



ENGINEERING RESEARCH IN DIABETES

SUMMER RESEARCH EXPERIENCES FOR UNDERGRADUATES

PRESENTATION DAY

August 9, 2007
9:30am – 1:00 pm



Funding for this program is provided by the National Science Foundation (Grant No. 0552896), the Pritzker Institute of Biomedical Science and Engineering, and a generous donation from Edward W. Ross ('43 BS ME).

Table of Contents

| | |
|------------------------------------|-----------|
| Thanks | 3 |
| Oral Presentation Schedule | 4 |
| Poster List | 5 |
| Oral Presentation Abstracts | 6 |
| Poster Abstracts | 11 |

We would like to thank the following for contribution to the program:

Funding

National Science Foundation Edward W. Ross
Pritzker Institute of Biomedical Science and Engineering

Mentors

| | |
|------------------------------|------------------------------|
| Mark A. Anastasio, Ph.D. | Sandra Whaley Bishnoi, Ph.D. |
| Eric M. Brey, Ph.D. | Ali Cinar, Ph.D. |
| Jennifer Kang Derwent, Ph.D. | Connie Hall, Ph.D. |
| Manami Hara, Ph.D. | David J. Mogul, Ph.D. |
| Emmanuel C. Opara, Ph.D. | Georgia Papavasiliou, Ph.D. |
| Victor H. Pérez-Luna, Ph.D. | Louis Philipson, M.D., Ph.D. |
| Ganesh Raman, Ph.D. | |

Seminar Speakers

| | |
|-----------------------------|---------------------------|
| Deborah Burnet, M.D., M.A. | Ali Cinar, Ph.D. |
| Marshall Chin, M.D., M.P.H. | Connie Hall, Ph.D. |
| Manami Hara, Ph.D. | David J. Mogul, PhD |
| Emmanuel C. Opara, Ph.D. | Lauretta Quinn, Ph.D., RN |

Ethics Program

| | |
|--------------------|---|
| Vivian Weil, Ph.D. | Michael Davis, Ph.D. |
| Amanda Jackson | LANGURE (Land Grant University Research Ethics) |

Activity and Tour Volunteers

| | |
|-------------------------|---------------------------|
| Suzanne Apsey | Dr. and Mrs. Bruce Bauer |
| Mary Hammes, M.D. | Timothy King, M.D., Ph.D. |
| William F. Mieler, M.D. | |

Everything

Cathie D'Amico

All graduate students, research staff, and employees who worked with the students this year.

Schedule

9:00-9:30 Set up posters/Load presentations on computer

9:30 Introduction (Brey)

Oral Presentations (10 minute presentations/3 minutes questions)

9:35-9:48 Quantitative Analysis of Optical Glucose Biosensor by the System of Catalytic Enlargement of Gold Nanoparticles via Redox Enzyme
James Bai (University of Rochester, NSF Scholar)

9:48-10:01 Near-Infrared Spectroscopy Imaging in Rat Brains
Maura Boyle (University of Texas, NSF Scholar)

10:01-10:14 Kv2.1 Phosphorylation in Insulin Secreting Cells
Shawn L. Call (Brigham Young University, NSF Scholar)

10:14-10:27 Procoagulant Microparticle Binding on Artificial Surfaces
Serena Chacko (IIT, Ross Scholar)

10:27-10:40 Imaging Islet Development
Amanda Feldman (Western New England College, NSF Scholar)

10:40-10:50 Break/Posters

10:50-11:03 Manipulating Protein Release from Outer Alginate Coat of Microcapsules.
Ogechi Omelogu (Loyola University Chicago, NSF Scholar)

11:03-11:16 FoodFit, a newly designed web application to illustrate food and physical activity choices.
Alyssa Rosenzweig (University of Pennsylvania, NSF Scholar)

11:16-11:29 Development of a Non-Invasive Glucose Monitor
Elise Springer (Vanderbilt University, NSF Scholar)

11:29-11:42 Effects of VEGF on retinal hemodynamics in normal rats.
Harry Tran (IIT, Ross Scholar)

11:42-11:55 Deciphering atherosclerotic plaque microstructures through Multiple Image Radiography
Sunil Vasireddi (IIT, Ross Scholar)

12:00-1:00 Poster Presentations/Lunch

Posters

1. Effect of Diabetes Mellitus on Adhesion Strength of Human Dermal Fibroblasts
 - a. Laura Datko (Clemson University, NSF Scholar)
2. Optical Nanoparticle-based Glucose Biosensor
 - a. Kaleyhia Flowers (Chicago State University, NSF Scholar)
3. Thermoresponsive hydrogels for sustained delivery of antiangiogenic drugs into the macula
 - a. Diana Gutierrez (University of Illinois-Chicago, NSF Scholar)
4. Effects of diabetic crosslinking on transport properties of model extracellular matrices
 - a. Brendan Inouye (IIT, Ross Scholar)
5. Role of fission in forming a pancreatic islet as a functional unit.
 - a. Uchenna Moka (Northwestern University, NSF Scholar)
6. Effect of Alginate Composition on capsule morphology.
 - a. Michael Morley (IIT, Ross Scholar)
7. Correlation of Hemodynamic Changes to Quantified Vasculature Alterations in Diabetic Retinopathy.
 - a. Arden Santoso (Rose-Hulman Institute of technology, NSF Scholar)
8. Near-Infrared Spectroscopy Imaging in Rats
 - a. Dawn Tian (IIT, Ross Scholar)
9. Poly(ethylene glycol) Hydrogel Crosslink Density Gradients through Interfacial Photopolymerization
 - a. Yanti Sumner (University of New Mexico, NSF Scholar)

Oral Presentations

9:35-9:48 Quantitative Analysis of Optical Glucose Biosensor by the System of Catalytic Enlargement of Gold Nanoparticles via Redox Enzyme

James Bai (NSF Scholar), Kaleiya Flowers, Sandra Bishnoi

The system of enlargement of Au Nanoparticles (NPs) by reducing AuCl_4^- onto Au colloid with hydrogen peroxide (H_2O_2) can be used for the development of a novel optical glucose monitoring technique. The change in size of Au NP solutions can be monitored using the change in the particle absorption wavelength and intensity as a function of peroxide concentration at 520nm. In a protocol similar to deposit of gold onto Au NPs with H_2O_2 , Glucose and Glucose Oxidase (GOx) are also employed to grow Au NPs. To determine glucose concentrations, GOx catalyze a redox reaction converting β -D-glucose into D-glucono-1,5-lactone. The reduced form of GOx is reoxidized by O_2 , producing H_2O_2 , which in turn acting as a reducing agent to deposit gold onto 30nm Au Colloid. The concentration of glucose can be determined by monitoring the Au NP solution using visible ultra-violet spectroscopy (UV-Vis) and Dynamic Light Scattering (DLS) spectroscopy. The present study was designed to quantitatively assess the Nanoparticle-GOx redox system, to evaluate its potential, and to explore its advantages and limitations. The system was demonstrated to be sensitive to wide range of glucose concentration and can be potentially applied to develop variety of noninvasive fluid glucoses monitoring techniques.

9:48-10:01 Near-Infrared Spectroscopy Imaging in Rat Brains

Maura Boyle (NSF Scholar), David J. Mogul

There exist certain disadvantages to current brain imaging modalities, specifically that many require equipment that is large, expensive, and non-portable. Developing a small, inexpensive, non-invasive system for research purposes that could image brain activity in rats in real time would solve many of the problems of the larger models in current use. Such an imaging system could be especially useful in the study of in vivo brain activity and oxygenation, which could provide insight into epilepsy and diabetes. We attempted to use the principles of near-infrared spectroscopy (NIRS) to create such a device. Light in the wavelengths between 600 and 950nm (the near-infrared range) is poorly absorbed by biological tissue, allowing it to penetrate the brain to a certain depth. Band-pass filters were used to study the reflectance and absorption of light with wavelengths of 615nm, 820nm, and 840nm. Hemodynamic signals could mark brain activity, showing where oxygenated blood is flowing in the brain. The 615nm filter was used to attempt to image these markers. Metabolic changes in the brain were also explored as a marker of brain activity. The 820nm and 840nm filters were used to attempt to detect this activity based on the detection of cytochrome c oxidase, which is involved in the electron transport chain. Preliminary experimentation produced images that may be useful but more experimentation needs to be done to determine the system's effectiveness. Through the use of this imaging modality, dynamics of seizures and neurovascular implications of diabetes could possibly be better understood.

10:01-10:14 Kv2.1 Phosphorylation in Insulin Secreting Cells

Shawn L. Call (NSF Scholar), David A. Jacobson, Louis H. Philipson

Secretion of insulin from the β -cell is regulated by complex electrical activity. Glucose-dependent insulin secretion can be increased when the channels responsible for repolarizing the β -cell are inhibited. Potassium delayed rectifier Kv2.1 channels are the primary current (I_K) repolarizing the β -cell following the initiation of an action potential. Kv2.1 demonstrates altered biophysical properties in a phosphorylation-dependent manner. Dephosphorylated neuronal Kv2.1 hyperpolarizes the voltage of activation, and suppresses action potential firings through faster I_K . Similar to pyramidal neurons, β -cells contain PKA, PKC, and Calcineurin that participate in modulating the phosphorylation state of Kv2.1. The goal of this study is to determine if Kv2.1 channels in the β -cell are also being phosphorylated and to what extent this process is modulated by glucose during insulin secretion. Insulin secreting cells were stimulated with Carbachol, Alkaline Phosphatase, Okadaic Acid, high glucose and KCl to activate maximum kinase and phosphatase activity. Western blots run with protein isolated under these conditions were probed with a Kv2.1 specific antibody. No change in phosphorylation status of protein from rat insulinoma cell lines (INS-1) was seen under these conditions, however preliminary observation of human islets and mouse insulinoma cell lines (MIN-6), indicate the possibility of multiple bands corresponding to differentially phosphorylated Kv2.1 protein. Kv2.1 phosphorylation plays a role in β -cell insulin secretion.

10:14-10:27 Procoagulant Microparticle Binding on Artificial Surfaces

Serena M. Chacko (Ross Scholar), S. Patchipulusu, D. Crandall, and C. Hall

Microparticles (MPs) are membrane vesicles released from cells upon activation and circulate in the bloodstream. Specifically, monocyte-derived MPs (MMPs) contain tissue factor (TF), the initiator of the extrinsic coagulation pathway. Previous studies demonstrated that MMPs adhere to and *impair TF dependent procoagulant* activity to biomaterials. This study used THP-1 monocytic cell-derived MPs as a model system. MPs were tested for TF and tissue factor pathway inhibitor (TFPI) activity using FXa generation assays, and then specific vs. non-specific mechanisms of interaction between MPs and surfaces were evaluated by measuring FXa generation. The majority of FXa generation was TF-dependent as confirmed using anti-TF MAb and a lack of significant TFPI activity was confirmed using anti-TFPI MAb. Biomaterial-MP interactions were evaluated by incubating THP-1 MPs with bare or protein (BSA or fibrinogen) coated glass and polystyrene surfaces. MPs adhered to all surfaces, with a clear preferential binding to glass surfaces over polystyrene in all cases, regardless of specific protein coat. This behavior is in contrast to MMPs that exhibited increased adhesion to adsorbed fibrinogen. CD18/CD11b is the putative fibrinogen receptor involved and the CD18 sub-unit has been identified on the THP-1 MPs. Current work involves the evaluation of its role in specific adhesion to materials.

10:27-10:40 Imaging Islet Development

Amanda Feldman (NSF Scholar), Uchenna Moka, Jikun Shen, Manami Hara

Due to the three-dimensional nature of the pancreas, as well as its structural organization, the accuracy of a two-dimensional pancreatic study is unreliable. To precisely monitor dynamic changes in beta-cell proliferation and islet formation in the developing pancreas with temporal and spatial information an imaging method was established. This method visualizes in three-dimensions, as well as quantifies the entire distribution of beta-cells and islets in the intact pancreas. Neonatal pancreata (E17.5, P0, P3, P6, and P10) were studied. The average total number of beta-cells in the fetal (E17.5) pancreas was $3,867 \pm 492$ (n=4). This number increased three-fold to $13,948 \pm 2,938$ (n=3) in the neonatal pancreas at P10. At P0 there were $4,313 \pm 1,120$ beta cells. Beta-cells proliferate contiguously without forming distinct islet structures in the fetal and newborn pancreas. This is followed by the development of conjoint clusters of spherical masses with islet-like structures in the periphery of the neonatal pancreas (P10). By weaning (3-wk), dumb-bell (two-conjoint) or peapod (several connected clustered) islets are observed mainly along the portal vein at the junction of the dorsal and ventral pancreas. These observations and analysis provides supporting evidence that islets undergo fission as a means of development. Islet formation occurs by these multiple fissions of such elongated beta-cell mass, which may be a protective mechanism for functionally immature beta-cell in the neonatal life. Statistical analysis, which was aided by a program written in MatLab, was completed on the islet distribution.

10:50-11:03 Manipulating Protein Release from Outer Alginate Coat of Microcapsules

Ogechi Omelogu (NSF Scholar), Monica Moya, Eric M. Brey, Emmanuel C. Opara

Type 1 diabetes is a chronic condition in which insulin producing beta cells of islets produce insufficient or no insulin. The disease often comes paralleled with other illnesses, leaving diabetics vulnerable to multiple complications. Transplantation of healthy encapsulated islets offers a promising alternative, which may preclude the use of immunosuppressive drugs. Co-encapsulation of islets with an angiogenic protein may increase the viability of the transplant by promoting localized neovascularization. Our research aims to investigate the release of protein from the outer layer of alginate microcapsules (MC). Our encapsulation procedure involves the use of MCs composed of 2 layers of alginate, an inner containing the islets and an outer layer that covers a poly-amino acid semi-permeable membrane. Release of angiogenic proteins was run by constructing empty MCs with BSA in the outer alginate coat. The concentration of outer alginate was varied (0.25%, 0.50% and 0.75%) as was the mannuronic acid (M) content of alginate. MCs were suspended in 1.5 mL of saline and BSA release was quantified each day for 7 days using a spectrophotometer. After 7 days, the MCs were broken to quantify the amount of unreleased BSA. Results indicate that higher concentrations of alginate and lower M content in the outer coat resulted in slower release. These results suggest protein can be released from the outer layer and release kinetics can be manipulated by altering alginate properties.

11:03-11:16 FoodFit, a newly designed web application to illustrate food and physical activity choices.

Alyssa Rosenzweig (NSF Scholar), Ali Cinar

FoodFit, an educational web application, is an interactive food and activity journal to illustrate food and activity choices, and their effect on daily energy balance. FoodFit is designed to teach healthy lifestyles, resulting in a promising preventative measure for obesity, type 2 diabetes and geriatric complications. This version will remain integrated with our previously developed glucose-insulin dynamics simulation software, GlucoSim, to specifically assist people with type 1 diabetes. Prior versions included only the nutritional analysis (as provided by the USDA National Nutrient Database) of food consumed, but this version takes into account calories burned from both exercise and everyday physical activities (using Metabolic Equivalent (MET) formulas) to give a more complete picture of the individual's daily energy balance. Example food and activity scenarios for different lifestyles are also being integrated as a means to quickly demonstrate caloric balance, whereas previous versions required the user to begin from scratch, a significant time investment. Much of the improvement lies in the new interface design and layout done with HTML and CSS. A fun game and tutorial are also under development to increase classroom application and educational value. These improvements to FoodFit are intended to improve the accuracy of energy balance predictions and enhance the user experience, making it a more appealing and thus a more effective tool than before.

11:16-11:29 Development of a Non-Invasive Glucose Monitor

Elise Springer (NSF Scholar), Praveen Panickar, Dr. Gamesh Raman

Diabetic patients are recommended to check their blood glucose at least three times daily, but because of the pain that current methods of blood glucose detection cause, most do not monitor their glucose as closely as they should. Consequently, a proposed non-invasive blood glucose monitor includes ultrasound to increase skin pore size, a piezoelectric crystal array vacuum pump to pull interstitial fluid from pores, and electrical impedance spectroscopy to determine the glucose level. Varying power supplied to the crystal array will vibrate and move the diaphragm, creating the appropriate suction to pull enough interstitial fluid from skin pores.

Simple COMSOL® models of a cross-section of skin, one with a flat top and one with a curved neck at the point of suction, were first developed to determine the necessary pressure. The simulation was run at various pressures to determine the maximum velocities and flow rates that would result if a vacuum pump at various levels of suction were applied to the hole. Calculated velocities between these two models were nearly identical, making their flow rates dependent on the size and geometry of the whole. To create a more accurate model of skin, the COMSOL® chemical engineering module was used to model a thin porous membrane through which fluid would be pulled (i.e., skin) and the simulation was repeated at various pressures. Despite the fact that most flow rates predicted in this experiment are very high and not practical for a biological application, we have tentatively determined from this data that the suction pressure used by the device should be below 100 Pa. Further development of the model and comparison of its data with a physical experiment will indicate a more specific pressure.

11:29-11:42 Effects of VEGF on retinal hemodynamics in normal rats

Harry Tran (Ross Scholar), S. Benac, A. Santoso, S. Tummala, and J.J. Kang Derwent

Vascular endothelial growth factor (VEGF) plays a significant role in angiogenesis and vascular permeability in normal and diseased states. The purpose of this study is to investigate the effects of VEGF on retinal blood flow and vessel diameters in a rodent model using a Scanning Laser Ophthalmoscope. The retinal vessel diameters were measured based on infrared reflectance images of fundus using a Matlab program. The velocities of retinal vessels were calculated using Matlab by tracking $1\mu\text{m}$ fluorescent microspheres. The cross sectional area of blood vessels and averaged velocities were used to calculate retinal blood flow. Fluorescent dye was also injected intravenously to examine presence of microaneurysms and vessel tortuosity. A $3\mu\text{l}$ solution of $29.6\text{ng}/\mu\text{l}$ or $50\text{ng}/\mu\text{l}$ VEGF was injected intravitreally. Data was collected at 0 hours (control), 48 hours and 1 week post injection. Additional data with $50\text{ng}/\mu\text{l}$ VEGF was collected at 15 and 30 minutes post injection. With the lower dose of VEGF, only small vessel velocities showed an increase of $\sim 17\%$ after 48 hours and $\sim 36\%$ after 1 week. With the higher dose of VEGF, the arterial blood flow rate increased $\sim 11\%$ by 30 minutes while venous blood flow rate remained constant. The small vessel velocities increased $\sim 27\%$ after 30 minutes. Vessel dilation and tortuosity were observed after 48 hours. Data suggest that alterations occurring due to VEGF mimic the early stages of diabetic retinopathy and may be a useful model to study the role of VEGF in vascular disease.

11:42-11:55 Deciphering atherosclerotic plaque microstructures through Multiple Image Radiography

Sunil K. Vasireddi (Ross Scholar), Cheng-Ying Chou, Eric M. Brey, Mark Anastasio

Multiple-image radiography (MIR) is an emerging phase-sensitive X-ray imaging method that holds great promise for biomedical imaging applications, and yields novel and more informative images reflecting tissue properties than conventional X-ray methods. MIR concurrently measures three distinct properties of tissue. In addition to virtually scatter-free attenuation images, MIR provides refractive gradient and ultra-small angle X-ray scatter (USAXS) images. The latter two images depict tissue properties that are not measured in conventional radiography, and do not rely on X-ray absorption contrast. In this study, we investigate the application of MIR for characterizing atherosclerotic carotid plaques. The plaques were obtained from human subjects and previously imaged at a dedicated MIR beamline at Brookhaven National Laboratory. Numerical algorithms for reconstructing MIR images were developed in the Matlab programming environment. Algorithms for implementing MIR in computed tomography (CT) mode were also developed. The algorithms were validated by use of computer-simulation studies and subsequently employed to process the experimental imaging data from the carotid plaque studies. Quantitative and qualitative measures of image quality were employed to demonstrate that the MIR images can accurately reveal features of plaque microstructure that were not revealed in a conventional radiograph.

Poster Presentations

Effect of Diabetes Mellitus on Adhesion Strength of Human Dermal Fibroblasts

Laura C. Datko (NSF Scholar), Megan E. Francis, Connie L. Hall, and Eric M. Brey

Diabetes affects over 20 million people in the US. Diabetics can experience many complications, such as peripheral vascular disease, chronic foot ulcers, and deficient wound healing, which can lead to non-traumatic limb amputations. Noting cellular differences between diabetics and non-diabetics can help explain the poor wound healing, and improve preventive measures for amputations. This study focuses on fibroblasts, critical cells in wound healing. Previous studies have shown an altered migration capacity in diabetic Human Dermal Fibroblasts (HDF). A complex process of adhesion to and release from substrates dictates cell migration, so a study of surface adhesion strength can help explain altered migratory traits. HDF were isolated from amputated limbs of diabetic patients and age-matched non-diabetics. Adhesion strength was assessed by observing HDF removal as a function of time and applied fluid shear stress. The HDF were plated on glass and exposed to 414 dyn cm^{-2} (wall shear stress) for 90 minutes. The percentage of diabetic or non-diabetic HDF that remained adherent was quantified to give relative adhesion strengths. Initial data show, after 60 minutes, a mean of $70.3 \pm 4.8\%$ of the non-diabetic HDF, while only $25.7 \pm 13.4\%$ of the diabetic HDF, remain adherent. Additional data are being acquired to improve current statistical significance, $p=0.09$ (Student's *t* test). However, these early results suggest that HDF isolated from diabetics have reduced adhesion strength relative to non-diabetics, which may explain the altered migration.

Optical Nanoparticle-based Glucose Biosensor

Kaleyhia Flowers (NSF Scholar), James Bai², Yu-Jen Lin³, Sandra Whaley Bishnoi

Diabetes has become extremely prevalent in Americans in the past decade. There are 20.8 million children and adults in the United States, or 7% of the population, who have diabetes.¹ Diabetics must monitor their blood sugar regularly, since their glucose tends to fluctuate throughout the day. Our group has focused on the creation of a nanoparticle-based optical glucose sensor. Different concentrations of hydrogen peroxide (H_2O_2) were added to reduce gold ions (Au^{3+}) present in solution onto small gold nanoparticles (2-3nm in diameter) which were stabilized with the protein bovine serum albumin (BSA). As the concentration of H_2O_2 increased, the particles enlarged and absorbed more of the incoming light. Many enzymes, called oxidases, generate H_2O_2 from molecular oxygen. In our experiments, glucose was reduced to gluconic acid by glucose oxidase, which produced peroxide. The presence of hydrogen peroxide caused the gold nanoparticles to grow. A linear relationship between the glucose concentration and the absorbance of the solution at a wavelength of 535 nm was observed. We have tested this system using physiological levels of glucose and have shown that this technique may be promising in the development of an optical glucose sensor.

Thermoresponsive hydrogels for sustained delivery of antiangiogenic drugs into the macula

Diana Gutierrez (NSF Scholar), Pawel Drapala, Jennifer Kang Derwent, Eric M. Brey, Victor H. Pérez-Luna

Due to the fact many patients diagnosed with diabetes are more prone to becoming blind, research to develop a drug delivery treatment with thermoresponsive hydrogels is currently being conducted. Currently, there are a series of drug treatments being investigated to decrease the amount of injections and dosages of Anti-VEGF drugs into patients who show damage in their retina, which could lead to potential blindness. Consequently, the usage of being able to inject a hydrogel in the back of the retina proves to be promising to reduce the amount of injections received by the patient in the drug delivery treatment process. Because hydrogels exhibit hydrophilic properties they do not exhibit any tendency to irritate any tissue, or become attached to a protein which it is injected with. Therefore, hydrogels were created by chemically crosslinking N-isopropylacrylamide, responsible for the sustained phase changes of the hydrogel to body temperature at 37°C with Poly (ethylene glycol) diacrylate, which were then combined with Bovine Serum Albumin (BSA) protein. The hydrogels were washed and placed at 37°C with Phosphate Buffered Saline (PBS), then tested for the release of protein within the hydrogel over the course of three days. The profiles were then tested under a bicinchoninic acid (BCA) assay to determine the percentage amount of protein which was recovered from the release profiles. At the moment, the Bradford Assay has been proven to be more beneficial for the determination of the amount of protein released and studies are still ongoing.

Effects of diabetic crosslinking on transport properties of model extracellular matrices

Brendan Inouye (Ross Scholar), Francis, M., Uriel, S., Brey, E.

Diabetes mellitus is a multi-faceted disease, which when unmonitored can result in chronic hyperglycemic conditions. Prolonged hyperglycemia may lead to increased crosslinking of stromal and basement membrane extracellular matrices (ECM). Abnormal crosslinking via the Malliard reaction increases membrane stiffness and may also alter transport. Our study utilized type 1 rat tail collagen and Matrigel™ as models for stromal and basement ECM, respectively, to analyze the effects of crosslinking on transport properties. Matrices were crosslinked by incubation with glucose-6-phosphate, producing similar crosslinking to *in vivo*. Swollen and dry gel weights were used to calculate the volume fraction. Partition coefficient values were obtained by dissolving gels in appropriate solvents and comparing the absolute fluorescence of the dissolved gel to the soaking solutions. Diffusion experiments are performed using a PermeGear™ side-bi-side diffusion chamber. Results from experiments indicate that crosslinking increases the volume fraction of both matrices. Average volume fractions for Matrigel™ were 0.0144±0.0003 and 0.0173±0.0003 for non-crosslinked and crosslinked gels, respectively (p<0.05). Average volume fractions for collagen were 0.0114±0.001 and 0.0142±0.0006 for crosslinked and non-crosslinked gels, respectively (p<0.05). Decreases in the partition coefficient were observed with crosslinking. These changes indicate a change in the physical structure of the matrix due to diabetic crosslinking. Measurements of diffusion coefficients are currently under investigation.

Role of fission in forming a pancreatic islet as a functional unit.

Uchenna Moka (NSF Scholar), Amanda Feldman, Manami Hara

Insulin-secreting pancreatic beta-cells play a key role in maintaining glucose homeostasis in the body. In the exocrine pancreas, endocrine cells aggregate and form islets, unique micro-organs that serve as a functional unit to secrete insulin in response to glucose. Through the combination of imaging, 3D mapping reconstruction and immunohistochemistry of the developing mouse pancreas, we have identified a stretch of coalescent islets along the large vessels, which is most prominent in the neonatal pancreas, followed by a reduced number of islet-clustering with further development. We hypothesized that the connected islets, also known as “dumb-bell islets”, are proliferative units that go through fission. Through advanced digital microscopy, morphological analysis and an image processing and analysis program in Java, we carried out a statistical analysis on dumb-bell and peapod islets, thus, allowing us to find the following: 1) The location of dumb-bell/peapod islets in the pancreas; 2) the average percentage of dumb-bell and peapod islets in the mouse pancreas from day 13 to adult mice; and 3) A specific islet’s area, circularity, and Feret’s diameter. The results suggest that islets are formed by multiple fission followed by contiguous endocrine-cell proliferation, rather than by local aggregation or fusion.

Effect of Alginate Composition on capsule morphology.

Michael Morley (Ross Scholar), Monica Moya, Eric M. Brey, Emmanuel C. Opara

In type I diabetes, pancreatic islets are absent or dysfunctional causing a lack of insulin production. Therefore, if not for rejection it would be ideal to treat type I diabetics with islet transplantation. Alginate encapsulation seeks to bypass this problem with a simplistic approach; by forming a barrier around transplanted cells through which nutrients may pass while preventing interaction between the islets and lymphocytes. An encapsulated cell could thus function in the body indefinitely without detection as long as the microbead integrity is maintained. It is therefore of interest to investigate the structural properties of alginate capsules in order to ensure the production of durable capsules. In our studies, alginate type (high mannuronic (M) or high guluronic (G)) and concentration (1%-3%) were varied and each set of beads was observed over the course of five months submersed in physiological saline and calcium concentrations. Beads were imaged and the perimeters of each was measured using Axiovision software. Shape factor (SF) determined the structural integrity, a measurement of the roundness of the bead based on the perimeter, of each capsule. No significant deterioration was seen in the alginate beads overtime. Beads of higher alginate concentration formed more circular beads than those of beads with lower concentration. No difference in SF over time was seen in beads formed with high G or high M content. This result is promising as it shows that capsules may last for extended periods of time in the body without deteriorating rapidly.

Correlation of Hemodynamic Changes to Quantified Vasculature Alterations in Diabetic Retinopathy.

Arden Santoso (NSF Scholar), S. Benac, H. Tran, S. Tummala, J.J. Kang Derwent

The purpose of this study is to design a grading classification system for retinal vasculature and to develop a correlation between retinal vasculature and retinal hemodynamics in a diabetic animal model. Streptozotocin (STZ)-induced diabetic Long-Evans rats (55mg/kg, 0.1M of citrate buffer, pH=4.5) were used to acquire vasculature images, blood velocity and vessel diameter measurements via Scanning Laser Ophthalmoscope (SLO). Fluorescent microspheres (1 μ m) were tracked with frame-by-frame analysis and average velocity was calculated by a custom made Matlab program. Infrared Reflectance (IR) and Fluorescein Angiography (FA) images were quantified by classifying the vasculature alterations into specific grades. Each grade (G0=no abnormalities to G4=severe) provided a quantifiable technique to track progressive change. The animals were examined weekly for six weeks. Up to four weeks, there were minimal changes (G1) based on the IR and FA images, which corresponded to slight tortuous vessels. At week seven, microaneurysms (G1), along with a decrease of ~13% in the arterial blood flow were observed. The venous flow decreased ~7% by week six. Using the grading classification system, clinical features of diabetic retinopathy appeared during week four, however, the blood flow changes measured by SLO were observed by week three. Data suggest that blood flow may change before structural changes can be detected. This may be an important factor when the effects of DR are examined.

Near-Infrared Spectroscopy Imaging in Rats

Dawn Tian (Ross Scholar), Maura Boyle, David J. Mogul

Noninvasive imaging techniques that can monitor hemodynamic and metabolic variances in the brain can provide an innovative method for looking at neural activity-dependent changes in the brain. This modality is ideally noninvasive and capable of monitoring blood flow and blood oxygenation using near-infrared spectroscopy (NIRS). Two different wavelengths were used, one in the visible light range (615nm) sensitive to oxygenated hemoglobin and deoxygenated hemoglobin and the other, in the near-infrared light range (820-840nm) sensitive to the metabolic changes of biological tissue. The relationship between blood utilization and neuronal activity is critical because blood supply is responsible for the constant refueling of the brain. By being able to measure the patterns of blood flow and blood oxygenation, we seek to better understand the events that precede and underline seizures and that may be linked to diabetic neuropathies that can result from vascular damage. The application of this model using near-infrared imaging will be useful in mapping changes in blood utilization and will focus mainly on the neocortex, responsible for our primary motor skills and control over language, learning, and complex thought. Preliminary experimental results suggest that the imaging system is successful at monitoring brain activity, however, supplementary experimentations is needed for further investigation. Through exploring this modality of imaging, the hope is to predict early signs of seizure onset and to handle seizure mechanisms that can be a consequence of low levels of glucose in diabetic patients.

Poly(ethylene glycol) Hydrogel Crosslink Density Gradients through Interfacial Photopolymerization

Yanti Sumner (NSF Scholar), M. Turturro, P. Bui, E. Brey, and G. Papavasiliou

Islet cell transplantation has proven to be a promising treatment for type I Diabetes. However, the lack of a microvascular supply has limited its success. *In vivo*, angiogenesis is regulated through extracellular gradients of proteins. Therefore, a potential solution would be to encapsulate islets within biocompatible crosslinked hydrogel networks containing gradients of adhesion and growth factor sites. This work focused on producing crosslink density gradients within PEGDA hydrogels using interfacial polymerization (IP). IP PEGDA hydrogels in the absence and presence of adhesion peptides were formed by immobilizing the photoinitiator onto a substrate followed by the addition of the precursor onto the substrate and exposure to visible light ($\lambda=514\text{nm}$). After photopolymerization, hydrogels were serially sectioned into 2 mm slices. Swelling experiments were performed for each section for which the crosslink density was quantified using the Flory-Rhener equation. As a control, hydrogels were similarly formed by bulk polymerization (BP) by the addition of the photoinitiator within the precursor. Experimental data indicate that IP results in a controlled gradient of crosslink density with a progressively decreasing crosslink density with increased hydrogel thickness. Conversely, hydrogels formed by BP did not display a gradient in crosslink density. Preliminary data indicate that 3T3 fibroblasts aligned on IP and not on BP hydrogels. Future work will focus on further characterizing the gradient and adapting hydrogels to promote neovascularization.