Welcome to the 13th Annual Biomedical Engineering Undergraduate Research and Design Conference!

Tulane University has one of the nation’s elite and most mature undergraduate departments of Biomedical Engineering. The department evolved from joint research efforts among faculty in the School of Engineering and the Schools of Medicine at Tulane and the Louisiana State University Medical Center in New Orleans. Research interests led to educational programs, and undergraduate BMEN degrees were first awarded at Tulane in 1972. In 1977 a separate Department of Biomedical Engineering was formed in the School of Engineering to offer the B.S., M.S., and Ph.D. degrees.

The undergraduate program, ABET accredited since 1981, is focused in four ‘domains’ of biomedical engineering: biomechanics, bioelectronics, cell/tissue engineering and biomaterials, and biomedical design. As one of the first and most highly acclaimed departments in the country, our faculty and students have pioneered many of the curricular innovations that set the standard for educational excellence in this exciting and rapidly growing field.

As is common for all accredited engineering disciplines, our students have participated in a required team design project since the department’s founding in 1977. Since 1987, these projects have all been related to assistive technology for local individuals with specialized needs. Biomedical Engineering students work on these designs during their senior year, culminating in a public design show in the spring. Starting in 2006, the outstanding design team has been presented with the Kenneth H. Kuhn Memorial Award, given in memory of our former Lab Coordinator/Instructor who served the department and assisted students with design projects from 1993-2005.

However, unique among the nation’s departments of Biomedical Engineering, all Biomedical Engineering undergraduates have also participated in a required year-long independent research or design project since the department’s founding in 1977. Students work with departmental faculty mentors or our counterparts throughout Tulane and at affiliated institutions in the New Orleans area and across the nation. The projects are not merely academic exercises – they are genuine and significant contributions to the field of biomedical engineering complete with scholarly undergraduate theses and, in many instances, peer-reviewed publications.

The presentations to follow today represent in a few minutes what has taken each student countless hours in the research and/or design laboratory to generate. The conference presentation is thus the punctuating culmination of a sustained effort of scholarship worthy of admiration at the highest levels. This conference will highlight the efforts and results of our undergraduates, and will serve to communicate the excitement and potential of the field to the larger university community.

As you look at the conference proceedings and attend the talks, I’m certain that you will be astounded by the breadth and depth of the educational experience each of our students has achieved, and I hope you will be excited about their potential as they
prepare to graduate this spring semester and begin to make their marks as leaders in the field of biomedical engineering.

We think you will agree that congratulations are due the members of the Biomedical Engineering Class of 2014 for their achievements, and their determination to leave a positive mark on biomedical engineering research and practice.

Thank you for attending!

Donald P. Gaver, Ph.D.
Alden J. ‘Doc' Laborde Professor
Department Chair of Biomedical Engineering

**Department’s Mission Statement**

*Our mission is to inspire and work with students as we develop and apply engineering methods to confront health science challenges.*

**Departmental Vision**

The Department of Biomedical Engineering is committed to being a global leader in biomedical engineering scholarship. Our faculty, staff, and students are all important parts of the team that provide distinctive and creative interdisciplinary solutions to biomedical engineering research and design problems. We aim for: excellence in undergraduate and graduate education, meaningful and innovative research, and service dedicated to advancing the field of Biomedical Engineering.

**Undergraduate Program Objectives**

Our undergraduate program provides students with the breadth required for participation in the interdisciplinary field of biomedical engineering and the depth required by engineers to advance the practice in our discipline. Our objective is to prepare graduates who are able to successfully pursue:

- advanced studies leading to research or professional practice in biomedical engineering
- advanced studies leading to research or professional practice in the health and medical sciences
- practice in biomedical engineering industries or related technical and professional fields
# Biomedical Engineering Senior Day

**PROGRAM – AT – A GLANCE**  
Friday January, 23, 2015  
Lavin-Bernick Center (LBC), Tulane University

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<tr>
<td>8:45 AM</td>
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<td>Dr. Donald Gaver, Chair of Tulane Biomedical Engineering</td>
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<td>Room 203</td>
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<td>9:00 AM</td>
<td>Model System Development</td>
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<td>Dr. Lee Murfee</td>
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<td>Liana Boraas</td>
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<td>Grand Challenges Presentations</td>
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<td>Room 203</td>
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<td>10:00 AM</td>
<td>Device Design and Computational Models</td>
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<td>11:15 AM</td>
<td>Stem Cells &amp; Vascular Bioengineering</td>
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### Room Assignments
- Model System Development: Room 208
- Device Design and Computational Models: Room 210
- Cancer: Room 208
- Stem Cells & Vascular Bioengineering: Room 210
- Grand Challenges Presentations: Room 203
SESSION ONE: 9:15 AM – 10:45 AM

**Model System Development**

9:15 – 9:30 AM
The Development of a Novel and High-Throughput Platform for the Electrophysiological Analysis of Neural Cultures in Hybrid Conductive Hydrogels
*Clayton Ford & Michael Moore*

9:30 – 9:45 AM
Voltage Sensitive Dyes for High-Throughput Neural Recording of *in vitro* Models
*Conor Vickers, Lowry Curley, Ashwin Sivakumar, Oliver Miller, Jackie Moran, Quincy Brown, Benjamin Hall & Michael Moore*

9:45 – 10:00 AM
Myelination of Neural Co-cultures in 3D Micropatterned Hydrogel
*Daniel Sazer, Parastoo Khoshakhlagh & Michael Moore*

10:00 – 10:15 AM
Enhanced Human-Computer Interface for Searching Large *ex vivo* Microscopy Images
*Mark Tortorich & Quincy Brown*

10:15 – 10:30 AM
Improvements to Structured Illumination Microscopy: Exploring Pattern Qualities that Provide for an Optimum Illumination Mask
*Chelsea Vaughtn, Quincy Brown & Mei Wang*

10:30 – 10:45 AM
Biological Impacts of a Cyclic *in vitro* Pulmonary Model for Recruitment and Derecruitment Events
*Alex Itin & Donald Gaver*

**Device Design and Computational Models**

9:15 – 9:30 AM
Designing a Novel Implant for the Treatment of Hill-Sachs Lesions of the Humeral Head
*Callie Clement, Stephen Cook, Samantha Salkeld & Lee Murfee*

9:30 – 9:45 AM
Hardware and Software Design to Study MSC-related Angiogenic Properties
*Richmond Van Winter, Erika Broadnax & Taby Ahsan*

Abstracts are listed alphabetically (by first author)
9:45 – 10:00 AM
Design, Manufacture, and Evaluation of a Hindlimb Suspension Cage
Natalia Sarmiento, Erika Broadnax & Taby Ahsan

10:00 – 10:15 AM
3-D Anatomical Models Based on Cross Sectional Imaging: A Potential Improvement to Surgery
David Weinstein & Jonathan Silberstein

10:15 – 10:30 AM
Computational Modeling of Non-linear Reversible Microfluidic Droplet Systems
Alex Kulick, Donald Gaver & Jason Ryans

10:30 – 10:45 AM
A Computational Model of Auditory Spatial Attention
Kyle Buschkoetter, Brent Venable, Nick Crowell, & Edward Golob

SESSION TWO: 11:00 AM – 12:00 PM

Cancer Room 208

11:00 – 11:15 AM
RP Thermoablation in 3D: optimizing the 3D Microenvironment for the Observation of the Cytotoxic Effects of Targeted Iron Oxide Nanoparticles on Breast Cancer Cells
Courtney Evans, Benjamin Vinson, Shiva Adireddy, Theresa Phamduy & Douglas Chrisey

11:15 – 11:30 AM
Platelet Adhesion to Breast Cancer Cells and its Role in Cancer Metastasis
Sabrina Lynch & Damir Khismatullin

11:30 – 11:45 AM
Synergy between High-Intensity Focused Ultrasound and Ethanol Injection in Thyroid Cancer Ablation in vitro and in vivo
Hakm Murad, Nguyen Hoang, Koji Tsumagari, Emad Kandil & Damir Khismatullin

11:45 – 12:00 PM
The Contribution of Host Cells in an Orthotopic Xenograft Model of Human Glioblastoma Multiforme
Gina Goorley & Luis Marrero

Abstracts are listed alphabetically (by first author)
Stem Cells & Vascular Bioengineering
Room 210

11:00 – 11:15 AM
Characterizing Mouse Embryoid Bodies of Various Densities
Leah Gerber, Taby Ahsan & Michelle Janaszak

11:15 – 11:30 AM
The Influence of Aging on Microvascular Density in Skeletal Muscle
Scott Stewart, David Sloas & Lee Murfee

11:30 – 11:45 AM
The Effects of Aging on Microvascular Resistance in the Rat Mesentery
David Christopher Sloas, Scott Stewart & Lee Murfee

11:45 – 12:00 PM
The Effects of Mediators Released by OXLDL and IFN-γ-Activated Tissue Resident Cells on the Progression of Atherosclerosis
Radhika Josi & Damir Khismatullin

Abstracts are listed alphabetically (by first author)
A COMPUTATIONAL MODEL OF AUDITORY SPATIAL ATTENTION
Kyle Buschkoetter, Dr. Brent Venable, Nick Crowell, Dr. Edward Golob
Department of Biomedical Engineering, Department of Neuroscience, Tulane University

Introduction: The auditory system is evolutionarily important because it allows animals to sense events occurring behind them, around corners, and in the dark. Auditory spatial attention can be defined as the focus of auditory cognitive resources to particular locations, directing the animal where to direct its actions. We are interested in understanding auditory spatial attention when a human has a predefined spatial goal and hears a sound.

Background: The brain directs auditory attention toward a particular space consciously and unconsciously. Unconscious, automatic attention shifts, like that which occurs with a sudden, loud sound behind a person, are referred to as bottom-up orienting since they originate at the brain stem. Conscious, top-down orienting, like that which occurs when listening for a sound “over there,” is caused by signals from your cortex, which is the top of the brain. The interplay between top-down and bottom-up orienting responses in the auditory modality has not yet been modeled, so the Tulane Cognitive Neuroscience Lab created a verbal model to describe this interplay.

Verbal models like these are good for establishing general understanding, but they are not quantitative like the scientific data they try to explain. The objective of this work in particular is to create a computational model of auditory spatial attention that corresponds with our verbal model and can predict and explain experimental data.

Materials and Methods: The computational core of the created Java computer model consists of four parts: the goal map, the saliency map, the priority map, and the working memory component. These run in a loop for the duration of the simulation, predicting attention gradients at every 100 milliseconds of the experiment. The goal map represents the top-down influence on attention and the saliency map represents the bottom-up influence on attention. The priority map is a simple addition of the goal map and the saliency map, and the location with the highest value in the priority map corresponds to the focal point of auditory spatial attention at a given time. This location serves as input for all higher level attention-associated cortical functions via working memory.

The model’s constant parameters were empirically defined by finding the best fit of the priority map’s shape to experimental data. Data came from a study conducted by the Tulane Cognitive Neuroscience Lab examining the attention paid to sounds occurring at different locations given an auditory goal at a single defined location.

Results: The experiment was composed of three blocks, one for an auditory goal directly to the left, one to the midline, and one to the right. Results are comprised of three models: one describing the data set at each of the three attended locations. The model fits the data very well in the “attend midline” and “attend left” goal locations ($R^2 = 0.98$ and 0.93 respectively). At the “attend right” goal location, $R^2 = 0.48$.

![Figure 1](image-url)

**Figure 1:** Comparison of model prediction and attentional data given goals at (A) -90 degrees, (B) 0 degrees, and (C) +90 degrees.

Discussion and Conclusion: The results above show that our computer program modeled the experimental attentional data accurately, except for the high relative error in the “attend right” predictions. Based on a recent experiment in the Cognitive Neuroscience Lab, we think that the attention gradient may be fundamentally different with a goal to the right since it does not shorten its standard deviation with a decrease in the range of presented sound locations like the “attend left” and “attend midline” gradients.
DESIGNING A NOVEL IMPLANT FOR THE TREATMENT OF HILL-SACHS LESIONS OF THE HUMERAL HEAD

Callie Clement, Dr. Stephen Cook, Samantha Salkeld, Dr. Walter Murfee.
Department of Biomedical Engineering, Tulane University; Fellowship of Orthopaedic Researchers.

Introduction: The shoulder joint has the greatest range of motion of any joint in the human body; it has the ability to move through a space greater than a hemisphere. Consequently, the shoulder joint is also the most commonly dislocated joint. Ninety-five percent of shoulder dislocations are anterior dislocations of the shoulder, causing large lesions of the humeral head known as Hill-Sachs lesions as the soft humeral head impacts the harder anterior glenoid rim. The goal of current treatments is to achieve a large range of motion while maintaining shoulder stability; however, none of these treatments have been shown to consistently and reliably restore the humeral head to its normal sphericity and the joint to its normal level of function. The goal of this project is to develop a novel implant to not only fill the Hill-Sachs lesion, but that restores the sphericity of the humeral head and the functional range of motion of the glenohumeral joint while exhibiting the properties of healthy bone and cartilage.

Materials and Methods: In order to determine the dimensions of the humeral head resurfacing implant, an in-depth literature search was performed to obtain data on the average size of the humeral head and Hill-Sachs lesions of the humeral head. With the data obtained from the literature search, a CAD drawing of an implant for the average male with an average sized Hill-Sachs defect was made using SolidWorks software. A CT scan file of a human humeral head was obtained from an internet database. Using Mimics image processing software, the CT scan was constructed into a 3D object. A depression with the dimensions of the average Hill-Sachs lesion found in literature was made in the SolidWorks humeral head object, and using the SolidWorks assembly setting, the implant part was placed in the defect of the humeral head to determine an initial fit.

Results and Discussion: The curvature of the implant was similar to that of the 3D model of the humeral head obtained from the CT scan; however, the implant was slightly recessed on the superior edge of the implant and slightly proud on the inferior edge. The implant size also corresponded well to the defect size on the humeral head.

Figure 1. The 3D model of the implant prototype placed into a humeral head model derived from a CT scan of a shoulder.

Conclusions: The measurements obtained from the literature corresponded well to the measurements of the CT scanned humerus; further, the prototype implant size fit in the Hill-Sachs lesion of the humeral head. Future studies will include testing the implant in additional humeral head models to further assess proper sizing and fit.

Acknowledgements: I would like to thank Dr. Murfee, the Tulane Biomedical Engineering Department, and Dr. Stephen Cook and Samantha Salkeld of the Fellowship of Orthopaedic Research for their support and guidance in this study.
RF THERMOABLATION IN 3D: OPTIMIZING THE 3D MICROENVIRONMENT FOR THE OBSERVATION OF THE CYTOTOXIC EFFECTS OF TARGETED IRON OXIDE NANOPARTICLES ON BREAST CANCER CELLS

Courtney Evans, Benjamin Vinson, Dr. Shiva Adireddy, PhD., Theresa Phamduy, Dr. Douglas B. Chrisey, PhD.
Department of Biomedical Engineering, Tulane University.

Introduction: Recent studies have shown promise for targeted nano-magnetic thermoablation as a potential therapy against breast cancer. When ferric oxide nanoparticles (Fe₃O₄ MNPs), conjugated with a specific cell penetrating peptide, are exposed to a radiofrequency inductive field (RF, 50-100 kHz), the resonance causes particle hysteresis loss, delivering a cytotoxic dose of thermal energy to the targeted cancer cell and inducing hyperthermia. 3D cell culture environments have proven to be model representations of native extracellular matrices by providing cells with the mechanical and biological integrity necessary for cell viability. By incorporating 3D cultures as an alternative ex vivo approach, magnetic thermoablation’s potential efficacy in vivo can be better understood. This study aims to design and optimize a 3-D microenvironment for observing the targeting ability of an endocytosis-inducing peptide conjugated to silica-coated Fe₃O₄ nanoparticles.

Materials and Methods: 3% Alginate and 3:1 Alginate/collagen microspheres, with diameters of ~200µm, were prepared via electric field driven fabrication, at varying humidity, using a syringe pump to dispense 1 mL of hydrogel solution through a 30-gauge dispensing tip, into a high voltage field (5 kV), atomizing the alg-gel and cross-linking the spheres in a 1% (w/v) calcium chloride (CaCl₂) bath below. Circularity morphometrics were analyzed using images taken with an inverted compound microscope. 2D diffusion analysis was conducted using time lapse fluorescence images of a 10 µL of 1 µg/mL nanoparticle solution diffusion through a 2 mL sample of bulk 3% Alginate. 3D analysis involved the incubation of nanoparticle solution with 3% alginate microspheres, with fluorescence images taken at 0 hr, 2 hr, and 24 hr time points.

Results and Discussion: 3% alginate microspheres demonstrated consistently higher circularity across all humidities, compared to alginate/collagen microspheres. Decreased circularity of the alginate/collagen copolymer can be explained by the increased surface tension caused by the interaction between the collagen component and the ejecting needle. Because uniformity is a necessary parameter for consistent 3D cell ingrowth and diffusion patterns, 3% alginate microspheres are the better choice for a 3D microstructure. Subsequent 2D diffusion analysis demonstrated the fast diffusion (average 11 seconds) capabilities of the nanoparticles through 3% alginate. 3D diffusion tests visualized aggregates of nanoparticles but the sub-micron size of the particles rendered fluorescence images inconclusive.

Conclusions: The reproducible size, circularity and fast diffusion characteristics of 3% Alginate microspheres suggests potential use as a pre-clinical model in future cytotoxicity studies. However, subsequent 3D diffusion analysis is necessary to confirm nanoparticle diffusibility into the 3D microstructure.
THE DEVELOPMENT OF A NOVEL AND HIGH-THROUGHPUT PLATFORM FOR THE ELECTROPHYSIOLOGICAL ANALYSIS OF NEURAL CULTURES IN HYBRID CONDUCTIVE HYDROGELS
Clayton B. Ford, Michael J. Moore, Ph.D.
Department of Biomedical Engineering, Tulane University

Introduction: Three-Dimensional hydrogel constructs are a more biomimetic platform for the evaluation of neural cultures in vitro than traditional two-dimensional cultures, making them an ideal system for the study of neurites. To that end, previous work has shown that the electrophysiological analysis of cultured hydrogel constructs is possible using traditional probe-based techniques. However, these techniques prove to be tedious, and have a low success rate. Therefore, a system was developed to facilitate the electrophysiological analysis process, employing hybrid conductive hydrogel regions into the constructs, and an associated system of deposited gold and snap-in components, to ensure facile electrical connection to the neurites without the use of probes.

Materials and Methods: Hybrid conductive hydrogel constructs were produced through the use of a novel 3-step photolithographic process using a Digital Micromirror Device (DMD). Three gels were present in each hybrid construct: a growth-permissive gel, Heparin (HP) or Hyaluronic Acid (HA), a growth restrictive gel, Polyethylene Glycol dimethacrylate (PEG), and a conductive gel consisting of the growth-permissive gel, and added conductive polymer, Polypyrrole (Ppy). Hybrid constructs were made by polymerizing the conductive regions with growth-permissive gel, polymerizing Ppy in-place in those regions, polymerizing the outer PEG, and then polymerizing growth-permissive gel in the remaining voids. Gold contacts were formed on the cell culture inserts through a sputtering process and use of a 3D-printed snap-in gold mask. Electrical connections were then made with a second snap-in, holding tapered pins against the gold contacts. Electrochemical Impedance Spectroscopy (EIS) measurements were conducted by loading a cylindrical sample of each gel into a parallel-plate testing apparatus, and performing a frequency sweep.

Results and Discussion: The novel three-step photolithography process consistently produced three-gel hybrid constructs with 0.5mm details. Gold deposition was accomplished with the snap-in mask, and electrical connections were successfully made through the use of the second snap-in, creating connections of 100-500Ω from the ends of the gold contacts to the tops of the tapered pins. EIS results showed that Ppy addition caused reliable dropping of resistivities in HP. HA-Ppy appeared to have the best signal conduction properties, as its resistivity was closest to that of PEG, and its phase angle was substantially lower, signifying lower signal distortion properties. This advantage would make HA-Ppy a robust choice for electrical connection with neurites.

Conclusions: Hybrid conductive hydrogel constructs, with the associated gold deposition and snap-in system, show potential for becoming a robust platform for the high-throughput analysis of in vitro neural cultures. Future studies will investigate hybrid gels with other conductive polymers, and the production of more robust snap-in components through the use of CNC techniques.
CHARACTERIZING MOUSE EMBRYOID BODIES OF VARIOUS DENSITIES
Leah Gerber, Taby Ahsan, Michelle Janaszak
STEM Laboratory, Department of Biomedical Engineering, Tulane University

Introduction: Stem cells possess the capability to differentiate into any cell type making them an attractive option for tissue therapies and organ regeneration. The common method of stem cell culture as embryoid bodies (EBs) yields inhomogeneous populations of different phenotypes. For use in in vivo therapies, pure populations of specific cell types are imperative. While there are many techniques currently used for sorting cells, they often prove costly, have a low throughput, and can sometimes damage the cells in the process. Here we propose that the pluripotency of cultured EBs increases directly with density and further, that a discrete Percoll™ gradient can be used to efficiently remove pluripotent EBs.

Materials and Methods: Murine embryonic stem cells were grown in rotating 3D culture and allowed to form EBs. After twelve days of spontaneous differentiation, they were sorted using a five-tiered Percoll gradient with densities of 1.030-1.050 g/ml in .05 g/ml increments. Photos were taken of each fraction for cross-sectional area comparative analysis and then they were fixed for flow cytometry to test for the pluripotency protein marker Nanog. A second experiment was performed in which, after the EBs were sorted, each fraction was dissociated and recultured as a single cell solution in ESC conditions for 5 days. Flow cytometry for Nanog was rerun on each recultured fraction as well as PCR for NANOG, SOX2, and OCT4 genes. Flow cytometry was collected analyzed using FCS and PCR was analyzed using StepOne.

Results and Discussion: Size analysis displayed a general trend of decreasing size with increasing density. Initial flow cytometry results indicated that there were subpopulations still expressing Nanog, an indicator that some cells had remained undifferentiated. This was especially evident in Fraction IV (1.045g/ml) and Fraction V (1.050 g/ml) (Figure 1, left). Repeated flow cytometry on the recultured samples found that Fractions IV and V expressed amounts of Nanog comparable to embryonic stem cells (ESCs) (Figure 1, right).

![Graph showing Nanog expression in Fraction IV and Fraction V compared to ESC expression](image1)

Figure 1: Nanog expression in Fraction IV and Fraction V compared to ESC expression (black) after 12 days of spontaneous differentiation (blue, left) and after 5 days of reculture (red, right). Areas to the right of the vertical line represent the positively selected cells.

These fractions also maintained a similar morphology to ESCs. Gene analysis using PCR also showed that Fractions IV and V expressed pluripotency markers NANOG and SOX2 at equivalent levels to ESCs. All of these traits indicate that there are populations of cells, particularly in the denser fractions that are remaining undifferentiated. This is an important conclusion for researchers looking to create stem cell therapies for use in vivo as undifferentiated cells could cause teratomas if placed in the body. Additionally, using density centrifugation in combination with flow cytometry could prove an effective method to increase the focus and throughput of cell sorting.

Acknowledgements: I would like to thank Dr. Taby Ahsan for providing me with the resources to carry out these experiments as well as Michelle Janaszak for mentoring and teaching me throughout this experience.
THE CONTRIBUTION OF HOST CELLS IN AN ORTHOTOPIC XENOGRAFT MODEL OF HUMAN GLIOBLASTOMA MULTIFORME
Gina Goorley, Luis Marrero.
Department of Biomedical Engineering, Tulane University
Department of Medicine, Louisiana State University Health Sciences Center

Introduction: Glioblastoma multiforme (GBM) is the most aggressive and lethal primary brain tumor of the central nervous system. A comprehensive understanding of cell dynamics involved in tumor growth is crucial for the development of cell-specific therapies to manipulate the microenvironment associated with GBM and similar pathologies. The objective of this study was to determine the fate of host cells in an orthotopic xenograft model of GBM, specifically as cancer stem cells (CSCs) and/or pro-tumoral cells.

Materials and Methods: Human GBM U87MH cells expressing a luciferase reporter gene were stereotactically implanted into the left striatum of eGFP transgenic mice. We employed biophotonic imaging with measurement of eGFP or luc reporter radiance to track tumorigenesis in vivo. For histologic analysis, brain tissues from the tumor-bearing mice were harvested 20 days after intracranial implantation. Sections of the xenograft were immunostained against eGFP, CD133(H+M) labeled for both human and mouse epitopes, CD133(M) specific to the mouse isoform, CD45, and the nuclear counterstain DAPI. Epifluorescence photomicrographs were captured at 200x, 600x, and 1000x magnification using an FV1000 confocal microscope.

Results and Discussion: Host cells in the xenograft were identified by eGFP labeling and accounted for 22.57% of the tumor. 59.8% of the xenograft cells, including some eGFP+ host cells, labeled for the classical CSC marker of GBM CD133(H+M). We confirmed that this was not a false positive in host cells by co-expressing CD133(M). On average, 19.33% of cells in the xenograft co-labeled with CD133(H+M), CD133(M), and eGFP. In order to evaluate the phenotype of CD133+ host cells, we immunostained xenograft sections with differentiation marker of hematopoietic lineage CD45 or leukocyte common antigen. 19.61% of the cells represented in the xenograft stained CD45+ (Fig.1A&C). To determine the fraction of CD45+ cells which fall into the category of CD133+ cells or CSCs, we co-detected CD45 with CD133(H+M) (Fig.1B). An average of 7.03% of all CD45+ cells co-expressed CD133(H+M). Since the average of CD133(M)+/eGFP+ cells was 11.56% (Fig.1C), we conclude that 4.53% of CD133+ host cells are CD45- and therefore not of hematopoietic origin.

Figure 1. Not all host-derived CD133+ cells are of hematopoietic lineage. (A) Hematopoietic cells labeled with a mouse-specific CD45 antibody (red) are abundant in the xenograft (200x magnification;50-µm bar). (B) Subsampling at 1000x magnification (10-µm bar) shows cells co-expressing eGFP, CD45, and CD133(H+M), which are host-derived and may have an immune or endothelial role (white arrows). Cells which co-express CD133(H+M) and eGFP but are CD45- (yellow arrows) are host derived CSCs with unknown phenotype but of potential mesenchymal or neuroectodermal origin which remains to be investigated. (C) Visual representation of cell phenotype percentage based on the current labeling strategy and total counts from 1x10⁵ cells.

Conclusions: This study confirmed that host stem cells in GBM can be categorized as CSCs that generally build vascular networks but may also have additional pro-tumoral effects. Although is a presence of CSCs that are unlikely to have an endothelial or immune phenotype, we still do not know what these other pro-tumoral effects are. Further studies will be required to determine the origin and functionality of these cells.
BIOLOGICAL IMPACTS OF A CYCLIC IN-VITRO PULMONARY MODEL FOR RECRUITMENT AND DERECRUITMENT EVENTS

Alex Itin, Donald Gaver.
Department of Biomedical Engineering, Tulane University

Introduction: The human lung consists of a highly bifurcated network of airways. According to the Weibel model, the respiratory bronchioles are approximately 1.6mm in diameter at generations 17-19. These respiratory bronchioles, which terminate in alveoli, can become occluded with fluid exudate in patients diagnosed with Acute Respiratory Distress Syndrome (ARDS). This airway occlusion results in impaired air transport and hypoxemia, which can be treated with mechanical ventilation. The mechanical forces generated during ventilation can lead to Ventilator Induced Lung Injury (VILI). Low-volume ventilation is commonly used to prevent the stretch-related injury occurring at high lung volumes; however, the cyclic closure and reopening of airways at low tidal volumes, termed derecruitment and recruitment, also causes lung damage and inflammation. The objective of this study is to develop a cyclic pulmonary recruitment and derecruitment model that uses human lung airway epithelial cells and to observe the biological impacts on this epithelium due to recruitment and derecruitment events.

Materials and Methods: For this in-vitro model, Silicone tubing with an inner diameter of 1.6mm was autoclaved and coated with 0.15 mg/mL of Human Plasma Fibronectin. The fibronectin coated tubes were placed in an incubator under standard culture conditions for 2 hours and rotated 180° after the first hour. Once coated, the tubes were seeded with human lung airway epithelial cells of cell line NCI-H441 at a concentration of 2.5 ·10^6 cells/mL (100,000 cells/cm²). The cell-seeded tubes were then incubated under standard conditions for a total of 36 hours. Using a syringe pump, a semi-infinite air bubble was introduced at two opening velocities: 2.5mm/s and 25mm/s. A staining solution of 5 µM Hoechst and 5 µM ethidium homodimer-1 (EthD-1) was introduced at 25mm/s immediately following. The percentage of red-emitting cells (EthD-1) relative to the entire quantity of blue-emitting cells (Hoechst) in each captured image was counted using a custom MATLAB code.

Results and Discussion: Due to several limitations, only one trial of a single recruitment event was conducted. The control with no recruitment event resulted in 19% cell death. The cellular damage increased from 26% to 43% when the recruitment velocity was decreased 10-fold from 2.5 mm/s to 25 mm/s. Past investigations of parallel plate systems have demonstrated that decreasing the velocity of recruitment leads to an increase in cellular damage, which fits the trend of the data. One limitation of this study is that we observed in several recruitment events that the layer of cells in the bottom curvature of the tube would lift from the tubing as the bubble tip approached. To prevent this, we designed a rotary incubator insert to promote the seeding of a full monolayer on the inner surface of the tube. In preparation for cyclic trials, we also designed a syringe holder that accommodates 4 syringes driven by an actuator. With these new tools, we plan to improve the current model to accommodate cyclic recruitment and derecruitment.

Conclusions: The current investigation confirmed that cells seeded inside of a cylindrical model experience an increase in cellular damage as the velocity of recruitment decreases. For future investigations, we plan to expand this single recruitment event to an actuator-driven cyclic recruitment and derecruitment experiment.

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THE EFFECTS OF MEDIATORS RELEASED BY OXLDL AND IFN-γ-ACTIVATED TISSUE RESIDENT CELLS ON THE PROGRESSION OF ATHEROSCLEROSIS
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Introduction: Atherosclerosis is the number one killer of Americans. The main culprit in Atherosclerosis is the formation of Oxidized Low Density Lipoprotein (OxLDL) from Low Density Lipoprotein (LDL). When LDL accumulates to a high amount, it begins to embed into the arterial wall where it oxidizes via Reactive Oxygen Species (ROS). A cascade of events begins to take place resulting in monocyte extravasation and the subsequent formation of a hardened globule called plaque. Recently, the role of mast cells has become an area of interest due to their presence at the shoulder regions of plaque. Furthermore certain mediators such as IFN-γ may trigger mast cells to degranulate and attract more monocytes to the damaged area. The objective of this study is to determine the effects of OxLDL and IFN-γ treated Mast Cells via quantification of monocyte adhesion to endothelial cells.

Materials and Methods: The three cell types used in this study were two non-adherent cell types: THP-1 (monocytes) and LUVA (mast cells) and one adherent cell type: HUVEC (endothelial cells). All cells were maintained at 37 °C and 5% CO2. In order to create OxLDL, the LDL was incubated with CuSO4 which acted as the oxidizing agent for 18 hours. The level of oxidation was measured by performing a Thioarbituric Acid Reactive Substances (TBARS) assay (Cayman Chemical). LUVA cells were allowed to incubate with OxLDL and IFN-γ. Four conditions were applied as follows: Control, OxLDL, IFN-γ, and OxLDL+ IFN-γ. A null was introduced into the experiments which utilized no LUVA. LUVA were incubated with IFN-γ for 4 hours before introducing OxLDL. After 24 hours of IFN-γ and 20 hours of OxLDL incubation, a static assay was performed using 96-well plates. HUVEC were seeded and maintained in the plate for 2 days before conducting experiments. The supernatant of each treated/untreated LUVA was added and allowed to incubate with the HUVEC for 4 hours. After 4 hours, the supernatants were removed, and the HUVEC were incubated with THP-1 cells for 25 minutes. Non-adhered THP-1 cells were removed via washing with PBS.

Results and Discussion: Images were obtained using the Nikon ECLIPSE Ti microscope under a 10x objective. The number of adhered THP-1 was counted. The results show that there is no statistical difference between the No LUVA group and LUVA treated with OxLDL+IFN-γ. The Oxidation of LDL was not successful which resulted in the experiment being conducted with LDL as opposed to OxLDL. If the LUVA groups are considered then there is a rise in the number of THP-1 adhered. In the presence of LDL, there is a slightly higher adhesion than the LUVA group. The IFN-γ group shows a slightly higher adherence than both the LUVA and LDL groups which may be due to IFN-γ being a crucial mediator in Atherosclerosis. Lastly, the LDL+IFN-γ group shows the highest amount of adherence due to possible synergistic effects.

Conclusions: The objective of this study was to determine the effects of OxLDL and IFN-γ treated mast cells via quantifying monocyte adhesion to endothelial cells. Besides the LDL not oxidizing, other limitations were present. The effective dose of IFN-γ on LUVA cells is not known; therefore a protocol for a different mast cell line was utilized in order to determine the concentration and incubation time. Furthermore, LUVA cells lose FcεRI, IFN-γ receptor, sensitivity the longer they are cultured which could prevent a response. For future work, it is necessary to determine how to efficiently oxidize LDL, and then go on from there.

Figure 1. A. Shown is an image of the OxLDL group. The round cells are the THP-1 and the elongated cells are the HUVEC. Three images were taken per well. B. Shown is the data collected. The mean values are taken. A one-way ANOVA gives a p-value of 0.6445 which shows no statistical significance. 3 independent trials. were conducted per group.
Computational Modeling of Non-Linear Reversible Microfluidic Droplet Systems
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Introduction: Droplet based microfluidic systems have seen a rise in use and importance in recent years, with applications ranging a broad spectrum, from fast analytical systems or the synthesis of advanced materials to protein crystallization and biological assays for living cells. Michael J. Fuerstman et al managed to construct a microfluidic device that simulates two notable phenomena; the transformation of linear periodic flow to non-linear aperiodic flow and reversible fluid flow, and it is this device which is the foundation for this research. A mathematical analysis of Fuerstman et al’s droplet-based microfluidic system was conducted by B.J. Smith and D.P. Gaver specifically focusing on the former aspect, which will also be the focus of this study. The objective of this study is, through the use of computational modeling software, gain a more in depth understanding of the mechanisms behind the transformation from simple periodic flow to aperiodic chaotic flow.

Materials and Methods: Construction of the model geometry was done in Comsol Multiphysics (COMSOL inc, Sweden), and was designed according to the specifications of the experimental apparatus designed by Fuerstman et al.; consisting of an inlet and outlet channel separated by two asymmetric loops, the bottom one 1.98mm long and the top one 1.78 mm long. There were three specific steps of construction for the model: creation of the asymmetric loops, creation of a droplet generator, and a combination of the two components. The first step was to establish a functioning model of the geometry seen in Figure 1, the second was to create a controllable method for droplet generation, and the third was to simply combine the loops and generator to create a single functioning system.

Results and Discussion: It is important to note what actual physics are at work in the model. The asymmetry of the loops is what leads to the transformation of periodic to aperiodic flow; due to initially having a higher pressure gradient thanks to its shorter length, the initial droplet will enter the bottom loop. The presence of the first droplet alters the resistance and pressure throughout the entire bottom loop, changing the pressure gradient. As this quantity changes, the top, and longer loop may end up having a higher pressure gradient than the bottom loop, and the second droplet can potentially flow through the top loop. The first phase of constructing the asymmetric loop geometry was tested by ensuring the inlet and outlet flow rates were identical, and that the initial pressures in the loops were identical. Both of these conditions were met. Analysis of the droplet generator was done qualitatively by observing volume fraction plots, and the input conditions for the final droplet generator are shown in Figure 2.

Figure 2. Input conditions: Bulk fluid=water droplet=oil W=0.08 L=0.6 \( v_b=0.04 \) \( v_d=0.04 \) \( \mu=0.0041 \) \( \gamma=0.052 \)
Combining this specific generator with the previously constructed asymmetric loops did not yield the expected results, and various input parameters were changed in hopes of modeling periodicity in the system. The most promising result is displayed in Figure 3.

Figure 3. Progression of droplet through system
It can be difficult to see, but Figure 3 shows very faint droplets exiting the system from both the inlet and outlet channels.

Conclusion: While an ideal model was not created, a significant amount of progress was made and, in the results displayed in Figure 3, we were even able to see the beginning of the transformation of linear flow to non-linear flow, which shows promise for future testing. Beyond attempting to perfect this transformation in the system, future studies should include methods for simulating reversible flow in the system like in Fuerstam et al’s device.
PLATELET ADHESION TO BREAST CANCER CELLS AND ITS ROLE IN CANCER METASTASIS
Sabrina Lynch, Damir Khismatullin, PhD.
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Introduction: Hematogenous metastasis, when breast cancer cells travel to distance sites via the cardiovascular system, is a feature of late stage breast cancer. It is important to develop therapies that impede the metastatic spread of tumor cells through the cardiovascular system. Circulating tumor cells are able to shield themselves from immune cells by activating and attaching platelets to the tumor cell surface. The objective of this investigation is to compare and quantify the adherence of platelets to breast cancer cells under static and flow conditions. A further objective of this study is to analyze how the metastatic potential of breast cancer tumor cells impacts the adhesion of platelets to tumor cells under both static and flow conditions.

Materials and Methods: MCF-7 and MDA-MB-231 breast cancer cells were cultured in T-75 flasks in full growth medium. A well-controlled system consisting of carboxylate-modified fluorescent microbeads was used to quantitatively describe GP Ibα-mediated platelet adhesion to breast cancer cells. Covalent coupling of the amino-terminal end of GP Ibα to the carboxylate group on the surface of latex microspheres (microbeads) was accomplished through carbodiimide chemistry, catalyzed by EDAC, using a previously established protocol. Static experiments were conducted by culturing confluent groups of breast cancer cells in a 24-well tissue culture. GP Ibα-coated microbeads were applied to cancer cells. After an incubation period the wells were washed and imaged. Flow experiments were conducted using the parallel plate flow chamber model.

Results and Discussion:

Figure 1. (A) Comparison of results from static assays with MCF-7 and MDA-MB-231 breast cancer cells combined with uncoated microbeads (control) or GP Ib-α coated microbeads. (B) Adhered microbeads to MCF-7 cells given Q = 0.01 mL/min

Conclusions: Through this investigation it has been shown that microbeads coated with the platelets protein GP Ib-α adhere to MCF-7 and MDA-MB-231 breast cancer tumor cells at a greater affinity than uncoated microbeads. Static experiments have also suggested that microbeads coated with the platelets protein GP Ib-α adhere more readily to MCF-7 than MDA-MB-231 breast cancer cells. This demonstrates a possible link between platelets and breast cancer cell in hematogenous metastasis.

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SYNERGY BETWEEN HIGH-INTENSITY FOCUSED ULTRASOUND AND ETHANOL INJECTION IN THYROID CANCER ABLATION IN VITRO AND IN VIVO

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Introduction: High-intensity focused ultrasound (HIFU) emerges as a powerful technology for noninvasive or minimally invasive non-ionizing treatment of cancer. HIFU deposits a large amount of acoustic energy at the focal region within the target tissue (i.e., tumor), causing tissue heating and necrosis, a process known as thermal ablation. In this study we test the hypothesis that HIFU and ethanol injection, a leading method for chemical ablation, have a synergistic effect on ablation of aggressive forms of thyroid cancer.

Materials and Methods: In the in vitro experiments, the suspension of FB1 anaplastic thyroid cancer cells (100 µl, 2.7 million cells/ml) were placed in a 0.2 ml thin-wall PCR tube and then exposed to HIFU alone, ethanol alone, or ethanol + HIFU. The focused ultrasound signal was generated by a 1.1 MHz transducer with acoustic power range from 4.1 W to 12.0 W. Ethanol was diluted in the FB1 cell growth medium to the concentration of 2%, 4%, or 10% (v/v) and applied to the cells before HIFU exposure. The viability of the cells was measured by flow cytometry and trypan blue exclusion 2, 24, and 72 h post-treatment. The in vivo study was performed using the xenograft mouse model of human thyroid cancer. K1 papillary thyroid cancer cells (2.0×10\textsuperscript{6}) were injected on both flanks of nude mice. Tumors were allowed to grow to 8-10 mm size and then separated into the following treatment groups: HIFU alone, ethanol (50%, 50 µl) alone, ethanol+HIFU (50%, 50 µl), and sham. Tumor sizes were recorded pre-treatment and 9 days post-treatment using a veterinary diagnostic ultrasound system.

Results and Discussion: Our in vitro and in vivo data indicate that thyroid cancer treatment with a combination of ethanol injection and HIFU stops tumor growth and reduces its size. Particularly, the exposure of FB1 cells to HIFU alone in vitro reduced the number of viable cells immediately after treatment, but their proliferation rate remained high. Both the viability and proliferation rate significantly decreased in the cells treated with a combination of ethanol and HIFU. Xenograft tumors in the ethanol alone, HIFU alone, and sham groups continued to grow post-treatment. Only tumors exposed to the ethanol + HIFU combination were reduced in size or completely eliminated.

Conclusions: Combination of HIFU and ethanol may provide an effective minimally invasive treatment option for patients with aggressive thyroid cancer. Combination of HIFU and ethanol shows synergistic effect at very low concentration of ethanol and lower acoustic power HIFU. Combination of HIFU and ethanol may reduce complications observed in clinical use of each method alone.
DESIGN, MANUFACTURE, AND EVALUATION OF A HINDLIMB SUSPENSION CAGE
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Introduction: Mesenchymal stem cells (MSCs) are thought to have angiogenesis effects on ischemic tissue; however, when used as therapy for revascularization, after a few days they are no longer present at the site of transplantation. This may be caused by the sudden change in the microenvironment of the MSCs. It is crucial to quantify how mechanical stresses/strains affect mesenchymal stem cell-mediated angiogenesis by developing a hindlimb suspension rodent model that will allow the modulation of these mechanical forces in situ. The objective of this thesis is to design, manufacture, and evaluate a hindlimb suspension cage that will offload the hindlimbs of rats in order to modulate mechanical stresses and strain on the posterior limbs, while still allowing the rodent to eat, groom, grow and move freely within its cage.

Materials and Methods: The hindlimb suspension cage and the suspension mechanism were designed using SolidWorks based on design criteria specific for the project. The cage and suspension brackets were then manufactured using power tools such as table saw, a router, and a drill press. The cage was later glued together and the suspension mechanism was assembled.

Results and Discussion: The final prototype produced for this thesis was a functional hindlimb suspension cage made out of polycarbonate plastic and a suspension mechanism made out of several different components, including a steel rod and a nylon pulley. The cage assembly was evaluated by doing engineering calculations to assess the stress on the polycarbonate plastic, for it was critical to make sure that the polycarbonate plastic was not going to crack or shatter due to the stress from the weight of the rat. A set of dead weights was used as well to model the weight of the rat, and showed that the cage worked effectively.

Figure 1. Complete Assembly and Final Product 1st Generation Prototype of the Hindlimb Suspension Cage

Conclusions: The evaluation of the cage was successful and showed that I was able to deliver the 1st generation prototype of the hindlimb suspension cage. Future work relevant for this project is the evaluation of the cage by suspending a rat and assessing how the rat can move and live about the cage, and assessing how easy it is for the researcher to work with the cage. Second generation cages could be made bi-axial by adding a set of pulleys that could allow movement of the rat in both x- and y-planes. This cage will be very useful in research, and it will be specifically used to modulate the mechanical stresses and strains of the rat’s posterior limbs in order to study the angiogenetic properties of MSC’s in ischemic tissue – ultimately aiding in the development of novel stem cell-mediated therapies to treat conditions such as myocardial infarction.

**MYELINATION OF NEURAL CO-CULTURES IN 3D MICROPATTERNED HYDROGEL**

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**Introduction:** In order to examine the therapeutic effects of neuropharmaceuticals, there needs to be a biomimetic tissue platform that accurately recapitulates the morphology and physiology of peripheral nerves *in vivo*. Recently, our lab has demonstrated that photolithographic hydrogel micropatterning can be used to support the three-dimensional (3D) growth of dorsal root ganglia (DRG) neurites *in vitro*. In order to improve the electrophysiological accuracy of our platform, this study aimed to determine whether or not the addition of Schwann cells (SCs) to a 3D DRG culture model would increase the presence of myelinated neurites.

**Materials and Methods:** A digital micromirror device was used to selectively photopolymerize hydrogel solutions into user-defined geometries. A longitudinal hydrogel channel was micropatterned using growth-restrictive poly(ethylene glycol dimethacrylate) and commercially available photoinitiator in order to direct longitudinal neurite growth that mimics *in vivo* morphology. DRG explants were then allowed to adhere to a permeable collagen-coated membrane within this channel. SCs were suspended in a solution of growth-permissive methacrylated dextran (MeDex) and riboflavin, a biocompatible photoinitiator, and this solution was used to completely fill the DRG culture channel. The MeDex solution was photopolymerized into a 3D hydrogel scaffold, and co-culture was allowed to proceed for time periods of 7, 10, 14, and 21 days. Neurite outgrowth was assessed by the presence of β-III tubulin, and myelin production was confirmed by the presence of myelin basic protein (MBP).

**Results and Discussion:** Confocal microscopy revealed that DRGs and SC populations were able to grow throughout the depth of each channel, and that co-culture viability was maintained for the entirety of each experiment. Figure 1C demonstrates the presence of myelinated neurites as early as day 10, and Figure 1D shows that myelinated neurite density continues to increase over time.

![Figure 1. Progression of neurite outgrowth and MBP production.](image)

(A, B) Staining for β-III tubulin illustrates longitudinal migration and increased neurite density over 21 days. (C, D) Staining for MBP reveals myelin accumulation. (E, F) Merged fluorescence. Scale bar: 500µm

**Conclusions:** Due to the persistent viability of DRGs and SCs over 21 days, it can be concluded that photolithographically micropatterned MeDex/riboflavin hydrogel is a suitable conduit for 3D neural co-culture experiments. Co-cultures were able to produce 3D myelinated neurites, indicating that successful SC-neurite interfacing had been achieved. This co-culture platform provides a morphologically relevant model for nerve tissue analysis, and will be employed in future studies to investigate the electrophysiological effects of various neuropharmaceuticals.
THE EFFECTS OF AGING ON MICROVASCULAR RESISTANCE IN THE RAT MESENTERY
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Introduction: A complete understanding of the mechanisms of microvascular network remodeling is crucial for the development of therapies for treating angiogenesis-related pathologies, such as myocardial infarction and hypertension. Many such pathologies occur more frequently in aged populations, and therefore there is motivation to understand how microvascular remodeling is impacted with age. In order to understand how the physiological function of microvascular networks change with age, the objective of this study is to compare the network resistances of mesenteric microvascular networks in aged and adult rats.

Materials and Methods: Mesenteric windows were harvested from adult (9 month, n=4) and aged (24 month, n=4) male Fischer-344 rats, immunolabeled for PECAM, and imaged. A MATLAB code was developed to allow for description of large networks in terms of segment diameters, segment lengths, nodal connectivity, and vessel type. Using this data, a modified Poiseuille relationship, and an assumed pressure drop of 65 mmHg, a computational model was used to calculate total network resistances as well as segmental flows and pressure drops. Additionally, capillary flow heterogeneity was compared between the two groups. This was done by examining the relative dispersion (standard deviation/mean) of capillaries, defined as all vessels with diameter less than 10 µm.

Results and Discussion: The results of our study show the network resistance of aged microvascular networks (1.90 ± 0.69 mmHg/(nL/min)) to not be significantly different than that of adult networks (2.48 ± 0.48 mmHg/(nL/min)) (Fig. 1a). To address the possibility that resistance from the Poiseuille calculation may be dominated by the diameter term (raised to the fourth power) rather than by the series/parallel nature of network patterning, the calculation was also performed for the case of uniform diameter 10 µm, again resulting in no significant change in resistance between the two groups (Fig. 1b). Therefore our model successfully isolates the effects of network patterning on resistance from the effects of segment diameter and suggests that there is no significant difference in resistance between aged and adult networks. Interpretation of results is limited by the networks chosen; although consistent with unpublished data from our lab, aged networks have a significantly higher vascular area. Further investigation into the complexities of network patterning will be required to understand if changes in microvascular remodeling occur with age.

![Figure 1. Quantification of total network resistance.](image)

Conclusion: In summary, our study helps elucidate the potential changes in vascular remodeling that occur with age. In the microvascular networks we analyzed, we have shown that network resistances are not significantly different. Our results suggest that network resistance is not altered in aged populations and provide motivation and direction for future studies on changes in microvascular network remodeling that occur with age.
THE INFLUENCE OF AGING ON MICROVASCULAR DENSITY IN SKELETAL MUSCLE
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Introduction: Microvascular remodeling and angiogenesis are closely related to many age-related pathologies. In some age-related pathologies, such as cancer and rheumatoid arthritis, it would be advantageous to prevent vessel growth. In others, such as myocardial infarction and hypertension, it would be beneficial to promote vessel growth. In such pathologies, it is imperative to have a comprehensive understanding of the age-associated vascular dynamics in order to develop an effective therapeutic treatment. Since these pathologies are characterized by vessel loss or excessive vessel growth, it important to examine the number of blood vessels in aging versus adult populations. There is a discrepancy in the literature about blood vessel density in aged adult skeletal muscle. Some studies show a decrease in blood vessel density with age while others show an increase or no change. The objective of this study was to investigate differences in vessel density between aged and adult populations by analyzing blood vessel density in skeletal muscle.

Materials and Methods: The rats used in this study were male Fischer 344 rats. The tissues were prepared in two experimental groups: 24 month (n = 6 rats) and 9 month (n=6 rats). The right soleus muscle was harvested, cryoembedded, and cut into 10µm sections that were labeled with anti-platelet endothelial cell adhesion molecule (PECAM) and anti-α-smooth muscle actin (αSMA). Representative 10X images were taken of the labeled slides and the positively labeled blood vessels were quantified in addition to autofluorescing muscle fibers. Significance was determined using a student’s t-test and any p-value under .05 was considered significant. All data is presented plus or minus the standard error of the means.

Results and Discussion: Comparing the number of PECAM positive blood vessels normalized to the number of muscle fibers (Fig. 1a) reveals that there is a significant difference (p-value = .022) of about 10% between the two groups. The aged (24 month) rats were found to have 2.24±.06 PECAM positive blood vessels per muscle fiber and the adult (9 month) rats had 2.00±.07 PECAM positive blood vessels per muscle fiber. There was no significant difference between the number of αSMA positive blood vessels per muscle fiber between adult and aged groups (adult = 1.56±.07, aged = 1.76±.07) (Fig. 1b). These results indicate that age related pathologies are likely not related to a decrease in blood vessel density in skeletal muscle.

Conclusions: While there is still much work to be done to fully understand the mechanisms of angiogenesis in aged populations (such as investigating changes that occur in stimulated tissues), this study helps clarify conflicting literature regarding skeletal muscle capillary density and indicates that there is not a significant decrease in capillary density associated with aging.
ENHANCED HUMAN-COMPUTER INTERFACE FOR SEARCHING LARGE EX VIVO MICROSCOPY IMAGES
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Introduction: There is a need for an imaging system with microscopic resolution in a clinical setting that can image large tissue samples in a reasonable amount of time in order to enable intra-operative analysis of the tissue such as tumor margin analysis. If a system like this were to be put in place, false negative rates would fall which would correlate to an increase in successful surgeries. Current manipulation of the images using a mouse and keyboard is cumbersome and slow, and could reduce the practicality of rapid assessment of ex vivo microscopy images in point-of-care timeframes. The objective of this study is to determine if the speed of user-interfacing is increased while maintaining accuracy when viewing an ex-vivo image when using a game controller as opposed to a keyboard and mouse.

Materials and Methods: In order to assess the speed and accuracy of a user while viewing an ex-vivo image, we first needed to obtain these images. Our images were actual images of prostate biopsies. We needed to determine if it was faster for a user to analyze these images using the standard method of a mouse and keyboard with ImageJ or using the new method of a PlayStation 2 controller and Brava! viewer. We did a trial where the subjects (Tulane undergrad students) were given as much time as necessary to count shape clusters in an image to measure their speed using both methods. We then did a second trial where the subjects had 30 seconds per image to identify as many clusters as possible in order to measure their accuracy using both methods. Once we obtained our results, we did both an unpaired and a paired t-test on the data to determine if they were significant. A p-value of less than 0.05 was considered significant.

Results and Discussion: We did two iterations of our experiment. Upon the first iteration, the results were not significant due to the fact that the controller was not best optimized for analyzing ex-vivo images. Once we better optimized the controller, we obtained a significant increase in time while obtaining a change in accuracy that was not significant. To make sure that our results did not stem from the varying sizes of the images, we decided to normalize our results by dividing the times by the number of pixels in each image making our units seconds per pixel. These new data points were also significant.

Figure 1. A bar graph of the average time per pixel of all of the images with standard error of the mean using each method. The Keyboard method is shown on the left while the controller method is shown on the right. The unpaired p-value was 0.037 and the paired p-value was 0.014. Both of these values are considered significant.

Conclusions: This study shows that the use of a game controller will speed up the process of user-interfacing. Therefore, this type of system could be integrated into a much larger imaging system and allow for quick diagnosis once the tissue has been imaged.

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HARDWARE AND SOFTWARE DESIGN TO STUDY MSC-RELATED ANGIogeneic PROPERTIES
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Introduction: Mesenchymal stem cells (MSCs), extracted from bone marrow, adipose tissue, and other various sources within the adult body, have positive effects on the revascularization of ischemic tissue. The ability of MSCs to promote angiogenesis is in part dependent on the mechanical microenvironment of the surrounding tissue, where microscopic forces within the tissue affect MSCs’ secretory activity and therefore the ability to revascularize the tissue. Another variable that affects angiogenic potential is the donor age of the stem cells, and the age-related changes in cellular stiffness that can affect the mechanical response of cells. The purpose of this project is design both hardware and software that will allow experiments to be conducted to test the effects of the mechanical environment and donor age on MSC-dependent angiogenesis in ischemic tissue.

Materials and Methods: Specifically, the first objective is to design a device that allows the researcher to decrease mechanical stresses in vivo by suspending rodents’ hindlimbs. The first step of the design process was to come up with valid Design Criteria. Merit and feasibility analysis of the design criteria helped determine the optimal choices for the critical components of the hindlimb suspension cage. The exterior portion of the cage was designed using SolidWorks and the remaining components were ordered from McMaster-Carr before the final hindlimb suspension cage was ultimately constructed. The second objective is to develop a software User’s Guide to function as a reference for researchers who need to use atomic force microscopy (AFM) to measure cellular stiffness. AFM is a high-resolution type of scanning probe microscopy and allows researchers to capture images and take measurements on the micro-scale. Prior to the development of the User’s Guide, the existing software program was dissected and analyzed. Additionally, it was necessary to study the specific mechanism in which AFM measures cellular stiffness in order to include this information in the User’s Guide.

Results and Discussion: The main categories for the Design Criteria of the hindlimb suspension cage are: robustness; adjustability; rodent health, safety, and comfort; and safety and convenience of handlers. The final design of the hindlimb suspension cage was evaluated to ensure it upheld the standards set for these criteria. Besides the health and safety of both the rodent and handler, the most important aspects of the hindlimb suspension cage are its adjustable features, one of which is demonstrated in the figure. Adjustability is a primary part of the design to enable the cage to account for rodent growth over the course of one experiment as well as to allow the cage to be reused throughout multiple experiments. The AFM Software User’s Guide is a comprehensive, multi-page flowchart that provides information on how to use AFM technology to perform a variety of applications. It is designed so that as the user moves through each of the tabs of the software, he/she is able to follow along on the User’s Guide and similarly proceed through each of the sections. Additionally, the User’s Guide provides information on AFM hardware and how one can use force/distance curves to measure cell stiffness.

Conclusions: Both objectives were met through the course of this project, providing the resources necessary to aid the completion of the overall objective of this project. Future work will qualify the viability of the hindlimb suspension cage by experimental testing using live rodents. Additionally, future work will attempt to quantify cellular stiffness using AFM technology with the help of the User’s Guide.

Figure 1: Hindlimb Suspension Cage.
The adjustable side walls of the cage pictured here allow for the height of the suspension device to be altered and provides more flexibility to the researcher.
Introduction: Due to time restraints and lack of technology at present time, removed tumors and biopsies cannot be immediately analyzed during surgical proceedings which often leads to the need for repeat biopsies and procedures. Structured illumination microscopy provides a way to obtain rapid, optically-sectioned images of thick tissue specimen. By improving the resolution and contrast of images obtained through video-rate SIM, it may be possible to allow for intraoperative analysis of tumors and their margins. The objective of this study was to experiment with new sparse-line pattern illumination masks and the previously used sinusoidal masks in order to determine which mask qualities allow for the best sound-to-noise ratios, signal-to-background ratios, and full-width-half-max of the axial optical response.

Materials and Methods: Using Matlab, 2 sparse-pattern illumination masks were created that displayed varying mark-to-area ratios. Instead of requiring 3 sequential images to be taken by phase-shifting the mask by 2\pi/3, the generated illumination patterns required the acquisition of 4 (MAR=1/4) and 8 (MAR=1/8) images. For each specimen (the 1 micrometer phantom, the 10 micrometer phantom, and the slice of bovine muscle), 20 images were taken using no SIM, the 18 frequency sine wave pattern, the 45 frequency sine wave pattern, the MAR=1/4 pattern, and the MAR=1/8 pattern. Resulting optically-sectioned images were analyzed for signal-to-noise ratios, signal-to-background ratios, full-width-half-max of the axial optical response, and overall acquisition time.

Results and Discussion: For both the SNR and the SBR image qualities, the sine wave with the 45 pixel pitch spatial frequency displayed the highest ratios among the illumination masks. However when looking at FWHM, the MAR=1/4 pattern displayed images with the thinnest optical sections, while the 45 spatial frequency sine wave illumination mask produced images with the thickest optical sections.

Figure 1. Mean signal-to-noise ratios, mean signal-to-background ratios, and FWHM data for images acquired of the 1 micrometer phantom using no SIM, the 18 frequency sine wave pattern, the 45 frequency sine wave pattern, the MAR=1/4 pattern, and the MAR=1/8 pattern.

Conclusions: When looking at cell and tissue morphology, it is a small FWHM that provides the best images because the thin optical sections allow for greater contrast and visualization of cellular structure. Through requirement of images with thin optical sections and moderately good SNR and SBR, the MAR=1/8 pattern was determined the optimum illumination mask in this study because it provided the overall best quality images for use by pathologists. In the future, a study of combined sine wave/sparse-line patterns will be necessary. Combining the patterns would not only be helpful in getting rid of banding artifacts present on a majority of SIM images acquired through the use of sparse-line illumination patterns, but it could also create images that contain high signal-to-noise and signal-to-background ratios while maintaining thin optical sections.
VOLTAGE SENSITIVE DYES FOR HIGH-THROUGHPUT NEURAL RECORDING OF IN VITRO MODELS

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Introduction: Neurological disorders affect us in a variety of ways. A comprehensive understanding of neuron growth, function, and interaction with various microenvironments is critical for treatment of these diseases. High throughput, biomimetic neural models will improve understanding of neurological characteristics and behavior. An effective neural model must exhibit both anatomic relevance as well as high throughput capability. Successful incorporation of voltage sensitive dyes as a form of recording biological function of the model will decrease acquisition time as well as reduce inconsistencies when compared to established field recording probe placement inaccuracies and other associated problems.

Materials and Methods: The neural cell cultures were made according to the protocol outlined previously in Curley et al. (2011). The construct pattern consisted of a DRG explant placed in a Puramatrix® bulb which is connected to a straight Puramatrix® channel. VF2.1.C1 (EWM07-048) was used as the voltage sensitive dye as described in Miller et al. (2012). A perfusion system was set up to deliver ACSF (Artificial Cerebral Spinal Fluid) to the construct. The Hamamatsu Orca-flash 4.0 (Hamamatsu Photonics, Shizuoka, Japan) was focused using the 10X dry objective lens on a section near the bulb of the construct. Camera acquired images at 50 ± 10 Hz. The images were captured using a ~475 nm light source. Custom software developed with MatLAB® (The Math Works Inc., Natick, MA) was used for further analysis of acquired images. ∆F / Fo matrices are calculated then compiled into an Audio Video Interleave (.afi file extension) movie file. The mean average, minimum value, and maximum value of each of the ∆F / Fo matrices are plotted for each trial.

Results and Discussion: The processed ∆F / Fo images’ minimum, maximum, and mean average pixel value was recorded and plotted over sequential frames. Trials either recorded no florescent activity for the entire duration or florescent activity after the onset of stimulation. A trial without florescent activity yielded a near-linear average pixel value of zero. A trial with florescent activity yielded significant spikes in average pixel value and rapid fluctuations of the max and min values. According to the characterization of the dye previously established in Miller et al. (2012), peaks in fluorescence may indicate biological, electrical activity of neural explant in the form of a compound action potential.

Conclusions: The changes in fluorescence observed in this study indicate biological activity of the neural culture and warrants further development of a voltage sensitive dye protocol for use as a recording method in a 3-dimensional, dual hydrogel, neural culture.

Miller, E. W., PNAS, 109.6 (2012): 2114-119
3-D ANATOMICAL MODELS BASED ON CROSS SECTIOANL IMAGING:
A POTENTIAL IMPROVEMENT TO SURGERY
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Introduction: An increase in laparoscopic and robotic surgeries has led to surgeons not having physical contact with a tumor until it has been removed. As a result, surgeons increasingly rely on visual clues and have almost abandoned tactile sensation as a tool for surgery. Converting computed tomography (CT) images of an organ with a tumor attached in situ into a 3D printed model will allow physicians physical contact prior to the operation. The objective of my project is to develop a method for creating three-dimensional models based on cross sectional imaging of kidneys with renal malignancies in-house.

Materials and Methods: To accomplish my goal feasibility of a process was shown by first loading DICOM files into a stacking and viewing medical software, OsiriX. Then, defining a region of interest based on intensity and spatial orientation of pixels, and converting that region of interest in to a 3D model. The pixels outside of the region of interest are then filtered out, and the file is exported as an .STL. The .STL was then loaded into a 3D mesh-altering program, Meshlab, in order to smooth the model. Finally the .STL is printed creating a physical 3D anatomical model.

Results and Discussion: The primary result for this project was developing a process for converting cross sectional images (CT scans) of organs with malignancies into 3-D printable models (Figure 1). The process demonstrates the feasibility of creating and printing accurate three-dimensional models in-house.

Figure 1. Shows the overview of the process starting with (figure 1.1) a series of CT scans then going to (figure 1.2) the isolation of the region of interest, then (figure 1.3) the model exported as an .STL and smoothed, and finally (figure 1.4) the model is printed with a Stratasys Dimension STT-1200 3-D printer.

Conclusions: The main contribution of this study is a process that was successful in printing a three-dimensional model of a kidney with a renal malignancy based on cross sectional images in-house. The region of interest was successfully isolated both spatially and by intensity, and the process was successful for an organ with and without a tumor. These 3D models can have benefits including; enhancing trainee and doctors’ education, improving patients’ comprehension of the disease as well as patients’ satisfaction, and most importantly improving surgical outcomes.

Acknowledgements: (Optional) I would to thank Dr. Jonathan Silberstein for the idea of this project as well as his guidance and assistance throughout the project. I would also like to thank the Tulane University Biomedical Engineering Department, especially Dr. Murfee for his help as an advisor within the department.
Plenary Session
Biomedical Engineering Grand Challenges
January 23, 2015

Stibbs 203 LBC
12:15 PM – 1:00 PM

Moderators:
Ronald C. Anderson, PhD, Michael J. Dancisak, PhD, and
Lars G. Gilbertson, PhD

Schedule:

12:15 – 12:50 Plenary Presentation
Jennifer Boudreaux, Samuel Gjerstad, Samuel Larson, Timothy
LeBlanc, Victoria Orlowski, Patrick Wolber, and Millie YU

12:50-1:00 Discussion and General Q & A

The Grand Challenge:

Grand Challenges is a two semester course in which a small group of students is
assigned the task of researching and developing a novel solution to a current
problem or “grand challenge” of medicine. This year’s grand challenges group was
assigned the task of identifying and solving a major medical complication resulting
from a pathology of the achilles tendon. Informed by further research, the group
decided to focus on the prevention of forefoot ulceration resulting in those with
diabetes mellitus who develop a tight achilles tendon in combination with
peripheral neuropathy. A tight achilles tendon, which is common in diabetics, causes
a change of the resting ankle flexion angle in such a way that the forefoot
experiences increased pressure during weight bearing activities. If this is combined
with peripheral neuropathy, another common side effect of diabetes mellitus which
results in a decreased nociceptive aptitude of distal parts of the anatomy, then the
forefoot is at an extremely high risk for ulceration due to prolonged, unintentional
exposure to high pressure loads. Current promising methods for reducing forefoot
ulceration in such patients with Diabetic Peripheral Neuropathy (DPN) focus on
intraoperative achilles tendon lengthening. However, though these methods are
successful, they are not designed to be reproducible; this lack of standardization
causes variance in patient care, reduces physician efficiency, and hinders efforts to
research procedure efficacy. In order to address this, the Grand Challenges team
developed a physical model of a foot and Achilles tendon to test tendon lengthening
procedures in order to determine which method resulted in the lowest pressure
transmission to the forefoot. These experiments indicated that the Hoke Triple
Hemisection procedure resulted in significantly less pressure than other accepted
and experimental methods of tendon lengthening. A preliminary computational model was developed in ABAQUS, a finite element analysis platform, using commercially available CT scan in order to corroborate such findings. The computational model will be furthered in future experiments. Lastly, the team developed a novel medical device called the QuikStik that would increase reproducibility of the Hoke Triple Hemisection procedure. The QuikStik includes scalpels placed according to the procedure chosen, as a way to eliminate the need for the physician to “eyeball” the cuts needed to be made by an additional blade.

Grand Challenges is a student driven course in which a current problem or “grand challenge” in medicine is investigated, and students work to develop a novel solution to the problem at hand. The “grand challenge” that was tackled this year is centered around ways to prevent forefoot ulceration caused by peripheral neuropathy in patients with diabetes mellitus. Tight Achilles tendons due to diabetes mellitus often cause increased pressure on the forefoot, leading to such ulceration and requiring lengthening of the Achilles; however, current methods for determining ideal slice location(s) for tendon lengthening are irreproducible and inherently different for every physician. This gap of standardization was taken as an indicator that there was room for innovation, specifically to help the surgeon perform the procedure as efficiently and effectively as possible. The Grand Challenges team developed a physical model of a foot and Achilles tendon that was used to test two accepted tendon lengthening procedures in order to determine that method which resulted in the lowest pressure transmission to the forefoot. After such experiments, it was evident that the Hoke Triple Hemisection procedure resulted in significantly less pressure on the forefoot when the same tensile force was applied to the tendon. A preliminary computational model was developed in ABAQUS to corroborate such findings. This model was composed of bones from a commercially available CT scan and were used to create a mesh; in addition, a custom-made foot pad was created to match the physical model. The computational model will be furthered in future experiments. Lastly, the team developed a novel medical device called the QuikStik that would increase reproducibility of the Hoke Triple Hemisection procedure by providing the user with a “stick-like” device. The QuikStik includes scalpels placed according to the procedure chosen, as a way to eliminate the need for the physician to “eyeball” the cuts needed to be made by an additional blade.