

University of Minnesota – Institute for Engineering in Medicine

1. Degree of electronic health record use and quality of care in MN-area clinics

Thompson Adevinkomi^{1,2} and Rebecca M. Prenevost²
Medical Industry Leadership Institute, Carlson School of Management, University of Minnesota¹,
www.Evidence.org²

To date, there has been limited study of the relationship between EHR use and healthcare quality, and in the studies that have been published, the results have been mixed. To help address this knowledge gap, this research brief analyzes two important publically-available datasets published by MN Community Measurement along with available county health statistics to assess the relationship between healthcare quality and HER use among healthcare clinics in the MN area.

2. Targeting $\alpha 5\beta 1$ on pig islets of Langerhans in culture with PR_b, a fibronectin-mimetic peptide, to increase islet yield and viability

Nicole Atchison¹, Wei Fan², Klearchos K. Pappas³, Bernhard Hering³, Michael Tsapatsis², and Efrosini Kokkoli²

¹Department of Biomedical Engineering, ²Department of Chemical Engineering and Materials Science,
³Schulze Diabetes Institute, University of Minnesota, Minneapolis, MN

Islet transplantation is a promising treatment for type 1 diabetes but the limited supply of human donor pancreases dictates the need for alternative islet cell sources. Pig islets have shown promise as an alternative to human islet allografts. However, stresses imposed during islet isolation and transplantation decrease islet yield and viability, leading to loss of graft function. Extracellular matrix (ECM) is lost during the islet isolation process and has been shown to cause islet cell apoptosis, decreasing the viability and number of islets recovered per pancreas. Additionally, transplanted islets suffer from hypoxic and other stresses that affect cell viability, leading to eventual loss of graft function. Therefore, to increase islet graft survival, the islets must be protected from apoptosis occurring from both isolation and transplantation.

This study investigated the ability of a fibronectin-mimetic peptide, PR_b, which specifically binds to the $\alpha 5\beta 1$ integrin, to reestablish the ECM around pig islets that is destroyed during isolation. Additionally, PR_b functionalized liposomes were investigated as possible drug-delivery vehicles to pig islet cells. Firstly, we demonstrated the presence of the $\alpha 5\beta 1$ integrin on the pig islet cells using confocal microscopy and Western blotting. We then found that islets cultured in medium supplemented with varying concentrations of free PR_b peptide had dose-dependent, increased levels of ECM fibronectin secretion compared to control islets. This technology could potentially lead to increased viable islet yields after isolation, decreasing the number of donors necessary per xenotransplantation. Using confocal microscopy and flow cytometry we found that PR_b functionalized liposomes delivered to the pig islets internalized into the cells in a PR_b concentration dependent manner, and non-functionalized liposomes showed virtually no internalization. PR_b functionalized liposomes targeted to the cells *ex vivo* could increase delivery of therapeutics or imaging molecules that could protect the cells from stresses encountered after transplantation, increasing graft function and survival, and allow for post transplant monitoring. These studies proved that the fibronectin-mimetic peptide, PR_b, is an appropriate peptide bullet for applications involving $\alpha 5\beta 1$ expressing pig islet cells. PR_b could provide a multi-faceted approach to protecting and maintaining islet viability, increasing the success of pig islet xenotransplantation.

3. Introduction of dimethylsulphoxide (DMSO) in a cell suspension using a microfluidic device

Rohini Bala Chandran¹, Jennifer Reinhart¹, and Dr. Allison Hubel¹
Department¹ of Mechanical Engineering, University of Minnesota, Minneapolis, MN

Cryopreservation solutions must contain some type of cryoprotective agent (CPA) to increase the survival of the cells following freezing and thawing. Conventional methods of cryopreserving blood cells for therapeutic applications involve the use of dimethyl sulfoxide (DMSO) as a cryo-protectant. Current methods of cryoprotectant introduction require trained lab technicians and are hence highly time consuming. We have already evaluated microfluidic platforms as effective means to remove DMSO from cell solutions and obtain favorable cell motions within the device. This automatically led us to evaluate the use of the microfluidic device for controlling the introduction of DMSO. A prototype of the microfluidic device has been designed to experimentally characterize the system dynamics where in two fluids flow in parallel through a constant rectangular cross-section channel, one being the cell suspension (without DMSO) and the other, a donor stream rich in DMSO. Two configurations of the stream flows in the channel have been extensively studied, one of them in which mass transfer is solely based on diffusion and the other involving gravity induced free convection in the channel. Atwood number, a dimensionless constant representative of density contrasts and flow rate fraction of the fluids, have been found to play a significant role in determining flow regimes. Extensive numerical and experimental studies have been performed to examine whether the designed device is able to handle clinical scale volumes. The ongoing investigation also focuses on visualizing cell motion in the channels for these configurations. The long term objective of the investigation is to design a fully automated clinical scale microfluidic device capable of gradually introducing DMSO into a cell suspension and minimizing osmotic stress on the cells to enable high recovery.

4. Computational motor-clutch model for substrate stiffness sensing by migrating glioma cells

Benjamin L. Bangasser¹, Clarence E. Chan¹, Steven S. Rosenfeld², and David J. Odde¹
¹ Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN, ² Cell Biology and Pathology and the Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY

The ability of cells to sense the stiffness of their environment is important for many cell properties including size, shape, migration, and proliferation. Different cell types have shown sensitivity to different ranges of stiffness, with glioma brain cancer cells sensitive at higher stiffnesses than other cell types. One proposed mechanism for cell stiffness sensing is the motor-clutch model whereby force generated by myosin motors is transmitted through actin filaments to the surrounding matrix by molecular clutches (e.g. integrins). A computational motor-clutch model was developed to simulate a motor-clutch system transmitting force to a substrate. By examining the F-actin retrograde flow rate in the model, it was found that certain combinations of parameters result in stiffness sensing while others do not. Firstly, the force generating capability of the motors must be approximately equal to the force resisting capability of the clutches. Secondly, the on-rate of clutches must be approximately ten times greater than the unloaded off-rate. Biphasic sensitivity of retrograde flow rate was also a key behavior in the model, and alteration of model parameters resulted in shifts of the minimum retrograde flow rate over a substrate spring constant range of 0.1-300 pN/nm. Additionally, regimes of "load and fail" were seen on soft substrates, and regimes of "frictional slippage" were seen on stiff substrates. These observations provide an explanation of the differences in stiffness sensing for different cell types, and may help explain the high range of stiffness sensitivity of glioma cells. Fluorescence microscopy experiments were done to measure the retrograde flow rate of U251 glioma cells on polyacrylamide gel substrates of Young's moduli ranging from 1.3-230 kPa. The experimental results showed a biphasic retrograde flow rate dependency on stiffness and demonstrated load and fail on soft substrates. Parameter adjustment in the model resulted in a quantitative match of experiment to the predicted results, and agrees with the observation of up-regulated myosin II and integrins in glioma cells.

5. Engineering monomer specificity of the *P. oleovorans* PHA synthase by site-directed mutagenesis and error-prone PCR

John Barrett and Friedrich Srienc

Department of Chemical Engineering and Material Science, University of Minnesota, Minneapolis, MN

Polyhydroxyalkanoates (PHAs) are polymers that are produced from renewable sources, are completely biodegradable, and biocompatible for use in medical applications. They offer an environmentally friendly and sustainable alternative to petroleum-derived plastics. PHAs can be synthesized from numerous monomer types depending on the specificity of the polymerase enzyme used for synthesis. In our work we have constructed several mutant polymerases with broadened substrate specificity that are capable of recognizing multiple monomer types. Using these enzymes we plan to synthesize novel block-copolymers with enhanced material properties. Novel material properties will expand the range of applications for PHAs in order to replace petroleum derived plastics.

6. The application of fluid power to meet the needs of surgical robotics

Devin R. Berg¹, Perry Y. Li¹, Arthur G. Erdman¹, Tianhong Cui¹, Timothy P. Kinney²

¹Department of Mechanical Engineering and ²Division of Gastroenterology – Hennepin County Medical Center, University of Minnesota, Minneapolis, MN

The use of robotics in surgery is becoming increasingly common with the advent of new minimally invasive surgical techniques such as natural orifice transluminal endoscopic surgery (NOTES), an experimental technique that involves performing traditional surgical procedures with a flexible endoscope through a natural orifice such as the mouth, rectum, or vagina. These sorts of novel techniques remove the need to create incisions across the abdominal wall, thus minimizing or even eliminating post-operative pain, abdominal scars, and complications such as incisional hernias. Other potential benefits include reducing postoperative recovery time, using moderate or deep sedation instead of general anesthesia, and the potential to perform many surgical procedures outside of traditional operating rooms. Because of these advantages, techniques such as natural orifice surgery appear to be the next natural step in the progression of minimally invasive surgery for routine procedures such as cholecystectomies and exploratory laparoscopies in hospitals around the world. Due to the complexity of these procedures and the natural limitations of space and site access, the development of advanced robotics becomes imperative for the future success of these sorts of surgical interventions. The research proposed here intends to identify the limitations of existing robotic surgical platforms and develop a means of overcoming them by taking advantage of fluid power as an enabling technology. The proposed approach differs significantly from conventional surgical robots in that in this case, the robot is embedded within the surgical device itself, whereas in the conventional system, a general-purpose robot is used to manipulate various surgical tools. The advantage of the proposed approach is that with proper technologies, the devices can have more degrees of freedom, be more cost effective, and have greater ease of mobility. The mobility and the relaxed requirements for the operating room cleanliness can be potentially useful for mobile clinics, out-patient clinical settings, and the battlefield. Being more cost effective and having a small overall size, the envisioned robotic assisted surgical devices can be widely deployed, even in rural or other less technology intensive environments.

7. Factors correlating to mechanically-induced disorder in pharmaceutical crystals

Sayantana Chattoraj¹, Chandan Bhugra², Chitra Telang², Zeren Wang² and Changquan Calvin Sun¹

¹Pharmaceutical Materials Sciences & Engineering, Department of Pharmaceuticals, University of Minnesota, ²Pharmaceutical Development-US, Boehringer-Ingelheim Pharmaceuticals Inc, Ridgefield, CT

The purpose of the research was to differentiate APIs depending on their amorphization tendencies, and 2) identify the critical material properties (thermodynamic and mechanical) of crystalline APIs that can predict their amorphization behavior. 11 crystalline APIs (acetaminophen, indomethacin, felodipine, griseofulvin, hydrochlorothiazide, piroxicam, phenacetin, dipyridamole, danazol, ketoconazole and terfenadine) were cryomilled and the % amorphous in the milled materials was quantified by DSC, using melt-quenched glasses as reference. The lattice stabilities of the starting crystals were compared by measuring crystal fusion energies using modulated DSC. Crystal hardness was measured with nanoindentation experiments. Non-isothermal recrystallization kinetics of amorphous phase for each API was measured using the heating rate dependence of recrystallization exotherms in DSC.

APIs with higher lattice stabilities (higher fusion energies) amorphize to a lower extent. Higher fusion energies correspond to stronger non-covalent interactions, which probably enhance resistance to amorphization. Crystal hardness is found to relate inversely with the extent of amorphization. Because crystal hardness also directly depends on the packing density of crystals, both the fusion energy and hardness dependence of amorphization suggest that crystal packing directly controls the extent of amorphization under mechanical energy input. No correlation is observed between the extent of amorphization and glass transition temperature (T_g). However, the kinetics of non-isothermal recrystallization from the amorphous phase produced by milling seems to control the extent of residual amorphous phase measured in DSC. The residual amorphous phase is the difference between the amount of amorphous produced during milling and the extent recrystallized post milling before we quantify it using DSC. Thus, APIs with higher activation energy barrier to recrystallization show a higher net residual amorphous post milling.

Results indicate that amorphization or disorder tendency can be correlated with material properties such as lattice stability, crystal hardness and non-isothermal recrystallization kinetics. It is possible to predict the sensitivity of a new crystal to processing-induced disorder by a comprehensive model that considers all these factors. Such a model may be further refined by incorporating more model drugs in the dataset, which we are currently working on in our laboratories.

8. Ionophore-based CO₃²⁻-selective sensors based on perfluoropolymeric matrixes: 100- to 1000-fold selectivity improvements

Li D Chen¹, Debaprasad Mandal², Gianluca Pozzi³, John A Gladysz², Philippe Buhlmann¹

¹Department of Chemistry, University of Minnesota, 207 Pleasant St. SE, Minneapolis MN 55455, USA,

²Department of Chemistry, Texas A&M University, PO Box 30012, College Station, Texas 77842

³CNR-Instituto di Scienze Tecno-logiche Molecolari, via Golgi 19, 20133, Milano, Italy.

In the clinical laboratory, the analysis of total CO₂ (comprising CO₂, HCO₃²⁻ and CO₃²⁻) is used to detect and monitor electrolyte imbalances. As part of routine blood analysis, an estimated 200 million measurements are carried out per year in the USA alone. To accurately and rapidly determine total CO₂, a CO₃²⁻ sensor with high selectivity, sensitivity and robustness is in need. For this purpose, we are using the fluororous matrix approach introduced by the Buhlmann group. Fluororous media are the least polar and polarizable condensed phases known. Since chemical species other than the target analyte are poorly solvated in fluororous media, electrochemical sensors with fluororous sensing membranes are exceptionally selective. Such sensors were reported for cations using fluorophilic anions and complexing reagents, and this work extends this approach to CO₃²⁻ analysis. Our work shows that several fluorophilic Mn(III) salen complexes are highly selective receptors that bind CO₃²⁻ by ligation to the metal center. In particular, one of these ionophores provides electrochemical sensors with selectivities that are orders of magnitude higher than those of comparable sensors based on trifluoroacetophenone derivatives, which were studied in the past extensively in view of use in routine clinical analyzers. In particular, the new CO₃²⁻ sensors are a hundred-fold more selective over Cl⁻ and a thousand-fold more selective over NO₃⁻ than the conventional trifluoroacetophenone-based sensors. Taking advantage of the unique properties of perfluorinated matrixes, fluororous membrane sensors show great potential for measurements of CO₃²⁻ in blood in the clinical laboratory and eventually, with implantable devices, in the living body.

Alley Institute Student Research Poster Session - LifeScience Alley Conference & Expo – December 8, 2010

9. The effect of the occlusal load angle on the stresses of a maxillary central incisor: A finite element analysis

YungChung Chen and Alex Fok, Minnesota Dental Research Center for Biomaterials and Biomechanics, School of Dentistry, University of Minnesota, MN

The incisor inclination has been of interest to various dental specialties. For orthodontic treatment, it plays an important role in reestablishing the esthetic facial profile. In prosthetic dentistry, it is a determining factor for the anterior guidance which controls eccentric movement of the mandible. Furthermore, from a mechanical viewpoint, an improper incisor inclination can be traumatic to the tooth structure. An improper incisor inclination may also jeopardize the periodontal tissue, including the gingival and alveolar bone attachment. Despite these important problems, investigation of the biomechanical behavior of the incisor with different inclinations under occlusal load has been limited. The aim of this study is, therefore, to evaluate the stress distribution of a maxillary central incisor with different inclinations under load.

A two-dimensional finite element model was constructed from the computed tomography images of a human maxillary central incisor. The bottom bone region of the model was fully fixed, and a load of 200 N was applied at the lingual surface of the crown. To investigate the effect of the incisor inclination on its stress distribution, the angle of the force with respect to the long axis of the incisor was altered incrementally. Thus, 19 models with load angles ranging from 0° to 90° were generated using the software Hyperworks 9.0 and exported to ABAQUS 6.9.1 for analysis. Mechanical properties were defined based on the literature review, with all materials and structures being considered as linear elastic, isotropic, and homogeneous. The model was meshed with plane-strain elements (CPE3 and CPE4I). Both the von Mises and the maximum principal stresses around the entire tooth surface were plotted for each load angle for comparison.

The results showed stress concentrations at the apex, lingual root surface, labial coronal surface and the cervical area in all cases. However, when the load angle was less than 30°, a sharp drop in stresses could be seen. This is also the average inclination observed clinically.

Using clinically observed inclination for the maxillary central incisor will provide not only the required aesthetics and anterior guidance, but also biomechanically favorable stress distribution around the whole tooth surface.

10. Cell surface engineering and controlled release of chemokine: Biomaterial approaches to enhance cardiac cell therapy

Daisy Cross, Sun-Young Choh, and Chun Wang
Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN

A number of promising developments have recently been made in the field of cell therapy for the repair of myocardial infarcts. However, a number of obstacles to successful therapy remain, including poor retention and survival of cells after transplantation into the myocardium.

We are currently investigating two parallel approaches to address these obstacles. In one approach, our goal is to improve adhesion of implanted cells to their host tissue. Typically, research regarding control of cell-substrate interactions has primarily focused on modifications of the substrate. In contrast, we chose to focus on the modification of cell surfaces rather than the substrate. We have developed methods by which polymeric biomaterials are employed to engineer the surfaces of cells. Using the layer-by-layer (LbL) polyelectrolyte assembly approach, we coated cell surfaces with charged polymers to promote or inhibit cell-substrate adhesion. The polymer coatings show minimal cytotoxicity.

In the other approach, we have encapsulated stromal cell-derived factor-1 (SDF-1) into biodegradable PLGA microspheres. SDF-1 is a chemoattractant of endogenous stem cells and has been used experimentally to encourage tissue regeneration after infarct injury. We studied various parameters in the microsphere fabrication process, and used our findings to produce a library of formulations that can be employed to tune the release of SDF from the microspheres. This approach has enabled us to gradually release SDF-1 from polymeric microspheres over a time period of more than one month. Current work is being performed to confirm the bioactivity of the released SDF-1.

We consider these proof-of-concept results as promising steps towards the enhancement of current cardiac cell therapy approaches.

Alley Institute Student Research Poster Session - LifeScience Alley Conference & Expo – December 8, 2010

11. Neural correlates of rule-dependent spatial categorization in primate prefrontal and posterior parietal cortex

Shikha Goodwin^{1,2}, Matthew Chafee^{2,3}

¹Department of Biomedical Engineering; ²Center for Cognitive Sciences, ³Department of Neuroscience, University of Minnesota, Minneapolis MN

Much of our behavior is governed by rules. Especially the rules governing the spatial maps are important for our understanding and use of space. To understand how different rules can classify space into different categories, we devised a dynamic spatial characterization task (DYSC). In this task, two rhesus macaque monkeys were shown a sample dot and a line, and were required to mentally place the dot into a spatial category based on its spatial relationship to the line (for example, all points falling to the left of the line comprise left spatial category, all points to the right form right category). By changing the orientation of the line, we forced the brain to recategorize the same sample position to fall into different spatial category. Neural activity from the two monkeys was recorded from parietal and prefrontal cortex simultaneously. Behavioral performance provided evidence that the monkey placed visual stimuli into spatial categories according to the variable rule. We found that the neural representation of the category and its executive control as evidenced by the rule dependence of that representation were distributed in the network. The neural signal coding spatial category exhibited rule-dependence in prefrontal cortex first however, consistent with prefrontal cortex leading in the network implementation of executive control. Together, these results substantially advance our understanding of the neural representation, control, and acquisition of categories in the brain and how categorical representations are dynamically computed to provide flexible behavioral control.

12. Comparison of in vivo and ex vivo dental implant stability

Shahrazad Grami¹, BSBE, and Wook-Jin Seong², DDS, MS, PhD

Departments of ¹Industrial and Systems Engineering and ²Restorative Sciences at the University of Minnesota, Twin Cities, MN

Osseointegration is the structural and functional connection between living bone and dental implant surface. Immobility is necessary for healing of bone and marrow tissues, long term health, and proper osseointegration, which in turn results in the success of final implant stability and strength.

This study investigated the relationship of implant stability in vivo at implantation and various healing times against ex vivo measurements of peak force and stiffness from mechanical testing. The aim was to characterize osseointegration, relate in vivo and ex vivo implant stabilities, and determine the post powerful ex vivo mechanical test: push-in, push-out or pull-out.

Two identical self-threading dental implants (3.3 x 8.5mm) were placed in the medial surface of the proximal tibia condyles in live, female New Zealand rabbits (n = 20). Rabbits were allowed to heal over 1, 4, 8, and 12 weeks, and five rabbits were sacrificed at each time point. Implant stabilities were measured at the time of implant (primary) and sacrifice (secondary), and tibia weights were recorded on sacrificed bones. Frozen samples were thawed and tested at a displacement rate of 6 mm/minute. Test setups were assigned to alternating rabbit legs. X-rays were taken before and after mechanical testing.

Force-displacement curves were produced and analyzed for peak force, displacement at peak force, and stiffness (slope of force-displacement). Only push-in data from this study showed statistically significant, increasing trends in peak force (p=0.01) and stiffness (p=0.005) over healing times, while pull-out showed no statistically significant trends in either parameter. Push-in reached 900N peak force and 2589N stiffness, while push-in maxed at 538N force and 1178N stiffness. Secondary implant stability was correlated to peak forces for both push-in and pull out but only stiffness on the push-in test. Push-out was eliminated during pilot studies.

Osseointegration was shown up to 12 weeks, further than previously believed for a rabbit model. The push-in test showed statistically significant results for peak force and stiffness, as well as the correlation with secondary implant stability. The pull-out test was less powerful, and push-out was not easily reproduced. Push-in is preferred and most clinically relevant.

13. Diffusion-based extraction of DMSO from cell suspensions in a vertical, three-stream microchannel

Jacob Hanna, Erin Lemke, and Allison Hubel
Department of Mechanical Engineering, University of Minnesota, Minneapolis, MN

Microfluidic channels have been proposed as a method for removal of cryoprotective agents (CPA) from cell suspensions. The device tested consists of a rectangular cross section channel of 500 μm thickness, 25 mm width, and 80 mm length. Three streams flow vertically downward in contact with one another through the device: a central stream containing a cell suspension, and a wash stream on either side. CPA diffuses from the cell stream to the wash streams. The three streams are subsequently separated by the device's outlet geometry. The CPA-containing wash streams are then discarded, and the washed cell stream is then ready for use. This device must be capable of removing 95% of the dimethyl sulfoxide (DMSO) from the cell stream to match current centrifugation techniques, with minimal cell losses. The design of the device focused on shortening processing times, reducing the device's footprint, and improving cell recovery over a range of conditions when compared to previous horizontal, two-stream devices. To test the device, Jurkat cells (lymphoblasts) in a 10% DMSO solution were flowed through the microfluidic channel in parallel with wash streams composed of phosphate buffered saline solution (PBS). Average cell stream velocities were varied from 2 to 24 mm/s (Re 1 to 12). Cell volume fractions (CVF) in the cell stream were varied from 0.5% to 16%. Cell viability at the outlet was high, indicating that cells are not damaged during their passage through the device. Cell recovery from the device was greater than 96% for all flow conditions tested, showing a marked improvement over centrifugation techniques. DMSO removal matched predictive models well. 95% removal of DMSO can be achieved using 2-3 stages in series. The faster DMSO removal afforded by the change to a three-stream geometry allows either an increase in processing speed or reduction of device length by as much as a factor of 4 when compared to two-stream geometries.

14. Decellularization of fibrin-based engineered tissue to build a small diameter vascular graft

Thanh N Huynh¹, Stefan M Kren^{2,3}, Doris A Taylor^{2,3}, Robert T Tranquillo^{1,4}
¹Department of Biomedical Engineering, ²Center for Cardiovascular Repair, ³Department of Integrative Biology and Physiology, ⁴Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN

Cardiovascular disease is the leading cause of death and small-diameter (<6mm) vascular graft availability remains the greatest challenge. Our lab has developed a tissue engineering approach to create a completely biological artificial artery based on entrapping cells in a tubular fibrin gel. In this study, we decellularized the engineered tissue while maintaining the extracellular matrix (ECM) integrity in order to remove any immunogenicity from the graft. The decellularization process was performed using 1% SDS for 24 hours and extensive PBS washes with constructs either remained on the mandrels or removed. Decellularization efficiency was investigated by Hoechst assay and ECM integrity was examined mechanically, histologically, and biochemically. Our data reveals that decellularization was achieved without extensive alteration of mechanical and biochemical properties of the ECM. The decellularized constructs maintained their original size if mounted on the mandrels throughout the process while compacted drastically without the mandrel. Decellularization of fabricated tissue constructs could be used to provide mass-produced acellular tissue grafts suitable for autologous cell engraftment and ultimately transplantation.

15. Surface plasmon resonance (SPR) biosensing with nanostructured materials

Hyungsoon Im, Antoine Lesuffleur, Nathan C. Lindquist, Nathan J. Wittenberg, and Sang-Hyun Oh,
Department of Electrical and Computer Engineering, University of Minnesota, Twin Cities, MN

We have demonstrated high-throughput biosensing using nanostructured materials as surface plasmon resonance (SPR) sensors in a real-time label free manner for medical diagnostics, drug discovery and single molecule detection. Surface Plasmons (SPs) are electromagnetic surface waves supported by a collective motion of the free electrons at the metal surface. The strong confinement of the field at the surface (within ~ 200 nm) allows a very high sensitivity for the label-free detection of molecular binding. In contrast to fluorescence spectroscopy, which is one of the most widely used techniques in bioengineering and medical research, the biosensors we develop provide unique advantages by directly measuring key biomolecular parameters—such as association and dissociation rates and the affinity of molecules—without the costly and time-consuming labeling process that may also interfere with the molecular binding interactions. This information is a prerequisite for proteomics research and drug discovery. In contrast to commercial SPR instruments, such as BIACore™ that measures the reflection of light incident on the sensor chip at certain angles, the biosensors we study are made with periodic sub-wavelength-sized hole arrays in a thin gold film and measure the transmission of normally-incident light. This co-linear optical transmission setup offers several key benefits in cost and simplicity. We have shown that the nanohole-based sensors have advantages over the commercial instrument in terms of high-resolution imaging, multiplexing capabilities, and high-throughput kinetic assays for protein microarray applications. The research will be to ultimately provide reliable, low-cost, and high-quality sensing devices to biomedical researchers.

16. What part of the iris dominates its behavior?

Sara Jouzdani¹, Julie E. Whitcomb¹, and Victor H. Barocas²
Departments of Mechanical Engineering¹ and Departments of Biomedical Engineering², University of Minnesota, Minneapolis, MN

We developed a study to determine the relative mechanical contributions of the iris components, i.e. the sphincter, dilator, and stroma, using experimental nanoindentation and histological analysis in combination with a three-dimensional finite element model (FEM). A total of 12 intact porcine irides were bisected into two equal C-shaped halves to allow indentation of the anterior and posterior surface of the same iris. The iris was pinned at the periphery to an aluminum surface. Nanoindentation tests were performed on both the anterior and posterior side of the iris at three locations - iris root (IR), mid-pupil (MP), and pupil margin (PM)-, using a Nanoindenter XP (MTS; Eden Prairie, MN) with a flat-end cylindrical tip of 1 mm diameter. An effective modulus was calculated using the slope of the load-depth curve during rapid indentation and assuming iris to be a homogeneous elastic material. The experimental indentation of the iris was modeled as a 3D trilayer material indented by a rigid flat-ended cylindrical indenter using ABAQUS (SIMULIA, Providence, RI). Histological analysis helped us create a realistic iris contour that defined the sphincter, dilator, and stroma. In the model, the iris components were modeled as incompressible neo-Hookean solids. Indentation simulations were performed at the three defined regions (IR, MP, & PM) similar to the experiments on both the anterior and posterior surfaces. We extended the method of Costa (Journal of Biomedical Engineering, 1999, 241:462-471) to determine region-specified elastic properties from indentation depth, force, and geometry information via comparison between our experiments and FEM. A significant difference was observed between the anterior and posterior effective modulus in all regions ($p < 0.05$). The posterior modulus was 1.3 times higher than the anterior modulus. The FEM results were in agreement with the experimental results, producing a higher force on the posterior side. Clinical studies suggest that the perceived floppiness of the iris in the IFIS disease is due to a weakening of the dilator muscle, which we have shown is significantly stiffer than the other two components of the iris.

17. Intelligent stethoscope

Adilbek Karaguishiyev and Dr. Marian Stachowicz,
Laboratory for Intelligent Systems, ECE Department, University of Minnesota, Duluth, MN

Auscultation of heart murmurs can detect various types of heart problems; however, this process is prone to human error because it involves a clinical evaluation and categorizing the heart sound via stethoscope. By implementing Soft Computing techniques to analyze the sound received from the stethoscope and classify the heart pathology, this project introduces the intelligent diagnosis system that is not based on subjective evaluations. The sound signal from the stethoscope is transformed into spectrogram. Color Reduction and Color Feature extraction methods are applied to confer the density plot to a manageable image characteristic and reduce the colors to eight. The new density plot is then matched with database to identify a possible pathology.

18. Characterization of the interaction of two essential putative glycoproteases in *Staphylococcus aureus*

Ting Lei, Li Zheng, and Yinduo Ji
Department of Veterinary Biomedical Sciences, College of Veterinary Medicine, University of Minnesota

In previous studies, we have demonstrated that a putative glycoprotease (Gcp) is essential for survival of *Staphylococcus aureus*, suggesting that Gcp is a potential target for developing novel antibacterial agents. However, the molecular mechanism behind the essentiality of Gcp remains unknown. In this study, we found that the *gcp* gene was co-transcribed with its paralog *yeaZ*, another essential gene for *S. aureus* survival, and *sa1855* and *sa1857* using a reverse-transcription PCR, indicating these genes may be located in one operon. We also revealed that Gcp and YeaZ interact using a yeast two hybrid (Y2H) screening system. However, no interaction was detected between other proteins encoded by the above genes located in the same operon. The interaction of Gcp with YeaZ was further confirmed by *in vitro* pull down assays. In order to identify residues critical for Gcp binding to YeaZ and/or residues required for Gcp's essentiality, we employed alanine scanning mutagenesis on the C-terminal segment of Gcp. Alanine mutagenesis of amino acids 317-322 abolished the capacity of Gcp to interact with YeaZ. In addition, the expression of the Gcp alanine scanning mutants of the amino acids 317-322, 324-329, or 332-336 *in trans* failed to complement the down-regulated wild-type Gcp. The results suggest that the C-terminal segment of Gcp may play an important role in the interaction of Gcp with YeaZ, which in turn may be associated with their indispensability for bacterial growth.

19. Physiologic parameters correlate to metabolomic markers in hemorrhagic shock

Dan Lexcen, Joseph Greenberg, Beth Luszczyk, Kristine Mulier, Greg Beilman
Department of Surgery, University of Minnesota, Minneapolis, MN

Metabolomics is a new field of study that looks at the entire profile of metabolites in a given sample. In our porcine model of hemorrhagic shock and resuscitation, nuclear magnetic resonance (NMR) is used to identify and analyze the flux of metabolites in a biofluid. Currently, clinical decisions are made using available measures of a physiologic and biochemical variables. Our hypothesis is that we can correlate known prognostic indicators with identified metabolic biomarkers. Thirteen animals underwent a standard hemorrhagic shock protocol and resuscitation and serum samples were collected at baseline (prior to hemorrhage), shock45 (after 45 min of shock), and 1, 4, 8, 23, and 48 h post resuscitation. Physiologic and biochemical measurements were taken at those same time points. ¹H-NMR spectroscopy was used to analyze serum collected from our porcine model of controlled hemorrhagic shock. Serine and Lactate, strong metabolic markers of shock were highly correlated to lactate, heart rate, oxygen consumption, base excess, oxygen saturation and urine output in either a positive or negative manner. The amino acids (Glutamine, Arginine, Histidine, Phenylalanine,) all shared strong positive correlations with oxygen saturation (StO₂) and urine output. Additionally, those same amino acids had a negative correlation with heart rate, oxygen consumption, and temperature. Several other metabolic markers of shock had correlations to relevant physiological/biochemical variables, including s-adenosylhomocysteine, adipate, and succinate. In this study we observed a correlation between the known markers and the metabolism of hemorrhagic shock. This may serve to validate the use of metabolomics as a tool to help elucidate the underlying biochemical pathways. Having linked known physiologic/biochemical variables of shock with changes in the metabolome, we hope to use metabolomics to isolate functional biomarkers indicative of poor prognostic outcomes.

20. Role of leukocyte ADAM17 in *E. coli*-mediated sepsis

Chunmei Long and Bruce Walcheck
Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN

Inflammation is the body's initial response to infection, which can be harmful when excessive, as exemplified in sepsis inflammatory syndromes. Ectodomain shedding directs both instantaneous and prolonged alterations in the activity of various cytokines, cytokine receptors, and adhesion molecules, and ADAM17 is a key membrane metalloprotease involved in this process. However, very little is currently known about the *in vivo* function of ADAM17 in regulating inflammation during infection. In this study, we generated *Adam17* gene targeted mice in which only the leukocytes lacked functional ADAM17, and then examined its role in the inflammatory and host responses during *E. coli*-mediated peritoneal sepsis. Conditional ADAM17 knock-out mice showed increased survival and bacterial clearance during *E. coli*-mediated peritoneal sepsis, which was associated with a reduction in systemic proinflammatory cytokine levels and bacterial burden. Of interest is that we observed a very rapid yet transitory infiltration of neutrophils into the peritoneal cavity of conditional ADAM17 knockout mice when compared with control mice. We are currently examining the mechanisms underlying the enhanced host response in conditional ADAM17 knockout mice, including the molecular processes involved in rapid recruitment of neutrophils. Overall, our study provides the first direct evidence of the instrumental *in vivo* role of leukocyte ADAM17 in modulating inflammation and host resistance during Gram-negative bacterial infection.

21. High-density EEG source imaging in partial epilepsy patients

Yunfeng Lu¹, Lin Yang¹, Greg Worrell², and Bin He¹

¹ Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN

² Comprehensive Epilepsy Center, Mayo Clinic, Rochester, MN

Epilepsy is a common chronic neurological disease that affects roughly 50 million people worldwide, and up to 30% of these patients do not respond to medication treatment. For medically refractory epilepsy patients, surgery is currently an effective option. Intracranial recording techniques such as electrocorticography (ECoG) are routinely used to aid pre-surgical planning in epilepsy patients, however, these recordings require opening the skull of the patients and is invasive, risky, and undesirable. EEG source imaging is a useful tool to localize epileptogenic foci because of its high temporal resolution at the millisecond scale and its noninvasive nature. A novel ictal source analysis approach was previously developed in our lab for spatial-temporal source localization and connectivity analysis. In this study, we evaluate the ability of the approach to identify epileptogenic foci for high-density EEG recordings in partial epilepsy patients. High-density EEG seizure data with 76 channels was recorded in five medically intractable partial epilepsy patients. All the patients had surgical resections, and were either seizure free or had significant seizure reduction one year after the operation. The EEG sources were modeled as the equivalent current dipoles. Patient-specific boundary element head models were created from their structural MRI. Subspace source imaging method (FINE) was used to localize the ictal sources in the 3-Dimensional brain. Source space connectivity analysis approach was then applied to identify the primary ictal sources. The results were evaluated by comparing the source locations with surgical resections. The source imaging results were well co-localized with the post-operative MRI lesions. Majority of the ictal sources identified were either overlapping with, or close to patient surgical resections. These results suggest the potential application of high-density EEG source imaging in localizing epileptogenic foci for pre-surgical planning.

22. B-scan based magnetoacoustic tomography with magnetic induction (MAT-MI)

Leo Mariappan, Gang Hu, Xu Li, and Bin He

Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN

Bioelectrical impedance imaging is of significance because it can provide unique impedance contrast of biological tissue, which is sensitive to physiological and pathological changes in a living system. Magnetoacoustic Tomography with Magnetic Induction (MAT-MI) is a recently introduced novel method for imaging tissue electrical properties by integrating magnetic induction and acoustic measurements. This approach has potential to become a non-invasive high spatial resolution impedance imaging modality that has possible applications such as early breast cancer detection. In MAT-MI ultrasound is generated in the object by placing it in a dynamic and a static magnetic field. Eddy currents are induced in the object due to the dynamic field. The static field leads to generation of acoustic vibrations from Lorentz force on the induced currents. The acoustic vibrations are at the same frequency as the dynamic magnetic field which is chosen to be in the ultrasound frequency range. These acoustic waves from the sources propagate in all directions and ultrasound transducers placed around the object are used to collect the acoustic waves. The measured acoustic signal is used to reconstruct the acoustic source distribution which is then used to estimate the conductivity distribution of the object. We have developed a MAT-MI system using focused transducer with B mode imaging. Computer simulation and phantom experiment studies demonstrate the merits of this approach. It is shown in our computer simulations that, as compared to the previous approach, the MAT-MI system using B-scan with a focused transducer allows MAT-MI imaging at a closer distance and has improved system sensitivity. In addition, the B scan imaging technique allows reconstruction of the MAT-MI acoustic sources with a discrete number of scanning locations which greatly increases the applicability of the MAT-MI approach especially when a continuous acoustic window is not available in real clinical applications.

23. Fibril alignment improves microvasculature formation by human blood outgrowth endothelial cells in fibrin gel

Kristen T. Morin and Robert T. Tranquillo
Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN

One of the major challenges in the tissue engineering of thick tissues is the provision of oxygen to the entire tissue. The formation of a microvascular network within tissue engineered constructs is a potential solution to this issue. The ability to align such a network is critical to the success of tissue engineering *in vitro*, because it creates natural inlet and outlet sides for perfusion of culture medium and, ultimately, blood. We have shown that mechanically-constrained cell-induced compaction and alignment of fibrin fibrils can be used to effectively align microvascular structures formed by human blood outgrowth endothelial cells (hBOECs), a potentially autologous source of endothelial cells. hBOECs and human lung fibroblasts were entrapped in a rectangle of 3.3 mg/ml fibrin gel attached to porous spacers at each end. The construct was cultured for 7 days, during which the cells compacted and aligned the fibrin in between the spacers. The structures formed by the hBOECs had distinct lumens, and their alignment increased over the course of the 7 days of culture. Therefore, cell-induced fibrin alignment provides a means to create an aligned microvascular network, an improvement over similar isotropic networks.

24. Comparing strip biaxial vs equibiaxial loading: The importance of biaxial testing in annulus fibrosus lamellae

Tina Nagel¹, Arin Ellingson², David Nuckley³, and Victor Barocas²
¹Department of Mechanical Engineering, ²Department of Biomedical Engineering, ³Department of Physical Medicine and Rehabilitation, University of Minnesota, Minneapolis, MN

About two thirds of adults in the United States experience low back pain at some point in their lives. The causes are generally unknown, although one theory attributes low back pain originating with intervertebral disc degeneration. The intervertebral disc is composed of an outer ring of layered collagenous tissue, the annulus fibrosus, and a gelatinous center, the nucleous pulposus. Biaxial testing of cadaveric annulus fibrosus lamellae has remained largely unexplored. Thus the objective of this research was to compare the suitability of strip biaxial and equibiaxial loading of cadaveric single annulus fibrosus lamellae to provide mechanical properties of the tissue. Five thawed, freshly frozen human cadaveric L1-L2 discs were excised and dissected to a single lamellae. The lamellae was immersed in a saline bath and biaxial tested using an Instron-Sacks biaxial tester. Paired t-tests were performed to compare the average force calculated at the peak strain in the axial and circumferential directions under the loading conditions. The force-stretch data show the most pronounced curves in equibiaxial loading conditions. Using a paired t-test, the final forces calculated in each protocol in the axial and circumferential directions were compared. Comparison of the circumferential data from the equibiaxial test and circumferential strip biaxial test produced a significant difference ($p=0.01$). Statistical analysis shows a moderate difference in the loading between the two directions in the equibiaxial test, while there is little difference between the two directions in the respective strip biaxial tests. In spite of fiber alignment in the tissue, no statistically significant mechanical anisotropy was observed. These data constitute a step towards characterizing the material properties of annulus fibrosus lamellae and support use of biaxial testing for characterization. The statistical strength of these data is limited due to the sample size; however given the observed trends, future work with a larger sample size is justified.

25. Polymersomes functionalized with the PR_b peptide promote targeted drug delivery to cancer cells

Todd Pangburn, Frank S. Bates, and Efie Kokkoli
Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN

Polymersomes, which self assemble in aqueous solutions from amphiphilic block copolymers, have been recognized as a promising system for encapsulation and drug delivery. In this work, peptide-functionalized polymersomes have been synthesized and their performance as a therapeutic tested. Polymersomes were functionalized via azide-alkyne "click" chemistry with two targeting peptides: GRGDSP and the recently designed fibronectin mimetic peptide, PR_b (KSSPHSRN(SG)₅RGDSP). The ability of these peptide functionalized polymersomes to promote targeted delivery to colon cancer cells was assessed by studying their delivery to cancer cells *in vitro*. The diblock copolymer poly(ethylene oxide)-*b*-poly(1,2-butadiene) was synthesized and self assembled in aqueous solutions to form polymersomes, which were subsequently functionalized with targeting peptides using a "click" conjugation reaction. The efficacy of targeted delivery to cancer cells was tested for these polymersomes, and PR_b functionalized polymersomes were compared to non-functionalized polymersomes and GRGDSP functionalized polymersomes. Additionally, the chemotherapeutic, doxorubicin, was loaded into these polymersomes and the cytotoxicity of the various targeted polymersome formulations to cancer cells was assayed. PR_b functionalized polymersomes are shown to significantly outperform both non-functionalized polymersomes and GRGDSP functionalized polymersomes as a targeted drug delivery system, both in terms of promoting binding and internalization and doxorubicin delivery and cytotoxicity. Thus, "click" functionalized PR_b targeted polymersomes are identified as an attractive targeted drug delivery vector, capable of delivering a therapeutic load directly into the interior of cancer cells, in an efficient and highly specific manner.

26. Handheld micro-tactile sensor for in-vivo tissue elasticity measurement

Peng Peng, Rajesh Rajamani, and Arthur G. Erdman
Mechanical Engineering, University of Minnesota, Minneapolis, MN

In biomedical applications, in-vivo measurement of tissue elasticity can facilitate doctors to reach a reliable palpation diagnosis, to evaluate the health of tissue, and to provide tactile perception during a minimally invasive surgery (MIS). Although in-vivo measurement of tissue elasticity is essential in physical examination, very few examples of elasticity sensors have been previously developed. A commonly used approach for elasticity measurement is to measure the force-displacement response of the tissue under examination. However, this type of device always includes an actuating element to provide a controlled deformation or force on the tissue, which becomes an obstacle for miniaturization of the device and also increases the fabrication complexity. This approach would become even more challenging in applications within a confined space (e.g. MIS).

A novel tactile sensor has been developed by this team for elasticity measurement. This novel approach enables elasticity measurement based on the relative deflection of two sensing diaphragms. These two diaphragms are designed to have different stiffness values. Neither displacement nor applied load needs to be measured during the contact. Further, prototypes of sensors have been fabricated through both a surface micromachining process and a polymer MEMS process. The sizes of the prototype sensors can be fabricated to be of the order of 1mm x1mm. Experimental characterization of the sensor shows that this tactile sensor can provide reliable elasticity measurement of polymer specimens with a resolution of 0.1MPa in the range of 0.1MPa to 2MPa (Young's modulus). At last, this type of sensor has been used to conduct measurements of porcine tissue of various types (cartilage, muscle, fat, kidney, esophagus and trachea).

27. Identification of regional mechanical anisotropy in soft tissues by the generalized anisotropic inverse mechanics method

Ramesh Raghupathy¹, Colleen Witzenburg¹, Spencer Lake², Margaret Thomas², and Victor Barocas²

¹Department of Mechanical Engineering, ²Department of Biomedical Engineering, University of Minnesota-Twin Cities, MN

Although mechanical testing of soft tissues is a field with a long and rich history, much remains to be learned about heterogeneous, anisotropic tissues such as the heart valve leaflet, ventricular wall, and arterial bifurcations. Advances in image correlation and inverse mechanical analysis now permit detailed analysis of the large-scale heterogeneity in tissue properties within an intact specimen. Mechanical anisotropy in the tissue can be related to the structural anisotropy, providing a means of exploring structures that are difficult to examine directly. Our method involves a series of symmetric and asymmetric biaxial stretches of tissues textured with dye or stain. The tissue is imaged during the loading steps and the displacement fields are calculated via image correlation. A general, linear elastic problem in two dimensions is solved for the unknown constants of the anisotropic, elastic tensor C using the displacements and measured boundary forces as inputs. Heterogeneity is evaluated by segmenting the domain into subdomains of uniform properties leading to a linear system with six unknown constants per segment. Use of a linear model allows rapid solution times and confidence intervals from linear-regression can be used to assess the performance of the model. The eigentensor of the linear elasticity tensor C corresponding to the largest Kelvin modulus is used to extract a major fiber direction. Fiber alignment maps and stiffness maps can be generated from the fiber directions and the Kelvin moduli respectively. We have validated our model with isotropic PDMS samples and anisotropic cell-compacted collagen cruciform of different arm dimensions, which led to different regional mechanical properties. Our model successfully captured the regional changes in mechanical anisotropy and stiffness. Further examples from various soft tissue analogs and cardiovascular tissues will be presented.

28. Encapsulation of mammalian cells in hybrid inorganic matrices for developing bio-detection applications

Eduardo Reátegui¹, Lisa Kasinkas¹, and Alptekin Aksan^{1,2}

¹ Department of Mechanical Engineering, University of Minnesota, Minneapolis, MN

² Biotechnology Institute, University of Minnesota, Minneapolis, MN

Certain prokaryotic or eukaryotic organisms can be encapsulated in nanoporous silica matrices, stabilized, and stored without loss of activity or cell viability. Interestingly, this technology has a great potential to be employed in the development of "on-site" biosensors for long term applications since using a living cells or cellular components opens a new era of functional diagnostics tools for pathogens, toxins or bioreactive compounds in clinical, pharmaceutical, and environmental applications. However, one major limitation of the silica encapsulation method is the highly specificity of the silica precursors with the biological species that causes detrimental effects on the performance of the cells.

In our research we have focus on the stability of mammalian cells encapsulated on hybrid silica matrices. We were able to maintain viable and metabolically active mammalian cells for a week by changing the chemical and physical properties of the silica matrix. This was possible by the incorporation of a biocompatible polymer (e.g. polyethylene glycol, PEG) into the matrix. PEG changed the surface properties of the silica matrix by reducing the interactions between the hydroxyl groups with the lipids of the cellular membrane. On the other hand, the incorporation of PEG reduced the mechanical stress of the forming matrix on the cells. Rheology test show that the elastic modulus (G') for a silica-PEG matrix reached a plateau value faster when compared to a pure silica matrix. Raman spectroscopy and electron microscopy showed the uniformity of the material (no phase separation or damage of the cells) and the increase of the porosity from 7 nm to 100 nm in silica and silica-PEG matrices, respectively. All these new features of the hybrid material allowed the cells to tolerate stress for longer periods of time. When the cells were encapsulated just in silica matrices they showed poor viability (~ 24 to 36 hours). Therefore, the present work shows the potential to integrate eukaryotic cells into hybrid silica matrices for the development of a wide range of biotechnology applications.

29. Estimating the microtubule GTP cap size in epithelial cells

Dominique Seetapun and David J. Odde
Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN

Microtubules (MTs) are intracellular biopolymers that play key roles in cellular trafficking, cell division and cell signaling. They are direct targets of the chemotherapy drug Taxol, which is also used in drug-eluting stents for the prevention of restenosis after angioplasty. MT dynamics are also responsible both for proper chromosome segregation during mitosis and for cell fusion during muscle differentiation. A hallmark of Alzheimer's disease is the lack of MT organization in neurons. Thus, understanding the regulation of MT dynamics is medically relevant and could hold the key to new therapies. MTs assemble when capped by GTP-tubulin subunits, but undergo catastrophe and rapid shortening when this cap is stochastically lost through hydrolysis and GTP-tubulin dissociation. Plus-end tracking proteins (+TIPs) specifically bind to and track growing MT plus ends, forming a comet-like distribution at the end of the growing MT, presumably via recognition of GTP-tubulin. EB1 is a +TIP thought to be a hub by which other +TIPs bind to the MT plus end and regulate MT assembly and signaling. In EB1-EGFP LLCPK1 epithelial cells we find that EB1 forms EB1 comets with a half-length of 340 ± 110 nm and MTs grow at a rate of ~ 130 nm/s. Using this information we estimate the GTP cap size to be around 600 tubulin dimers. This was corroborated using a chemical model of MT growth (Van Buren *et al.*, 2002). In LLCPKs, a comet comprises of 270 ± 140 EB1 dimers, which serves as a lower bound for the size of the GTP cap *in vivo* that is far larger than the single-layer cap proposed in numerous previous studies based on *in vitro* measurements. This means that the concentration of EB1 at the MT plus end *in vivo* is >100 μ M, which creates a potent signal that could be recognized by signaling molecules *in vivo*.

30. Effect of surface charge of Gold nanoparticles: an *in vivo* biodistribution study.

Neha Shah¹ and John Bischof^{1,2}

¹Department of Biomedical Engineering, ²Department of Mechanical Engineering, University of Minnesota, Minneapolis, MN

Intravenously injected nanoparticles (NPs) hold great promise for clinical diagnostic and therapeutic applications. While several NPs for such clinical applications have emerged in various designs (metallic, polymeric, quantum dots etc.), a critical issue in their *in vivo* use is NP accumulation in untargeted (healthy) organs, including the reticuloendothelial system. Recent calls by NIH, FDA, CDC and NCI emphasize the importance of defining the mechanism of biodistribution of NPs to avoid toxicity and side effects *in vivo*. However, to our knowledge a detailed *in vivo* study of the interaction of metallic nanoparticles with blood and tissue components as a function of their surface properties does not yet exist.

Here we look at the effect of gold NP (GNP) surface charge on blood cell interactions *in vivo*. 30nm GNPs were coated with either m-PEG 5000 or cm-PEG 5000 to give particles a net neutral or negative charge respectively (as measured by zeta potential). Human prostate tumor (LNCaP) bearing nude mice were injected with a single intravenous dose of GNPs. Blood samples collected after 1 hour were analyzed via ICP-MS, for the quantification of gold. ICP-MS showed that freshly synthesized neutral and negative GNPs show similar retention in blood at 1 hr. However, lyophilized and/or GNPs that were synthesized and stored for a long time show five-fold lower retention in blood suggesting possible degradation of PEG layer overtime. Additionally, both GNPs seem to be taken up by circulating monocytes while neutral GNPs show preferential uptake in platelets (demonstrated by TEM and SEM analysis). There was higher clearance of anionic GNPs (with RBCs) in spleen suggesting possible involvement of the complement system. However, both GNPs showed similar accumulation in the tumor which could be further increased several fold by attaching specific ligands to the neutral particle (i.e. TNF- α).

These results suggest that NP surface charge yields specific interactions with blood components thereby guiding if not determining biodistribution within the reticuloendothelial system (i.e. liver vs. spleen) and ultimately the tumor.

31. Fibronectin-mimetic peptide-amphiphile nanofiber gels support increased cell adhesion and promote ECM production

Kamlesh Shroff, Emilie L. Rexeisen, Manickam Adhimoolam Arunagirinathan, and Efosini Kokkoli
Department of Chemical Engineering & Materials Science, University of Minnesota, Minneapolis, MN

The design of nanofiber scaffolds has been a key objective in tissue engineering as they structurally mimic the natural extracellular matrix (ECM) found in tissues. The continuous cross talk between cells and the surrounding matrix ligands plays a crucial role during development and wound healing which leads to tissue regeneration and repair. In an attempt to provide a nanofiber scaffold with a ligand that can promote cell adhesion and ECM production, we propose the use of our peptide-amphiphile nanofibers as a potential scaffold for tissue engineering. A fibronectin-mimetic peptide sequence containing both the cell-binding GRGDSP domain and the synergistic PHSRN domain has been recently developed in our group. It has been shown to be a specific ligand for the $\alpha5\beta1$ integrin, and has been synthesized to a C16 single-tailed peptide-amphiphile, PR_g (with a peptide headgroup of GGGSSPHSRN(SG)5RGDSP). The PR_g peptide-amphiphiles self-assemble into nanofibers in an aqueous environment and form hydrogels. In this study, the PR_g hydrogels were characterized with cryo-scanning electron microscopy (cryo-SEM) and rheology measurements, and their surfaces were evaluated in terms of sustaining long term human umbilical vein endothelial cell (HUVEC) adhesion and proliferation, cytoskeleton formation, and secretion of extracellular fibronectin. PR_g gels were compared to polyethylene glycol (PEG) gels, PEG gels functionalized with fibronectin as well as commercially available peptide hydrogels (PuraMatrix). Our results show that the PR_g hydrogels outperform the other gels evaluated in this study and therefore hold promise as scaffolds for tissue engineering.

32. Novel synthetic inducible transcription regulators for prokaryotes

Kat Vö Izing and Yiannis Kaznessis
Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN

We're designing, constructing and characterizing novel synthetic gene regulators. They range from individual inducible fusion protein-promoter pairs to complex nonlinear regulatory networks. All are compatible in bacteria and control expression by direct activation. Our investigations have experimental and computational components, allowing us to build and characterize these complex and robust synthetic systems while reducing the expense and effort to construct future networks.

Our focus has been on two two-part synthetic systems, proTetON and proTetOFF, composed of an inducible fusion regulatory protein and a complementary fusion promoter. The systems are based on *tet*- and *lux*-operon components. We're experimentally optimizing and characterizing the systems' gene expression regulation using qPCR, immunoblotting and flow cytometry. qPCR and cytometry results show that proTetON upregulates transcription, increasing protein production upon anhydrous tetracycline (aTc) induction. After aTc induction, a cell population emerges with GFP expression 2 orders of magnitude above the uninduced control. The activity of proTetOFF is inversely related to aTc levels. In cells with this protein, GFP upregulation occurs in the absence of aTc, while only low expression is observed in its presence. In the absence of aTc, a second cell population emerges with GFP expression one order of magnitude above the untreated cultures. Next, we will investigate the behavior of each regulator in the presence of transcription repressors, aiming to reduce background GFP expression.

In parallel, we're building and testing stochastic models to characterize the systems' context independent behavior. Each system is represented by a network of biochemical reactions that describe the bimolecular interactions of bacterial transcription and translation, while also accounting for molecule transport into the cell and degradation. We're using kinetic parameters and species concentrations from the literature, and integrating these networks, their parameters, and the simulations with Synthetic Biology Software Suite (www.SynBioSS.org). As we fine tune the unknown kinetic parameters to fit the models' behaviors to experimental results, we identify the appropriate values of these parameters.

In summary, we're producing novel synthetic inducible transcription regulators that can be used to improve industrial bioprocesses and academic research. We're combining experimental and computational approaches to quickly and thoroughly build and characterize these tools.

33. A scaffold free approach to tissue-engineered vascular grafts

Nathan K. Weidenhamer¹ and Robert T. Tranquillo^{1,2}

¹Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN

²Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN

Coronary heart disease has become one of the leading health issues in the United States. Many patients in need of coronary bypass surgery lack autologous grafts due to age and/or disease. Consequently, there has become a need for a completely biological tissue-engineered vascular graft (TEVG). In one approach, which is currently in clinical trials, vascular cells seeded on tissue culture plastic (TCPS) are stimulated to secrete extracellular matrix. After several weeks, the resulting tissue is rolled around a cylindrical mandrel to form a TEVG. Several more weeks of culture are required for the tissue layers to fuse to form a homogeneous TEVG that can be implanted.

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The work presented here represents a new approach to scaffold-free vascular tissue-engineering. Rather than being cultured on TCPS, the cells are seeded directly onto a silicone tube and allowed to grow directly around the tubing, bypassing the need to allow extra time for tissue layer fusion. Neonatal human dermal fibroblasts (NHDF) were seeded onto fibronectin-coated silicone tubing by rolling the tubing through a cell suspension. We show that 7 days of static culture results in a confluent monolayer entirely around the circumference and length of the tubing. Furthermore, after 4 to 8 weeks of static culture, a mechanically stable tubular tissue is obtained. The resulting tissue is highly cellular and contains a significant amount of collagen. The tissue is approximately 30 μm thick and has an elastic modulus of 10.2 MPa and 26.7 MPa at 4 and 8 weeks, respectively. The tissue also has an ultimate tensile strength (UTS) of 1.0 MPa and 2.7 MPa at 4 and 8 weeks, respectively.

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Chin-Ann Yang

Using 3D cube-based features for automated focal cortical dysplasia lesion detection

Poster #34

34. Using 3D cube-based features for automated focal cortical dysplasia lesion detection

Chin-Ann Yang and Mostafa Kaveh

Department of Electrical and Computer Engineering, University of Minnesota, Minneapolis, MN

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Focal cortical dysplasia (FCD) is the most frequent malformation for patients with pharmacoresistant epilepsy that require surgical treatment. Due to its subtlety and minor structural changes, the FCD lesions may easily go unrecognized by standard radiological analysis even with the availability of high-resolution MRI. Providing automated procedures to detect FCD lesions is greatly desirable because visual diagnosis is often challenging, time consuming, and highly relies on the individual's expertise. Several works have been dedicated to this specific topic for the past two decades. These works mostly integrate computational image processing operators and machine learning algorithms on a voxel-based analysis to detect lesional areas. While voxel-based analysis methods report to have good results on the detection task, we believe that spatial neighboring information are also crucial in determining whether an area is lesional or not. We propose using three-dimensional (3D) cube-based features instead of voxel-based features, which allows us to process and analyze several neighboring voxels at a time. Some preliminary experimental results on the newly proposed 3D cube-based features tested on a total of 21 FCD subjects' data provided by the Radiology Department of Mayo Clinic.

Zhaoye Zhou

Noninvasive imaging of 3-D ventricular activation in an in-situ swine model during pacing

Poster #3535. **Noninvasive imaging of 3-D ventricular activation in an *in situ* swine model during pacing**

Zhaoye Zhou¹, Michael G. Bateman², Chengzong Han¹, Chenguang Liu¹, Paul A. Iaizzo², and Bin He¹

¹Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN

²Department of Surgery, University of Minnesota, Minneapolis, MN

Noninvasive 3-dimensional (3-D) electrical imaging of cardiac activation is an area of intense research relative to its potential importance for assisting in the clinical diagnoses and interventions of cardiac diseases. The aim of this study was to evaluate the transmural cardiac electrical imaging in a swine model, while employing a pacing protocol utilizing intramyocardial 3-dimensional mapping.

The heart and torso anatomical structures were digitally reconstructed from pre-operative MRI. On the anesthetized swine, up to 90 ECG electrodes were put on the anterior chest of the animal for recording the body surface potential maps (BSPMs) during different paced rhythms. Multiple pacing locations were identified by using a non-contact intracavitary mapping system EnSite 3000 (St. Jude Medical). Additionally, intracardiac electrograms were simultaneously recorded at up to 128 sites by using customized intramural electrodes. The sites of paced activation were estimated using a noninvasive 3-D cardiac electrical imaging technique previously developed at the Biomedical Functional Imaging & Neuroengineering Laboratory at the University of Minnesota. The noninvasive localization results were compared with the identified pacing sites. The localization error (LE) for left ventricular lateral (LVL) free wall pacing was 9.0 mm and the LE for left ventricular apex (LVA) pacing was 8.6 mm. Global 3-dimensional ventricular activation sequences were also reconstructed from BSPMs.

The present pilot study suggests that the 3-D cardiac electrical imaging technique can non-invasively well localize the sites of initiation of ventricular activation during paced rhythm in a large animal model. Further, the feasibility of simultaneously performing body surface potential mapping and intramyocardial mapping has been experimentally tested. These validation studies of body surface mapping should help progress the use of these technologies in clinical settings.

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Yang Zhou

Intelligent helmet with collision detection

Poster #3636. **Intelligent helmet with collision detection**

Yang Zhou and Dr. Marian Stachowicz,

Laboratory for Intelligent Systems, ECE Department, University of Minnesota, Duluth, MN

According to National Highway Traffic Safety Administration research, "There is a dramatic increase of fatalities for cyclists during last a few years. Approximately three-fourths of motorcycle accidents involved collision another vehicle". So it's extremely helpful to develop an intelligent helmet with collision detection, which could alert the motorcyclist at certain circumstances and allow more time for them to react on an emergency.

Many motorcycles die each year because of their failure to detect and recognize motorcycles in traffic. Therefore, we design an intelligent helmet to help the riders recognize the potential danger ahead, especially in some extreme situation such as fog, or snow. We successfully found a way to calculate the precaution time and give rider options to adjust the precaution time at their comfort. This could largely reduce their likeness to collies with another vehicle.

University of Minnesota - College of Veterinary Medicine

37. Role of enteric oxalate-degrading bacteria in canine calcium oxalate urolithiasis

Josephine S. Gnanandarajah¹, Juan E. Abrahante¹, Carl A. Osborne², Jody P. Lulich², and Michael P. Murtaugh¹,

¹Department of Veterinary and Biomedical Sciences and ²Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN

Supersaturation of calcium and oxalate ions in the urine leads to calcium oxalate (CaOx) urinary stone formation in dogs. Presence of enteric bacteria that metabolize oxalate in the intestine is correlated with absence of hyperoxaluria and/or CaOx urolithiasis in humans and laboratory animals. Therefore, we hypothesize that decreased colonization of enteric oxalate-degrading bacteria is a risk factor for CaOx urolithiasis in dogs. Fecal samples were collected from dogs with CaOx uroliths (n=20), clinically healthy, age, breed and gender matched-dogs (n=20) and healthy non-stone forming breed dogs (n=20). The fecal samples were screened for the presence of three potential oxalate-degrading bacteria (ODB), *Oxalobacter formigenes*, *Lactobacillus acidophilus*, and *Bifidobacterium animalis* by quantitative PCR to detect the species-specific oxalyl CoA decarboxylase (Oxc) genes of these bacteria. Oxalate degrading activity also was measured in fecal cultures using biochemical methods. We found that the presence of Oxc genes of *O. formigenes*, *L. acidophilus*, and *B. animalis* was significantly higher in healthy non-stone forming breed dogs than in the dogs with calcium oxalate stones. However, there was no significant difference in the presence of Oxc genes between dogs with CaOx stones and their matched controls. Further, the dogs with calcium oxalate stones and the matched controls showed comparable levels of oxalate-degrading activity in oxalate-supplemented fecal culture. In this study, we also isolated *O. formigenes*, *L. acidophilus*, *L. reuteri*, *B. animalis* and *B. pseudolongum* from canine fecal cultures that showed higher oxalate degradation. In addition, the fecal samples were analyzed to determine the differences in the enteric bacterial populations between healthy and CaOx stone-forming dogs. In conclusion, enteric colonization of ODBs may have a preventive effect on CaOx urolithiasis.

38. Isolation and characterization of methicillin resistant *Staphylococcus aureus* from bulk tank milk in Minnesota dairy farms

K. P. Haran¹, S. M. Godden¹, J. B. Bender¹, and S. Sreevatsan^{1,2}.

¹Veterinary Population Medicine, ²Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN

Staphylococcus aureus is a major cause of mastitis in dairy cattle and is frequently associated with antibiotic use on farms. The current study was undertaken to determine the prevalence of methicillin resistant *S. aureus* (MRSA) in dairy farms around Minnesota. Farm prevalence of *S. aureus*, including MRSA, was estimated from bulk tank milk (BTM) samples and isolates were characterized genotypically and phenotypically. A total of 150 pooled BTM samples from 50 farms spanning the 3 seasons (spring, summer and fall of 2009) were collected. The prevalence of any *S. aureus* was: 62% while that of MRSA was: 5.3%. All 101 isolates were subjected to antibiotic susceptibility testing using the Kirby Bauer method. Of these 54 were pansusceptible, 2 were resistant to a single antibiotic, 37 MSSA were resistant to >2 antibiotics. All the 8 MRSA isolates were multidrug resistant. Staphylococcal protein A or spa typing Alley Institute Student Research Poster Session - LifeScience Alley Conference & Expo – December 8, 2010

identified that spa types t529 and t034 were most prevalent among MSSA and types t034 and t359 among MRSA. All MRSA isolates were also genotyped using MLST and PFGE profiling. Of the 8 MRSA, one isolate had a composite profile of - ST 5-USA 100-spa 2 type which has been reported among HA MRSA, while a second carried - ST 8-USA 300- t121 type commonly identified amongst CA-MRSAs. The third had: ST 398- untypable by PFGE-t034, reported for LA-MRSA particularly of porcine origin. Four other isolates carried genotype combinations similar to those reported for CA-MRSA. This suggests that MRSA associated with hospitals, community and livestock can all be found in dairy farms. Large-scale studies on MRSA are needed to understand factors associated with their emergence and persistence in dairy environments.

39. A membrane associated serine protease of *Mycobacterium avium* subspecies *paratuberculosis* plays a role in resistance to phagosomal acid stress

Abirami Kugadas¹, Elise A Lamont¹, and Srinand Sreevatsan^{1,2}

¹Department of Veterinary Population Medicine, ²Department of Veterinary Biomedical Sciences, University of Minnesota, St Paul, MN

Pathogenic mycobacteria successfully survive in the acidic microenvironment of the phagosome. We hypothesize that *Mycobacterium avium* subspecies *paratuberculosis* (MAP) over expresses a membrane associated serine protease (MAP0403) in response to phagosomal acidification in macrophages. Expression of serine protease by MAP K-10 was studied at 10, 30, and 120-min. post infection of bovine monocyte derived macrophages treated with or without baifilomycin to block phagosomal acidification. Gene encoding the MAP serine protease was significantly up regulated exclusively in the acidified phagosomes. Highest level of MAP0403 expression coincided with the timing of peak phagosome acidification in macrophages. Inasmuch as *Mycobacterium smegmatis* cannot resist and persist in the acidified phagosome, we cloned the open reading frame of MAP0403 into *M. smegmatis mc² 155*. Compared with controls, *M. smegmatis mc² 155* transformants carrying the MAP serine protease show increased survival during *in vitro* acid stress and in monocyte derived macrophages. Our studies suggest that MAP serine protease is critical in resisting the phagosomal acidification by MAP.

40. Glycan analysis of GP5 on porcine reproductive and respiratory syndrome virus

Juan Li¹, Shujuan Tao², Ron Orlando² and Michael Murtaugh¹

¹Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN

²Complex Carbohydrate Research Center, University of Georgia, Athens, GA

Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiologic agent of porcine reproductive and respiratory syndrome, which is the most severe infectious disease facing the swine industry worldwide. Understanding the molecular structure of PRRSV is expected to facilitate the study of viral infection mechanism and the development of disease prevention strategies. PRRSV is a RNA virus and has a smooth spherical envelope embedded with major envelope proteins (GP5/M heterodimer) and minor envelope proteins (E, GP2a, GP3, GP4), most of which are highly glycosylated. In particular, GP5/M have been shown to be involved in viral binding and entry into the permissive cells. GP5 has two short ectodomains outside of the envelope; the molecular weight of GP5-linked glycans exceeds that of its ectodomain amino acid sequences. Thus the broadly distributed viral glycans are likely to cover the virion surface, or even stretch out as antennae, suggesting a potential role in virus-cell interactions and possible targets for virus neutralization in virucidal design. Herein, PRRSV was purified by iodixanol self-generated gradient ultracentrifugation. Endoglycosidase digestion of purified PRRSV suggested that GP5 contained most of the viral glycans and the major glycan type was complex type N-glycans. The viral proteins were then separated by running through SDS-PAGE; the GP5 bands were cut and trypsinized in gel. After ID confirmation using mass spectrometry (MS/MS), the glycopeptide products were treated with PNGase F to release all the N-glycans. After glycan extraction and purification, the N-glycan products were finally analyzed by LC-MS/MS to obtain the potential glycan compositions and structures on PRRSV GP5. We found 58 potential N-glycan structures in total and 47 were complex type N-glycans, consistent

with the endoglycosidase digestion assay. Furthermore, 20 out of 47 complex type N-glycans contained 3 - 5 closely grouped N-acetylglucosamines (GlcNAc) in glycan structures. The importance of (GlcNAc)₃₋₅ in PRRSV infection was demonstrated by the data that lectins specific for (GlcNAc)₃ were able to inhibit viral binding and infection in MARC-145 cells. Finally, lectin co-precipitation assay was performed to show that lectins specific for (GlcNAc)₃ could directly bind PRRSV.

41. Characterization of PCV2 growth in R1BL cells

Sumathy Puvanendiran and Michael P. Murtaugh.
Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN

Porcine Circovirus type 2 (PCV2) is a small, non-enveloped virus with a single-stranded DNA genome. It causes a variety of disease syndromes collectively referred as Porcine Circovirus Associated Disease (PCVAD) and has been identified in most swine producing countries worldwide. The mechanism of pathogenesis is unclear, though it is believed that high viral loads contribute to histological lesions. PK-15 and ST cells are widely used to grow PCV2, but the virus does not grow to high titer in either cell line, thus complicating the study of cellular pathogenesis. R1BL, a cell line, obtained from porcine fetal retinal tissue, also is permissive to PCV2. We hypothesize that R1BL cells support efficient PCV2 replication, and thus will serve as a model to study PCV2 cellular biology. As a first step, we report here the comparative growth characteristics of PCV2, both genotypes A and B, in PK15 and R1BL cell lines by quantitative PCR, TCID₅₀ and immunofluorescence. Our results reveal that PCV2 growth is significantly higher in R1BL compared to PK15 cells. Although, the pattern of PCV2 capsid distribution is similar in both cell lines, 96 h after infection there was fragmentation of nuclei, which was co-localized with PCV2 capsid, in R1BL cells but not in PK15 cells. The growth characteristics of both cell lines were analyzed by monitoring the amount of DNA at different time points. Our results show that R1BL cell line grows slowly and that a high density of cells is necessary at the time of seeding compared to PK15 cells. Although R1BL cells grow slowly, DNA content during first 48 h is higher than PK15 cells. Since PCV2 is a DNA virus and depends on the host cell for replication, early cell cycle events could influence its replication. R1BL cells are considered as mesenchymal in origin and permissive cells for PCV2 in pigs include lymphocytes, which are also mesenchymal in origin. We conclude that R1BL cells are a good model for elucidation of PCV2 growth and cellular pathogenesis in vitro.

University of Minnesota - College of Biological Sciences

42. TGF- β signaling regulates cytochrome P450 proteins during developmental transitions in *Drosophila*

Ying Y. Gibbens¹, James T. Warren², Lawrence I. Gilbert² and Michael B. O'Connor^{1,3}

¹Department of Genetics Cell Biology and Development, University of Minnesota

²Department of Biology, University of North Carolina, Chapel Hill, NC

³Howard Hughes Medical Institute, Minneapolis, MN

In insects, initiation of metamorphosis requires a surge in the production and release of the steroid hormone 20-hydroxyecdysone from the prothoracic gland, the primary endocrine organ of juvenile larvae. Here we show that blocking TGF β /Activin signaling specifically in the *Drosophila* prothoracic gland results in developmental arrest prior to metamorphosis. The terminal, giant third instar larval phenotype results from a failure to induce the large rise in ecdysteroid titer that triggers metamorphosis. We further demonstrate that Activin signaling regulates competence of the prothoracic gland to receive PTTH and insulin signals and that these two pathways act at the mRNA and posttranscriptional levels respectively to

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control ecdysone biosynthetic enzyme expression. This dual regulatory circuitry may provide a cross-check mechanism to ensure that both developmental and nutritional inputs are synchronized before initiating the final genetic program leading to reproductive adult development. Since steroid hormone production in *C. elegans* and mammals is also influenced by TGF β /Activin signaling, this family of secreted factors may play a general role in regulating developmental transitions across phyla.

University of Minnesota - College of Pharmacy

43. ADME characteristics of novel catalytic inhibitors of topoisomerase II

Aaron M. Teitelbaum,¹ Rajan Giri,² Adam R. Benoit,¹ David M. Ferguson,¹ Jose L. Gallardo,³ Jessica Bedi,³ John R. Ohlfest³ and Rory P. Remmel¹

¹Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN

²Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Chicago, IL

³Department of Pediatrics University of Minnesota Medical School, Minneapolis, MN

Dr. David Ferguson's group at the University of Minnesota recently discovered a series of substituted 9-aminoacridines with potent anti-proliferative activity toward several pancreatic and brain cancer cell lines. In collaboration, we are characterizing the ADME (Absorption, Distribution, Metabolism, and Excretion) properties of selected 9-aminoacridines by investigating their metabolic stability, Caco-2 permeability, MDCK cell accumulation, Pgp and BCRP efflux transport, plasma protein binding, and tissue distribution in mouse pharmacokinetic studies. Metabolic stability experiments in pooled human liver microsomes indicated good metabolic stability ranging from 2.2 - 4.1 hrs for oxidation and glucuronidation. In addition, all substituted 9-aminoacridine compounds accumulated in MDCK cells and were also shown to be relatively weak substrates for the Pgp efflux transport pump. Interestingly, it was discovered that the 9-aminoacridines were substrates for the organic cation transporter 2 (OCT-2). A mouse pharmacokinetic study following a 60 mg/kg oral dose with Acridine 1 and 2 demonstrated low penetration into the brain (C_{max} = 0.25 μ M and 0.6 μ M), but high uptake in kidney (C_{max} = 30 μ M and 300 μ M) and liver (C_{max} = 125 μ M and 225 μ M) relative to peak concentrations in plasma (C_{max} = 2.25 μ M and 20 μ M). Subsequently, an intravenous pharmacokinetic study with Acridine 2 following a 15mg/kg dose produced peak concentrations in the brain (1.7 μ M), kidney (212 μ M), and liver (78 μ M) at 2.0 hours relative to a 2.0 μ M peak concentration in the plasma. Acridine 2 bioavailability was 83.8%. Further evaluations of these compounds in liver cancer models are currently in progress.

University of Minnesota - Medical School

44. State of the art of pre-clinical evaluation of surgical cardiac valve replacements

John P. Carney, John L. Schomburg, Matthew T. Lahti, Richard W. Bianco
University of Minnesota, Minneapolis, MN

The purpose of this historical study was to analyze and evaluate the state of preclinical surgical cardiac valve replacements, using a decade of valve replacement data generated by Experimental Surgical Services of the University of Minnesota.

We compiled surgical and pathology data generated in nearly five hundred cardiac valve replacements performed by Experimental Surgical Services. Utilizing descriptive statistics, we attempted to determine success trends in appropriate animal model choice, surgical technique, valve design and

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positioning. We relied on surgical notes, post-operative care records, mortality rates and physical description of the valve at necropsy to make our assessment.

We have determined that there has been a steady improvement in surgical technique with each valve implantation, based on decreased incidence of surgical complications and increased rate of full term implants. We have also found significant evidence relating the success of specific valve designs to animal models.

Our study indicates that pre-clinical surgical cardiac valve replacement is continuing to evolve and adapt to new device technology. We have found that results from pre-clinical implantation of cardiac valves serve as an accurate predictor for sustainability and evaluation in clinical trials.

45. Cytomegalovirus-induced sensorineural hearing loss with persistent cochlear inflammation in neonatal mice

Scott J. Schachte¹, Manohar B. Mutnal¹, Mark R. Schleiss², and James R. Lokensgard¹
Center for Infectious Diseases and Microbiology Translational Research, Departments of Medicine¹ and Pediatrics², University of Minnesota, Minneapolis, MN

Congenital cytomegalovirus (CMV) infection is the leading cause of sensorineural hearing loss (SNHL) in children. During murine (M)CMV-induced encephalitis, the immune response controls viral dissemination and facilitates clearance from the brain. While the importance of CMV-induced SNHL has been described, the mechanisms surrounding its pathogenesis and the role of inflammatory responses remain unclear. This study presents a neonatal mouse model of profound SNHL in which MCMV preferentially infected both cochlear perilymphatic mesenchymal cells and spiral ganglion neurons. Surprisingly, MCMV infection induced cochlear hair cell loss by 21 d post-infection, despite a lack of direct hair cell infection. Flow cytometric, immunohistochemistry, and quantitative PCR analysis of MCMV-infected cochleae revealed a robust and chronic proinflammatory response, including an increase in macrophage reactive oxygen species production. These data support a pivotal role for inflammation during MCMV-induced SNHL.

46. CUGBP1 and GU-rich element represent a novel target pathway for cancer therapy

Irina St.Louis^{1,2}, Daniel J. Beisang^{1,2}, and Paul R. Bohjanen^{1,2,3}
Center for Infectious Diseases and Microbiology Translational Research¹, Department of Microbiology², Department of Medicine³, University of Minnesota, Minneapolis, MN

Our research group has measured mRNA decay rates in normal and malignant T cells, on a genome-wide scale, using microarray technology. We identified the group of thousand of growth-regulatory transcripts with defective mRNA decay rate in malignant T cells compared to primary human T lymphocytes. Twelve percent of these mRNA transcripts contained AU-rich element, and twenty percent contained a GU-rich element (GRE) in the 3' untranslated regions (3'UTR), according to results of bioinformatics search. GRE is a novel conserved regulatory element which mediates rapid decay of target RNA transcripts. We have reported that GREs function to mediate mRNA decay by binding to CUG-binding protein 1 (CUGBP1) in normal T cells and Hela Tet-off reporter system. However, CUGBP1 is hyperphosphorylated in some cancer cell line, which decreases its binding to GRE.

Recently, we performed an RNA-immunoprecipitation followed by microarray assay (RIP-CHIP) with Hela cell cytoplasmic extracts. We identified near five hundred CUGBP1-target transcripts containing different [GUUUG]_n- and [GUGU]_n motifs, despite a much larger number of GRE-containing transcripts, expressed in Hela cells. To find out why some, seemingly genuine targets of CUGBP1 were not immunoprecipitated with aCUGBP1 antibody, we selected a set of GU-rich transcripts, that were: (1) abnormally stable in malignant T cell lines and (2) were absent in the CUGBP1-RIP-CHIP target checklist. RNA-IP followed by PCR assays were performed with cytoplasmic extracts from Jurkat (leukemia cell line), H9 (T cell lymphoma cell line) and normal T cells, and were compared with each other. Interestingly, there were no PCR products amplified if primers were designed against distal portion of 3'UTR of target mRNAs in malignant T cells. However, alternatively processed isoforms of same

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transcripts were present, when amplified with primers, designed against proximal portion of 3'UTR, which did not contain GRE. Our results suggest, for several growth-regulatory transcripts and oncogenes, that isoform switch allows their mRNAs escape destabilization by CUGBP1. These results represent an additional level of complexity to abnormal post-transcriptional regulation in cancer, on one hand. On the other hand, this observation revealed the opportunity for bimodal therapeutic design to target additional step of abnormal post-transcriptional regulatory pathway in cancer.

47. Beyond the electronic health record: The clinic workbench

Elisabeth Sandberg Tan, Mark Janowiec, and Kevin A. Peterson
University of Minnesota – Center of Excellence in Primary Care, Minneapolis, MN

Information from a clinic's Electronic Health Record (EHR), laboratory system, patient management system, spreadsheets, and/or other data sources, is commonly not standardized in order to facilitate use by clinicians, researchers and public agencies. The major EHR vendors and software corporations are developing proprietary tools to be sold to the leading hospitals and research centers but not to small clinics. A more integrated architectural vision is needed to support community and rural health services to standardize data, to provide point-of-care decision support, flexible electronic health records for new discoveries and devices, and easy access for consumers to their health information. The Center of Excellence in Primary Care at the University of Minnesota is currently developing a scalable network which standardizes clinic and hospital information to meet these needs. Using a flexible design for managing electronic health records (dimensional data warehouses), standard messaging formats (XML with CCR/CCD), enterprise vocabulary services (UMLS, EVS) and a federated architecture, the network promotes "Meaningful Use", interoperability, and research on the national and international level.

The poster presents the basic design of this scalable network and focuses on the major open-source component, the Clinic Workbench. The Clinic Workbench is a suite of new methods and HIPAA compliant, locally installed applications, tools, and services. The five basic activities include: 1) import of patient data – electronic health records (EHR), 2) transformation and standardization of data with vocabulary services, 3) population management, performance measuring, and quality reporting, 4) patient recruitment administration, and 5) the export of patient data – personal health records (PHR). Integration of these activities into a comprehensive system at the clinic level will aid in performance improvement and allows the clinician and researcher to participate in the larger goal of creating a learning healthcare system. Versions of the Clinic Workbench are currently part of the design of the electronic Primary Care Research Network (ePCRN), the UK National Health Service, the European TRANSFoRm project, and various Practice Based-Research Networks (PBRN) in the US.

University of Minnesota - Carlson School of Management

48. Experiential learning in healthcare industry consulting

Ruchika Aggarwal and Phil Miller
Carlson School of Management, University of Minnesota, Minneapolis, MN

The Consulting Enterprise (CCE) at the Carlson School is a student delivered management consultancy providing strategy and operations advisory services. Students have a significant interest in healthcare related fields, with recent graduates having taken jobs in a wide range of healthcare firms. As a result, the CCE has evaluated a wide range of healthcare industry projects with clients representing providers, med device/technology firms, distributors and payers.

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49. Healthcare technology in grey areas of the law: How innovation is hampered by legal tradition

Rachel Brucker
Medical Industry Leadership Institute, Carlson School of Management, University of Minnesota,
Minneapolis, MN

Advancements in healthcare technology can impact standards of care. Many times, the impact of innovation on existing standards of care is negligible. At other times, however, the governing standard of care will become obsolete in the face of new technology and the processes developed to implement it. During these transitions, there is a period of time where no "governing standard of care" will exist for that new product or treatment. As the success of medical malpractice claims hinges on a doctor's observance of a governing standard of care, this lull in regulation may impact judicial decisions.

The current evaluation method for evaluation of medical malpractice claims routinely fails to properly take into account the use of new technologies; often imposing negative outcomes on those who embrace innovation. This increased risk of liability can have a chilling effect on the adoption of innovative technologies and procedures. Furthermore, introduction of liability increases physician premiums that may be associated with shortages in particular medical specialties and rises in the overall cost of health care.

This research will explore the use of medical "custom" as the basis for determining standard of care, the impact of this standard on doctors, medical technology companies, and health care administration. In conclusion, recommendations are offered to mitigate issues of liability that may arise when a doctor elects to use a new medical procedure or product in the treatment of his patients. Given the widespread impact of technological development on the healthcare marketplace, understanding potential malpractice will require constant attention in the future.

50. MILI Valuation Laboratory: The process of medical technology business evaluation

Roy Bryan Jr^{1,2}, Stephen Parente², and Matt Stoll²
Medical School¹, Carlson School of Management², University of Minnesota, Minneapolis, MN

Trillions of dollars are spent towards scientific research or commercial R&D each year. Often these projects explore an innovator's idea with the goal of ultimate commercialization. The projects are often conducted in a "shotgun" approach without a clinical need or end commercial target in mind at the start of the research. Great ideas can languish in technology commercialization offices of university's or fall unfunded in large corporations. The Medical Industry Leadership Institute at the University of Minnesota created the Valuation Laboratory as an experiential learning course that brings together an interdisciplinary collaboration of students, faculty and industry leaders working to speed the evaluation process of the multitude of promising opportunities in the Minneapolis/St. Paul community.

Graduate students from as many as eight collegiate units can be involved in the Lab, including students from the Institute of Technology, the Carlson School of Management, the School of Law, College of Liberal Arts, and the Academic Health Center. The multidisciplinary teams get real-world experience, acting as a team of a university's Office of Technology Commercialization or a corporation's new product development team. Working under the supervision of experienced faculty members and mentors, Alley Institute Student Research Poster Session - LifeScience Alley Conference & Expo – December 8, 2010

students produce a comprehensive valuation report covering all aspects of the medical innovation. This interdisciplinary approach assesses intellectual property issues, regulatory challenges, market size and growth trends, the effectiveness of and need for the technology, and financial issues and strategic positioning. The final product is an unbiased market analysis and a recommendation for or against continuing product development.

This poster will provide an overview of the Medical Industry Valuation Laboratory, lay out the typical process used in valuation, and provide an example of the team and outcome of a recent project.

51. Reverse innovation capital sourcing model for medical technology

Ge Yan, Medical Industry leadership Institute, Carlson School of Management, University of Minnesota

Two years after the collapse of Lehman Brothers and the official start of the economic recession, Medtech Start-Ups are still experiencing the most challenging capital environment in United States. Facing a weak IPO market and increasing regulatory requirements, VCs as a group stepped back and tried triage their existing portfolio instead of funding new opportunities. Meanwhile, the Chinese healthcare market and medical device industry is rock solid. Huge population base, aging demographics, and additional insured lives coming into the public health care system provide growth opportunities for the medical products industry. However, the R&D engine and pipeline of device industry is still in its infancy. Leading companies and investment community are proactively searching for cutting edge medical technology and products to satisfy the enormous market demands.

Reverse Innovation Capital Sourcing Model (RICS) provides an effective framework that enables Medtech Start-Ups to source foreign capital investment to fund its U.S. operation, and to penetrate foreign market at the same time. Under RICS model, Start-Ups will raise capital from Chinese investment community (VC and PE) to continue their business and product development efforts in the U.S. market. In return, Chinese investors will entitle equity in the Start-Up and be granted exclusive distributorship and first refusal right of patent licensing in Greater China Region. Chinese investors will cooperate with U.S. Start-Ups to establish highly localized China strategy and facilitate clinical trials, regulatory path, reimbursement, marketing, distribution channel, and other business development process within China market. Based on the local regulatory environment, the technology and product will most likely to hit Chinese market first and bring additional cash flow and profits as a steady augmentation to U.S. strategy. RICS model will not only match capital demands with technology needs, but also address common pitfalls of international business and marketing strategy, including governance of foreign investment, oversea IP protections, government relations, and localization to foreign market.

University of Minnesota - College of Science & Engineering

52. Training laser prostate surgery with an advanced simulation based curriculum: The VicTOR[®] Greenlight Trainer

Divya Ambadipudi^{a,c}, Yunhe Shen^{a,b}, Vamsi Konchada^{a,c}, Nan Zhang^{a,b}, Xiangmin Zhou^{a,d}, Daniel Burke^{a,b}, Saurabh Jain^{a,c}, Omer Argun^{a,b}, Carson Wong^e, Culley Carson^f, Claus Roehrborn^g and Robert Sweet^{a,b}, ^aCenter for Research in Education and Simulation Technologies, Univ. of Minnesota, ^bDepartment of Urologic Surgery, University of Minnesota, ^cDepartment of Computer Science and Engineering, University of Minnesota, ^dDepartment of Mechanical Engineering, University of Minnesota, ^eDepartment of Urology, University of Oklahoma Health Sciences Center, ^fDepartment of Surgery, University of North Carolina ^gDepartment of Urology, University of Texas Southwestern Medical Center at Dallas

Photo-selective vaporization of the prostate (PVP) is an established and effective method to treat lower urinary tract symptoms secondary to benign prostatic hyperplasia. Virtual Reality (VR) simulation opportunities for PVP may facilitate an objective, learner-centered method for rapid and safe skills-

acquisition and maintenance. Our objective was to build a modular computer-based PVP training curriculum guided by a consortium of key opinion leaders and verified by AUA members.

A clinical advisory panel consisting of 4 key opinion leaders for PVP defined learning objectives and performance metrics. The curriculum includes 6 core prostatic anatomic full cases, as well as part-task training exercises for training proper sweep speed, tissue distance, power setting management and landmark identification. Fast and robust computation algorithms were developed to resolve challenges in modeling directional force-feedback, tissue vaporization and deformation, contact/collision modeling, and to optimize the simulation at speeds not previously seen in desktop surgical VR simulators to date. The early prototype was formally evaluated by 40 urologists at the 2010 AUA meeting, San Francisco.

We successfully developed and integrated the training system. Simulation rates with realistic collision detection, deformation, and haptics force-feedback for a half-million tetrahedral elements was successfully rendered at over 30 frames/second (industry standard) on a mid-range desktop computer. As part of this project, we designed a web-based learning management platform (LMP) that facilitates instantaneous, coordinated data acquisition, access, display and analysis across institutions worldwide.

VicTOR[®] is a state-of-the art VR trainer worthy of further development and more extensive validation studies. Verification data was critical for refinement of the trainer for release at this year's AUA.

University of Minnesota - Institute for Health Informatics

53. Improved biodegradation pathway prediction

Junfeng Gao¹, Lynda B.M. Ellis², and Lawrence P. Wackett³

¹Institute for Health Informatics, ²Department of Laboratory Medicine and Pathology, and ³Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, St Paul, MN

As xenobiotic chemicals increasingly enter the environment, it is becoming imperative to understand their fate in soil and water. The environmental fate of chemicals is largely predicated on their biodegradation by microbes. Conducting biodegradation studies for all new chemicals would be prohibitively expensive. In this context, scientists and non scientists are increasingly relying on computational tools that predict biodegradation. Well-curated information on microbial biodegradation is the basis for developing knowledge-based systems for *in silico* predictions of metabolic pathways and accumulating end-products.

The University of Minnesota Pathway Prediction System (UM-PPS) (<http://umbbd.msi.umn.edu/predict/>) has been in existence since 2002. It is a rule-based system that predicts plausible pathways for microbial degradation of organic compounds. Its rule-base is based on information found in the University of Minnesota Biocatalysis/Biodegradation Database (UM-BBD, <http://umbbd.msi.umn.edu/>) that contains information on over 1300 compounds, over 1200 reactions, over 800 enzymes, and over 500 microorganism entries. Currently, the knowledge base of the UM-PPS contains 275 biotransformation rules and five types of metabolic logic entities.

The original system predicted up to two levels of prediction at a time. Users had to choose a predicted product to continue the prediction. This approach provided a limited view of prediction results and heavily relied on manual interventions. The new UM-PPS produces a multi-level prediction within an acceptable time frame, and allows users to view prediction alternatives much more easily in a directed acyclic graph.

With the increase of UM-PPS rule-base, the system predicts more products at a level and may face a combinatorial explosion in a multi-level prediction. Knowledge based approaches were explored to address the combinatorial explosion issue, and to improve the prediction accuracy.

A prediction may take more time to complete based on the growing rule-base. The time-cost on a multi-step prediction may not be affordable for an online application. New computing method is developed to optimize prediction performance, and simulation studies were conducted to eliminate queuing interference. These improvements lead to smarter and faster UM-PPS.

54. Development of a personalized web-based data management system for physiological and metabolomic data integration in systems biology research

Priyaranjan Tokachichu¹, MS, Gregory J Beilman², MD
Department of Health Informatics¹ and Department of Surgery², University of Minnesota, Minneapolis, MN

The Surgical Critical Care laboratory at the University of Minnesota conducts pre-clinical research that involves integration of physiological and metabolomic data. Managing the data using flat file data management systems has become increasingly difficult as the research progressed. Diversity of research groups and differences in data generation timeline makes data sharing between research groups difficult. This project explains the development of a data management system that can fulfill the data needs of the Surgical Critical Care laboratory at the University of Minnesota, and other research with similar data needs. Each systems biology research project has unique data needs and requires a personalized data management system. A web-based data management system would be independent of any computer operating system and makes data access and sharing easier. A relational database management system manages enormous amounts of data more effectively than a flat file data management system. These are some of the factors that influenced the development of a personalized web-based data management system with a back end relational database management system. Multi-disciplinary teams' involvement, short-term goals, need for continuous changes/development, pre-clinical research, etc., are some of the factors that have driven the hybridization of open-source/commercial software products, and the usage of a health informatics model in combination with an agile software development model, in developing this software product named c-Surge. A combination of the Extreme programming (XP) which is a type agile software development model and health informatics model with three basic components data, information and knowledge (DIK) is used in developing the cSurge software. A thorough review of data management needs at SCC laboratory, technological choices, and a development plan are the core building blocks for this software. The software was evaluated by user satisfaction survey and testing the accuracy of data collection in real-time. The c-Surge data management system's development process shows easier ways to develop a home grown application that can fulfill unique data needs of a systems biology research project within a reasonable economic expense.

St. Cloud State University

55. Particle size dependent in vitro toxicity studies of Iron oxides/hydroxides

Yong Heng Lee, Prasad Mawilmada, and Kannan Sivaprakasam
Department of Chemistry, St. Cloud State University, St. Cloud, MN

Nanosuspensions of iron oxides/hydroxides (IOH) were recently approved for iron supplement therapy for anemic conditions associated with iron deficiency. But the toxicity of these nanoparticles in terms of their particle size is not well understood. The main objective of our research project is to study the toxicity of IOH as a function of their particle size both at the cellular and at subcellular levels. In vitro toxicity of nano
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/ macroparticles of iron oxides / oxyhydroxides were tested as a function of concentration of IOH in human embryonic kidney (HEK) cells. Cytotoxicity of the IOH was monitored by morphological changes using Scanning Electron Microscopy (SEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and defragmentation of DNA of HEK cells. The in vitro toxicity assays were also carried out in presence of varying concentrations of surfactants: poly(vinyl alcohol) – PVA, poly (ethylene glycol) – PEG and oleic acid. We will present the results of toxicity assays with and without surfactants. This is the first report of size dependent toxicity of IOH.

56. Diffusion dynamics of combination of drugs in hydrogels

Prasad Mawilmada¹, Yong Heng Lee¹, Lee Schaefer¹, Kannan Sivaprakasam¹, and Jeff Schoess²

¹Department of Chemistry, St. Cloud State University, St. Cloud, MN

²Eden Medical Inc, Howard Lake, MN

Hydrogels are smart drug delivery system that can respond to a variety of physical, chemical and biological stimuli. However, the potential of hydrogels as a novel drug delivery system of combination of therapeutics has not been explored in detail. The main objective of our research project is to understand transport and diffusion kinetics of different combination of drugs in hydrogels. We have synthesized and characterized copolymer hydrogels using N-isopropyl acrylamide and acrylic acid. We were able to relate the low critical solution temperature, microstructure and the swelling properties of the different chemical composition of hydrogels. The microstructure, studied scanning electron microscopy, was also examined to understand the diffusion kinetics of different drug candidates that were chosen for this study (aspirin, warfarin, and acetaminophen).

57. An investigation into the role of chlorophyll in leaf senescence

Prasad Mawilmada, Abraham Yorek, Yong Heng Lee, Sagar Chaulagain, and Kannan Sivaprakasam
Department of Chemistry, St. Cloud State University, St. Cloud, MN

Autumnal senescence is a spectacular change in color of the leaves from green to yellow to brown. The variation in colors arises from the programmed degradation of chlorophylls accompanied by the synthesis of red-colored pigments like anthocyanins. Autumn senescence has not been well characterized at the cellular and molecular levels. Our main objective of this project is to understand the molecular events associated with the degradation of chlorophyll using fluorescence techniques. Fluorescence and chromatographic studies done with hexane extracts of green, yellow and brown maple leaves indicate clearly that levels of chlorophyll level is correlated with the color change of the leaves. The onset of appearance of anthocyanins in yellow and brown leaves correlates with the disappearance of chlorophyll pigments. We will also discuss the results of our investigation into the variation of fluorescence properties of chlorophylls in presence of substances that are present in leaves (e.g., vitamin B, vitamin C, waxes).

[St. Mary's University](#)

58. Expression optimization and purification of baculovirus FGFs

Nicole Clammer, Sean Ohl and Casey M. Finnerty, Ph.D.
St. Mary's University of Minnesota, Biology Department, Winona, MN

Fibroblast growth factors, also known as FGFs, are a large family of cytokines found in almost all animals. FGFs have many functions including angiogenesis, wound healing, embryogenesis, and many other
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regulatory effects, but these roles fundamentally result from proliferation and/or cell motility. Some viruses have evolved to express viral cytokines, such as FGF, from their own genome to use for pathogenesis. Baculoviruses, a large family of occluded, double-stranded DNA viruses that infect insects in the family Lepidoptera, contain an FGF homologue which may aid in the systemic infection of their host. In this research, the expression optimization of six recombinant FGF constructs from the *Autographa californica* nucleopolyhedrovirus and the *Choristoneura fumiferana* nucleopolyhedrovirus was investigated. An affinity purification procedure was also completed using two of the homologues that were tagged with maltose binding protein (MBP). Expression of several untagged FGFs was also performed in order to produce ample amounts of protein for crystallization studies.

Bethel University

59. The effects of diazepam (Valium) on skeletal muscle contractile strength *in vitro* and *in situ*

David Johnson and Teresa DeGolier (advisor), Bethel University, St. Paul, MN

Various skeletal muscle relaxants are used to treat musculoskeletal conditions in medicine today. Among some of the most widely used drugs is diazepam, a benzodiazepine derivative. Diazepam functions in the central nervous system by binding to a specific subunit of the GABA receptor. GABA receptor activation results in decreased neuronal activity, or inhibitory effects, due to the receptors' control of chloride ion influx which hyperpolarizes the post-synaptic membrane. Diazepam is predominately thought of as a centrally-active drug, but evidence exists which suggests substantial effects on the periphery and the skeletal muscles themselves, namely, skeletal muscle relaxation. The aim of this study was to examine the effects of diazepam on skeletal muscle contractile strength both *in vitro*, using psoas muscle tissue isolated from *Rattus norvegicus*, and *in situ*, administering diazepam via the sciatic nerve and measuring contractions of frog gastrocnemius, *Rana pipiens*. *In vitro* experiments were carried out by situating isolated portions of the psoas muscle (without nerve tissue) in an organ bath mimicking physiological conditions. *In situ* experiments utilized the exposed sciatic nerve and frog gastrocnemius severed at the Achilles tendon with the rest of the organism intact. For both experiments contractile strengths were measured using a force transducer and percent changes between trials were measured. Findings suggested no statistical difference in contractile strength between control and experimental groups, indicating that, with respect to the tissues studied, diazepam mechanism of action requires central nervous system interaction and descending neuronal pathways.

University of Manitoba

60. Total shoulder arthroplasty and the *in vivo* measurement of implant loosening with radiostereometric analysis

Trevor Gascoyne^{1,2}, Martin Petrak², Peter MacDonald^{3,4}, Eric Bohm^{2,4}, Jeff Leiter³, Sheila McRae³, and Jason Morrison¹; ¹University of Manitoba, Biosystems Engineering, ²Concordia Joint Replacement Group, Concordia Hospital, ³Pan Am Clinic, ⁴University of Manitoba, Department of Surgery

Radiostereometric analysis (RSA) is a highly accurate imaging technique used primarily for 3-dimensional measurement of implant micromotion in joint arthroplasty. Detection of implant micromotion is used to predict early loosening of implant components. Total shoulder arthroplasty (TSA) is a well established joint replacement procedure, but there remain concerns about proper fixation of the glenoid component into the bone due to cyclic edge loading, particularly in components with keeled fixation. A newer pegged fixation design is reported to reduce the incidence of component loosening compared to keeled fixation. *In vivo* assessment of glenoid-implant micromotion was performed with radiostereometric analysis (RSA) in a controlled randomized pilot study.

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Thirteen patients (14 shoulders) were enrolled for this study. It was expected that less micromotion, particularly varus/valgus rotation (resulting from edge loading), would occur with pegged components than with keeled components. In order to improve the detection of RSA markers, the orientation of the RSA setup was changed from a medial-lateral direction (x-rays across the patient's shoulder) to a proximal-distal direction (x-rays above and below the patient's shoulder).

Translation and rotation of most of the glenoid components was found to be less than 0.5 mm and 1 degree, respectively. One patient with a pegged glenoid component experienced valgus rotation of 5.0 degrees after 1 year. Due to patient drop out and poor distribution of markers, only 8 patients (8 shoulders) had useable data at 1 year follow-up.

The use of RSA in hip arthroplasty is well established and there is promise for its use to be extended to shoulder arthroplasty as well. Adjustment of the RSA setup was beneficial to the study and newly recruited patients will be examined in this manner. Our preliminary data suggests that new implant designs need to be tested extensively before survivorship can be accurately determined. Further follow-up of all patients in this study is required to draw conclusions between these two glenoid component designs.

Michigan Technological University

61. Magnetoelastic sensor-based chemical dosimeter

Sam Bredeson and Keat Ghee Ong
Biomedical Engineering, Michigan Technological University, Houghton, MI

We are developing a wireless, passive dosimeter based on magnetoelastic sensors for tracking personal exposure to toxic gaseous chemicals. The magnetoelastic sensors vibrate at a characteristic frequency under the excitation of a magnetic field. This creates a secondary magnetic flux that can be monitored by a set of magnetic coils. Detection of a specific chemical is achieved by applying an absorptive coating which gradually absorbs the target chemical. The increased mass of the sensor as it absorbs the toxin causes a shift in its resonant frequency, which can be determined and evaluated by measuring the frequency at selected time intervals. These sensors are incorporated into dosimeters to be worn by users, with the detection coil system built into a doorway, much like RFID scanners near the doorways of large stores. The goal of this project is to create a dosimeter system that can be used to measure long-term exposure to a variety of chemical toxins through the use of different coating types. The toxins being tested in this study are volatile organic compounds (VOCs) and mercury vapor, which are detected by polymer and gold coatings, respectively.

62. Characterization of novel photoinitiated nitric oxide releasing S-nitrosothiols for use in coating implantable devices

Beatrice M. Burgess and Megan C. Frost
Michigan Technological University, Houghton, MI

While in some cases provoking a host response is the goal of a biomedical device, it is more often a hurdle that needs to be overcome. One particularly negative response on devices implanted into blood pathways is thrombus formation, which causes the proper function of the instrument to be impaired. Nitric oxide (NO) is known to inhibit platelet adhesion and activation and has been implicated in mediating the inflammatory response and promoting wound healing. S-nitrosothiol based compounds are light sensitive, and can be made to release a precise amount of NO by controlling the wavelength and intensity of light they are exposed to. Currently we are able to successfully synthesize three novel compounds, S-nitroso-2-methoxybenzene, S-nitroso-3-methoxybenzene and S-nitroso-2-chlorobenzene, using tert-butyl nitrite as a nitrosating agent. Characterization of these compounds is being done, including the wavelength they are most sensitive to, the surface flux of NO that is released when exposed to specific doses of light, as well as access the stability of the compounds. Other organonitrites are also being explored as effective nitrosating agents including butyl nitrite and iso-butyl nitrite. Since the S-nitroso-2-methoxybenzene seems exhibit the most desirable stability and NO-release properties of the S-nitrosothiols under investigation, it is being used to compare the effects of the nitrosating agents on formation rates. Once the properties of these compounds are known and optimized, select compounds will be attached to polymer fillers and formed into a hydrophobic polymer matrix for use in coating

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implantable devices. A model system that will be used to demonstrate the utility of this approach will be a multi-element fiber optic sensors that will contain sensing elements capable of measuring blood gases and NO-releasing fibers that locally generate enough NO to inhibit clot formation on the sensor surface, thus allowing the sensor to function reliably *in vivo*.

63. Optical nitric oxide sensing polymer using sol-gel encapsulated cytochrome-c

Ben Cottrill, Matthew Nielsen, and Megan C. Frost
Michigan Technological University, Biomedical Engineering, Houghton, MI

Nitric oxide (NO), a highly reactive, free radical gas, has attracted the attention of many biologists and scientists due to the identifications of its numerous roles in the human body, including acting as an intracellular messenger, maintenance of normal blood pressure, fighting infection and as an indicator of human organ transplant failure. A great many detection schemes have been developed for the detection of NO including electrochemical, chemiluminescent and fluorescence-based systems. Many of these systems have draw backs such as irreversible, slow response times, high detection limits and various interferents. As a biosensor, the fluorescence-based systems have been the most successful. The metalloprotein cytochrome-c has been one of the forerunners used in different fluorescence-based sensors. The cytochrome-c structure creates an environment that has a high selectivity when binding with NO. The cytochrome-c was encapsulated by an acid-catalyzed sol-gel. The sol-gel created an extra layer of selectivity to further filter out interferents. Preliminary characterization of this optical NO detecting polymer in a planar format will be presented. The ultimate goal is to develop an implantable fiber optic sensor that will be used to probe real-time NO production *in vivo*.

64. Development of photoinitiated nitric oxide releasing plastic optical fibers for smooth muscle cell investigation

Henry Durnwald and David Michael Smeenge
Michigan Technological University, Polymeric Biomaterials Lab, Houghton, MI

Nitric oxide has been shown to inhibit smooth muscle cell proliferation. Definitive levels of nitric oxide surface flux by natural sources or a biomaterial have not been well documented. Work described here is aimed at quantitatively determining what surface fluxes (level and duration) of NO inhibit smooth muscle cell proliferation. Our laboratory has developed photosensitive NO-releasing polymers that release NO in response to wavelength and intensity of irradiation. These polymers are used as a substrate upon which cells are cultured.

MOVA SMC's are grown to 70-80% confluence in culture. A 500 micrometer diameter plastic optical fiber is then coated with modified polydimethylsiloxane (PDMS) that releases NO. The coated fiber is then illuminated with an LED light source that produces appropriate light to release NO from the PDMS polymer. These coated fibers will then be placed inside of a cell culture dish (affixed to the surface cells are growing on) and the fibers will be attached to the LED source to allow NO to be released from the fiber. Four cell cultures are needed to provide evidence on how the cells will respond to the NO:

1. A modified PDMS polymer attached to an LED that will release NO over time.
2. A similar control PDMS polymer that will not be releasing NO.
3. A control optic fiber placed in the cell culture.
4. A control SMC cell culture

The cells will then be incubated for 24, 48, and 72 hours. After each 24 hour increment the cells will be imaged. This will allow the cells to be able to respond to the NO. Different levels of light will be used to

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generate different NO surface fluxes, which will establish therapeutic levels of NO efficacy to inhibit SMC growth in vitro.

65. Direct measurement of real-time nitric oxide production from lymphatic endothelial cells

Kylin Hoehn and Megan Frost
Michigan Technological University, Houghton, MI

Nitric oxide (NO) is released by blood vascular endothelial cells (BEC) to prevent platelet adhesion and activation and to maintain normal blood pressure. It has also been found to be necessary for angiogenesis. NO release in lymphatic endothelial cells has been measured by indirect methods, such as measuring gas release after chemical reactions, but little is known of its function, duration, or level of generation in the lymphatic system.

We are using a Seivers Nitric Oxide Analyzer (NOA) that directly measures NO produced via chemiluminescence detection. A T-25 tissue culture flask was attached to the NOA using a modified flask cap. Nitric oxide surface fluxes from cultured human dermal lymphatic endothelial cells (HDLEC) were directly measured. Factors that are known to affect NO production in BECs (calcium, arginine, and NADPH) are being tested as triggers for NO generation in HDLECs.

Preliminary testing has shown promise for real-time measurement of NO with an NOA, but the system must be improved on to make the measurements more accurate. Currently, NO gas is measured when bubbles form and break through the surface of the culture medium. This leads to random spikes, making surface flux difficult to measure. The length of time it takes the bubbles to escape the medium may be increasing the amount of NO that reacts before reaching the surface, making it unmeasurable. To counteract this problem, we are investigating ways of forcing Nitric oxide out of solution more rapidly.

Quantitative knowledge of NO release in lymphatic endothelial cells will provide a basis for further understanding of the role of NO in lymphangiogenesis and other lymphatic functions. Breakthroughs in this area could greatly improve the quality of life for patients who have had sections of their lymphatic systems removed and are no longer able to drain fluids effectively.

66. Magnetoelastic materials as means to control and monitor cellular adhesion

Hal Holmes, Eli Vlaisavljevich, Keat Gee Ong, and Rupak Rajachar
Department of Biomedical Engineering, Michigan Technological University, Houghton, MI

The service life of percutaneous implants, such as bone anchored prosthetics and catheters, is substantially reduced by the onset of interfacial fibrosis. Currently, means to achieve a stable implant-tissue interface include anti-fibrotic drugs and bioactive coatings, however these methods are transient and ultimately failure at the implant site will occur. This work looks to supplement the transient nature of current bioactive coatings using magnetoelastic (ME) materials that can be remotely activated via a magnetic field to produce localized sub-micron vibrations. Initial work suggests ME vibrations can be used to control cell adhesion and further as a means to monitor and treat subsequent changes at the implant surface in real-time. In this work we demonstrate the ability to control fibroblastic cell attachment and the monitoring capacity via secondary magnetic field assessment. Direct adhesion experiments performed on fibroblasts and marrow-derived macrophages seeded on poly-L-lactic acid (PLLA) coated ME materials showed that frequency and amplitude controlled ME vibrations applied for 1 hour allowed for the control

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of surface cell adhesion. Further, vibration response monitoring (secondary field measures) was able to track cell adhesion in real-time. The findings that ME materials can control as well as monitor cellular attachment via sub-micron vibrations, suggests a possible novel approach to address interfacial fibrosis and ultimately the long-term service life of percutaneous devices. Ongoing work addresses the effect of ME vibrations on the adhesion behavior of clinically relevant bacteria and other cells involved in the host response to implantable biomaterials; as well as a detailed analysis of cell morphometry and gene expression in response to sub-micron vibrations. Funding: DoD-CDMRP Grant-OR090762.

67. Leaching of nitric oxide donor from silicon rubber

Matthew L. Nelson
Department of Biomedical Engineering, Michigan Technological University, Houghton, MI

Nitric Oxide (NO) releasing polymers are currently being developed in our lab. These materials are useful due to their anti-thrombogenic properties which can be used in blood contacting devices to help reduce the host response. Since these materials will be in contact with fluid, and a controlled release is the goal, leaching of our NO donor is of concern. The goal of this study was to develop a procedure that allows us to quantify the amount of NO donor leaching from our modified silicon rubber materials. The S-nitrosothiol (the NO donor), SNAP was dissolved in an RTV-3140 solution in toluene. Films were casted by pipetting 400 μ L of SNAP/silicone rubber solution three times allowing for 1.5 hours for layer drying. The films were casted in 31mm Teflon discs on a block of Teflon. 5.7mm diameter punches were removed from cured films for testing. Film punches were then agitated in phosphate buffered saline solution (PBS) at pH 7.4 for 24 hours. Sample PBS solution was drawn from the test vials and prepared for Ellman's test for free thiols. Samples were then split into two different groups, one containing a reducing agent, 10mM ascorbic acid, and the other without the reducing agent. This was used to check for disulfide bond formation of SNAP molecules in solution. The absorbance test was carried out using BioTek Synergy HT microplate reader at a wavelength of 412nm. Results showed that the samples with the ascorbic acid had a lower absorbance than those without, but both were in the range of the cysteine standard. This demonstrated that disulfide bond formation does not appear to be a factor in quantitating sulfur containing leachables. Further testing will be carried out to confirm these results.

68. Wireless passive force mapping system for above knee prosthetics

Brandon D. Pereles, Sam Bredeson, Eugene Kim, and Keat Ghee Ong
Department of Biomedical Engineering, Michigan Technological University, Houghton, MI

Following a lower-limb amputation many patients are fit with a prosthetic replacement. Unfortunately, the remaining tissue and skin forming the stump (residuum) are not naturally exposed to the stresses and strains that a healthy limb experiences. As a result, many patients develop skin blisters, cysts, ulcerations, etc. A patient displaying one or more of these problems may be required to stop using the prosthetic until the residuum has healed and if healing does not occur, further amputation may be required. The focus of this project is to develop a sensor system for real time monitoring of forces at the socket-residuum interface of a lower-limb prosthetic to predict and prevent issues relating to excessive force on residuum tissue such as soars, ulcers, and tissue death. The sensor system consists of strips of stress-sensitive magnetoelastic material forming a grid between the socket and residuum interface. Under the excitation of an AC magnetic field, the magnetoelastic material magnetizes and, due to its magnetic softness, generates higher order magnetic field components (magnetic fields at multiple frequencies of the excitation magnetic field) which can be captured with an external detection coil. In addition, as a result

of the magnetoelastic property of the material, these higher-order magnetic field components vary with stress/strain, allowing for remote pressure mapping of forces at the socket-residuum interface.

69. Development of a stress-sensitive, magnetoelastic coating for detecting coronary stent failure.

Hannah M. Smith, Ee L. Tan, and Keat G. Ong
Michigan Technological University, Houghton, MI

During balloon angioplasty, a stent is placed within a coronary artery to help retain the tubular shape after the balloon is withdrawn. Recent studies suggest that arterial stent placement carries a low risk of complications, but scar tissue-like cellular over-growth and blood clots may develop, causing in-stent restenosis that restricts arterial blood flow. Although angiography and imaging techniques can diagnose in-stent restenosis, they are invasive or expensive. This project focuses on the developing a low-cost technology for the non-invasive detection of in-stent restenosis by applying a layer of stress-sensitive material on standard coronary artery stents. While this technology will not replace current techniques for confirming in-stent restenosis, it is ideal as a low-cost, convenient screening method, where patients can have regular checkups for early detection of this condition. The stress-sensitive layer will be made of a magnetoelastic material, which when under the excitation of an AC magnetic field, generates a secondary magnetic field at higher frequencies that can be remotely detected with a coil antenna. Due to the magnetoelastic property of the material, its magnetic response is also stress dependent, allowing remote measurement of stress through changes in the secondary magnetic field. In this project, in-stent restenosis will be characterized by examining the difference in the response of the magnetoelastic layer during systole and diastole. In the case of in-stent restenosis, the stent will be covered by overgrown cells and tissues to reduce the sensitivity, and consequently, the response, of the magnetoelastic layer. Previous research has shown that when the magnetoelastic material was directly exposed to pressure changes, the signal variation from low to high pressures was significantly larger than the situation when the material was covered by a rubbery coating. The goal of this project is to develop the magnetoelastic material and examine its biocompatibility.

University of North Dakota

70. Expression of Arginase II in human urothelial cells (UROtsa) exposed and malignantly transformed by cadmium or arsenite

Jenny L. Canine, Seema Somji, Mary Ann Sens, Donald A. Sens, and Scott H. Garrett
Department of Pathology, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, ND

The extrahepatic isoform of arginase, Arginase 2 (ARG2), is localized in the mitochondria and cleaves L-arginine to ornithine. ARG2 is essential in the biosynthesis of polyamines, glutamate and proline, and the modulation of nitric oxide synthesis. Previous studies have found elevated ARG2 activity in patients with thyroid, prostate, breast, lung, and colon cancers. The increased enzyme activity may be responsible in maintaining necessary levels of polyamines and altering the intracellular redox environment for tumor
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development and growth. However, the role of ARG2 in bladder cancer has not been defined. Previous studies from our laboratory have shown that cadmium (Cd^{2+}) and arsenite (As^{3+}) can malignantly transform the human urothelial cell line UROtsa and these transformed cells can form subcutaneous as well as intra-peritoneal tumors. The goal of this study was to determine if ARG2 was expressed in the parent as well as the transformed cell lines and the tumor heterotransplants. It was demonstrated that the level of ARG2 expression was low in the UROtsa parent, whereas all the arsenite transformed cell lines and one of the cadmium transformed cell line expressed ARG2. In addition, the level of expression of ARG2 in the subcutaneous tumor heterotransplants was variable. The three arsenite transformed cell lines and one of the cadmium transformed cell line that produced intra-peritoneal tumors also expressed ARG2. These results suggest that environmental exposure to As^{3+} and Cd^{2+} can modify the expression of ARG2 in normal human urothelium that may contribute to bladder carcinogenesis.

71. Effect of EGF and insulin on the expression of keratin 6 in arsenite and cadmium transformed UROtsa cells

Ling Cao, Scott H Garrett, Donald A Sens, Mary Ann Sens, Xudong Zhou, Jane R. Dunlevy, and Seema Somji
Department of Pathology, University of North Dakota School of Medicine, Grand Forks, ND

Arsenite (As^{3+}) and cadmium (Cd^{2+}) are carcinogens that have been implicated in the development of bladder cancer. Previous work from this laboratory has shown that normal urothelial cells (UROtsa) do not express keratin 6, however malignant transformation of these cells by the heavy metals As^{3+} and Cd^{2+} results in an over expression of keratin 6 mRNA as well as protein. Tumor heterotransplants generated from these cells also express keratin 6 as determined by immuno-histochemistry. Growth factor deletion and addition studies in the normal UROtsa cell line indicated that the level of keratin 6 expression was regulated by the presence of both insulin and epidermal growth factor (EGF). The first goal of this study was to determine if EGF and insulin had an effect on the expression levels of keratin 6 in UROtsa cells malignantly transformed by As^{3+} and Cd^{2+} . For this purpose six independent As^{3+} transformed urothelial cell lines and seven independent Cd^{2+} transformed cell lines were exposed to EGF, insulin or EGF and insulin for 24 hr. The level of keratin 6 mRNA expression was determined by real time PCR and protein levels were measured by western analysis. The data obtained indicates that the process of transformation of the urothelial cells by As^{3+} or Cd^{2+} induces the expression of keratin 6 in some of the transformed cell lines. Furthermore, exposure to EGF, insulin or EGF plus insulin only had a modest effect on the expression of keratin 6 in some of the transformed cell lines whereas all the tumor heterotransplants generated from these transformed cell lines over expressed keratin 6. This suggests that the in-vivo environment may be necessary for the up regulation of keratin 6. The second goal of the study was to determine the signal transduction pathways involved in the overexpression of keratin 6 in bladder cancer. The data obtained suggest that the ERK1/2 pathway is involved in the upregulation of keratin 6 in the parental as well as transformed UROtsa cells.