Multi-Scale Bioengineering

Controlled One-on-One Encounters between Immune Cells and Microbes Provide a New Window into the Mechanisms of the Innate Immune Response, **VOLKMAR HEINRICH** (Department of Biomedical Engineering, University of California Davis, 451 East Health Sciences Drive, Davis, CA 95616; vheinrich@ucdavis.edu).

Infectious, autoimmune, and chronic inflammatory diseases present a rising threat, underlining the need for a deeper understanding of how the immune system recognizes—or fails to recognize—pathogens. Many prevailing gaps in our knowledge can be attributed to an imbalance between the cross-disciplinary nature of the immune response and a lack of truly interdisciplinary studies of the underlying mechanisms. Moreover, there is mounting evidence that insight into the human immune system cannot be reliably inferred from animal models or cell lines.

Immune cells are perhaps the most relevant functional units of the immune system. Mechanistic analyses of single-live-cell encounters with microbes deserve a front-and-center place in the study of host-pathogen interactions. Such enquiries should be integrative, encompassing the spectrum of mechanisms by which immune cells home in on (by chemotaxis), take hold of (through adhesion), and internalize (by phagocytosis) microbial pathogens. We here discuss new, interdisciplinary approaches to study independently single-cell chemotaxis, adhesion, and phagocytosis by analyzing one-on-one encounters between immune cells and bacteria or fungi. These experiments use non-adherent cells, thus preventing premature cell activation. They offer unprecedented control over cell-microbe contacts and have a time resolution of fractions of a second. They also facilitate an essentially axisymmetric configuration of the cell-microbe pair, which is viewed from the side, allowing us to visualize the interaction with unique clarity. These experiments have been validated with various types of human immune (and other) cells, and have already revealed insight into cellular behavior that had been inaccessible to traditional techniques.

Scalable Drug Infusion Technologies, **ELLIS MENG** (Department of Biomedical Engineering and Ming Hsieh Department of Electrical Engineering, University of Southern California, 1042 Downey Way, DRB-140, Los Angeles, CA 90089-1111; ellis.meng@usc.edu).

Controlled drug administration is a critical tool for scientific investigation, drug discovery, and most importantly, the treatment of chronic diseases and conditions. Animal models, typically starting in rodents, are commonly employed in the early investigation of potential therapies. However, methods of and technologies for administration available for use in small animals are limited and do not allow for advanced temporal and spatial control of delivery. These factors severely limit the ability to maximize the therapeutic efficacy of infused agents and management of associated side effects. Thus, a new paradigm for studying and treating diseases using next generation delivery pumps enabled by biomedical microelectromechanical systems (bioMEMS) was introduced. These pumps feature on-demand dosing with operation over a wide dynamic range of flow rates, remote and wireless operation, accurate electrolysis-based pumping, a refillable drug reservoir, and broad drug compatibility. Our advanced bioMEMS pumps infuse drugs directly to the site of therapy and enable chronic drug therapy with only a single surgical procedure. The drug infusion technologies developed were specifically designed to be scalable so as to enable infusion pumps for miniaturized for pre-clinical research and devices large enough to accommodate clinical needs. This talk will present the infusion approach and different applications enabled by the technology.

Biological Insights from Measuring the Physical Properties of Cells and Organisms, **WILLIAM H. GROVER** (Department of Bioengineering, University of California, Riverside, 219 MS&E Building, 900 University Ave., Riverside, CA 92521; wgrover@engr.ucr.edu).

The fundamental physical properties of a cell or microorganism—its mass, volume, and density—provide a unique window into the biology of that cell or microorganism. Since a cell's physical properties are largely determined by its biological state, changes in cell state can manifest themselves as measurable changes in the cell's physical properties. And since all cells have these physical properties, techniques for measuring these properties are truly "label free" and suitable for studying all types of cells and microorganisms.

In this presentation, I will share results from our most recent measurements of the physical properties of different cell types. We measure the mass, volume, and density of single living cells using Suspended Microchannel Resonator (SMR) mass sensors. The SMR consists of a vibrating silicon cantilever with an embedded microfluidic channel. When a cell passes through the channel, the resonance frequency of the vibrating cantilever changes by an amount proportional to the mass of the cell.

By using the SMR to weigh a cell in two fluids of different densities, we can determine the mass, volume, and density of the cell. Using this technique, we have found that clear changes in cell density accompany many biologically important processes, including cell growth, apoptosis, and reaction to toxicant exposure. These cell density changes are often statistically more significant than the underlying mass and volume changes, suggesting that the density of a cell or microorganism is a unique and meaningful biomarker for a wide range of biological processes.

A Kinetic Model of Multivalent Nanoparticle Binding, **JERED B. HAUN[1,2,3*] and MINGQIU WANG[1]** ([1]Department of Biomedical Engineering, [2]Department of Chemical Engineering and Materials Science, [3]Chao Family Comprehensive Cancer Center, University of California Irvine, 3107 Natural Sciences II, Irvine, CA, 92617; jered.haun@uci.edu).

Targeted delivery of imaging or therapeutic agents holds tremendous potential to transform detection and treatment of diseases such as cancer and atherosclerosis. However, this potential has remained largely untapped clinically because molecularly-targeted agents have failed to provide sufficient delivery yield and/or specificity. Thus, new strategies are needed not just to improve targeting results, but radically shift the paradigm. Nanomaterial carriers offer numerous advantages as a delivery platform, but targeted nanoparticle agent development has focused simply on generating specificity. This means that binding performance is only evaluated based on equilibrium behavior. But adhesion within the body is a dynamic process, and thus we believe that a kinetic treatment will be far more powerful. In previous work, we developed a framework to study multivalent nanoparticle adhesion from a kinetic standpoint and studied the influence of numerous design

parameters. One of the major findings was that multivalent binding causes the kinetic binding rates to vary on a time-scale that can be captured experimentally. The focus of our most recent work is on developing a computational simulation of nanoparticle docking that includes stochastic and biophysical components to capture the time-variant nature of nanoparticle binding kinetics. Previous simulation treatments have focused on how the formation of multiple bonds affects thermodynamic energy. Our approach will enable recapitulation of the temporal components of experimental binding studies while adding valuable information about bond dynamics and providing predictive power for future experimental investigations.

In-situ Advanced Optical Spatiotemporal Analysis of Collagen Systems, **JULIA LYUBOVITSKY[1]*, YU-JER HWANG[2], XUYE LANG[3], CASSANDRA TURGMAN[1], and JOSEPH GRANELLI[1]** ([1]Department of Bioengineering, University of California, Riverside, 239 MSE, Riverside, CA 92521; [2]Cell, Molecular and Developmental Biology Program, University of California, Riverside, Riverside, CA 92521; [3]Department of Biochemistry, University of California, Riverside, Riverside, CA 92521; julial@ucr.edu).

Recently, we applied *in-situ* multiphoton imaging that employs intrinsic contrasts of second harmonic generation (SHG) and two-photon fluorescence (TPF) to evaluate structural states of fibrillar collagen within collagen hydrogels. We prepare these systems while employing different ions, incubate them at different temperatures and modify them with reducing sugars. The investigations of how the reactions between the reducing sugars (prevalent in diabetes mellitus) and collagen affect the 3D collagen architecture had been revealing. For example, we incubated 37 °C polymerized collagen hydrogels with fructose – a simple sugar in honey, fruit and high-fructose corn syrup. After about 20 days of incubation at 37 °C, there was a rather significant induction of *in situ* fluorescence. The two-photon fluorescence emission was detected at about 460 nm for 730 nm excitation wavelength and shifted to 480 nm when we changed the excitation wavelength to 790 nm. The one-photon fluorescence emission was centered at about 416 nm when excitation was 330 nm. It red shifted and split into two peaks centered at about 430 nm and 460 nm for 370 nm excitation; 460 nm peak became predominant for 385 nm excitation and further shifted to 470 nm for 390 nm excitation. SHG and TPF imaging showed restructuring of hydrogels upon fructose modification. Our studies on collagen hydrogels create a solid foundation in understanding of how reducing sugars affect the collagen and its 3D architecture. They can possibly aid in diagnosing the complications of a chronic disorder such as diabetes mellitus *in situ.*

Label-free Imaging of Neural Activity from Brain to Single Neurons, **M. REZUANUL HAQUE, MICHAEL C. OLIVEIRA, MELISSA M. EBERLE, CARISSA L. RODRIGUEZ, CHRISTIAN M. OH, and B. HYLE PARK*** (Department of Bioengineering, University of California, Riverside, CA; hylepark@engr.ucr.edu).

Current technology largely allows for detection of neural activity on two distinct spatial scales: in ensemble collections of neurons as in MRI, and in a limited number of individual neurons using electrodes and optical microscopy. More recent techniques, including multi-electrode arrays and genetically-encoded calcium indicators, have started to allow detection of activity on an intermediate scale. However, these methods require some level of invasiveness, either in the form of near or direct contact with electrodes or requiring introduction of some exogenous contrast agent.

We have been exploring the potential of optical coherence tomography (OCT) for label-free imaging of neural activity on a range of spatial scales from the brain to single neurons. On a larger spatial scale (4x4x2mm), we have observed the temporal evolution of localized changes in backscattered intensity in three dimensions in both global (induced with PTZ) and focal (induced with 4-aminopyridine (4-AP)) murine seizure models and analyzed the differences in seizure propagation in the time-resolved 3D functional maps. At an intermediate range (0.3x0.3x1.0mm), OCT can be used to detect changes associated with activity in acute hippocampal brain slice that can be correlated with recordings from multi-electrode arrays. On a single cell level, OCT can be used to detect transient changes in the thickness of single cells in the optic nerve associated with the limulus compound eye. These results demonstrate the potential of OCT as a single, label-free optical imaging platform to detect neural activity on a range of spatial scales.

Mechanism of Bone Remodeling in Normal and DMP-1 Deficient Mice, **MEGAN VELTEN[1], JIAN Q. FENG[2], and PRANESH B. ASWATH[1]*** ([1]Materials Science and Engineering Department, Box 19031, University of Texas at Arlington, Arlington, TX 76019; [2]Department of Biomedical Sciences, Texas A&M Health Science Center, Baylor College of Dentistry, Dallas, TX; Aswath@uta.edu).

Differences in apparent and actual density have been noted in the mineralized tissue of postnatal dentin matrix protein-1 (DMP1) mice. In order to investigate this difference at the lowest hierarchical level, a XANES (X-ray Absorption Near Edge Structure) spectroscopy investigation of DMP1 null mice was performed. Female wild type (WT) and DMP1 null mice (KO) were sacrificed (21 d), stored, and the femurs (n=5) were collected, sectioned, and dehydrated. The K- and L-edges of Ca and P and the L-edge of O were obtained at the Canadian Light Source synchrotron facility. The P L-edge data (50x50µm spot size, ~10-20 nm penetration depth) showed evidence of soluble phosphate species in the KO samples, but the higher energy P K-edge data (200x50µm, ~500 nm penetration depth) showed no significant difference in the phosphate bonding structures of the WT and KO mice. The low energy Ca L-edge data showed evidence of a local depletion of Ca in the KO mice while the O L-edge data showed increased carbonate content of the KO bone. Taken together this data creates a picture of a unique local coordination of phosphorus in the mineralized tissue of the DMP1-null bone at an earlier stage in the lifecycle than the phenotype is generally observed. These changes observed in the crystal structure of the mineral matrix help explain DMP1 role in the mineralization of tissue.

Engineering Nutrient Derived Alloys for Medical Applications, **HUINAN LIU** (Department of Bioengineering, Materials Science and Engineering Program, and Stem Cell Center, University of California, Riverside, CA 92521, USA; huinanliu@engr.ucr.edu).

Current medical implants and devices are mostly made of titanium alloys or stainless steel – permanent materials that potentially increase patients' risk in infection and chronic pain, and often require secondary surgeries for removal. Because of clinical

problems associated with these traditional materials, a novel class of biodegradable metallic materials, i.e., magnesium-based alloys, attracted great attention and clinical interests. Controlling the interface of Mg with the biological environment, however, is the key challenge that currently limits this biodegradable metal for broad applications in medical devices and implants. This talk will particularly focus on how to create nanostructured interface between the biodegradable metallic implant and surrounding tissue for the dual purposes of (1) mediating the degradation of the metallic implants and (2) simultaneously enhancing bone tissue regeneration and integration. Nanophase hydroxyapatite (nHA) is an excellent candidate as a coating material due to its osteoconductivity that has been widely reported. Applying nHA coatings or nHA containing composite coatings on Mg alloys is therefore promising in addressing the challenges in commercializing biodegradable metallic implants. The composite of nHA and poly(lactic-co-glycolic acid) (PLGA) as a dual functional interface provides additional benefits for medical implant applications. Our results indicate that nHA and nHA/PLGA coatings slow down Mg degradation rate and enhance adhesion of bone marrow stromal cells, thus promising as the next-generation multifunctional implant materials. Further optimization of the coatings and their interfacial properties are still needed to bring them into clinical applications.

Multi-step Self-organization of Tissue-scale Tubules, **CHIN-LIN GUO** (Division of Engineering and Applied Science, Caltech, Pasadena, CA; guochin@caltech.edu).

The ability of cells to self-organize into multi-scale architectures raises the possibility that understanding such processes can help engineer organs for regenerative medicine. Here, we engineer in vitro microenvironments to study how epithelial cells can self-organize into tissue-scale tubules without the guidance of morphogen gradients. We find two distinct behaviors. When cells are surrounded by pre-assembled 3-D extracellular matrix, they spontaneously develop long-range mechanical interactions (up to 600 microns) to promote tumor cell invasion. In contrast, when ECM assembly is limited and broken into multiple stages, cells form two tissue architectures of distinct scales: low cell density leads to the formation of isotropic acini (50-100 microns), and high cell density leads to the self-organization of long tubules (few centimeter-long, hundreds of micrometer-wide). Our results pave the way for a quantitative study of how cell-microenvironment interactions lead to multi-scale self-organization and/or tumor invasion.

Modulation of Stem Cell Fate via Engineered Mechano-environment, **MARICELA MALDONADO, LAUREN WONG, KAREN LOW, GERARDO ICO, and JIN NAM*** (Department of Bioengineering, University of California, Riverside, CA; jnam@engr.ucr.edu).

Despite the promising potential of induced pluripotent stem cells (iPSCs) for personalized regenerative medicine and tissue engineering, their tendency of tumorigenesis and teratoma formation poses a major challenge for therapeutic applications. In this regard, controlling the differentiation of the cells to specific target cell types *in vitro* provides a means to overcome such risks. Traditionally, the formation of embryoid bodies has been used to enhance differentiation of stem cells, but the low efficiency with heterogeneous differentiation of the method remains a major limitation. Considering significant effects of microenvironments on stem cell differentiation, therefore, tightly controlled scaffolding may provide a tool to maximize the differentiation efficiency. In this study, electrospun nanofibrous scaffolds with varied mechanical properties were used to differentiate human iPSCs to mesendodermal or ectodermal lineages. During pre-culture of the iPSCs, a flat, two-dimensional colony morphology was observed on stiffer scaffolds accompanied by enhanced proliferation. In contrast, a round, three-dimensional colony morphology was observed for iPSCs cultured on softer scaffolds with retarded self-renewal. Such differences in colony morphology depending on the mechanical properties of scaffolds, resulted in significant alterations in stem cell differentiation. For mesendodermal differentiation, its differentiation was positively correlated to increased stiffness of the scaffolds while it was inversely correlated for ectodermal differentiation. These results offer promising solutions to enhance the efficiency of iPSC differentiation towards specific lineages by means of modulating micro-mechanical environments of the cells.

Engineering the Neural Microenvironment to Promote Spinal Cord Regeneration, **STEPHANIE K. SEIDLITS[1,2]*, RYAN M. BOEHLER[2], ALINE M. THOMAS[3], DOMINIQUE SMITH[3], DANIEL J. MARGUL[2], ASHLEY G. GOODMAN[2], TODOR V. KUKUSHLIEV[2], TING HE[2], DYLAN A. MCCREEDY[2], JAIME PALMA[2], DONNA M. HASSANI[4], BRIAN J. CUMMINGS, AILEEN J. ANDERSON, and LONNIE D. SHEA[2]** ([1]Department of Bioengineering, University of California Los Angeles, 420 Westwood Plaza, Los Angeles, CA 90095; [2]Department of Chemical and Biological Engineering, [3]Department of Biomedical Engineering, [4]Department of Psychology, Northwestern University, 2145 Sheridan Rd, Evanston, IL 60208; [5]Department of Physical Medicine and Rehabilitation, Anatomy and Neurobiology, University of California Irvine, Sue and Bill Gross Hall, Irvine, CA 92697; seidlits@ucla.edu).

The local environment after spinal cord injury (SCI) lacks cues support axon growth, cell survival, and remyelination and exhibits an abundance of cues that inhibit these processes. Development of clinically effective strategies to restore function after SCI will require consideration of multiple aspects of this inhibitory environment. The goal of this research is to develop a multifaceted therapy for SCI repair which uses a biomaterial platform to present an architecture that guides regenerating axons across the injury site and gene delivery vectors encoding for growth factor cues that enhance cell survival, reduce inflammation, prevent formation of a dense glial scar and promote axonal growth and remyelination. Previously, we have reported that porous bridges with a defined channel architecture that significantly encourage axons to regenerate across the injury site and can be used to deliver lentiviral vectors.

This research builds upon the success of these bridges by adding a gene delivery component to enable localized, sustained expression of multiple factors designed to simultaneously address different barriers to spinal cord regeneration. Moreover, these factors were selected to target various barriers to spinal cord regeneration. First, we report that delivery of lentivirus encoding for interleukin-10 (IL10) significantly reduces the presence of specific inflammatory cells thought to be detrimental to repair. In addition, we demonstrate that tandem delivery of sonic hedgehog (SHH) and neurotrophin-3 (NT-3) significantly increases the number of myelinated, regenerated axons in a mouse model of SCI.

Physical Determinants of Endothelial Inflammation, **KAUSTABH**

GHOSH (Department of Bioengineering, University of California, Riverside, CA 92521; kghosh@engr.ucr.edu).

Chronic endothelial inflammation contributes significantly to the development of pathological conditions such as atherosclerosis and diabetic retinopathy. Aging and diabetes, important risk factors for these diseases, are also characterized by chronic endothelial inflammation. Importantly, the stiffness of sub-endothelial extracellular matrix (ECM) *is significantly altered* in such inflammatory conditions. However, whether a causal relationship exists between aberrant ECM stiffness and chronic endothelial inflammation remains unknown. Here we show that excessive ECM stiffening or softening alone can significantly enhance leukocyte-endothelial cell (EC) adhesion, the earliest step in endothelial inflammation. Further, the preferential increase in leukocyte-EC adhesion on soft and stiff ECM correlates inversely with levels of nitric oxide, an endogenous anti-inflammatory factor, but directly with the activation of NF-kB, the master inflammatory transcriptional switch. Importantly, these studies have revealed a mechanosensitive ion channel that plays a critical role in this ECM stiffness-dependent biphasic regulation of endothelial inflammation. Our ongoing work is aimed at delineating the underlying mechanotransduction pathway that mediates this process as it has the potential to offer a new therapeutic target for immunomodulation and cardiovascular normalization.