and imaging agents. We have developed a method to synthesize monodispersed colloidal yttrium-90/gadolinium nanoparticles that will allow simultaneous radiation treatment and MRI imaging. Adding the MRI imageability to the therapeutic particle is crucial for assessing localization of the treatment. Yttrium-90/gadolinum particles were prepared by hydrothermal synthesis method as insoluble phosphate salt colloids. We have determined variables controlling hydrothermal synthesis of colloidal yttrium/gadolinium phosphate particles, and optimized the synthesis towards particle monodispersity and increased yields. We have demonstrated in vitro stability of fibrin surgical adhesive (Tisseel , Baxter Healthcare Corporation, Glendale, CA) in contact with the radiocolloid. We have also investigated different application modes for the radiocolloid/fibrin composite and optimized the delivery mode. The composite would be used as an intraoperative, topical therapeutic agent for eradicating residual cancer cells left in tissues after local excision of tumors. Preliminary in vivo studies in rodent animal models demonstrated MRI imaging of the particles and localization of the radiocolloid at the injection site.

11:15 AM AA6.9
Formation of Titania Submicron-Scale Rod Arrays on Titanium Substrate and In Vitro Biocompatibility. Yongxing Liu[1], Kazuya Okamoto[1], Satoshi Hayakawa[1], Kanji Tsuru[1,2] and Akiyoshi Osaka[1,2]; [1]Biomaterials Laboratory, Okayama Univeristy, Okayama, Japan; [2]Research Center for Biomedical Engineering, Okayama University, Okayama, Japan.

One-dimensional nanostructures of polymers, metals and semiconductors in forms of rods, tubes and others, have attracted much attention for a broad range of potential applications, including catalysts, electronics, sensors, photonics, micromechanical devices and biomedical devices, etc. However, to obtain scale-up functional devices with highly ordered nanorod or nanotube arrays are essential. A number of fabrication techniques have been employed or challenged. Many of these fabrication processes were also applicable for TiO_2, but it is complicated because of the using templates or the chemical process involved. Commercially available pure titanium and titanium alloys have been used for circulatory assist devices such as artificial hearts, and has been employed in the mechanical components of the pump and a blood-contacting surface. However, they have problems with blood-clot formation in the devices. Recently, Takemoto et al. (Biomaterials, Vol. 25/17, pp. 3485-3492, 2004) reported that cp. Ti substrates treated with hydrogen peroxide solution and subsequently heated at 550°C showed good blood compatibility and that the specimens consisted of rutile and anatase phases of titania, and had hydrophilic surface. In this study we prepared titania submicron-scale rods array on metallic titanium (α-Ti) surfaces by coating a layer of sodium tetraborate on titanium substrates and subsequent thermal treatment. Thin-film X-ray diffraction analysis indicated that the sodium tetraborate gave rutile (TiO_2: PDF# 21-1276) submicron-scale rod array. This novel technique promises us one simple method to prepare submicron-scale arrays of various functional materials on various substrates. The in vitro biocompatibility of the rod array was examined by the immersion experiment in a simulated body fluid in terms of the apatite-forming ability, and by the platelet adhesion experiment.

11:30 AM *AA6.10
Microengineering of materials to control cell fate and function. Christopher Chen, Johns Hopkins University, Baltimore, Maryland.

Adhesive interactions between cells and scaffolds play a central role in regulating cell function. Many approaches to fabricate well defined surfaces and substrates have enabled us to develop an understanding of how cell adhesion controls cell migration, proliferation, differentiation, and death. Here, we describe how we have used microfabricated systems to study cellular processes related to wound healing, cell growth, and stem cell differentiation, and demonstrate that not only the chemistry but also the structure and mechanics of the adhesive interactions are critical components of the cell-material interface that affect cell function. In the future, a major challenge will be to integrate these approaches into tissue engineering on larger length scales.

SESSION AA7/Z7: Joint Session: Biomimetic Surfaces/Cell-Material Interactions/Biofunctional Peptides
Chairs: Phillip Messersmith and Molly Stevens
Wednesday Afternoon, December 1, 2004
Room 304 (Hynes)

1:30 PM *AA7.1/Z7.1
Biointerfaces Mimicking Extracellular Matrices to Engineer Cell Function. 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. In addition to anchoring cells, 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 surfaces that control the presentation of integrin-binding domains and mimic the primary, secondary, and tertiary protein structure of fibronectin and type I collagen. These surfaces promote specific integrin binding and focal adhesion assembly and signaling as well as osteoblast cell adhesion and bone-specific gene expression, alkaline phosphatase activity, and deposition of a biologically equivalent mineralized matrix. A recombinant fragment of fibronectin spanning the 7th-10th type III repeats of fibronectin and encompassing the PHSRN and RGD motifs was tethered to non-fouling supports to specifically bind alpha5beta1 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 incorporating the GFOGER motif 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 levels than conventional culture supports. Finally, we have engineered mixed ligand surfaces presenting varying fibronectin-/collagen-mimetic ligand densities to independently target alpha5beta1 and alpha2beta1 integrins. These mixed ligand surfaces synergistically modulate cell adhesive activities. These surface engineering strategies provide a basis for the rational design of robust biospecific interfaces 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.

2:00 PM *AA7.2/Z7.2
Interactions of Biomimetic Peptide-Amphiphiles with Their Receptors. Efrosini Kokkoli, Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, Minnesota.

This study involves the use of a model biomimetic system that allows us to investigate collective and single-molecule forces between receptor-ligand pairs that reveal details of the molecular recognition mechanisms of multiple and individual pairs. An Atomic Force Microscope (AFM) is used to provide high resolution images and

direct adhesion measurements at the piconewton level. In this work, bioartificial membranes that mimic the cell adhesion domain of the extracellular matrix protein fibronectin (GRGDSP) are constructed from peptide-amphiphiles. The receptors of choice are the $\alpha_5\beta_1$ integrins that are immobilized onto the AFM tip. The effect of different parameters such as different ions, synergistic effects from other peptides (PHSRN) and the loading rate have been investigated on the dynamics of the $\alpha_5\beta_1$-GRGDSP interaction using the AFM.

2:30 PM AA7.3/Z7.3

Engineering Communication Between Mammalian Cells and Materials Surfaces Via a Novel Cell-Surface Protein.

Joel H. Collier[1] and Milan Mrksich[2]; [1]Biomedical Engineering, University of Cincinnati, Cincinnati, Ohio; [2]Chemistry, University of Chicago, Chicago, Illinois.

Establishing communication pathways between cells and materials surfaces is important in the development of cell-based sensors and devices, electronic components, and biomaterials. Here we describe a system in which mammalian cells have been engineered to display a non-native chimeric protein on their cell membranes that enables them to enzymatically process self-assembled monolayer (SAM) substrates. This specific processing produces changes in the redox characteristics of the SAM that can be readily measured with cyclic voltammetry, in short transducing cellular activity into an electrical signal. The functionality of the chimeric protein is endowed by the enzyme cutinase, which catalyzes the hydrolysis of esters on the SAM surface. The cutinase is presented away from the cell membrane by a rigid stalk domain, and the entire construct is anchored by the transmembrane domain and an intracellular fragment of the β1 integrin. Transfection with this construct produces cells with a high cutinase activity on their surfaces. We describe the construction of the chimeric protein, transfection of Chinese hamster ovary (CHO) cells, and characterization of a cell line that stably expresses this engineered construct. On the SAM component of the system, we have displayed hydroquinone esters that serve as good substrates for cutinase. In the absence of cutinase activity (e.g. in cultures with untransfected cells) this hydroquinone remains protected, and no redox peaks are detectable with cyclic voltammetry (CV). When these surfaces are exposed to transfected cells displaying cutinase, however, the CV signal increases dramatically. We also monitored this deprotection of the hydroquinone with MALDI-TOF mass spectrometry. This approach constitutes a novel way to transduce cellular activity into measurable electronic signals.

2:45 PM AA7.4/Z7.4

Self-assembly of Peptide Amphiphiles and its Implications for Bioelectronic Nanostructures.

John D. Tovar[1,2] and Samuel I. Stupp[1,2,3]; [1]Department of Materials Science and Engineering, Northwestern University, Evanston, Illinois; [2]Institute for Bioengineering and Nanoscience in Advanced Medicine (IBNAM), Northwestern University, Evanston, Illinois; [3]Department of Chemistry and Feinberg School of Medicine, Northwestern University, Evanston, Illinois.

Supramolecular architectures based upon self-assembling molecules are emerging as powerful tools for bionanotechnology. Our laboratory has used the self-assembly of peptide amphiphile (PA) molecules to form nanofiber networks capable of directing the crystallographically oriented growth of hydroxyapatite and inducing selective differentiation of neural progenitor cells. These systems consist of a hydrophobic alkane tail coupled to an oligopeptide sequence. In their assembled state, the aggregated alkyl tails form a well-defined hydrophobic region within the nanofiber while the exterior of the nanofiber is decorated with bioactive peptide epitopes. We report here on a spectroscopic examination of self-assembled PAs tailored with rational placement of tryptophan residues and pyrenyl-amidated lysines. Fluorescence measurements with the environmentally sensitive tryptophan probe indicate that the peptidic segments of the PA molecules remain well-solvated regardless of their location within the self-assembled aggregate. Extrinsic fluorescence quenching also confirms that the chromophores are accessible in their aggregated states, although this accessability is expectedly hindered relative to the unassembled state. In fact, we see trends indicating a progression towards increased quencher access within the nanostructures that have chromophores placed closer to the outer fiber periphery. We are currently applying this knowledge to the study of electrically active PA nanofibers designed as novel bioelectronic components. We have synthesized PAs that bear covalently attached electroactive moieties (such as terthiophene) and studied their electrochemical behavior once assembled into nanofibers. We have also demonstrated that the PAs may be used to sequester hydrophobic monomers that then undergo polymerization to form conducting polymers.

3:30 PM *AA7.5/Z7.5

Interfacial Biomaterials.

Mark Grinstaff[1] and Daniel Kenan[2]; [1]Departments of Biomedical Engineering and Chemistry, Boston University, Boston, Massachusetts; [2]Department of Pathology, Duke University, Durham, North Carolina.

Advances in implant technology have revolutionized health care practices over the last twenty years, with abundant examples ranging from prosthetic joints to vascular grafts. Yet, today one of the pressing challenges is the development of prosthetic implants or devices that integrate appropriately with tissues. Existing materials can either generate an overly robust inflammatory reaction that compromises function, or fail to integrate appropriately, resulting in suboptimal performance. Thus, new materials, tools, and procedures are needed to promote better integration at the interface between synthetic materials and biologics. We describe a general protocol for the design and preparation of bio-mimetic coatings termed "interfacial biomaterials." Interfacial biomaterials represent a novel coating technology capable of directing biological processes at the interface between a synthetic surface and a biologic. The approach relies on screening combinatorial libraries to identify unique peptides that adhere to a synthetic target such as a plastic or metal, or to a biological target such as a protein or cell. Next, two or more adhesion peptides are synthetically coupled to create an interfacial biomaterial that mediates the interaction of the protein or cell with the synthetic material. Preparation of multi-functional interfacial biomaterials provides one strategy to mediate the biological process at the critical interfacial site between biologics and synthetic surfaces. Importantly, these materials expand the current repertoire available for designing and developing new biomaterials for applications ranging from proteomics to tissue engineering.

4:00 PM AA7.6/Z7.6

Dynamic Assembly of Nanostructures: Exploiting Peptide-Peptide Recognition.

Molly M. Stevens[1,2], Nolan T. Flynn[4], Chun Wang[2], David A. Tirrell[3] and Robert Langer[2]; [1]Materials, Imperial College London, London, United Kingdom; [2]Chemical Engineering, Massachusetts Institute of Technology, Boston, Massachusetts; [3]Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California; [4]Chemistry, Wellesley College, Wellesley, Massachusetts.

There is growing interest in the ability to actively direct the assembly of inorganic nanoparticles using biomolecular recognition for the creation of new materials and nanotechnology devices. This area of research exploits the high specificity exhibited by biomolecular recognition systems to achieve assembly. Here we report the coiled-coil peptide based assembly of nanoparticles and demonstrate that the system can be dynamically controlled under mild conditions (near-neutral pH and ambient temperature). The development of new methods such as this to control dynamic nanoparticle assembly may impact on certain applications in medical science such as the generation of novel tunable and/or switchable materials. In particular, the ability to dynamically assemble and dis-assemble such structures under physiologically accessible environmental conditions, as triggered for example by changes in pH would be valuable for materials to be utilized for sensing in vivo and drug delivery. Self-assembly of gold and other nanoparticles was investigated utilizing nanoparticles functionalized with acidic or basic leucine zipper-like peptides. The peptides adopt an alpha-helical conformation when they form a homomer or heterodimer coiled-coil structure, the stability of which is modulated by electrostatic interactions across the interface of adjacent helices. Circular dichroism spectroscopy of the free peptides in solution revealed changes in the helicity of the peptide, which indicates the association or dissociation of coiled-coil structures, as the pH is varied. Reversible pH-induced transitions were observed (e.g. between pH 8.5 and 7 for the acidic peptides). Transmission electron microscopy images of the peptide-coated nanoparticles revealed that well-dispersed populations of nanoparticles could be caused to dynamically assemble in response to mild pH triggers.

4:15 PM AA7.7/Z7.7

Design of Peptides with Collagen High Frequency Triplets Sequence for Templating of Bone Mineralization.

Yang Zhang[2,1], Jie Song[2,4] and Carolyn Bertozzi[1,2,3]; [1]Department of Chemistry, University of California, Berkeley, Berkeley, California; [2]Materials Sciences Divisions, Lawrence Berkeley National Laboratory, Berkeley, California; [3]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, California; [4]Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California.

As the main extracellular matrix protein in bone, type I collagen is only thought to serve as the structural scaffold for bone minerlization, while other acid proteins binding to their gap region may play a main role that prompts the nucleation and deposition of bone minerals. By analysing the repeating Gly-Xaa-Yaa triplets sequence of type I collagen molecules, it is found that most of high frequency triplets

sequences contain uncharged or positive charged amino acid residues. Therefore, three peptides containing with repeating high frequency triplets sequences in human type I collagen but with different charged side chain (positive, uncharged and negative) of amino acid residues were designed and synthesized to probe their mineral-templating abilities. It shows that peptides with different charged side chain have different behaviors on Ca-P mineralization. And the most interesting thing is that the triplets-sequence with positive charged amino acid residues could facilitate the formation of Ca-P crystals, as observed by HRTEM. This result may display the information of protein-minerals interaction encoded in the sequence information of collagen molecule, and provide a better understanding for that the replacement of one glycine in collagen molecule could present disorder of minerals deposition, which as shown in lots of collagen defect diseases such as osteogenesis imperfecta.

4:30 PM AA7.8/Z7.8

Molecular Modeling of Engineered Polypeptides.

Urartu O. S. Seker[1], Ersin Emre Oren[2], Selcuki Cenk[1], Candan Tamerler[1,2] and Mehmet Sarikaya[2,1]; [1]Materials Science and Engineering, University of Washington, Seattle, Washington; [2]Molecular Biology and Genetics, Istanbul Technical University, Maslak-Istanbul, Turkey.

In biological hard tissues, proteins control inorganic materials assembly, morphogenesis and formation through molecular recognition and specific binding. Adapting both cell-surface and phage display protocols, we have selected and isolated short oligopeptide sequences (7-12 amino acids) that bind to noble metals and oxide semiconductors. The understanding of the nature of molecular recognition, binding and kinetics of the inorganic specific peptides is essential for an effective utility of the selected polypeptides in materials assembly and formation. Considering that protein recognition of an inorganic surface may originate both from chemical (e.g., polarity, H-bonding, polarity and charge effects) and physical (structural size, surface crystallography & morphology) interactions, we have developed/modified an array of experimental techniques to quantitatively characterize binding characteristics of the peptides and also to investigate the interactions of the selected sequences with various materials surfaces through computational methods. The initial step in our conformational analysis consists of generating conformers of selected oligopeptides in vacuum. The CHARMM force field has been used to generate the conformers. Next, the most stable conformers have been re-optimized with the same force field. Results obtained are used to compare the hydrogen bonding patterns, which is one of the major factors determining the conformational preference. Our preliminary calculations have shown that there are significant differences in the fully optimized conformers compared to the initial geometries taken from the conformational analysis. The most important part of the modeling bioinorganic hybrid systems is the choice of the force field that will be used to investigate the interactions at the peptide-metal interface. Initially, these hybrid systems have been investigated by using the CHARMM Molecular Mechanics program in vacuum as implemented in HyperChem 7.5. The most stable conformer has been brought onto the metal surface and than the system is fully optimized allowing the interactions of both systems. The same calculations have been repeated for each system by using different force fields implemented in different suit of programs. The similarities and/or differences between the calculated results have been discussed in terms of energies, geometries and electronic properties as well as the nature of the metal surface and the oligopeptides. Since these interactions occur usually under physiological conditions, further calculations will focus on modeling of these systems in solution (in water). The nature of solvent and the solute-solvent interactions could play an important role on the type and strength of interactions between these two components. Research supported by ARO-DURINT and SPO-Turkey.

4:45 PM AA7.9/Z7.9

Controlling Viability and Osteogenic Differentiation of Human Mesenchymal Stem Cells Photoencapsulated in Poly(Ethylene Glycol)-Based Hydrogels.

Charles Raymond Nuttelman[1], Margaret Claire Tripodi[1], Sean Michael Langelier[1] and Kristi Sue Anseth[1,2]; [1]Chemical and Biological Engineering, University of Colorado, Boulder, Colorado; [2]Howard Hughes Medical Institute, University of Colorado, Boulder, Colorado.

Human mesenchymal stem cells (hMSCs) have many properties that make them ideal for tissue engineering applications, including their ease of isolation, high proliferative capacity, and ability to form new tissue. By rationally designing a poly(ethylene glycol) (PEG)-based, photocrosslinkable hydrogel scaffold, we aim to provide a three-dimensional environment that maintains viability of encapsulated cells and actively promotes their osteogenic differentiation. Due to the highly hydrophilic nature of PEG hydrogels and the fact that proteins do not adsorb strongly to this material, encapsulated hMSCs are presented with a blank environment upon encapsulation. As a result, initial cell viability is low. By incorporating either the well-known RGD cell adhesion peptide sequence or charge into the scaffold, cell viability in vitro at least up to four weeks is dramatically improved. Charge is incorporated into the hydrogel network by copolymerizing diacrylated PEG with ethylene glycol methacrylate phosphate (EGMP) or methacrylic acid (MA). In addition to maintaining cell viability in these PEG hydrogels, we also aim to develop an osteogenic scaffold, which induces the differentiation of encapsulated hMSCs in the absence of added osteogenic factors in the surrounding media (i.e., differentiation factors are released from within the gel). Dexamethasone, a synthetic corticosteroid that causes osteogenic differentiation of hMSCs, was conjugated to a photoreactive methacrylate group through hydrolytically degradable ester bonds. During network formation, this molecule is covalently linked into the network, resulting in pendant dexamethasone, which, over time, can release from the network due to hydrolysis of the ester bonds. Released, soluble dexamethasone can then interact with the encapsulated hMSCs, causing osteogenic differentiation as indicated by gene expression using real-time PCR and other techniques.

SESSION AA8/Z9: Joint Session: Nano Structured Surfaces and Materials in Biology and Medicine
Chairs: Joel Collier and Craig Halberstadt
Thursday Morning, December 2, 2004
Room 304 (Hynes)

8:30 AM *AA8.1/Z9.1

Self-Assembling Nanomaterials for Regenerative Medicine.

Samuel I. Stupp, Materials Science, Chemistry, and Medicine, Northwestern University, Evanston, Illinois.

The molecular and nanoscale design of synthetic environments that emulate extracellular matrices is critical for the future of regenerative medicine. These matrices need to manage cells into regenerative events that recapitulate development. Ideally, they should also hone in to the right tissues by self-assembly and be programmed to disappear into nutrients after completing their tasks. Chemistry's role lies in the supramolecular crafting of synthetic matrices that will allow cells to survive, control their proliferation, guide them in space or recruit them into the space of the matrix, and, most importantly, control their differentiation into a desired lineage. This lecture describes the design of peptide amphiphiles that form solid, cylindrical nanofibers designed to present artificially high densities of epitopes to cells with interesting biological consequences. This will be illustrated with experiments using neural progenitor cells that demonstrate how chemically designed matrices could help promote the regeneration of the central nervous system. Other systems are able to guide the differentiation of human stem cells and trigger events important in angiogenesis. Finally, systems of supramolecular nanofibers can be used to template the formation of inorganic crystals like those found in bone, leading to biomineralization in vivo under conditions that would not normally promote bone regeneration.

9:00 AM AA8.2/Z9.2

Self Assembled Monolayers: A Versatile Tool for Investigating Immune Cell Signaling on the Submicron Scale.

Wageesha Senaratne[1,2,3], Prabuddha Sengupta[2], Vladimir Jakubek[1], David Holowka[2,3], Barbara Baird[2,3] and Christopher K. Ober[1,3]; [1]Materials Science and Engineering, Cornell University, Ithaca, New York; [2]Chemistry and Chemical Biology, Cornell University, Ithaca, New York; [3]Nanobiotechnology Center, Cornell University, Ithaca, New York.

We utilize self-assembled monolayers (SAMs) as molecular templates to engage and cluster IgE-receptors on RBL mast cells with sub-micron scale spatial resolution. Bioactive templates were fabricated using electron beam lithography, and these consisted of gold arrays on silicon with patterns from 1 μm down to 200 nm. These gold arrays served as molecular tethering sites, enabling covalent binding of functionalized self-assembled monolayers of alkanethiols. The free ends of the monolayers were functionalized with 2,4-dinitrophenyl(DNP)-caproate-based ligands which interact specifically with anti-DNP IgE bound to its high affinity cell surface receptor, FcϵRI on RBL mast cells. Present results indicate that these patterned SAM arrays can function as a powerful tool for visualization and systematic characterization of submicron scale co-redistribution of membrane and cytosolic components in IgE receptor mediated immune cell signaling.

9:15 AM AA8.3/Z9.3

Topographically-controlled Orientation of Tobacco Mosaic Virus on Nanopatterned Substrates. Matthew J. D'Amato[1],

Nicholas L. Abbott[2,1], Barbara A. Israel[3], Mark A. Eriksson[4,1] and Robert W. Carpick[5,1]; [1]Materials Science Program, University of Wisconsin, Madison, Wisconsin; [2]Chemical and Biological Engineering, University of Wisconsin, Madison, Wisconsin; [3]Pathobiological Sciences, University of Wisconsin, Madison, Wisconsin; [4]Physics, University of Wisconsin, Madison, Wisconsin; [5]Engineering Physics, University of Wisconsin, Madison, Wisconsin.

Understanding the interaction of virus particles with surfaces containing engineered nanoscale topography is important for answering fundamental questions in biology as well as many applications in nanobiotechnology. The latter includes sensors for rapid and sensitive detection of viruses. We use intermittent-contact mode atomic force microscopy (IC AFM) to characterize the orientation and distribution of tobacco mosaic virus (TMV), an anisotropically-shaped rigid virus. TMV is adsorbed to nanopatterned polyurethane substrates with corrugated line patterning of various widths (30-2000 nm) and depths (5, 40, and >200 nm). On 40 nm deep, 200 nm wide patterns, we observe an unexpected bimodal distribution of orthogonal alignment angles that correlates with whether the TMV particle resides on a ridge or in a trench. While TMV particles in the trenches are preferentially aligned parallel to the long axis of the lines, particles on the ridges are aligned, surprisingly, transverse to them. A normal distribution of ridge particles exhibits a standard deviation of 38 degrees whereas the control surface (particles on flat polyurethane) has a 52 degree standard deviation – a much flatter distribution, as expected. IC AFM images of samples in air and liquid (aqueous buffer), and of samples with tailored surface chemistry (an amine-terminated self-assembled monolayer, with an appropriate isoelectric point to adsorb the charged virus particles from solution) are used to understand the effects of sample preparation and nanoscale patterning on orientation. We will discuss the mechanisms that lead to this alignment, including capillary- and flow-driven alignment and particle motion kinetics, and how this new effect can be exploited for virus sensing and assembly applications.

9:30 AM AA8.4/Z94

Antimicrobial Thin Films Produced via Polyelectrolyte Self-Assembly. Jaime Grunlan[1,2], John Choi[3] and Albert Lin[3]; [1]Mechanical Engineering, Texas A&M University, College Station, Texas; [2]Avery Research Center, Avery Dennison Corporation, Pasadena, California; [3]Biological Science, Biola University, La Mirada, California.

Antimicrobial wound dressing technologies currently used in the marketplace suffer from a variety of drawbacks that include slow activation, instability of ingredients, opacity, and staining of skin. In an effort to improve upon these shortcomings, highly effective antimicrobial thin films were prepared using a technique known as layer-by-layer (LBL) or electrostatic self-assembly (ESA). The LBL process is a thin film deposition technique involving the buildup of oppositely charged bilayers (i.e., anionic-cationic pairs) that are 1-100 nm thick, depending on a variety of factors. This technique has been used to successfully produce electrochromic, photovoltaic, and biocompatible thin films beginning with water-based starting materials. The ability to control coating thickness at the nanometer-level, easily insert variable components without altering the process, and deposit under ambient conditions are some of the key advantages of this technique. In most cases, these multilayer thin films are more transparent, higher performance, and easier to manufacture than current competitive techniques. In the present case, films were produced using polyethylenimine (PEI) as the polycation and poly(acrylic acid) (PAA) as the polyanion. Two antimicrobial agents, silver and cetrimide, were added to separate PEI solutions prior to deposition onto poly(ethylene terephthalate) (PET) film. Thin films containing 16-bilayers (anionic-cationic pairs) were tested for antimicrobial activity using the Kirby-Bauer method. PET with no antimicrobial coating showed no zone of inhibition (ZOI) against S. aureus or E. coli, which indicates that PET has no intrinsic antiseptic properties. With the addition of 20mM silver nitrate to the PEI solution, the resulting 16-bilayer film showed a 1 mm ZOI against S. aureus and a 2 mm ZOI against E. coli. This result was very comparable to that observed for commercial wound dressings that use silver as their active ingredient and the LBL films have %T > 96% across the visible spectrum. By producing the same 16-bilayer film with cetrimide instead of silver, the ZOI grew to 11 mm against S. aureus and 3 mm against E. coli. Cetrimide is a quaternary ammonium molecule with a small hydrocarbon tail and is believed to be much more mobile than silver in a moist environment. In shake flask testing, however, the silver-based film lasts three days in contrast to the cetrimide film that is no longer active after one 24-hour test period. This indicates that silver is a better agent for sustained release. The ESA technique is particularly advantageous because multiple ingredients can be easily deposited into one thin film to create hybrid functionality (e.g., silver combined with cetrimide for strong initial kill along with sustained antimicrobial release). The versatility in antimicrobial performance demonstrated here has the potential to extend into drug delivery and other biomedical applications requiring hybrid functionality.

9:45 AM AA8.5/Z9.5

Effect of Nano- to Micro-Scale Surface Topography on the Orientation of Endothelial Cells. Pimpon Uttayarat[1], Peter I. Lelkes[2] and Russell J. Composto[1]; [1]Materials Science and Engineering, University of Pennsylvania, Philadelphia, Pennsylvania; [2]School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, Pennsylvania.

A model vascular graft of cross-linked polydimethylsiloxane (PDMS) has been modified with protein and varied topographically to promote endothelial cell attachment as well as to guide cell-substrate interactions. PDMS with a smooth surface (RMS roughness 0.5 nm) and grating-textured surfaces, having channel depths of 100 nm, 500 nm, 1 μm and 5 μm, and lateral width of 4 μm, are fabricated. While the pre-adsorbed fibronectin promotes cell adhesion, the underlying topographic features provide a contact guidance that influences cell morphology and cell orientation. Using phase contrast microscopy after seeding cells for 1, 4, 24 and 48 h, cell elongation and alignment parallel to the grating direction increases monotonically with increasing channel depth, reaches maximum orientation at 1 μm, and then slightly decreases at 5 μm. By fluorescence staining of F-actin and vinculin, cytoskeleton and focal contacts are observed to preferentially orient parallel to the grating direction on textured surfaces having depths of 1 and 5 μm. Confocal and scanning electron microscopies show that cell protrusions extend into channels and also along the side walls of the channels. Cell proliferation is found to be independent of surface topography. At confluence, cell orientation is retained on textured PDMS surfaces. Using surface topography to create contact guidance provides an alternative pathway to obtain endothelial cell alignment, similar to flow in the natural blood vessel.

10:30 AM AA8.6/Z9.6

Competitive Adsorption of Plasma Proteins on Dextran-Modified Silicon Surfaces. Michela Ombelli[1], Samuel Bernard[1], Lauren Costello[2], Qing Cheng Meng[1], Russell J. Composto[2,3] and David M. Eckmann[1,4]; [1]Department of Anesthesia, University of Pennsylvania, Philadelphia, Pennsylvania; [2]Department of Materials Science and Engineering, University of Pennsylvania, Philadelphia, Pennsylvania; [3]Center for Bioactive Materials and Tissue Engineering, University of Pennsylvania, Philadelphia, Pennsylvania; [4]The Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, Pennsylvania.

The initial response of blood exposed to an artificial surface is the adsorption of blood proteins which triggers a number of biological reactions such as inflammation and blood coagulation and competitive protein adsorption plays a key role in the hemocompatibility of the surface. The synthesis of nonfouling surfaces is therefore one of the major prerequisites for devices for biomedical applications. Dextran, a highly hydrophilic, neutral polysaccharide, is one of the main components of the endothelial cell glycocalyx and has the ability of reducing nonspecific protein adsorption and cell adhesion and, therefore, is generally coupled with a wide variety of surfaces to improve their biocompatibility. We have developed a procedure for covalently binding dextran on silicon wafers pre-activated by amine terminated APTES and we have been able to reach a high level of control on the thickness, wettability and roughness of the coatings by varying the molecular weight, polydispersity and the degree of chemical oxidation of the dextrans. We have also demonstrated that monodisperse, high molecular weight dextran coatings applied on microcapillary glass tubes show bubble adhesion properties almost identical to the values found for *invivo* and *exvivo* experiments of microvascular gas embolism. In the present research effort we focus on a detailed investigation of competitive plasma protein adsorption on dextran-modified silicon surfaces so that we can use our ability of tuning the physical and morphological properties of the coatings to optimize their long-term ability to control biofouling. Adsorbed mixtures of bovine serum albumin and bovine fibrinogen are eluted from the surfaces by exposure to acetonitrile and/or sodium dodecyl sulfate solutions. The freeze-dried desorbed protein fractions are then separated and quantified by high-performance liquid chromatography. Competitive adsorption of proteins from more complex mixtures and eventually from dilute bovine plasma on the dextran-grafted silicon surfaces has been evaluated using the same methodology. Supported by NIH Grants R01 HL60230 and R01 HL67986

10:45 AM AA8.7/Z9.7

Surface-Patterned PEO Nanohydrogels to Control Cell-Substrate Interactions. Peter Krsko[1], Ye Hong[1], Keri Vartanian[2], Herbert M. Geller[2] and Matthew R. Libera[1]; [1]Chemical, Biomedical and Materials Engineering Department, Stevens Institute of Technology, Hoboken, New Jersey; [2]National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland.

Signal transduction to cells by extracellular matrix occurs at the molecular level, and there is mounting evidence that the nanoscale spatial distribution of ECM signaling proteins can significantly affect cytoskeletal organization, gene expression, and process development. Our goal is to use surfaces whose affinity for proteins and cells is modulated at nano and micro length scales to control cell-substrate interactions at the subcellular level. In contrast to monolayer surface-patterning technologies such as soft lithography and dip-pen nanolithography, we are patterning hydrogel films and using these to control the presentation and spatial distribution of adhesion-promoting ECM proteins such as laminin and fibronectin. We are creating nanohydrogels and microhydrogels using low-energy focused electron beams to locally crosslink PEO films and have made thin-film gels on silicon substrates with gel diameters as small as 100 nm and as large as 1 cm. By varying the incident electron dose, we control the nano/micro gel swelling, and we can control the one-dimensional swelling anywhere from unity to as much as fifteen times. Consistent with the large body of literature on PEG and PEGylated surfaces, we find that fibronectin and laminin do not adsorb onto lightly crosslinked, high swelling PEO microhydrogels. Neurons, macrophages, and fibroblasts are also repelled by these surfaces. However, Fn and Ln both adsorb significantly onto heavily crosslinked gels, and, in turn, these proteins signal for cell adhesion. We have also demonstrated the alternate approach of covalently binding Fn and Ln to high-swelling patterned functionalized PEG. By exploiting the ability to modulate these surfaces at micron and sub micron lengths scales together with the flexible patterning capabilities of modern electron-optical systems, we have been able to create surfaces which control whether, where, and what shape neurons, macrophages, and fibroblasts adhere.

11:00 AM AA8.8/Z9.8

Multicomponent protein patterning with photogenerated polyelectrolyte bilayers. Junsang Doh[1] and Darrell J. Irvine[2,3]; [1]Department of Chemical Engineering, MIT, Cambridge, Massachusetts; [2]Department of Material Science and Engineering, MIT, Cambridge, Massachusetts; [3]Biological Engineering Division, MIT, Cambridge, Massachusetts.

Fabrication of surfaces patterned with multiple proteins organized on more than one length scale can be useful for mimicking cell surfaces or the extracellular environment. Such surfaces can be used to address fundamental biological questions related to cell-cell interactions or cell-extracellular matrix interactions, since protein ligands can be presented to cells in a controlled manner. To achieve this goal, we synthesized a novel photoresist polymer that can be processed under mild aqueous conditions, and developed a new patterning strategy based on the unique properties of this photoresist. A random terpolymer composed of o-nitrobenzyl methacrylate (o-NBMA), 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. Upon UV exposure (250nm) of films of the resist, the o-nitrobenzyl group of the resist was cleaved and carboxylic acid was generated. Polyacids generated by UV irradiation showed pH dependent solubility in water; exposed material was insoluble in water at low pH, but dissolved above pH 6.6. In addition to its pH-dependent development, the resist could be used to create photogenerated polyelectrolyte bilayers: When the photoresist (PR) was spincoated over a poly(allylamine)-coated substrate, exposed to UV, and developed with PBS (pH 7.4, 10mM sodium phosphate and 140mM NaCl), the bulk of the PR film was dissolved, but a polyelectrolyte bilayer formed in situ at the PR/polycation interface remained bound to the substrate. Using these novel characteristics, we developed a photolithographic process using a lift-off approach to pattern two proteins into two different micron scale domains without exposing either biomolecule 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 particles (from nanometer size quantum dots to submicron size polymeric particles) to implement multiple length scale ligand presentation. Currently, we are applying this patterning methodology to create surfaces presenting T cell-stimulating protein ligands, in order to study the effect of ligand density and spatial distribution on T cell function.

11:15 AM AA8.9/Z9.9

Specific Antibody-Antigen Interaction on a Functional Lipid-Membrane Vesicles Modified Surface. Hea-Yeon Lee, Ho-Sup Jung and Tomoji Kawai; ISIR-SANKEN, Osaka Univ., Osaka, Japan.

Recently, the possibility of analyzing high-throughput proteomics using microarrays has attracted great interest. Especially, a specific array of capture antibody that maintains its bio-reactivity is a critical milestone for applications of future immunosensors because immobilization is based on very specific interaction between the immobilized ligand and its counterpart We will present a strong specific antibody-antigen interaction on a functional lipid-membrane vesicle (liposome, FLVs) modified gold surface using streptavidin-biotin interaction. Real-time quartz crystal microbalance (QCM) response and surface plasmon resonance (SPR) after reaction of a target antigen HSA was observed only for a specific immobilized anti-HSA antibody. Electrochemical immunoassay by specific antibody-antigen (Ag-Ab) interactions showed variation of redox peak current only on a specific-array electrode. This specific protein array system using FLVs may be useful for immune disease therapy. Acknowledgements: Financial support from the New Energy and Industrial Technology Development Organization (NEDO) is gratefully acknowledged.

11:30 AM *AA8.10/Z9.10

Nanofeaturing biomaterials for specific cell responses. Adam Sebastian Curtis, Centre for Cell Engineering, University of Glasgow, Glasgow, United Kingdom.

A range of fabrication methods including electron beam lithography or polymer demixing have ben used to create masters from which nanotopography can be embossed or molded onto a range of polymer surfaces. Related methods can be used to print nanochemistry onto such surfaces. These materials have been used to examine the behaviour of a variety of mammalian (including human) cell types to nanofeatures. Effects on cell adhesion,cell movement, cytoskeletal organizarion amd gene expression will be reported and reviewed. Effects on adhesion are related to the dimensions and spacing of nanofeatures. The same applies to the organization of the cytoskeleton. Extensive changes in gene expression can be detected with some types of nanofeature resulting in partial phenotype shifts. These phenotype changes are reversible. Random arrangements of nanofeatures are little different in their effects on these cells from those seen on ultraflat surfaces. The mechanisms that may produce these responses will be discussed and the possible uses of such surfaces in medical devices will be outlined.

SESSION AA9: Nano and Micron Biomaterials
Chairs: Edward Botchwey and Helen McNally
Thursday Afternoon, December 2, 2004
Room 304 (Hynes)

1:30 PM *AA9.1

Living Three-Dimensional Microfabricated Constructs for the Replacement of Vital Organ Function. Cathryn A. Sundback, [1]Surgery, Massachusetts General Hospital, Boston, Massachusetts; [2]Harvard Medical School, Boston, Massachusetts.

Tissue engineering is a multi-disciplinary field that combines the principals of engineering, material science, and cell biology to address the problem of donor organ shortage for an increasing population of patients with end-stage organ failure. To date, most tissue engineering systems have relied on de novo vascularization from the host to support the implanted graft, an approach that has been successful for thin tissues with low metabolic activities, like cartilage and skin, but has not been promising for thick and highly metabolic tissues such as liver, kidney, or heart. The primary challenge for tissue engineering of vital organs is the requirement of establishing a vascular supply for the exchange of oxygen and nutrients at the time of implantation, as significant cell death occurs when cells are more than several hundred microns away from a vascular supply. To overcome this limitation, three-dimensional living tissue constructs have been created based on a microfluidics model to simulate the fractal topology of the native microvasculature. Advanced microfabrication technologies have been applied to fabricate microvascular networks from thin sheets of replica molded polydimethylsiloxane (PDMS) and other biocompatible and biodegradable polymers. Polymeric scaffolds are created by bonding these vascular network compartments to parenchymal compartments, which house organ-specific cells; these compartments are separated by nanoporous polymer membranes, which permit effective exchange of oxygen and nutrients yet maintain separation of blood cell components from organ-specific cells. Complementary in vitro and in vivo tests have been conducted with these devices. Endothelial cells have been seeded into the vascular compartments and have attached in monolayers to the walls of these structures. In both in vitro and in vivo studies, flow through the microvascular network has been shown to be uniform and predictable and the flow path to be patent. Additional studies have been conducted which demonstrate long-term human hepatocyte (HepG2/C3a) viability and function. During in vitro studies with continuous perfusion of the vascular network, hepatocytes remained viable and functional, as exhibited by increasing levels of hepato-specific proteins, like albumin, transferrin, and alpha-fetoprotein, as well as by effective cytochrome P450 function in drug metabolism studies. When these devices were implanted, HepG2 cells maintained viability and demonstrated

increasing levels of alpha-fetoprotein. Together these results represent a promising step towards the ultimate goal of creating readily available, living tissue engineered organ replacements.

2:00 PM AA9.2
Attachment of Osteoblasts to Nano-Textured Surfaces.
Alvaro Mata[1], Aaron Fleischman[1], Shuvo Roy[1], Sharon Miller[2], Bruce Banks[2] and Ronald J. Midura[1]; [1]The Cleveland Clinic Foundation, Cleveland, Ohio; [2]Electro-Physics Branch, NASA Glenn Research Center, Cleveland, Ohio.

The viability of musculoskeletal cells is highly dependent on their attachment to the extracellular environment. Cell attachment is determined by the interaction of cell membrane receptors with specific amino acid sequences of preadsorbed or cell secreted proteins (e.g. R-G-D sequence from fibronectin (Fn)). The present study investigated whether osteoblasts could attach to a culture substratum through a surface texture-dependent mechanism.

Chlorotrifluoroethylene (Aclar®) substrates were textured by atomic oxygen bombardment (NASA Glenn Research Center, Cleveland, OH). Four test groups were used: un-textured (A); and three texture groups with maximum feature sizes of < 0.5 μm (B), 2 μm (C), and 4 μm (D). Substrates were coated with Fn to serve as positive control, and the non-adhesive protein bovine serum albumin (BSA) to serve as the experimental group. X-ray photoelectron spectroscopy (XPS) was used to analyze the surface oxygen concentration after protein coating. Osteoblasts were inoculated in BSA media and allowed to adhere for either 1 or 4 hours (h), at which time non-adherent cells were removed. At 16 h cells were fixed, counted, and immunostained for nuclei, focal adhesion contacts, and actin cytoskeleton. XPS revealed no significant difference in oxygen surface concentration between all four Fn and BSA coated surfaces. Fn coated surfaces exhibited similar cell number on A, B, and C (80%), and lower cell number on D (53%). On BSA coated surfaces, un-textured surface A exhibited no cell attachment, while textured surfaces B, C, and D exhibited 9%, 32% and 16% cell attachment, respectively. At 16 h of incubation, adherent osteoblasts on textured surface C exhibited focal adhesion contacts and microfilament stress-fiber bundles. These results indicate: a) that nano-textured surfaces in the absence of exogenous adhesive proteins can facilitate osteoblast adhesion, and b) that there was a preference for cell attachment on the intermediate size textures (0.5-2 μm).

2:15 PM AA9.3
Fabrication of Novel Porous Chitosan Matrices as Scaffolds for Bone Tissue Engineering.
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The biopolymer chitosan is receiving much research interests for biomedical applications recently1. A number of studies have demonstrated the feasibility of fabricating porous chitosan matrices for bone tissue engineering using a freeze-dried method. However, these scaffolds presented low mechanical properties. The objective of this study was to develop porous 3-D chitosan matrices based on microspheres with interconnected pore structure and having high mechanical properties ideal for bone tissue engineering. Chitosan matrices were fabricated using sintered microsphere technique developed in our laboratory2. Briefly, chitosan microspheres were prepared by an ionic crosslinking method using tripolyphosphate ions as the crosslinking agent3. Microspheres were then mixed with a small amount of 3% acetic acid and packed in appropriate mold and heated at 85 °C for 100 min. Mechanical test on matrices (5mm×10mm) was performed using an Instron 5544 mechanical tester. Student/s t-test was used for statistical analysis. For cell studies, matrices (5mm×10mm) were sterilized with 70% ethanol and seeded with MC3T3-E1 osteoblast like cells and maintained under standard cell culture conditions. Cell adhesion and proliferation on matrices were visualized using SEM. We found that chitosan solution concentration has a significant effect on microsphere formation. At low concentration (<1%) no microsphere formation was observed. Concentrations above 1.5% resulted in the formation of uniform solid spheres as evidenced from SEM. Furthermore, we observed that increase in chitosan solution concentration resulted in a significant increase in microsphere size however the 3-D matrices showed high mechanical strength with no significant variation in compressive modulus with microsphere diameters (Table 1). Furthermore, the 3-D matrices showed high osteocompatibility as evidenced by the adhesion and proliferation of MC3T3-E1 cells with time. By 4 days, the cells adhered on the matrices with an apparent spreading, however, a more robust cell spreading was observed after 7 days. After 14 days, SEM revealed cellular infiltration and cell migration between individual microspheres within the 3-D chitosan matrices. The present study demonstrated the feasibility of developing porous 3-D chitosan matrices with high mechanical properties and osteocompatibility as scaffolds for bone tissue engineering. This work is supported by NSF INT-0115595. References 1. E. Khor. Chitin: fulfilling a biomaterials promise. Elsevier. 2001. 2. M.D. Borden et al. Biomaterials 23: 551-559, 2002 3. F.L. Mi et al. J Appl Polym Sci 74: 1868-1879, 1999

2:30 PM AA9.4
Ex Vivo Study of C2C12 (Skeletal Muscle) Myoblasts Seeded on an Electrospun Membrane of a Biodegradable Polyurethane for Use in Tissue Engineering.
Danielle Nicole Rockwood[1], Joanna Fromstein[2], Kimberly A. Woodhouse[2] and John F. Rabolt[1]; [1]Materials Science and Engineering, University of Delaware, Newark, Delaware; [2]Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada.

The goal of tissue engineering is to mimic natural systems for therapeutic treatment of failing tissue. Polymers are being studied to either induce tissue growth or to provide temporary scaffolds for cells to be implanted . In vivo, the extracellular matrix (ECM) is a network of fibrous proteins and adhesion molecules that provide structural support for cells. In order to mimic the ECM architecture, electrospinning has been used to create membranes of isotropically oriented polyurethane fibers. Fibers produced by electrospinning offer many advantages that comply with tissue engineering parameters. Some of these benefits include high-surface to volume ratio, interconnected porous network, and small diameter fibers. Each of these properties promotes cell adhesion and migration. The segmented-polyurethane used in this research has been synthesized using an amino acid-derived diisocyanate and a phenyalanine-based chain extender . The chemical architecture of this polymer joins the necessary functional components (i.e., hydrolyzable groups and enzymatic cleavage sites) to promote in vivo degradation in order to provide space for transplanted cells to excrete their own matrix proteins and develop into tissue. Due to the amino acid-based architecture, it is expected that the material will prove to be non-toxic to cells . In addition, the segmented nature of the polyurethane allows for elastomeric behavior thus providing the mechanical properties required to respond to physiological stresses. The processing parameters for electrospinning the polyurethane have been tailored to this specific application. Because it is important that the processing does not alter the polymer before beginning skeletal muscle cell attachment studies, Raman spectroscopy has been used to ensure the conformational integrity of the polymer before and after processing. Preliminary cell adhesion and cytotoxity tests show that these membranes can support cells and work is being done to co-spin the polyurethane with laminin to further enhance cell adhesion.

3:15 PM AA9.5
Nanolithography with Peptides on GaAs Surfaces.
Albena Ivanisevic[2,1] and Youngnam Cho[2,1]; [1]Chemistry, Purdue University, West Lafayette, Indiana; [2]Biomedical Engineering, Purdue University, West Lafayette, Indiana.

Atomic force microscopy (AFM) was used to fabricate well-defined peptide templates onto GaAs surfaces via Dip-Pen Nanolithography (DPN). DPN is a powerful technique to write specific organic and/or inorganic molecules onto a surface with an AFM tip. In this work, DPN was used to construct arrays of peptides with nanometer features. TAT peptides (e.g. CGISYGRKKRRQRRR) which exhibit rapid uptake in cells, were patterned onto the surface in either contact or tapping mode. Several techniques were used for the characterization of the modified surfaces: X-ray photoelectron spectroscopy (XPS), Fourier Transforms Infrared (FT-IR) spectroscopy, contact angle, and Ellipsometry. Transmission FT-IR provided structural information such as peptide conformation. The complementary analysis of XPS, ellipsomentry, and contact angle confirm the binding of the peptide onto the substrates and allowed to quantify the density of immobilized peptides on a given surface. Furthermore, the nanoscopic features were successfully used in recognition experiments where an RNA sequence with a loop structure, known for its specific interaction with the peptide, was tested. The results in this report indicate that one can use nanolithographic strategies to pattern GaAs surfaces, and therefore provide a proof-of-concept experiment that can be transferred in complex microfabricated semiconductor architectures.

3:30 PM AA9.6
Detection of Epidermal Growth Factor Receptor by Affinity Microcontact Printing and Liquid Crystal Signal Amplification.
Chang-Hyun Jang[1], Matthew Tingey[1], Paul Bertics[2] and Nicholas Abbott[1]; [1]Chemical and Biological Engineering, University of Wisconsin, Madison, Wisconsin; [2]Department of Biomolecular Chemistry, University of Wisconsin, Madison, Wisconsin.

The over-expression and the constitutive activation of epidermal

growth factor receptor (EGFR) are associated with a variety of cancers. Many efforts to develop anti-cancer agents have been limited by efficient methods for assessing alterations in EGFR kinase activity and expression in tissues from treated individuals. This presentation will report a new method based on affinity microcontact printing (αCP) and liquid crystal (LC) that can detect EGFR from crude cell lysates using small amounts of sample. The PDMS stamp is functionalized by the covalent attachment of capture molecules (anti-EGFR). The affinity-captured EGFR is then stamped in an oriented manner onto solid substrates. The orientational behavior of the thermotropic LC, 4-cyano-4'-pentylbiphenyl, provides high optical contrast between regions of the surface supporting and not supporting printed EGFR. Ellipsometric measurements show an increase in optical thickness on antibody-modified surfaces upon binding of EGFR as well as on amine SAMs after stamping. The high sensitivity of LC and the capability provided by αCP to detect binding events within localized regions of the surface provide the potential for this method to be used for future clinical cancer research.

3:45 PM AA9.7
Processing and Development of Nano-Scale HA Coatings for Biomedical Application. Brent Thomas[2] and Afsaneh Rabiei[1]; [1]Mechanical and Aerospace Engineering Department, North Carolina State University, Raleigh, North Carolina; [2]Materail Science and Engineering Department, North Carolina State University, Raleigh, North Carolina.

The material proposed in the present study as a more effective orthopedic/ dental implant coating is a functionally graded Hydroxyapatite coating with graded Crystallinity across the thickness of the film. The present study aims to increase the service-life of an orthopedic/ dental implant by creating materials that form a strong, long lasting, bond with the Ti substrate as well as juxtaposed bone. To approach this goal, the HA coating has been produced with larger grain size and Crystallinity at the interface of the coating with the substrate. The Crystallinity and the grain size have been decreased towards the surface of the coating. The health relatedness of the new material is to increase bonding between an implant and juxtaposed bone so that a patient who has received joint or dental replacement surgery may quickly return to a normal active lifestyle. The desire to introduce this innovative technology to the field of implantology is based on three primary advantages of the process over existing technology used to apply HA coatings to implants. First, the chemical composition, Crystallinity and grain structure of the applied coating can be precisely controlled over a wide range of values, without the need for post deposition annealing. This can save both processing time and cost. Second, the HA coatings can be deposited as thin films from 1 to 2 micron thick, much thinner than coatings applied using plasma spray technology. Thinner coatings can provide a higher interfacial strength and better fracture resistance than thicker coatings. Additionally, this processing technique will cause the highest adhesion bonding between the HA coating and substrate as well as the coating and the juxtaposed bone that will lead to longer lifetime of the implant. Microstructural and mechanical properties of the new coating material have been studied as well as the bioactivity and osseointegration properties.

4:00 PM AA9.8
Development of Porous Hyaluronic Acid Scaffolds for Wound Healing Applications. Ezra Katz[1], Eric Mansfield[2], Kaustabh Ghosh[3], Miriam Rafailovich[3], Glenn D. Prestwich[4] and Richard Clark[3]; [1]South Shore Yeshiva High School, Hewlett, New York; [2]Smithtown High School, Smithtown, New York; [3]Biomedical Engineering, Stony Brook University, Stony Brook, New York; [4]Medicinal Chemistry, University of Utah, Salt Lake City, Utah.

Successful tissue repair and regeneration using an appropriate biomaterial depends on the ability of cells to migrate into the 3D-engineered scaffold. Hyaluronic acid (HA) has been implicated in normal wound healing and has therefore been successfully used in wound healing applications. Native HA is, however, rapidly degraded in vivo. Chemical cross-linking of derivatized HA is therefore routinely employed to increase its residence time. Although engineered scaffolds are rendered biologically active, cell migration is severely impaired in the absence of an appropriate porous network. We therefore aim to create a biologically active 3D porous HA scaffold that allows for invasive cell migration. In this study, thiol-fucntionalized HA was crosslinked using poly(ethylene glycol) diacrylate to obtain a 3D hydrogel scaffold. Four different methodologies were employed to introduce porosity: phase separation, lyophilization, gelation around a template and electrospinning. To verify if a porous network was created, 1 micron fluorescent beads were allowed to diffuse into the scaffold. Images of this diffusion were obtained by confocal microscopy every 60 s for 30 minutes, over a depth of up to 1000 microns. All formulations resulted in a porous network, with a direct relationship between pore diameter and bead flow rate. Additionally, human dermal fibroblasts were seeded on each of the scaffolds and then imaged with confocal microscopy to determine the extent of invasive migration. It was found that phase separation allows for the creation of pores of at least 10-15 microns as quantified by cell migration data. Lyophilization created large interconnected pores; the method. However, did not allow for a precise control over pore location or diameter. Gelation around the template proved highly reproducible but was limited by size restrictions that hindered microscopic porosity. Electrospinning will be further explored as a method for introducing porosity, as initial studies have shown it to be a viable technique. The ability of these four methods to introduce pores allows these HA scaffolds to be successfully used as templates for wound healing applications. Work supported in part by the NSF-MRSEC program.

4:15 PM AA9.9
Selective Laser Sintering of Polycaprolactone Bone Tissue Engineering Scaffolds. Suman Das and Brock Partee; Mechanical Engineering, University of Michigan, Ann Arbor, Michigan.

Present tissue engineering practice generally requires the use of porous, bioresorbable scaffolds to serve as temporary 3-D templates to guide cell attachment, differentiation, proliferation, and subsequent regenerate tissue formation. Such scaffolds are anticipated to play an important role in allowing physicians to simultaneously reconstruct and regenerate damaged human tissue such as bone, cartilage, ligament and tendon. Recent research in the area of tissue engineering strongly suggests that the choice of scaffold material and its internal porous architecture significantly influence the type of regenerate tissue that is grown and its resulting mechanical properties. However, a lack of versatile biomaterials processing and fabrication methods capable of meeting the complex geometrical and compositional requirements of tissue engineering scaffolds has slowed progress towards fully testing these promising findings. It is widely accepted that layered manufacturing methods such as selective laser sintering (SLS) have the potential to fulfill these needs. Polycaprolactone (PCL) is one of the most widely investigated biocompatible, bioresorbable materials for tissue engineering applications. We have used SLS to fabricate complex tissue engineering scaffolds composed of PCL and its composites with hydroxyapatite (HA) and tri-calcium phosphate (TCP). Such scaffolds were found to be dimensionally accurate to within 3-8% of design specification and near fully dense (>99%). Tensile and compressive mechanical properties of SLS processed PCL were evaluated and found to be comparable to published data. As a proof of concept, SLS was then used to fabricate minipig and human condyle scaffolds directly from computationally optimized digital designs based on computed tomography of actual bone. These scaffolds incorporated complex porous internal architectures with 0.7-2mm features. In this work, we report on porous scaffold design and fabrication, optimal SLS processing parameter development, mechanical property measurements, and structural characterization via optical microscopy and micro-computed tomography.

4:30 PM AA9.10
Two Photon Excitable Fluorescent Dye and/or Infrared Emitting Dye Encapsulated in Biodegradable Polymer Nanoparticles: Novel Preparation Technique of Polymeric Nanoparticles for Nanomedical Application. Koichi Baba[1], Tymish Ohulchanskyy[1], Qingdong Zheng[1], Tzu-Chau Lin[1], Hachiro Nakanishi[2] and Paras N. Prasad[1]; [1]Chemistry, Institute for lasers, photonics and biophotonics, Buffalo, New York; [2]Chemistry, Institute for Multidisciplinary Research for Advanced Materials, Sendai, Miyagi, Japan.

Nanomedicine is an emerging field that deals with utilization of nanotechnology to develop new methods of minimally invasive diagnostics for facilitating targeted drug delivery, effective therapy, etc. Many formulations of anticancer drugs or therapeutically attractive dyes (e.g. multiphoton excitable fluorescent dyes, near infrared range fluorescent (IR) dye) are poorly soluble in water. Because of their hydrophobicity, the parenteral administration is highly hampered. Nanoparticles provide a novel mechanism for delivery of hydrophobic formulations of these drugs/dyes. We have developed an effective preparation technique of organic nanoparticles which can encapsulate dye/drug using a well-known biodegradable polymer. Our synthetic technique is simple, produces ultrafine poly(DL-lactide-co-glycolide) nanoparticles which are dispersible in aqueous media. The size of particles can be controlled within few tens of nanometers to several micrometers. A hydrophobic two-photon dye was encapsulated into the biodegradable nanoparticles during synthesis. These dye encapsulated nanoparticles were 50 nm in diameter as determined by SEM. Subsequent laser scanning microscopy revealed the *invitro*uptake of these particles into cultured cells (KB, oral adenocarcinoma cell line). This polymeric nanoparticle formulation can potentially serve a biodegradable drug delivery methodology for other entities such as photosensitizers (visible and IR dyes etc). These biodegradable nanoparticles offer new opportunities for diagnostic imaging and therapy.

4:45 PM AA9.11
Enhanced Mineral Deposition by Osteoblasts Cultured on Nanophase Metals. Brian C Ward and Thomas Jay Webster; Biomedical Engineering, Purdue University, Lafayette, Indiana.

Many engineers and surgeons trace orthopedic implant failure to the current inability of biomaterials to match the physical properties of surrounding bone. For instance, immature bone has an average inorganic mineral grain size of 10-50 nm while mature bone has an average inorganic mineral grain size of 20-50 nm and is 2-5 nm in diameter. However, most modern implants are smooth at the nanometer level since they are composed of constituent micron grain sizes. Importantly, researchers have shown that nanostructured, or materials composed of grains/particles less than 100 nm, enhance cell function. Materials well-tested to date include ceramics, polymers, and composites thereof. In a recent study, researchers have added metals (specifically, c.p. Ti, Ti6Al4V, and CoCrMo) to the list of materials that when created at the nanoscale, increase the adhesion of osteoblasts (bone-forming cells). However, long-term (days to months) functions of osteoblasts on nanostructured metals have yet to be determined. This was the focus of the current study. Results of this study provided the first evidence of increased calcium and phosphorus mineral deposition on nanophase compared to respective conventional Ti, Ti6Al4V, and CoCrMo metals. Since the metals prepared in the present study were composed of nanometer particles instead of conventional particles, these alloys were identical to current orthopedic implant alloys except for surface topography. By doing so, this study continues to provide justification for the pursuit of nanophase metals to design the next generation of more successful orthopedic implants.