TEXAS A&M UNIVERSITY

Texas A&M University ENG-LIFE Workshop

At the Interface of Engineering and Life Sciences

November 15, 2013

8:00 a.m. – 4:30 p.m.

Emerging Technology Building (ETB) 2005

Welcome!

We would like to welcome you to the 1st Texas A&M University ENG-LIFE Workshop: At the Interface of Engineering and Life Sciences. The purpose of this workshop is to promote multidisciplinary interaction and scientific communication in the field of engineering and life sciences. This event will also offer a venue for graduate and undergraduate students to gain valuable experience by presenting their latest research results as well as interacting with fellow students and prominent researchers from Texas A&M University. We hope that you all enjoy this workshop.

Sincerely,

The 2013 Symposium Organizing Committee

Acknowledgements

Symposium Organizing Committee

Arum Han
Associate Professor, Dept. Electrical and Computer Engineering

Arul Jayaraman
Ray Nesbitt Professor, Dept. Chemical Engineering

Allison C. Rice-Ficht
Associate Vice President for Research, Texas A&M Health Science Center
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Program:

Time	Activity	Speaker
8:00 - 8:30	Registration/Breakfast	
8:30 - 9:00	Opening Remark	**Dr. M. Katherine Banks: Vice Chancellor for Engineering, Texas A&M University System; Dean, Dwight Look College of Engineering** **Dr. Brett P. Giroir: Interim Executive Vice President and CEO, College of Medicine, TAMHSC** **Dr. Glen A. Laine: Interim Vice President for Research (VPR), TAMU** **Dr. David S. Carlson: Vice President for Research, TAMHSC; Dean of School of Graduate Studies, TAMHSC**
9:00 - 9:45	Keynote Speech	**Center for Translational Environmental Health Research (CTEHR): At the Nexus of Biology, Medicine and Engineering** **Dr. Cheryl Walker** *Director of IBT (Institute of Biosciences & Technology) & Robert A. Welch Professor of TAMHSC*
9:45 - 10:00	Presentation 1	**Cancer Therapy Design Based on Pathway Logic** **Dr. Aniruddha Datta** J. W. Runyon, Jr. '35 *Professor of Electrical & Computer Engineering*
10:00 - 10:15	Presentation 2	**Chemotaxis of Enteric Bacteria to Norepinephrine and Its Metabolites** **Dr. Arul Jayaraman** *Ray Nesbitt Development Professor of Chemical Engineering*
10:15 - 10:45	Coffee Break	
10:45 - 11:00	Presentation 3	**Naturally Occurring Canine Spinal Cord Injuries** **Dr. Jonathan Levine** *Associate Professor of Small Animal Clinical Sciences*
11:00 - 11:15	Presentation 4	**GI Tract Ecology and Immune Homeostasis - A Metabolomic Linkage** **Dr. Robert Alaniz** *Assistant Professor of Microbial and Molecular Pathogenesis, TAMHSC*
11:15 - 11:30	Presentation 5	**Knife-Edge Scanning Microscopy for Brain Connectivity Mapping** **Dr. Yoonsuck Choe** *Associate Professor of Computer Science*
11:30 - 11:45	Presentation 6	**Expanding the Design Landscape for Lymphocyte Antigen Receptors With Sharks** **Dr. Mike Criscitiello** *Assistant Professor of Veterinary Pathobiology*
11:45 - 12:00	Presentation 7	**Core-shell Nanoparticles for Drug and Vaccine Delivery** **Dr. Michael Pishko** *Stewart and Stevenson Professor of Biomedical Engineering*
12:00 - 1:30	Lunch	
1:30 - 1:45	Presentation 8	**A New Functionalizable Protein-Based Material** **Dr. Sarah Bondos** *Assistant Professor of Molecular and Cellular Medicine, TAMHSC*
1:45 - 2:00	Presentation 9	**Synthetic Biology to Deliver Disruptive Solutions for Biofuels** **Dr. Joshua Yuan** *Assistant Professor of Plant Pathology and Microbiology*
2:00 - 2:15	Presentation 10	**Microfluidic Lab-on-a-Chip Accelerating Life Science Research** **Dr. Arum Han** *Associate Professor of Electrical and Computer Engineering*
2:15 - 2:30	Closing Remarks	**Dr. Arum Han**
2:30 - 4:30	Poster Session	

Poster Session Abstracts
Emerging Technology Building (ETB) 2005
2:30 – 4:30 PM

(Arranged in alphabetical order by title)

1. A Bayesian Multivariate Poisson Model for RNA-Seq Classification

Jason M. Knight[1], Ivan Ivanov[2], Robert S. Chapkin[3] , Johanna W. Lampe[4], and Edward R. Dougherty[1,5]

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[4] Dept. Epidemiology, University of Washington & Fred Hutchinson Cancer Research Center, Seattle, Washington, USA
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Sequencing datasets consist of the number of reads found to map to specific regions of a reference genome. They are often modeled with a discrete distribution, such as the Poisson. For this reason, Gaussian and multinomial distributions are not ideal for modeling sequence-based datasets. Thus, we introduce a multivariate Poisson model (MP) and the associated optimal Bayesian classifier (OBC) for classifying samples using sequencing data. Lacking closed-form solutions, we employ a Monte Carlo Markov Chain (MCMC) approach to perform classification. We demonstrate superior or equivalent classification performance compared to typical classifiers for two synthetic datasets and over a range of classification problem difficulties. We also introduce the Bayesian minimum mean squared error (MMSE) conditional error estimator and demonstrate its computation over the feature space. In addition, we demonstrate superior or leading class performance over the TCGA tumor RNA-Seq database and a RNA-Seq data from a dietary intervention study in a model organism.

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2. Circadian Clock Regulation of MAPK Pathway Activation

Teresa Lamb, Stephen Caster[2], Nikita Ojha, Rigzin Dekhang, Oneida Ibarra, Nirmala Karunarathna, and Deborah Bell-Pedersen[1,2]

[1] Dept. Biology, Texas A&M University, USA
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We study how the circadian clock functions in organisms to regulate daily rhythms in behavior, physiology, and biochemistry. Defects of the human clock are associated with sleep disorders, and for unknown reasons epilepsy, cerebrovascular disease, multiple sclerosis, headaches, cardiovascular disease, and cancer. In addition, daily changes in metabolism and cell division rates influence the efficacy and toxicity of many pharmaceuticals, including cancer drugs. Therefore, knowing how clocks work and what they regulate at the molecular level is important for the development of new ways to improve human health. Using *Neurospora crassa* as a model system, we discovered that the clock regulates the activity of conserved MAPK signaling pathways involved in stress responses and the control of cell growth and division. This finding provides a rationale for observations that deregulation of the clock in humans contributes to cancer, and suggests novel approaches for treatment of circadian disorders. In addition, we are investigating the link between MAPK activity and mRNA translation control through phosphorylation of eEF2 as a mechanism to regulate rhythmic translation. Knowing the rhythmic proteome will help to understand why certain drugs are more effective or toxic depending on the time of administration.

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3. Comparative Proteomic Analysis of Two Stages of Sexual Development in *Neurospora crassa* Reveal an Association between Secondary Metabolites and Fruiting Body Maturation

A.V. Suescún[1], B. Russell[2], and R. Aramayo[1]

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[2] Russell's Research Group, Laboratory of Biological Mass Spectrometry, Dept. of Chemistry, Texas A&M University, USA

Filamentous fungi are well known for the production of secondary metabolites and their presence is association with fungal development and cell differentiation. Usually the function of these metabolites for the producing organism is unknown; however the majority of these compounds are of medical, industrial and/or agricultural importance with potent and diverse biochemical actions. In order to control the production of these metabolites we need to elucidate the stages at with those metabolic pathways are activated. *Neurosporacrassa* is a well-understood filamentous fungus and an important eukaryotic model for studying cell differentiation. During *N. crassa* sexual development several morphological and physiological changes take place. Although some genes required for sexual development has been determined, little is known about the metabolic pathways that dictate these morphological changes or the secondary metabolites that are produced. We applied a proteomic approach to gain insights into the metabolic pathways operating at two phases of sexual differentiation (female reproductive structure formation and fruiting body maturation). The goal of the present study was to develop a comprehensive proteomics base data set for *N. crassa* to better understand, at the molecular level, the morphological changes observed during sexual development. By examining the protein profile of different sexual tissues we had been able to determine the proteome and estimate metabolic pathways require for progression of sexual development. We found significant productions of secondary metabolites associated with sexual development, specifically during fruiting body maturation. This knowledge serves as the foundation for the future of synthetic biology in the filamentous fungi.

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4. Comparative Transcriptome Analysis of the Serpentine Endemic Plant *Caulanthusamplexicaulis*var. *barbarae* (Brassicaceae)

A.K. Hawkins[1] andA.E. Pepper[1]

[1] Department of Biology, Texas A&M University, USA

Serpentine soils are derived from ultramafic rock and usually have extremely low levels of essential plant nutrients (e.g. N, P, Ca) and very high to toxic levels of heavy metals (e.g. Ni). Serpentine soil distribution is rare and patchy, and outcrops are often home to many endemic plant species. In California, serpentine soils account for approximately 1.5% of the total land area however this area is home to 13% of all endemic flora. The Streptanthoid Complex (Brassicaceae, tribe Thelypodieae) is a highly diverse group with approximately 60 taxa, representing at least 6 genera, many of which are adapted to harsh or extreme environments. The mechanisms by which *Caulanthusamplexicaulis*var. *barbarae* (CAB), a serpentine endemic plant, has not only adapted to, but thrives in such soils are being investigated. By comparing CAB to its non-serpentine sister species, *Caulanthusamplexicaulis*var. *amplexicaulis*(CAA), the genetic and phenotypic variation that underlies adaptation to serpentine environments will be elucidated. Using both ecologically distinct parents, reference transcriptome assembly has been undertaken. Plants were grown in a variety of environmental conditions (both optimal and stressed) and several different tissue types were harvested for use in subsequent RNA extractions. Reversed transcribed cDNA was amplified and normalized using a duplex-specific nuclease technique, then paired-end sequenced using the next generation Illumina platform. Preliminary comparative analysis of these two closely related but ecologically distinct taxa is presented here.

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5. Crystallographically Templated Topologies: Toward Novel Packings for Microfluidic Separation

D.Han[1] and V.Ugaz[1]

[1]Artie McFerrin Department of Chemical Engineering, Texas A&M University, USA

We describe a novel method to imprint complex surface topographies on biodegradable substrates by exploiting the sensitivity of enzymatic activity to a substrate's degree of cyrstallinity. This process is illustrated in an enzyme/substrate system involving proteinase K and poly(lactic acid) (PLA), where a strong etch rate selectivity to PLA crystallinity is observed. By establishing a laminar flow of the enzyme solution through a template microchannel, morphological features associated with the substrate's crystalline domains can be embedded into the sidewalls. The PLA crystalline morphology is governed by its thermal history (annealing time and temperature, cooling rate) and material properties (molecular weight), enabling the size and density of the imprinted features to be manipulated. We identify conditions under which post-like arrays of tunable size can be produced, making it possible to embed a variety of complex architectures within a micro- or nano-scale fluidic channels in a lithography-free manner. The simplicity and robustness of this approach may offer advantages for producing barrier or packing structures relevant for filtration and chromatography applications. The highly-specific nature of the governing interactions and wide range of enzyme/substrate combinations lays a foundation for broad control over the templatednano-scale morphologies.

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6. Designing Experiments for Optimal Reduction of Uncertainty in Gene Regulatory Networks

Roozbeh Dehghannasiri, Byung-Jun Yoon, Edward R. Dougherty

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Gene regulatory networks (GRNs) typically contain inherent uncertainty. The uncertainty in GRNs may be due to the complex nature of living organisms, insufficient training data, and so forth. Researchers aim to reduce the uncertainty by conducting additional biological experiments. However, the high cost of biological experiments, limited resources for conducting experiments, and dealing with large amount of uncertainty make it necessary to devise a cost-effective plan for conducting experiments. Our proposed method is based on the concept of mean objective cost of uncertainty (MOCU). MOCU, which measures the expected increase of cost induced by the uncertainty, is an objective-based measure for quantifying uncertainty. In our proposed method, we want to choose from potential experiments such that the expected reduction of the cost of uncertainty is maximized.

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7. Development of Novel Injectable Biomaterials for Tissue Regeneration

Z. Li, T. Qu, C. Ma, C. Ding, and X. Liu

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Injectable biomaterials are desirable for many clinical tissue repair and regeneration. Here we report a unique injectable system in which the biomimetic nano-fibrous microspheres (NF-MS) serve both as an injectable cell carrier and as a growth factor carrier for bone tissue regeneration. The NF-MS were fabricated by a novel approach, which integrated an emulsification and a thermally induced phase separation technique. The NF-MS mimicked the architecture of natural collagen fibers at a nanometer scale and had many unique properties including extraordinarily high surface area and extremely low density. Growth factors were effectively encapsulated into the microspheres through a simple process without using any organic solvent. In vitro release kinetics indicated that the encapsulated recombinant human bone morphogenetic protein 7 in the NF-MS was released in a temporal controlled manner. When used as an injectable scaffold for repairing the rat critical-size calvarial defects, the rhBMP-7-loaded NF-MS induced a much more significant amount of new bone formation than the rhBMP-7-free NF-MS control. We conclude that the biomimetic NF-MS combined with the controlled growth factor release is an excellent cell carrier for bone regeneration.

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8. Development of the wild tomato *Solanumpennellii* as a novel and sustainable biofuel feedstock

Sabyasachi Mandal, WangmingJi, Chika Okonkwo, Thomas D. McKnight

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Abstract: *Solanumpennellii,* a wild relative of tomato, is native to arid regions of Peru and is a potential new feedstock for biofuels. *S. pennellii*is drought tolerant and can be grown on marginal lands unsuited for typical crops. This plant secrets 2,3,4 tri-O-acylated glucose esters through trichomes on its leaf surface, presumably to reduce water loss. Transesterification of the secreted compound yields one molecule of glucose and three molecules of C4 to C12 fatty acid esters. These esters are analogous to biodiesel, but with carbon chains in the range of bio-gasoline. This bio-gasoline should be compatible with current fuel transport and storage technologies and with conventional gasoline engines. The biosynthetic pathway of the glucolipid involves only four enzymes, making it a good candidate for transfer into other plants. We have cloned genes encoding the first two enzymes of the pathway and we are analyzing their expression in transgenic plants. We are using comparative transcriptomics between high-producing accessions and low-producing accessions to identify the remaining genes in the pathway. Our goal is to transform these genes into tobacco and other plants with large leaf surfaces to improve yield of this novel biofuel to fill the critical niche between ethanol and biodiesel.

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9. The Dictyostelium protein Tpp1 is a functional ortholog of a human protein involved in NCL, a childhood onset neurodegenerative disease

Jonathan E. Phillips[1] and Richard H. Gomer[1]

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Neuronal ceroid lipofuscinosis (NCL) is the most common neurodegenerative disease with a childhood onset. Individuals with this disease experience a gradual decline in vision and motor function beginning at age 2-10, the onset of a vegetative state, and eventually, death. There is currently no therapeutic treatment for the disease. NCL is a recessive disorder caused by mutations in a set of genes thought to play a role in lysosomal funtion, and the disease correlates with autofluorescent lipopigment buildup within lysosomes. Mutations responsible for a juvenile onset form of NCL (JNCL) map to a gene encoding tripeptidyl peptidase 1 (Tpp1), a lysosomal peptidase that cleaves the three N-terminal amino acids from polypeptides. Little is known about the physiological role of this protein, due in part to the fact that Tpp1 is not present in many traditional model organisms, such as yeast, flies, or nematodes. We have disrupted the *tpp1* gene in the social amoeba *Dictyostelium discoideum*. When starved, *Dictyostelium* cells aggregate and undergo multicellular development, forming a ball of spores supported by a stalk. Tpp1 is strongly upregulated during development, and we found that the loss of Tpp1 results in a reduction in the number of spores formed during development. Starved cells lacking Tpp1 show an increase in vesicular fluorescence, resembling the fluorescent lipopigment seen in JNCL. The identification of mutations that suppress the phenotype of *tpp1*$^-$ cells might lead to approaches to suppress JNCL disease progression. We have conducted suppressor screens and found 3 mutants that suppress Tpp1 mutant phenotypes.

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10. Dipeptidyl-peptidase IV is a human and murine neutrophil chemorepellent

Sarah E. Herlihy, Darrell Pilling, Anu S. Maharjan, and Richard H. Gomer

Texas A&M University, Department of Biology, College Station, TX 77843

Acute respiratory distress syndrome (ARDS) affects 200,000 people per year with a 40% mortality rate. The disease is initiated by an insult to the lungs where neutrophils migrate into the lung tissue in response to the insult causing further damage, which in a positive feedback loop recruits more neutrophils. In *Dictyostelium discoideum*, AprA is a secreted protein that causes chemorepulsion of *Dictyostelium* cells. AprA has little sequence similarity to any human proteins. We found that a predicted structure of AprA has similarity to the structure of human dipeptidyl-peptidase IV (DPPIV). DPPIV is a serine protease present in extracellular fluids that cleaves peptides with a proline or alanine in the second position. Defined DPPIV gradients encompassing concentrations below, similar to, and above the human serum DPPIV concentration cause movement of human neutrophils away from higher concentrations of DPPIV. A 1% DPPIV concentration difference between the front and back of a neutrophil is sufficient to cause chemorepulsion. Neutrophil speed and viability are unaffected by DPPIV. Conditioned media experiments reveal that DPPIV is not acting by inactivating a neutrophil chemoattractant. Alexa-fluor647-labeled DPPIV binds to isolated human neutrophils, indicating the presence of a receptor. Inhibitors of DPPIV reduce the ability of DPPIV to bind neutrophils and cause chemorepulsion. In a murine model of ARDS, aspirated DPPIV inhibits the bleomycin-induced accumulation of mouse neutrophils. These results indicate that DPPIV functions as a chemorepellent of human and mouse neutrophils, and suggest new mechanisms to inhibit neutrophil accumulation in ARDS.

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11. Distinct receptors mediate the effect of Serum Amyloid P on monocytes and neutrophils

N. Cox[1] , D. Pilling[1] , and R.H. Gomer[1]

[1] Dept. of Biology, Texas A&M University,USA

Fibrosing diseases such as cardiac fibrosis, pulmonary fibrosis, and end-stage kidney disease involve aberrant scar tissue formation in organs. The scar tissue forms whenneutrophils and monocytes enter the affected organ and monocytes differentiate into macrophages and fibrocytes. The secreted pentraxin Serum Amyloid P (SAP) inhibits fibrosis in animal models by inhibitingneutrophil adhesion, enhancing phagocytosis bymacrophages,and inhibiting fibrocyte formation. All known SAP functions are mediated through the Fcγ receptors (FcγRI, FcγRIIa, FcγRIIb,and FcγRIII).To understand the molecular mechanism undelaying SAP's anti-fibrotic effects,we carried out site directed mutagenesis on SAP.Wethen examined the effect of these mutations on SAP function and binding to the Fcγ receptors. We identified SAP variants that had alterationsin one or more functions.SAPvariants that have altered effect on fibrocyte formation and macrophage phagocytosis have changes in FcγRI binding.However, SAP variants that impact neutrophil adhesion differently than the WT SAP have altered binding to FcγRIIa.Our results indicate the presence of a novel Fcγ receptor binding site on SAP that is distinct from the previouslyidentified one.The presence of thisnovel site suggests that SAP could bind multiple Fcγ receptors simultaneously.Furthermore, the results from our mutagenesis studies indicate the possibility of modulating specific SAP functions without altering other functions. This allows us to fine tune the different SAP functions for therapeutic uses and also study the contribution of different immune cells types to fibrosis.

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12. Effect of strain rate on cell morphology and collagen fibril alignment within hybrid tissue

C. Haase, H. Hsu, A.Braz, R. Sawhney, R. Kaunas

Department of Biomedical Engineering, Texas A&M University, College Station

Nearly all cells in the body are embedded in a 3-dimensional microenvironment. Cyclic stretch experiments are usually performed by culturing cells on 2-dimensional silicone sheeting coated with extracellular proteins. However, when cells are grown in a physiological 3-dimensional environment made of proteins such as collagen I, this helps understand cell morphology, cell-matrix interactions, signaling, and cytoskeletal reorganization in vivo. In this study we showed the effect on the strain rate of cyclic stretch on cell and collagen fibril alignment within hybrid construct. The hybrid tissue consisted of collagen-U2OS microspheres encapsulated in a PEGDA hydrogel. We hypothesized that this hybrid tissue can bear comparable levels of stress as pure PEGDA, while the reduced stress transmitted to the cellular microenvironment is sufficient to induce cell and collagen remodeling. In these experiments the hybrid construct was subjected to 6 hours of 7.5% cyclic stretch at 0.01 and 1 Hz. At 1 Hz, the cells in the center of the microsphere were elongated parallel to the stretch direction while cells at the interface aligned along the boundary. This shows that cells at the center of the microsphere respond to the stretch signal while cells at the boundary respond to the PEGDA stiffness. These research findings offer new knowledge into the mechanisms by which cells sense the mechanical properties in their microenvironment.

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13. Electron Beam Technology and Microbiological Safety of Alfalfa Sprouts

J.A. McCoy, S.D. Pillai

National Center for Electron Beam Research,Texas A&M University, USA

In recent years, the demand for minimally processed foods, such as fruits and vegetables, has increased due to people seeking healthier diets. This increase has in turn led to increasing numbers of foodborne illnesses and outbreaks . Advanced technologies such as Electron Beam (eBeam) together with the application of new packaging material could reduce the risk of these foodborne outbreaks without compromising the sensory and nutritional quality of fresh produce. The research focuses on the use of eBeam at low dose (≤1 kGy) on the microbiological safety and sensory attributes of alfalfa sprouts when packaged in perforated polyethylene terephthalate (PET) clam shells. Even at low eBeam doses, a 2-log reduction of microbial bioburden can be achieved without any significant loss of textural or color attributes. Additional studies evaluating the quantitative microbial risk reduction associated with specific pathogens such as E.coli O157:H7 and other serotypes, as well as consumer taste panel studies are planned. These findings and studies highlight the importance of integrating life sciences with engineering technologies to address 21st century challenges facing the food industry.

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14. Electron Beam Technology in the Food Industry – A Nexus between Food Science and Engineering

S. Shayanfar, S.D. Pillai

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In order to achieve the expected requirements of the increasingly demanding global market all industrial sectors are expected to embrace innovative actions and food industry is not an exception. The food industry is evolving beyond solely providing nourishment and takes a step forward in the quest to encompass novel consumer expectations such as convenience, sustainability, nutraceuticals, etc. that are not met with the current practices. Electron beam (eBeam) is a non-thermal technology with diverse functions which can be deployed as a toolbox for a variety of applications that range from improving food safety to food waste management. The novel applications of eBeam have not been widely known to the food industry. The food industry has to acknowledge the potential to improve the current practices in different food related industries. The application of eBeam in biodegradable packaging, food waste management, and value-added food products will span food science to engineering.

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15. Endocytosis and Polarized Growth in Filamentous Fungi

Z. Schultzhaus, B.D. Shaw[1]

[1]Program for the Biology of Filamentous Fungi, Department of Plant Pathology and Microbiology, Texas A&M University, USA

Filamentous fungi are characterized by extreme polarized growth, which occurs at the tips of their cells. This type of growth is also typical of neurons and plant root hairs, and is a highly regulated process maintained through directional secretion. Endocytosis is known to be essential for growth in several model filamentous fungi, but its importance outside of the phylum Ascomycota has not been resolved. Here we document the importance of actin and endocytosis in the growth of fungi from two different phyla to present a possible conserved mechanism for polarized cell growth.

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16. ENHANCED ELECTROPHORETIC TRANSPORT VIA NOISE-SYNCHRONIZED NANOSCALE ENTROPIC TRAPPING

N. Shi[1] and V.M. Ugaz[1]

[1]Dept. Chemical Engineering, Texas A&M University, USA

Macromolecules confined within nanoporous surroundings experience entropic trapping (ET) when their dimensions approach the average pore size, leading to emergence of transport behavior that can be immensely beneficial (e.g., a counterintuitive trend of increasing separation efficiency with DNA size during gel electrophoresis) [1]. But the noisy uncorrelated process by which the embedded macromolecules discretely hop from pore to pore contributes additional dispersion that detrimentally impacts most practical applications. Here show how the same dynamics governing phenomena as diverse as global climate change and sensory perception can be exploited to direct macromolecular transport through nanoporous surroundings. We demonstrate this in the context of gel electrophoresis by establishing a resonance condition that synchronizes the otherwise noisy uncorrelated motion of DNA between pores in the matrix. Surprising consequences include simultaneous transport of different-sized molecules in opposite directions, and a counterintuitive inverted size dependence of separation efficiency. We further show how DNA binding interactions can be sensitively probed by exploiting conformation dependence of resonance. These phenomena can be easily accessed in ordinary hydrogels (as opposed to idealized planar nanomachined topologies), offering a direct pathway to implement them in a host of useful settings.

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17. Engineering Elastomeric and Mechanically Stiff Nanocomposite by Covalently Crosslinking Poly(glycerol sebacate) and Silicate Nanoplatelets

Punyavee Kerativitayanan[1] and Akhilesh K Gaharwar[1]

[1] Dept. Biomedical Engineering, Texas A&M University, USA

Poly(glycerol sebacate) (PGS) has been proposed to engineering soft tissues due to its elastomeric properties, biocompatibility, linear degradation rate and non-toxic byproducts. However, one of the problems with PGS is low mechanical stiffness, which limits its application to musculoskeletal tissue engineering. Here we report the synthesis and characterizations of highly elastomeric and mechanically stiff nanocomposites by covalently reinforcing PGS network with synthetic silicate nanoplatelets. Synthetic silicates are plate-like polyions composed of simple or complex salts of silicic acids and are recently shown to induce osteogenic differentiation of stem cells without using any growth factors. We hypothesize that adding nanoparticles and tailoring the crosslinking density, it is possible to engineer scaffolds with customized mechanical stiffness, hydration properties and degradation characteristics. The addition of silicate nanoplatelets also enhances the bioactivity of polymeric scaffolds. The unique combination of high mechanical stiffness, elastomeric nature and bioactive character can be processed to design scaffolds for musculoskeletal tissue engineering.

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18. Fusion of bacterial cells using phage spanins

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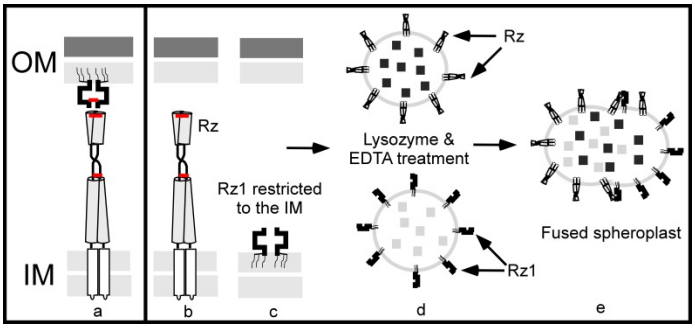
In phage lysis, holins initiate the lytic pathway by permeabilizing the cytoplasmic membrane, allowing endolysins to attack the peptidoglycan (PG). In Gram-negative hosts, a third class of proteins, the spanins, are required to disrupt the outer membrane (OM). There are two types of spanins: unimolecular spanins and, by far the most common, two-component spanins. The best-studied two-component spanins are lambda Rz and Rz1, an integral inner membrane (IM) protein (i-spanin) and an OM lipoprotein (o-spanin), respectively. Rz and Rz1 interact by their C-termini to form a complex spanning (thus the term spanin) the periplasmic space of its host (**a**). Previous studies with the purified periplasmic domains of Rz and Rz1 revealed large conformational changes associated with the formation of heteroligomeric complexes. These and other results suggested a model in which removal of the PG by endolysin would free the spanin complex to undergo a condensing conformational change, causing IM-OM fusion. To test this model, we constructed an *Rz1* variant that was restricted to the outer surface of the IM (**c**). When we spheroplasted mixtures of these cells and cells expressing Rz, ~5-10% of the spheroplasts underwent fusion, as judged by mixing of differential cytoplasmicfluorescent labels (d&e). The method used provides a new approach for studying membrane fusion systems without artifical membrane bilayers. These results may point the way to a general method for creating intercellular fusions between spheroplasts of heterologous cells; i.e., bacterial “hybridomas”.



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19. GC-MS Analysis of Metabolites

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The Laboratory for Biological Mass Spectrometry (LBMS) serves TAMU research communities with expertise in mass spectrometry methodology and instrumentation. Here we discuss the use of GC-MS in some of our latest collaborative projects that highlight the capabilities of LBMS. Lignocellulosic biomass is the most abundant renewable resource that is suitable for biofuel production. However, the efficiency of biofuel production is often limited by the presence of lignin degradation products, some of which are known to inhibit the activity of biofuel-producing microorganisms. Thus, bacteria that can grow in the presence of these inhibitory compounds as well as accumulate triacylglycerol (TAG) are of great interest. In collaboration with the Kung-Hui Chu Laboratory we will show the identification of inhibitory compounds and sugars present in the hydrolysate of three different biomasses. In addition, the amount of each sugar detected was quantified using a GC-MS single quadrupole mass spectrometer. Evaluation of food chemicals is essential to make appropriate feeding decisions. The molecular genetic analysis of Gustatory receptor (Gr) genes and the characterization of the neural circuits that they engage has led to a broad understanding of taste perception in adult Drosophila. Gr43a, which was recently shown to function as a hemolymph fructose sensor in adult flies, was identified as the major larval sugar receptor. In collaboration with Professor Hubert Amerein's group, we examined the post-ingestive mechanism of converting carbohydrates into fructose; including quantifying fructose, sorbitol and glucose in larval hemolymph.

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20. Handheld Fluorescence Lifetime Imaging (FLIM) System with Real-time Image Processing

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A handheld system for simultaneous multispectral FLIM imaging with real-time image processing is presented here. The handheld endoscope consists of a 7x13x5 cm^3 enclosure with a rigid probe (1.7 cm diameter, 14 cm length). The probe includes a relay lens pair and a third achromat working as an objective. The emission is collected through the same three lenses combination and launched to a detection unit outside of the handheld box. In this detection unit, we use a set of dichoric mirrors and filters to separate the emission into three spectral bands and then collect them with multimode fibers of different and specific lengths to provide an optical delay. Thus, for single excitation pulse, three decays corresponding to three spectral bands are simultaneously detected. In this design, lateral resolution and maximum field of view (FOV) can be trade off by changing of the excitation fiber and the objective in order to tailor the system for a given application. The range for lateral resolution and maximum FOV are tested to be ~35 μm to 140 μm and ~6.5 mm to 13 mm, respectively. Real-time image processing was also accomplished by embedding FLIM deconvolution algorithms within the imaging instrumentation. A maximum pixel acquisition and processing of 30 kHz was demonstrated. The system was validated by imaging both fluorescent dyes (NADH, FAD and POPOP) and human oral mucosa *in vivo*.

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21. A High-Throughput Light-controlling Microfluidic Microalgae Culture Platform for Growth and Oil Production Analysis

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Microalgae are envisioned as a future source of renewable oil. The feasibility of producing transportation grade hydrocarbons from microalgae is strongly dependent on developing better algal strains, understanding algal gene regulation, and optimizing growth conditions for higher oil production. We present a scalable high-throughput microfluidic microalgal photobioreactor array capable of applying 64 different light conditions (combination of 8 different light intensities and 8 different light-dark cycles) to arrays of microscale algal photobioreactor chambers to investigate how light conditions influence algal growth and oil production. Continuous perfusion of nutrients to arrays of single-colony-trapping microstructures allowed time-course single-colony-resolution analysis. Analysis of growth and oil production for the green microalga *Botryococcus braunii* under various light conditions revealed the light intensity and light-dark cycle inducing maximum oil accumulation. This screening test was accomplished by the developed photobioreactor array at 250 times higher throughput and 850 times lower reagent consumption compared to conventional photobioreactors. We expect that this platform will serve as a powerful tool to investigate optimum algae growth and oil-producing conditions at significantly lower costs and shorter times, which can dramatically enhance the development of renewable algal energy systems.

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22. High-Throughput Microfluidic Screening Platform Capable of Selective Single Cell Extraction

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Conventionally, screening and selection of mutagenized cell populations are conducted by culturing them at low dilution on culture plates and manually picking cells that show desired trait. Although this process is useful and widely used, it is time-consuming and quite labor intensive. Also, a large number of mutants to be screened make this strategy costly and challenging. Here, we have developed a high-throughput mutagenized cell screening platform that enables easy monitoring of mutagenized cell properties through light/fluorescence microscopy followed by selective extraction of a particular genetic variant of interest for further off-chip analysis and sampling. The platform is composed of two functional components; a microfluidic cell analysis layer and pneumatic control layers. The cell analysis layer has 1024 trapping sites where a single cell can be captured, cultured, analyzed, and selectively extracted to off-chip reservoir. Combination of two pneumatic control layers, each comprising 32 microchannels, allows individually addressing and controlling each of the 1024 trapping sites underneath. By pressurizing either of the two pneumatic control channels or releasing pressure from both channels, the underlying trapping site was opened or closed, respectively. Cells inside a particular trapping site were successfully extracted and collected at the reservoir with backflow by selectively opening only this particular site. The performance of this screening platform was characterized using green microalga *Tetraslimis suecica.*

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23. Intein-mediated protein hydrogel and its application as bioreactor scaffold

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Hydrogels made entirely of proteins have become an important field of research for applications in tissue engineering, drug delivery, and in vitro biosynthesis. Here, we describe a self-assembled protein hydrogel that is stable at a wide range of pH (6-10), temperature (4-80℃) and solvent conditions (aqueous and organic solvent). The building blocks of the hydrogel are two protein triblock copolymers expressed separately in E.coli. Each copolymer contains a bioactive protein flanked by one half of a split intein and a crosslinker comprising of a multimeric protein subunit. Upon mixing, split intein reactions covalently link the crosslinker/bioactive protein from the two building blocks, resulting in the formation of a protein polymer capable of self-assembling into a hydrogel. Proteins/enzymes can be stably immobilized in the hydrogel via high-affinity protein-ligand interactions. We will discuss the use of our hydrogel as immobilization scaffolds for enzymatic reactions in organic solvent and bioelectrode synthesis.

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24. Low-Concentration Oil Detection and Separation by Acoustic Standing Wave

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Detection and quantification of extremely small amount of oil on site and at low cost has broad applications in environmental monitoring, both in response to oil spills as well as in routine marine/costal ecosystem monitoring. For example, dispersed oil (typically less than 100 μm), generated through natural weathering as well as the use of chemical dispersants in oil spills to break up oil slick into small droplets are the greatest concern and poses the most challenges in detection. Fluorometry is the current standard method, however mostof the current fluorescence based oil detectors have trade-offs between detection sensitivity and portability/cost.Here we demonstrate for the first time the development of an acoustic standing wave based microfluidic platform capable of processing large amount of liquid samples from which dispersed oil can be concentrated to a detectable level by acoustophoretic force and separated for further off-chip analysis. The microfluidic platform consists of a circular oil droplet trapping chamberand a downstream separation region.Two piezoelectric transducersare attached at the bottom of the silicon-glass microchipto generate acoustic standing wave that facilitates oil droplets trapping and separation. An optical detector measures the presence of concentrated oil droplets by their distinct fluorescent signatures.This system holds the promise as a portable highly efficient and highly sensitive field-deployable oil spill detector.

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25. The *Magnaporthe grisea* PTH11-Like G Protein-Coupled Receptor PLG1 is Required for Rice Blast Pathogenicity

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Magnaporthe grisea is the causative agent of rice blast disease and was recently reviewed as a plant fungal pathogen of highest economic importance. Successful infection relies on fungi's ability to sense both environmental and host surface signals at different stages of the disease cycle. Signal transduction is carried out in part by G-Protein Coupled Receptors (GPCRs). This project is focused on the functional analysis of PLG1, a member of the *M. grisea* PTH11 class of GPCR-like proteins. Targeted deletion of *PLG1*generated mutants that are unable to infect rice and barley plants, but are capable of invasive growth on wounded leaves. *M. grisea* requires a hard and hydrophobic surface such as a Teflon membrane for infection-related morphogenesis. Exposure of spores on Teflon showed that germ tube hooking and apical swelling occurred in the *Dplg1* mutants. However, they developed melanized appressoria, the main infection structures at a lower frequency (~10%) compared to the wild-type 70-15 strain. When *Dplg1* conidia were incubated on Teflon, with inducers, such as cAMP or 1,16-hexadecanediol, a significant increase in the formation of appressoria was observed after 24 hours. This suggests that PLG1 lies upstream the cAMP signaling pathway. As a predicted receptor, PLG1 may not be necessary for penetration or invasive growth, but may function during the pre-infection process possibly by interacting with a ligand that enables the fungus to recognize the plant host or any suitable surface. Further characterization of PLG1 is being done to confirm its cellular location and its effect on downstream targets largely involved in the pre-penetration stage.

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26. Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry Imaging (MALDI-TOF-MSI) Applications

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The Laboratory for Biological Mass Spectrometry (LBMS) serves the TAMU research communities with expertise in mass spectrometry and is currently equipped with high mass accuracy and high resolution mass spectrometers. LBMS research scientists are actively involved in the development of new analysis methods and development of next-generation instrumentation for analysis and sample handling. We will discuss several of our latest projects. Soil streptomycetes are saprotrophic bacteria that secrete numerous secondary metabolites and enzymes for extracellular functions. Many streptomycetes produce antibiotics that are thought to protect vegetative mycelia from competing organisms. In collaboration with the Straight Laboratory we report that an organism isolated from soil, *Streptomyces*sp. Mg1, actively degrades colonies and causes cellular lysis of*Bacillus subtilis*when the organisms are cultured together. We use a MALDI-TOF-MSI strategy to map the positions of metabolites secreted by both organisms to show that*Streptomyces*sp. Mg1 produces the macrolide antibiotic chalcomycin A, which contributes to inhibition of*B. subtilis*growth in combination with other, as yet unidentified factors. A new separation platform using heterogeneous supported lipid bilayer (SLB) electrophoresis was developed to investigate and isolate membrane components within their native environment. In collaboration with the Cremer Laboratory, we report the SLB electrophoretic separation of a five-lipid mixture; two of which are biological important receptors. Vesicles containing the ortho- and para- isomers of Texas Red-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (TR-DHPE), monosialoganglioside(GM1), disialoganglioside(GD1b), and 1-palmitoyl-2-oleoyl-3-phosphocholine (POPC) were deposited and separated using heterogeneous SLB electrophoresis into five resolved bands as determined by MALDI-TOF-MSI.

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27. Micron-Size Particle Deposition Scenarios by Reflection Interference Contrast Microscopy

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Different deposition scenarios are expected when micron-size particles are deposited on a surface depending upon environmental conditions (relative humidity, temperature), deposition medium (air, liquid), and time effects. For instance, when using a liquid as deposition medium, a liquid meniscus can form between the particles and the substrate at the end of the drying process; depending on the conditions, the meniscus can dry out completely or partially, and the particles might undergo deformation due to capillary forces, in addition to regular adhesion forces, creating a variety of deposition scenarios. Reflection Interference Contrast Microscopy (RICM) offers a unique and convenient view of the deposition phenomenon as the minimum separation distance between particle and substrate, contact area, and particle contour can be accurately quantified when looking at the sample from below using monochromatic light. This information collected from hundreds of individual particles can be used to describe the microscopic effects of the aforementioned variables on particle deposition which represents valuable information for resuspension and adhesion models.

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28. Mechanical device for creating microwounds to facilitate gene transfer into germline cells within shoot apical meristem of cotton embryos.

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Direct or *Agrobacterium*-mediated transformation of germline cells within the L2 and L3 layers, present within the shoot apical meristem of plants, has been a great challenge. Transformation of cells within these layers requires direct penetration of naked DNA or contact with the *Agrobacterium* cells. However, the outermost L1 layer and the surface cuticle act as barriers and prevent penetration/contact with the inner L2 or L3 cells. We have established an efficient protocol for isolating the embryo axes manually from presoaked cottonseeds. We are examining various means such as sonication, ‘sand-blasting’, etc. to generate microwounds into the shoot apical meristem in order to provide access to the L2 or L3 cells.Transformation of these germline cells will allow us to generate transgenic cotton plants in a matter of few months rather than a year when using the cell culture-based system. An efficient mechanical method to create microwounds in the shoot apical meristem without causing extensive damage to the tissue is needed to help improve and accelerate generation of transgenic cotton and other important crops.

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29. Microfluidic Neural Progenitor Aggregate Culture Platform for *in vitro* Myelination Study

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We present a microfluidic PDMS culture platform for neuralprogenitor aggregates, capable of culturing 40 neural aggregates in spatially controlled environment for potential testing of drugs that impact neural development and CNS myelination. The proposed microfluidic culture platform enables neural aggregates to be cultured in a spatially controlled environment, thereby maintaining aggregate-to-aggregate distances throughout the culture period. The platform is composed of four culture chambers each containing 10 horseshoe shaped trapping sites. Neuralprogenitorsprepared from E16 rats were cultured inside an array of microwells with 150 μm openingfor three days to form size-controlled aggregates, which were then dispensed through the cell loading port into the culture chambers. Neural aggregates were trapped inside each trap,and robust neurite outgrowth and glial migration were observed during the first week of culture. At DIV 14, when abundant astrocytes and oligodendrocytes (OLs) were generated and dense axonal networks were formed among neighboring aggregates, the cultures were treated with retinoic acid (500 nM) to investigate its effect on myelin formation. After two weeks of treatment, increased number of myelinating OLs and myelin segments were found in retinoic acid-treated group as compared to controls, demonstrating the capabilities of this microfluidic culture platform. In summary, we developed a microfluidic neural progenitor aggregate culture platform that can be exploited as a powerful tool to investigate neural development and myelination *in vitro* and to test potential drug candidates capable of promoting CNS myelination.

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30. Microfluidically Enabled High-throughput Monitoring of Environmental Nanoparticles

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Increased environmental exposure is an unavoidable consequence of the growing prevalence of nanomaterials, posing new and largely unknown risks to human health. Few methods integrate both sampling and detection with sufficient throughput to perform continuous environmental monitoring of room-sized volumes. We describe a new approach that enables continuous environmental sampling of airborne nanoparticles with online detection and quantification of the collected species. Our method uniquely combine the high flow rate sampling capability of wetted wall cyclone (WWC) collectors (up to > 1,000 L/min) with a microfluidic component that permits sensitive quantitative measurement of nanoparticle concentration. By coupling these components, we demonstrate detection of airborne ultrafine Al_2O_3 nanoparticles at environmental concentrations below 200 μg/m^3 in air sampled at a 200 L/min, well within established toxicity limits.

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31. A New Method for Surface Enhancement of Bones in Ultrasound Images Based on the Doppler Effect

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Ultrasound (US) techniques are becoming an attractive modality for orthopedic applications. 3D ultrasound reconstruction techniques have shown significant potentials for the visualization of bone surface defects. In many cases, however, the soft tissue/bone interface may not be clearly identifiable in the B-mode images due to the presence of hyperechoic tissue and artifacts by acoustic attenuation, multi-reflection, transducer motion, etc. Thus, the application of an image enhancement technique is usually a necessary step prior performing 3D US bone imaging reconstructions.

In this paper, we present a novel technique to acquire and process ultrasonic signals from bones. Inspired by elastography and Doppler imaging, this technique takes advantage of both the distinctive mechanical and acoustical properties of bones with respect to soft tissue to make bone surfaces more distinguishable in noisy ultrasound images. This method is not affected by multi-reflection artifact, which is typical when dealing with ultrasonic bone imaging. Image quality performance of the proposed method is investigated using data obtained from controlled experiments in vitro and experimental data in vivo. The preliminary results obtained from the controlled experiments suggest that the proposed enhancement technique can significantly increase the bone-tissue contrast, which in turns allows for easier and faster bone surface segmentation. This algorithm can achieve real-time performance and has the potential to provide more accurate and faster 3D bone reconstructions.

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32. The NEW Route of Chitosan Extraction from Waste Shrimp Head to Use as a Flocculant

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The chitosan can be used in waste water treatment as flocculant which can bind the sediment particles and subsequently removed by settlement. Most studies of chitosan extraction from the shell of crustaceans propose that it is converted from chitin by deacetylation through the process of demineralization and deproteinization using chemical or microbial treatment. Two different attempts suggest in this proposal to test the new route of chitosan extraction from waste shrimp head on effectiveness of flocculation for anaerobic digestion: 1) The chitosan extractes using lime treatment (deproteinization) followed by heterogeneous 50 % NaOH treatment (deacetylation), 2) The chitosan extractes using mixed anaerobic microorganisms followed by heterogeneous NaOH treatment. The extracted chitosan dissolves in 2 % acetic acid solution which forms chitosan-acetate, and then examines to flocculation. The removal of about 78% solids from anaerobic digestion was achieved by the addition of 10 ml/L chitosan-acetate. The optical density was also significantly decreased from 2.84 to around 0.5 after flocculation. The purity % (g acids/ g solids) was reached about 82% by the same dosing flocculant level. The results of this study verified that the lime treatment for deproteinization should generate protein-rich materials and increase the chitin extraction yield. Moreover, mixed anaerobic microorganisms should ferment the protein and mineral during the incubation which helps to generate chitin from shrimp head. These two new routes of chitosan extraction should be a useful method for making flocculant in the industrial waste water and anaerobic digestion treatment.

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33. Non Mechanical Axial Scanning in Confocal Microscopy

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We present the use of an electrically tunable focal length lens to perform axial scanning in a reflectance confocal microscopy and show results from ex vivo human oral tissues. The field of view is 625 microns in diameter with a lateral resolution ranging between 1 to 2.4 microns and an axial resolution ranging between 4.5 to 14 microns over an axial scan range of 255 microns. Sub-cellular morphologic features are seen throughout the depth of the epithelium while axially scanning using the focus tunable lens.

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34. A Novel Method to Identify Subnetwork Markers for Cancer Classification Based on Message-Passing Algorithm and Relative Gene Expression

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The utilization of additional biological knowledge, such protein-protein interaction (PPI) data, has been shown to improve cancer classification in terms of prediction accuracy as well as reproducibility of the predicted biomarkers. We propose a novel method for identifying subnetwork markers from a human PPI network that can be used to predict the cancer prognosis. The proposed method utilizes a message-passing algorithm called affinity propagation to identify subnetworks of discriminative and synergistic of genes based on their relative expression. Our experimental results based on two large-scale breast cancer datasets show that the proposed method can simultaneously identify reliable and non-overlapping subnetwork markers that may potentially lead to more accurate prediction of breast cancer prognosis.

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35. A Novel Random Walk Model for Comparing Protein Interactions Networks

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In this work, we propose a novel method for comparing large-scale protein-protein interaction (PPI) networks. The proposed method is based on a Markov random walk model that performs a simultaneous random walk on two PPI networks to estimate the node-level similarity between the given networks. At each step, the random walker examines the neighboring nodes to adjust its mode of random walk, where it can switch between a simultaneous walk on both networks and an individual walk on either network. Our simulation results based on synthetic PPI networks show that the proposed method yields more effective node-correspondence scores that can lead to more accurate alignment of PPI networks.

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36. Optical Biomedical Sensing Applications

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Over the past several decades, a variety of optical sensing schemes have been employed toward the detection and quantification of biologically relevant analytesforboth *in vivo* and *in vitro*applications.Our lab, Optical Bio-Sensing Laboratory (OBSL),focuses on the design, development, and testing of optical systems for a variety of biomedical sensing applications. We have developed polarimetric and fluorescent based techniques capable of monitoring physiologic glucose concentrations. These non-invasive and minimally invasive optical sensors can potentially assist individuals with diabetes in the tighter regulation of their blood sugar levels. Also, weare currently exploring the use of Surface Enhanced Raman Spectroscopy (SERS) toward the development of a platform technology capable of quantifying the level of specific analyte or biomarkers present in blood. Such a technology would be a powerful tool in early diagnosis of several medical conditions such as cardiac events and blood biotoxins. In addition to these techniques we also developnear infrared (NIR) and visible optical spectroscopy sensors to evaluate perfusion and oxygenation of biological tissue and quantify oxidative stress for a variety of applications such as monitoring of implantable organs.

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37. PD 404,182 is a novel and promising anti-HIV microbicide

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We recently identified a virucidal small molecule, PD 404,182 (PD) that disrupts HIV. PD possesses multiple attributes that make it an attractive candidate anti-HIV microbicide. PD 1) exhibits a unique mode of action – irreversible disruption of HIV through interaction with a yet unknown structural component; 2) exhibits antiviral activity against a broad range of primary HIV-isolates, HIV-2, and SIV at submicromolar to micromolar concentrations; 3) retains its antiviral activity for at least 8 hours in cell culture at 37°C prior to the addition of HIV to the cells; 4) is effective against both cell-free and cell-associated HIV and inhibits the transmission of dendritic cell-associated HIV to T cells; 5) is potent at neutral and low pH and fully active in seminal plasma and cervical fluids; 6) is extremely efficacious since less than 5 min of incubation with virus results in >99% loss of viral infectivity; 7) is non-toxic to human cells; 8) is non-toxic to the commensal bacteria Lactobacilli; 9) is specific – being ineffective against other enveloped viruses including Sindbis and Dengue virus, setting PD apart from non-specific surfactant/polyanion-based anti-HIV microbicides; 10) is viral-envelope-protein-independent; and 11) does not foster the emergence of resistant HIV variants at sub-neutralizing concentrations in cell culture for 60 days. These data suggests that PD is a potent anti-HIV agent with a novel mode of action.

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38. $PDMS_{star}$–PEG Hydrogels for Osteochondral Tissue Engineering

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Tissue engineering (TE) presents a strategy for repair of osteochondral defects - damage extending from cartilage to subchondral bone. A “materials-guided TE approach” relies on the physical and chemical properties of the scaffold to direct cell behavior and the ultimate regeneration of tissues in a desired 3-D geometry. Conventional poly(ethylene glycol) diacrylate (PEG-DA) hydrogels, prepared via photocure of aqueous precursor solutions, have been extensively studied for this purpose. However, their potential to generate tissues is inhibited by a somewhat narrow range of properties and a lack of bioactivity and osteoinductivity, which limits osteochondraltissue regeneration. Previously our lab fabricated, via solvent induced phase separation (SIPS), inorganic-organic hydrogels based on methacrylated star polydimethylsiloxane ($PDMS_{star}$-MA) and PEG-DA. Along with exhibiting bioactivity and osteoinductivity, SIPS $PDMS_{star}$-PEG hydrogel scaffolds displayed increased pore size and modulus versus analogous conventional scaffolds. However, the organic solvent of the precursor solution prohibits photoencapsulation of cells into SIPS scaffolds. Thus, in this study,$PDMS_{star}$-PEG SIPS scaffolds were formed with interconnected pores via salt leaching to facilitate the infiltration of seeded cells post-fabrication. The influence of the total macromer concentration, weight percent ratio of $PDMS_{star}$-MA to PEG-DA and average salt particle size on morphology, modulus, and hydration were evaluated.

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39. Positive Contrast for Brachytherapy Seeds in MRI Using Quantitative Susceptibility Mapping

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Brachytherapy is a method to treat certain cancers by directly inserting radioactive seeds. MRI can provide high-resolution visual assistance to physicians for seeds insertion and evaluation. However, the brachytherapy seeds usually show as dark spots, i.e. negative contrast, on the MRI images. Here we propose an improved QSM method for seed identification. A unique feature of the proposed method is the use of an automatically defined mask to leave out the unreliable data in the regularized model fitting. Experimental results show that the proposed method can correctly locate the seeds and differentiate them from the tissue voids/structures.

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40. A Possible Application of Biomimetics in Optimizing the Quantum Efficiency of Photovoltaics

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The most effective circumnavigation of the challenges arising from responding to the economics and politics of climate change is achieved by utilizing non-fossil sustained energy sources such as sunlight, which is a huge source of energy (120,000 TW/year). Currently, the most advanced low-cost organic photovoltaic cells have a quantum efficiency of ~8% in stark contrast to plant/bacterial light-harvesting systems, which offer a quantum efficiency of ~95%. The biomimetic project is concerned with how one could make use of the underlying principal components of photosynthesis in developing highly efficient photovoltaic devices from man-made materials. Of particular interest is the highly effective quantum coherence-enabled energy transfer. Noting that quantum coherence is promoted by charged residues and local dielectrics, classical atomistic simulations and time-dependant density functional theory (DFT) are used to identify charge/dielectric patterns and electronic coupling at energy transfer interfaces that have to be accurately defined both in terms of their chemistry and their locale. The latter is particularly critical as locations, distances, and orientations matter across the scales and vary in response to the environment. Therefore the calculations make use of structural information obtained on photosynthetic protein-pigment complexes while still in the native membrane making it possible to establish a link between supramolecular organization and quantum coherence in terms of what length scales enable fast energy transport and prevent quenching. Calculating energy transfer efficiencies between components based on different proximities will permit the search for patterns that enable defining material properties suitable for advanced photovoltaics.

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41. Proteases and fibrocyte differentiation: A possible triggering mechanism for fibrosis and wound healing

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Trypsin-containing topical treatments can be used to speed wound healing, although the mechanism of action is unknown. Tryptase and thrombin are up-regulated in scar tissue and in wound healing, respectively. To help form scar tissue and heal wounds, monocytes leave the circulation, enter a wounded tissue, and differentiate into fibroblast-like cells called fibrocytes. We find that 20-200 ng/ml trypsin (concentrations similar to those used in wound dressings) potentiates the differentiation of human monocytes to fibrocytes in cell culture. Tryptase (28-56 ng/ml) and thrombin (7-14 ng/ml), proteases with substrate specificity similar to that of trypsin, also potentiate fibrocyte differentiation at biological concentrations. Proteases with other site specificities such as pepsin, endoprotease GluC, and chymotrypsin do not potentiate fibrocyte differentiation. Adding protease inhibitors increases the amount of trypsin protease to potentiate fibrocyte differentiation, suggesting that the potentiating effect is dependent on the enzyme's proteolytic activity. An inhibitor of protease-activated receptor-2 (PAR-2) inhibits the effect of trypsin and tryptase on fibrocyte differentiation, suggesting these proteases signal through PAR-2. Tryptase and thrombin compete with serum Amyloid P (SAP) and human serum to potentiate fibrocyte differentiation. Each protease also biases macrophage differentiation towards an M2a phenotype associated with scar tissue. Together, these results suggest that certain proteases speed wound healing and scar tissue formation by activating PAR-2 receptors to potentiate the differentiation of monocytes into fibrocytes and M2a macrophages even in a competition with SAP and human serum, raising the intriguing possibility that proteases play a role in the initiation of a fibrotic lesion.

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42. Protein Resistance and Surface Restructuring of PEG-Silane Amphiphiles with Variable PEG Segment Lengths

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Protein adsorption on blood-contacting medical devices leads to thrombosis and infection. Therefore, protein-resistant biomaterials have the potential to improve device safety and efficacy. Poly(ethylene glycol) (PEG) has been incorporated into biomaterials (e.g. silicones) to confer protein resistance, but its performance *in vivo* is limited. To improve protein resistance, we prepared PEG-silane amphiphiles with a siloxane tether and variable PEG segment repeat unit length (m = 3, 8, & 16). Surface-grafted coatings having similar graft densities were prepared on silica wafers to eliminate restructuring effects and compared to those based on "PEG-controls" (i.e. no siloxane tether) as well as "siloxane controls" (i.e. no PEG segment). While surface hydrophilicity of PEG-silane amphiphile coatings increased with PEG segment length, the "PEG-control" surface-grafted coating was the most hydrophilic and exhibited the greatest resistance to fibrinogen adsorption. Next, medical-grade silicone was bulk-modified with PEG-silane amphiphiles as well as the controls. While silicones modified with the "PEG-control" were hydrophobic, those modified with PEG-silane amphiphiles were more hydrophilic and demonstrated a high capacity to restructure at aqueous interfaces. These results indicate that the siloxane tether imparts PEG with an important ability to restructure to the surface of polymeric networks. It was also found that PEG segment length influenced surface restructuring, where silicones modified with PEG-silane amphiphiles of m=8 were the most hydrophilic.

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43. Regulatory T-Cell Differentiation Plasticity Model

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Naïve T helper cell differentiation comprises different pathways to type 1, 2, 17 and regulatory, among other, phenotypes. However, these differentiation pathways are not a dead end, and regulatory T cells are able to take on an inflammatory phenotype after therapeutic adoptive transfer. Therefore, a quantitative understanding of T helper cell differentiation has the potential to advance the use of cell-based therapeutics in a wide variety of conditions characterized by a dysregulated immune response. Here, we study Th2, 17 and regulatory T cell differentiation in response to a range of cytokine and metabolite conditions. We utilize a feed-forward neural network to model T helper cell differentiation *in vitro*. By surveying a range of different network architectures for the model, we identify a model that has a good trade-off between parameterization and generalizability and produces experimental hypotheses to optimize regulatory T cell differentiation cultures. In future work, we will plan to expand this modeling technique to an *in vivo* model of regulatory T cell adoptive transfer in order to identify optimal culture conditions for stability of the regulatory phenotype.

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44. Resimulation of Noise: an algorithm for measuring the reliability of minimum least square error estimated temporal elastographic parameters

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Novel temporal ultrasound elastography techniques typically involve the estimation of the temporal mechanical behavior of tissues using curve-fitting methods. Parameters estimated from sampled noisy experimental data using the minimum least square error curve fitting method are more meaningful when a measure of reliability is presented for the estimated parameters. Typically, the correlation coefficient between the data points and the fitted curve, which is suitable for linear curve-fitting, is used. However, estimation of temporal elastographic parameters often involves fitting noisy data points in time to non-linear (decaying exponential) mathematical models. In these cases, a measure of reliability of an estimated parameter *X* written in the form of *X*±*Error* may be easier to interpret. If several trials of *X* are available, then *Error* can simply be equal to the standard deviation of X. However, the nature of elastographic parameter estimation (and in general imaging in clinical settings) seldom allows for such experiment retrials to be done. We have developed and tested the Resimulation of Noise (RoN) algorithm that simulates the process of trialing experiments, using only the noisy data from a single trial and its resultant fitted curve to generate an estimate of standard deviation of X. Simulations show that the RoN algorithm provides an estimate of the standard deviation of X that is linearly related to the actual standard deviation with near 1 slope and near 1 correlation coefficient. Compared against other commonly used reliability measures, RoN'sstandard deviation estimate willmore consistently detect unreliable estimates.

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45. The role of *fit* in *Drosophila melanogaster* starvation response, eating behavior, and lipid storage

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Novel genes that contribute to male courtship behavior were identified from microarray analyses on the heads of males that had courted females[1]. Decreased expression of *socially responsive gene 1* (*srg1*), which increased during courtship, led to homosexual courting displays, indicating that *srg1* is important for wild-type courtship behavior. *srg1* expression decreases in starved animals, increases in mated females, and is fat body-biased. To test whether *srg1* signals satiety from the fat body to the brain, we evaluated food consumption as well as preference for sugar or protein sources, as mated females are known to switch preference to protein-based food sources. We concluded that Srg1 relays information about future nutrient demands in response to current sexual status and results in altered feeding behavior.

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46. Self-assembling protein hydrogel for enzyme incorporation onto electrodes in biofuel cells

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- Biocompatible protein-based hydrogels provide a promising platform for many medical and industrial applications.
- We developed a bioaffinity-based, self-assembling protein hydrogel that forms upon mixing of two soluble protein components.
- Each component consists of a trimer protein (CutA) and a PDZ domain containing protein (Tip1) or a PDZ domain-recognizing peptide ($Tip1_{lig}$).
- Disulfide bond between Tip1 and $Tip1_{lig}$ was introduced via rational design to stabilize the protein interaction.
- Hydrogel is
  - Highly stable in acidic and neutral pH (6-8) and a wide range of temperature (4-50 °C) conditions.
  - Highly elastic and is able to completely recover elasticity after shear-thinning.
  - Compatible with tissue culture growth medium.
- Incorporation of a docking station peptide (Dsp) into the hydrogel building block facilitates stable immobilization of docking protein (Dp)-fused target proteins.
- Incorporation of carboxylated multi-walled carbon nanotubes (cMWNT) and a small laccase (Slac) converts the hydrogel into a functional biocathode.
- Our protein hydrogel should serve as a multifunctional scaffold for diverse applications in tissue engineering, drug delivery, biocatalysis and biosensing.

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47. Self-powered biosensors driven by flexible thermoelectric power generators made ofp- and n-type carbon nanotubes

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Flexible organic thermoelectric modules made of both p- and n-type single wall carbon nanotubes were developed to generate high voltage and power enough to operate biosensors. In order to prepare n-type films, nanotubes were functionalized by both diethylenetriamine (DETA) and polyethyleneimine (PEI), and subsequently further reduced by using $NaBH_4$. The thermopower and electrical conductivity of optimized n-type nanotubes were measured to be -86 μV/K and 5,200 S/m, respectively. The p-type nanotube samples have a thermopower and electrical conductivity value of +100 μV/K and 11,000 S/m, respectively.Electronic band structures with the Fermi level and carrier mobilities of the CNTs were experimentally investigated, which have elucidated the carrier type and relatively large thermopower values. The as-synthesized 72 p- and n-type pairs were assembled by stacking them alternatively to complete the power generation module. The Seebeck voltage of the thermoelectric module was measured to be 9.3 mV/K leading to a 460 mV output at a temperature gradient, ΔT = 49 K, which are significantly larger than other polymer based organic composites. A glucose sensor was operated by using 1.8 μW from the thermoelectric module at ΔT = 32 K. We demonstrated that flexible organic thermoelectric devices are promising for portable and self-powered sensors.

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48. Sexually experienced *Drosophila melanogaster* males are better at courting and competing for mates

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Competition for mates is a widespread phenomenon that affects reproductive success. Gaining a mating advantage over competitors is therefore a priority for increasing individual fitness. The ability of animals to adjust their behaviors in response to changing social environment is important and well documented. *Drosophilamelanogaster* males compete with one another for copulations with females and vary their reproductive behaviors based upon prior social interactions. However, it remains to be determined how male social experience that culminates in mating with a female impacts subsequent male reproductive behaviors. In this study we quantified the effects of prior mating experience on subsequent *D. melanogaster* male courtship behavior and mating success. Males with previous sexual experience performed less courtship but extended their wings and attempted to mount the females more often compared to sexually naïve males. When a sexually experienced and a naïve rival competed for mating with a female, sexually experienced males won more often by increasing the effort they directed towards component courtship behaviors. Interestingly, males with only courtship experience or with incomplete copulations did not out-compete naïve males; therefore courtship experience alone was not sufficient in providing this competitive advantage, indicating that copulation plays a role. Our results demonstrate the ability of previously mated males to learn from their sexual experience and modify their behaviors to gain a mating advantage. These experienced-based changes in behavior reveal learned strategies that animals likely use to increase their fecundity in natural competitive environments.

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49. A Sol-Gel Derived Silver Nanoparticle Embedded Thin Film for Mass Spectrometry-Based Biosensing

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Matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) has proved invaluable for the analysis of a number of high molecular weight compounds; however its utility for analysis of small molecules (<1000 m/z) such as steroids and lipids has been limited due to significant background noise from matrix clusters that congest the low mass region of the spectra. Here, we describe a sol-gel based silver nanoparticle (AgNP) embedded thin film for matrix free detection of several analytes in various chemical classes.In these experiments, UV laserirradiation (337 nm) of the AgNP facilitates desorption andionization of a number of peptides, triglycerides, andphospholipids. Preferential ionization of sterols from vesiclescomposed of olefinic phosphosphatidylcholines is alsodemonstrated, offering the possibility for a simplified approach for sterol analysis from complex mixtures.Moreover, the platform displays an excellent shelf life and retains its properties for overa year without compromising LDI-MS function, an appealingquality from a diagnostic standpoint where minimal samplepreparation is often required for rapid analysis.

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50. Split intein mediated ultra-rapid purification of tagless proteins (SIRP)

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- ❖ Rapid and efficient tag removal remains a significant problem in recombinant protein purification.
  - Proteases, as the most common tool, are either non-specific, inefficient, rather expensive, or leaving certain amino acids at the N-terminus of the target protein after cleavage.
  - Most engineered inteins exhibit low catalytic efficiency, requiring prolonged incubation to achieve significant N-/C-terminal cleavage.
- ❖ We engineered a DnaEintein from *Nostocpunctiforme* (*Npu*) that enables rapid C-terminal cleavage efficiency.
  - Extraordinarily rapid thiol-activated C-terminal cleavage with ~50% completion within 30 seconds at both 22℃ and 6℃.
  - >90% cleavage completion is achieved within 30 minutes at 22℃, or within 3 hours at 6℃.
  - Target proteins are cleaved efficiently regardless of the identity of the N-terminal amino acid except in the cases of threonine and proline.
  - The C-terminal cleavage reaction can be effectively inhibited by divalent Zn^{2+} under non-reducing conditions.
- ❖ We developed a split intein mediated ultra-rapid purification (SIRP) method using the engineered *Npu*intein.
  - SIRP enables rapid purification of tagless protein from *E. coli* lysate in < 1hr.
  - The column-bound bait intein can be regenerated and recycled for multiple usages.

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51. Superior Methods to Examine Bone Tumor and Host Tissue Interactions Using Micro-Gravity Bioreactors

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Malignant bone disease can occur by metastasis or as a result of a primary bone tumor. When tumors establish in bone, catastrophic tissue damage occurs as a result of accelerated bone destruction and inhibition of repair. The resultant osteolytic lesions cause pain and fractures, but more importantly, they provide an ideal niche for tumor propagation. Tumors maintain this microenvironment by secreting Wnt inhibitors that prevent mesenchymal stem cells(MSCs) from differentiating into osteoblasts. We have demonstrated that inhibiting peroxisome proliferator activated receptor-γ(PPARγ) by small molecule GW9662, reduces negative cross-talk on the Wnt pathway resulting in the establishment of a pro-osteogenic MSC phenotype(OEhMSCs). The OEhMSCs secrete extracellular matrix that mimics the composition of anabolic bone tissue (hMatrix). When hMatrix is co-administered with OEhMSCs the cell-matrix composite has a unique capacity for rapid repair of bone defects in rodents. To discover ways to inhibit the activity of Wnt inhibitors, it is necessary to mimic the interactions between bone tumors and MSCs. The rotating wall vessel is an excellent tool for this purpose because it permits the growth of 3D tissue constructs while facilitating excellent fluid and gas exchange. The hMSCs and tumor cells will be seeded separately onto microspheres that mimic the surface of bone tissue. The hMSCs will be induced to differentiate into osteoblasts and generate bone in the presence of osteosarcoma cells. Successful execution of these studies will lead to a range of new methods for the investigation of tumor-MSC interactions without the limitations of 2D tissue culture or in vivo approaches.

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52. Thermally Targeted Adsorption and Enrichment in Microscale Hydrothermal Pore Environments

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The unique ability of chaotic advection under micro-scale confinement to direct chemical processes along accelerated kinetic pathways has long been recognized. But practical applications have been slow to emerge because optimal results are often counter-intuitively achieved in flows that appear to possess undesirably high disorder. Here we demonstrate how thermally actuated chaotic phenomena within these microenvironments are capable of establishing a continuous convey or transporting chemical compounds from the bulk to targeted locations on solid boundaries where they become greatly enriched. These findings intriguingly suggest that microscale chaotic advection may offer a new mechanism to explain emergence of biomolecular complexity from dilute organic precursors in the prebiotic milieu—a key unanswered question in the origin of life.

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53. Ultra Strong, Thermoresponsive Double-Network Hydrogels

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An implanted glucose sensor would not only eliminate the inconvenient and painful finger prick test, but would also provide diabetics with more precise control over blood sugar levels thereby improving their quality of life. A sensor membrane that controls the host response (i.e. biofouling) is crucial to the sensor's long-term functionality. We propose a "self-cleaning" membrane based on a thermoresponsive poly(N-isopropylacrylamide) (PNIPAAm) hydrogel, which reversibly switches from a water-swollen, hydrophilic state to a deswollen, hydrophobic state at unique volume phase transition temperature (VPTT, ~33-35oC). PNIPAAm hydrogels are limited by their poor mechanical properties and slow responsiveness. Two major PNIPAAm hydrogel designs were explored in these studies overcome the above issues: (1) a double-network (DN) nanocomposite membrane containing polysiloxane nanoparticles and (2) a DN membrane containing an electrostatic comonomer2-acrylamido-2-methyl-propane-sulfonic acid (AMPS). The resulting changes to morphology, deswelling/re-swelling behavior and mechanical properties were evaluated and compared to single network (SN) and DN hydrogels containing no AMPS and polysiloxane nanoparticles.

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54. Ultrasound Elastography as a New Assessment Tool for Orthopedics

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The mechanical properties of the soft tissue surrounding a bone change when the bone undergoes fractures or in the presence of defects. Phenomena such as tear in the tissues, callus formation or a gradual hardening of the callus during bone regrowth are directly correlated with changes in the mechanical properties of the soft tissue at the soft tissue/bone interface. These changes may contain valuable diagnostic and prognostic information. At the present time, there is a lack of imaging methodsthat can allowvisualization of these changes. Ultrasound imaging modalities, on the other hand, have been gaining interest for bone imaging applications by virtue of their ability to assist with rapid diagnosis of bone fractures or abnormalities in a portable, interactive, real time, safe and repeatable fashion. In this study, we show that it is possible to use novel ultrasound strain elastography techniques to assess intact and fractured bones, the severity of fractures and to monitor bone healing. Elastograms obtained from *ex vivo* intact and fractured canine tibias show that it is possible to accurately detect changes in the stiffness and connectedness of the tissue in the proximity of a bone or at the soft tissue/bone interface, and that such information can be of clinical value. In the future, new bone elastography techniques might be used to provide an insight into the health of musculoskeletal tissues and for better diagnosis and prognosis of bone abnormalities in real-time and without the use of radiations.

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Notes