DEPARTMENT OF BIOMEDICAL ENGINEERING
TULANE UNIVERSITY

Biomedical Engineering Undergraduate Research and Design Conference

Conference Proceedings

April 19, 2008
Welcome to the 6th 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 five ‘domains’ of biomedical engineering: biomechanics, bioelectronics, bioelectricity, biomaterials, and cell and tissue engineering. 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 will be 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.

Until recently, the presentation of their independent projects occurred during the Department’s Spring Semester Seminar Series. Since 2003 we have instead chosen a presentation format modeled on a professional research conference.

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. We are hopeful that 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 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 2008 for their achievements, and determination to leave their mark on biomedical engineering research and practice.

Thank you for attending!

Donald P. Gaver, Ph.D.
Alden J. ‘Doc’ Laborde Professor and 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 opportunities for creative 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
OPENING REMARKS
Donald P. Gaver
Department Head and Laborde Chair
Biomedical Engineering
8:30 – 8:45 AM, Boggs Room 104

MORNING SESSION A – ROOM 104

MODERATORS: Michael J. Moore, J. Lowry Curley

8:45-9:00 THE EFFECT OF FLUID SHEAR STRESS ON NITRIC OXIDE PRODUCTION IN OSTEOCYTES
Peter J. Navarro and Yuefeng Han

9:00-9:15 APOPTOSIS OF OSTEOCYTES PREVENTED BY NITRIC OXIDE
Karl M. Runbeck, Yuefeng Han, and Russell Wolfe

9:15-9:30 ANALYSIS OF THE EFFECTS OF NITRIC OXIDE ON HYPOXIA OF OSTEOCYTES USING WESTERN BLOTS
Brent M. Smith and Yuefeng Han

9:30-9:45 A STUDY OF ANTIOXIDANT CYTOTOXIC EFFECTS WITH MESENCHYMAL STEM CELLS (MSCS) FOR THE DEVELOPMENT OF AN IN VIVO OSTEOGENIC SARCOMA TUMOR XENOGRAFT MODEL
Trivia P. Fazier, Radhika R. Prochampally, Patrice Penfornis, Michael Harris, and Zheng Chen

9:45-10:00 DETERMINATION OF MESENCHYAL SYEM CELL FATE DURING ANGIOGENESIS
Brooke L. Lovett and Walter L. Murfee

10:00-10:15 A QUANTITATIVE COMPARISON OF THE ANGIOGENIC RESPONSE IN ADULT HYPERTENSIVE AND NORMOTENSIVE ANIMALS
DéJeune Antoine and Walter L. Murfee

10:15-10:30 BREAK
MODERATORS: Yuefeng Han, Hongzhi Lan

10:30-10:45 EVALUATION OF LYMPHATIC/BLOOD VESSEL CONNECTIONS AND THE PRESENCE OF PERICYTES ALONG LYMPHATIC SPROUTS DURING MICROVASCULAR REMODELLING
Samantha J. Warren and Walter L. Murfee

10:45-11:00 COMPUTATIONAL INVESTIGATION OF PRESSURE LOSS FROM FLOW ENERGY DISSIPATION: RELAVANCE TO FONTAN-TYPE MODIFICATIONS
David C. Ladd and Donald P. Gaver

11:00-11:15 DUEL HYDROGEL SYSTEM FOR THE GUIDANCE OF RETINAL NEURITES
Eric W. Franca and Michael J. Moore

11:15-11:30 DESIGNING A FUNCTIONAL HYDROGEL WITH EMBEDDED PHOTOLABILE PROTEINS FOR THE GUIDANCE OF RETINAL GANGLION CELL GROWTH
Elaine L. Horn and Michael J. Moore

11:30-11:45 GENETIC TRANSFECTION TO TARGET RETINAL GANGLION CELLLS
Anne Marie Norman, Guoyong Wang, and Michael J. Moore

11:45-12:00 DEVELOPMENT OF A MULTI-SCALE LIGHT BASED NEUROSTIMULATOR
Lee W. White and Michael J. Moore

12:00-1:30 LUNCHEON GALA – BOGGS ATRIUM
OPENING REMARKS
Donald P. Gaver
Department Head and Laborde Chair
Biomedical Engineering
8:30 – 8:45 AM, Boggs Room 105

MORNING SESSION B – ROOM 105

MODERATORS: Darryl R. Overby, Stephen J. Foltz

8:45- 9:00  THE EFFECT OF BACTERIAL COLONIZATION ON THE MATERIAL STRENGTH OF BIOLOGICAL TISSUE GRAFTS
Benjamin M. Wheatley, Charles F. Bellows, and Lisa A. Morici

9:00-9:15  HYDROPHOBIC/HYDROPHILIC ANALYSIS OF CONTACT LENS
Karthik K. Kura and Jean T. Jacob

9:15-9:30  IDENTIFICATION OF SURFACE BIOFOULING ON CONTACT LENS USING A BLINK MODEL SYSTEM
Taylor E. Moss and Jean T. Jacob

9:30-9:45  DESIGN AND TESTING OF A TALAR IMPLANT FOR THE TREATMENT OF OSTEOCHONDRAL DEFECTS
Shiob Bajaj and Stephen D. Cook

9:45-10:00  DESIGN AND TESTING OF A MEDIAL CONDYLE IMPLANT FOR THE TREATMENT OF OSTEOCHONDRAL DEFECTS
Chaitanya R. Nandipati, Stephen D. Cook, Samantha L. Salkeld, and Laura Patron

10:00-10:15  FORCE GENERATION OF HEAT TRANSFORMABLE SHAPE MEMORY (NITINOL) IMPLANTS
Jay M. Mattappally, Richard B. Ashman, Ronald C. Anderson, and Megan Ohar

10:15-10:30  BREAK
MODERATORS: Donald P. Gaver, Bradford K. Smith

10:30-10:45 CORRELATION OF MICRO-CT AND HISTOMORPHOMETRIC MEASUREMENTS OF BONY DEFECT HEALING AND THE BONE-IMPLANT INTERFACE IN A CANINE ACETABULAR DEFECT MODEL
    Gina M. Sequeira, Laura P. Patron, and Stephen D. Cook

10:45-11:00 FATIGUE EFFECTS ON SURGICAL HAND TREMOR
    Mark W. Young, David A. Rice, and Michael Dancisak

11:00-11:15 CARACTERIZING FATIGUE IN THE SURGEON’S PHYSIOLOGIC HAND TREMOR
    Roy Dory, David A. Rice, and Michael Dancisak

11:15-11:30 DESIGNING A REMOTELY MONITORED PULSE OXIMETER
    Jesse D. Ranney and Cedric F. Walker

11:30-11:45 DESIGN AND APPLICATION OF A 3-AXIS CONTROL DEVICE
    William C. Kethman and Cedric F. Walker

11:45-12:00 ACOUSTICAL DEMONSTRATIONS IN EDUCATION
    William D. Sprott and David A. Rice

12:00-1:30 LUNCHEON GALA – BOGGS ATRIUM
AFTERNOON SESSION A – ROOM 104

MODERATORS: David A. Rice, Jenae Guinn

1:30-1:45  
**G LUCOSE AND OXYGEN TRANSPORT IN OSTEONS**
Laurin T. Buettner and Yuefeng Han

1:45-2:00  
**I SOLATION OF SCHLEMM’S CANAL ENDOTHELIAL CELLS**
K. Noel Schexnayder and Darryl R. Overby

2:00-2:15  
**Q UANTIFYING THE HETEROGENEITY OF AQUEOUS HUMOR OUTFLOW PATTERNS IN HUMAN EYES**
Stephanie M. Roberts, Steven J. Foltz, and Darryl R. Overby

2:15-2:30  
**Q UANTIFYING CELLULAR DEFORMATION WITHIN ENDOTHELIAL MONOLAYERS DURING IN VITRO PERFUSION**
Samantha B. Weil, David D. Simon, and Darryl R. Overby

2:30-2:45  
**D YNAMIC APPLANATION TONOGRAPHY APPLIED TO ENUCLEATED EYES**
Raeanna L. Poplus, Ronald C. Anderson, and Darryl R. Overby

2:45-3:00  
**I NVESTIGATION OF THE STRESS DISTRIBUTION AT THE CORNEO-SCLERAL JUNCTION**
Bryan D. Molter and Ronald C. Anderson
AFTERNOON SESSION B – ROOM 105

MODERATORS: Cedric F. Walker, Justin P. Cooper

1:30-1:45  DROPLET TRAINS IN MICROFLUIDIC NETWORKS: A COMPUTATIONAL ANALYSIS OF PERIODIC BEHAVIOR
Cynthia N. Lumby, Bradford Smith, and Donald P. Gaver

1:45-2:00  DISTINCTION OF SINGLE NUCLEOTIDES FOR THE PURPOSE OF DNA SEQUENCING USING A NANOPORE-BASED DETECTOR
Molly E. Oehmichen, Stephen Winters-Hilt, and Amanda Alba

2:00-2:15  MODELING OF CA2+ WAVE PROPAGATION IN OSTEOCYTES
Scott R. Jennings, Hongzhi Lan, and Yuefeng Han

2:15-2:30  ANIONIC EFFECTS ON THE KINETICS OF BETA-GLUCOSIDASE
Westbrook M. Weaver and Larry D. Bryan

2:30-2:45  SYNTHESIS OF A STRUCTURAL ANALOGUE OF THE MONONUCLEAR COPPER ELECTRON TRANSFER SITE IN PLASTOCYANIN
Ashok K. Manepalli and James P. Donahue

2:45-3:00  INVESTIGATION INTO THE ROLE OF M. TUBERCULOSIS TRANSCRIPTION REGULATORS RV0158 AND RV2745C
Joseph R. Kramer and Deepak Kaushal
Department of Biomedical Engineering
Tulane University

Morning Session A

Lindy Boggs Center
Room 104

Biomedical Engineering Undergraduate Research and Design

Conference Abstracts
**Introduction**

Bone is a porous tissue that is constantly perfused by interstitial fluid driven by vascular pressure and mechanical loading. This fluid flow generates significant shear stress through the canaliculi, and once the fluid reaches the lacuna, fluid shear stress is applied to the osteocytes. This shear stress causes the osteocytes to release nitric oxide. Nitric oxide plays a critical role as a molecular mediator of a variety of physiological processes, including blood-pressure regulation and neurotransmission. Also, nitric oxide plays anti-hypoxial and anti-apoptotic roles in several cell lines.

Osteocytic hypoxia and apoptosis increase in both disused and overused bone, however the mechanisms are unknown. Investigators have observed that hypoxia and apoptosis of osteocytes show up earlier than bone resorption while their occurring sites always coincide, and thus, believe that these osteocytic responses mediate local bone resorption by recruiting and activating osteoclasts. A question of debate is exactly how hypoxia and apoptosis are initially induced. We hypothesize that nitric oxide mediates the observed hypoxial and apoptotic responses observed in both disused and overused bone. When fluid flow in the local bone environment diminishes, osteocytes are not stimulated, nitric oxide release stops, and the cells are left without any protection from the nitric oxide to prevent hypoxia and apoptosis.

This study aims to quantitatively measure the effect of fluid shear stress on the production of nitric oxide from osteocytes. Based on the hypothesized connection between nitric oxide and hypoxia and apoptosis, a sound understanding of the quantitative relationship between fluid shear stress and nitric oxide production will help our understanding of the signaling system as a whole. It will also better our understanding of the system’s role in bone remodeling. Nitric oxide is essential for mechanically induced bone remodeling and is an important parameter for osteocyte activation after mechanical loading, and therefore, it is important to understand its regulated release from osteocytes.

**Materials and Methods**

An osteocyte-like cell line, ML0-Y4 cells, were cultured, including the process of passing and splitting them, for their use in these experiments. The culturing consisted of keeping the cells in α-MEM media in a CO₂ incubator kept at a constant temperature of 37 °C. The growth and health of the cells were evaluated by qualitative observation under a compound microscope.

In order to quantitatively measure the concentration of nitric oxide released from the osteocytes, a Shimadzu UV-visible spectrophotometer was used. The spectrophotometer measured the absorbance of the sample being tested, but this absorbance does not directly indicate the concentration. In order to make this correlation, a calibration curve which related absorbance to concentration was necessary. This calibration curve was generated through the use of a Molecular Probes Griess Reagent Kit for Nitrite Determination, in which the absorbance of solutions with known concentrations was determined. The calibration curve is a linear graph of absorbance vs. concentration. The concentration of nitric oxide can be determined from its measured absorbance in the spectrophotometer by corresponding it to the absorbance vs. concentration curve.

![Absorbance Calibration Curve](image)

**Figure 1.** The curve above was generated from data obtained from the measurement of the absorbance of a Griess reagent which had a known concentration and absorbance wavelength. The curve was fit to a linear trend. This linear relationship between concentration and absorbance allows for the correlation between absorbance and nitric oxide concentration in osteocytes.

Fluid shear stress was initially applied to the osteocytes through the use of an orbital shaker (New Brunswick Scientific C24 Incubator/Shaker). The osteocytes were used in experiments once they reached a confluency of approximately 70%. Two sets of experiments were conducted using an orbital shaker as the means of implementing fluid shear stress. The cells were placed on the orbital shaker for times varying from 15 to 60 minutes at speeds of 80 and 100 RPM’s. Another set of experiments was conducted using a VWR Rocking Platform (Model #100) at 0.825 Hz to implement a more linear force as opposed to the circular force applied by the orbital shaker.

**Results**

The calibration curve was the first result obtained, having a high coefficient correlation of 0.9971 (Figure 1). It will be used throughout the study as the basis of comparison to relate the absorbance and nitric oxide concentration values as measured in the spectrophotometer. The nitric oxides concentrations measured thus far have been relatively inconclusive. While several procedural errors have been identified and corrected, dependable results are actively being pursued.

**Discussion**

The cause of the unreliable results obtained from the experiments that have been conducted are currently being investigated. Through the use of square, rather than round, cell plates, the cells closer to the center of the plate will be exposed to more shear stress and should therefore release more nitric oxide. A second calibration curve will also be created to reassure the quality of the technique being used. The media volume within the cell plate, as well as the RPM’s of the shaker, are also variables that can be adjusted to obtain more desirable results.

**Acknowledgements**

Research was supported by the Department of Biomedical Engineering at Tulane University.
APOPTOSIS OF OSTEOCYTES PREVENTED BY NITRIC OXIDE

Karl M. Runbeck, Yuefeng Han, Russell Wolf
Biomedical Engineering Department, Tulane University

Introduction

Homeostasis in bone requires the balance between bone formation and resorption, yet aging and bed rest disturb this balance and, thus lead to osteoporosis. It is important to understand how disuse and overuse of bone leads to the enhanced resorption of bone and the eventual loss of bone mass. Osteocytes are believed to be responsible for signaling the increase in active osteoclasts number and eventual resorption of bone. Studies have been conducted on how mechanical load on bone affects osteocytes. These studies show that increased number of osteocytes die through apoptosis in disused bone and the apoptosis of osteocytes trigger bone resorption. Presumably, normal loading conditions on bone induces a fluid shear force on osteocytes that stimulates the release of nitric oxide (NO). The NO in turn prevents the osteocytes from undergoing apoptosis, and therefore inhibiting the activation of bone resorption. However, under unloading or high loading situations the fluid shear is either disrupted as in the case of unloading, or the cells become desensitized to the force in over loading. Hence, the cells release insufficient or no NO to prevent apoptosis. The goal of this thesis is to analyze the effects that varying concentrations of NO will have on osteocytes that undergo apoptosis induced by Tumor-Necrosis-Factor-alpha.

Materials and Methods

We used MLO-Y4 murine bone derived osteocytic cells, which were provided by Dr. Linda Bonewald (University of Texas Health Center at San Antonio, San Antonio, Texas, USA). Cells were cultured in αMEM supplemented with 4% FBS, 4% bovine calf serum, 1% P/S. Cells were plated at a concentration of 360 x 10⁶ cells/ml. Cell passaging was done using Trypsin EDTA 10mm.

MLO-Y4 osteocytic cells were cultured for several days and then TNF-α (Recombiant Mouse TNF-α 10 ug, purchased from BioSourced: Invitrogen Camarillo, Ca USA) was added to obtain final concentrations of 1ng/ml, 10ng/ml, 100ng/ml. At varying time intervals apoptosis was assessed by different methods of quantifying apoptosis were used. The first experiment involved quantification of the effects of NO on osteocyte apoptosis. Six wells were set up for two different time points of 5 hours and 20 hours. Different concentrations of TNF-α were used, and there was a set of controls for each time point. Using trypan blue and the hemocytometer cell concentrations of live cells and dead cells could be determined. The results are shown in table 1. These results reveal the effect of TNF-α on osteocytes. The greater the concentration of TNF-α and the longer the time period, the greater the percentage of dead osteocytes. Similar results were shown when using the YO-PRO-1 fluorescence kit.

Table 1: Quantification of Osteocyte Apoptosis due to TNF-α with Trypan Blue

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control</th>
<th>10ng/ml</th>
<th>100ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 hours</td>
<td>Live(cells/ml)</td>
<td>62*10⁴</td>
<td>32*10⁴</td>
</tr>
<tr>
<td></td>
<td>Dead(cells/ml)</td>
<td>2*10⁴</td>
<td>10*10⁴</td>
</tr>
<tr>
<td>24 hours</td>
<td>Live(cells/ml)</td>
<td>74*10⁴</td>
<td>13*10⁴</td>
</tr>
<tr>
<td></td>
<td>Dead(cells/ml)</td>
<td>12*10⁴</td>
<td>48*10⁴</td>
</tr>
<tr>
<td>24 hours</td>
<td>Live(cells/ml)</td>
<td>14*10⁴</td>
<td>14*10⁴</td>
</tr>
<tr>
<td></td>
<td>Dead(cells/ml)</td>
<td>2*10⁴</td>
<td>37*10⁴</td>
</tr>
</tbody>
</table>

Discussion

We hypothesize that nitric oxide release from the SNAP donor, will lower the amount of apoptotic osteocytes. Using different assays, including a YO-PRO-1 fluorescence kit, and Trypan blue it has been demonstrated that the greater the concentration of TNF-α the higher the number of cell deaths. Also the concentration of nitric oxide directly affects the percentage of apoptotic osteocytes. The higher the concentration of nitric oxide the less the effect that TNF-α has on osteocytes.

Acknowledgements

I want to thank the Tulane Biomedical Engineering Department for the resources provided to conduct my thesis.
ANALYSIS OF THE EFFECTS OF NITRIC OXIDE ON HYPOXIA OF OSTEOCYTES USING WESTERN BLOTS

Brent M. Smith1, Yuefeng Han1, Erica N. Nierth-Simpson2
1Tulane University, Department of Biomedical Engineering
2Tulane Cancer Center

Introduction
The processes of bone modeling and remodeling allow for the skeleton to constantly adapt to its surroundings. Remodeling is often triggered as a response to bone disuse or overuse. When in disuse, bone is reabsorbed at a quicker rate than it can be formed. This results in thinner, weaker bones. However, how bone resorption is initiated in bone disuse is poorly understood. Recent studies show that bone disuse increase hypoxial and apoptotic responses of osteocytes, and these responses are temporally and spatially correlated with bone resorption. Microrcracks can occur in bone structures when they have been subjected to overuse. When a microrcrack occurs, osteocytes, which are believed to be the main sensory cells within bone, surrounding the area exhibit a hypoxial response and go through apoptosis later. Similarly, these osteocytic responses are believed to mediate bone targeted remodeling. The hypoxial response here is characterized by upregulation of HIF-1α and VEGF yet its causes are unknown.

We hypothesize that the hypoxial response by osteocytes is due to the reduced release of nitric oxide in disused bone.

Theoretical studies have shown that dynamic loading on bone induces oscillating interstitial fluid flow in the lacunar-canalicular porosity of cortical bone, and generates fluid shear on osteocytes in the order of 10 dyn/cm². It is well established that fluid shearing of this magnitude triggers cellular signaling in osteocytes, such as increase of intracellular Ca²⁺, release of PGE₃, (most importantly) release of nitric oxide, etc. However, how this signaling exactly functions in bone modeling and remodeling remains unknown. Interestingly, nitric oxide has been shown to reduce cells from hypoxia in many types of cells. We propose that nitric oxide has the same effect on osteocytes. In the case of disused bone, there is no oscillating interstitial fluid that stimulates osteocytes to release nitric oxide and, thus, release of nitric oxide by osteocytes is reduced. As a result, osteocytes are less protected from hypoxia in disused bone. This explains the observation that disuse of bone enhances hypoxia in osteocytes.

The goal of this thesis is to analyze the effects various concentrations of nitric oxide have on osteocytes in a hypoxic environment. Cobalt chloride is used to simulate a hypoxic environment and will trigger the upregulation of hypoxia inducing factor 1-alpha (HIF-1α). To quantify the effects of nitric oxide on osteocytes in hypoxia, we will treat the osteocytes with different concentrations of cobalt chloride over varying time intervals. Western blot analysis is then used to detect the expression level of HIF-1α. Eventually, we will test our hypothesis that NO will inhibit hypoxia of osteocytes.

Materials and Methods
The MLO-Y4 osteocyte-like cell line was cultured in α-MEM supplemented with 4% FBS, 4% CBS, and 1% PBS on 100 mm Petri dishes. New media was placed in the dishes every 48-36 hours. The cells were then split in 35 mm dishes for experimentation. Two different media were used in the experimental dishes. Initially the α-MEM supplemented with 4% FBS, 4% CBS, and 1% PBS was used. Due to unclear results, a new media containing 5% charcoal-stripped FBS in Dulbecco’s modified media (DMEM) supplemented with essential amino acids, non-essential amino acids, sodium pyruvate and the antibiotic gentamicin was used in an attempt to clarify the results.

Experimental dishes were prepared using 20μM, 100μM, and 500μM of CoCl₂ in sterile PBS solution. A plate with each concentration was created in addition to a fourth as a control. An incubation period of 2, 6, 12, and 24 hours were tested for each set of concentrations. The cells were then collected and stored in a -80°C freezer until further experimentation.

A protein assay was then conducted to normalize the amount of protein per sample. Upon completion, either a western blot or a PCR was performed on each sample. The western blot was performed using a HIF-1α antibody provided by Cell Signaling Tech (Danvers, MA). The PCR was conducted using a primer designed by Beacon Builder from Premier Biosoftware (Palo Alto, CA).

The experiments will then be repeated with the addition of varying concentrations of SNAP from Invitrogen (Carlsbad, CA) to the previously described setup.

Results
The results of the first trial of western blots show increases in expression levels of HIF-1α with increasing CoCl₂ concentrations during the 6 and 12 hour trials. The 2 and 24 hour results were inconclusive as they did not follow any trends.

Discussion
This thesis is designed to analyze the effects of NO on osteocytes in a hypoxia environment. Initial results have been inconclusive. Potential reasons for this could include poor technique and the initial media used. These are being corrected by practice and using the new media as described in the materials section. Another sources or error is the use of a poor quality antibody. PCR analysis will be used to support the western blot results in future experiments.

Acknowledgements
This work was supported by the Department of Biomedical Engineering at Tulane University.
A study of antioxidant cytotoxic effects with mesenchymal stem cells (MSCs) for development of an in vivo osteogenic sarcoma tumor xenograft model

Trivia P. Frazier1, Radhika R. Pochampally2,3, Patrice Penfornis2, Michael Harris2, Zheng Chen2
Tulane University Biomedical Engineering Dept1, Tulane Center for Gene Therapy3, Department of Pharmacology and Tulane Cancer Center3, TUHSC, New Orleans, LA-70112

Introduction

There is increasing evidence suggesting that human Mesenchymal Stem Cells (MSCs) play a major role in the establishment of solid tumors, and that antioxidant-specific doses inhibit cancer cell viability. We hypothesized that MSC niche in bone marrow plays a role in initiation and/or progression of osteogenic sarcoma (osteosarcoma, OS), and that we can block this process through drug treatment at the proper lethal doses. The goal of this project is to determine the concentration of antioxidants that differentially induces apoptosis in SD-MSCs and determine the effect of drug in an in vivo osteosarcoma tumor xenograft model.

Materials and Methods

In phase I, cancer cells were established from biopsies of osteogenic sarcoma tumors. The cells were cultivated until 70% confluence in standard conditions (37°C, 95% air; 5% CO2); 1 million cells in a 15cm2 dish, in a solution consisting of 90% Dulbecco’s Modified Eagle Medium (DMEM) 1X and 10% fetal bovine serum (FBS) for OS cells, and 80% alpha minimum essential medium (αMEM) with L-glutamine, 20% FBS, and 1% penicillin-streptomycin (CCM) for MSCs. The cells were treated with antioxidant-specific concentrations in triplicates of 11 drugs, each in a 12-well plate. The cells were counted using a CyQUANT assay and a fluorescence microplate reader.

OS cells and MSCs were separately cultivated, resuspended, counted using a hemocytometer, and then diluted to a concentration of 1 million cells per ml of Hanks Balanced Salt Solution (HBSS) media. The cells were then subcutaneously co-injected into one side of female athymic nude mice (nu/nu). The other side, the control, was injected only with OS cells. In phase II, OS cells were subcutaneously injected into nude mice. Following tumor development, antioxidants were prepared and the dosages obtained from phase I.

Results

First, we demonstrated that subcutaneous co-injection of human OS cells with MSCs have no effect on tumor initiation occurrence, but induced a 2 to 14 fold increase of tumor volume in a nude mouse model. As drug treatment could lead to the inhibition of the OS tumor growth, we tested the effect of various antioxidants on cell viability of MSCs, serum deprived MSCs (SD-MSCs; which present features of very early progenitor cells) and OS cell lines. Of the eleven antioxidants tested, curcumin, from the Indian spice turmeric, dramatically reduced osteosarcoma cell and MSC viability by >90% at a concentration of 100 µg/ml. Interestingly, SD-MSCs are very sensitive to curcumin: concentrations as low as 10µg/ml decreased the cell viability by >50% (Figure 1).

Investigations into the acute effect of curcumin on tumorigenesis (Figure 2) reflect a decrease in tumor progression after 24-hour periods of intra-tumoral injection of curcumin into ongoing Osteosarcoma mouse xenograft models.

Discussion

These results suggest that MSCs support osteosarcoma tumor growth, that SD-MSCs seem to be more sensitive to curcumin than the MSCs, and that an antioxidant-specific treatment approach could decrease or inhibit tumor progression through a pro-apoptotic mechanism.

Acknowledgements

This research is supported by the Louisiana Cancer and Gene Therapy Research Consortium, HCA, the Health Care Company; and The W. M. Keck Foundation.
DETERMINATION OF MESENCHYMAL STEM CELL FATE DURING ANGIOGENESIS

Brooke L. Lovett, Walter L. Murfee
Department of Biomedical Engineering, Tulane University, New Orleans, LA

Introduction
Angiogenesis, the formation of blood vessels through the process of capillary sprouting, is critical for maintaining homeostasis in adult tissues.

Peri-endothelial cells, which include fibroblasts and pericytes, support capillary sprouting through paracrine signaling and provide mechanical stability to newly formed capillaries. Recent studies have focused on the origins of pericytes and their contributions to angiogenesis, suggesting that they develop from mesenchymal stem cells (MSCs). However, MSC fate and the origin of pericytes remains unclear.

The objective of this study is to examine the fate of MSCs in wound healing induced angiogenesis. A further understanding of the relationship between MSCs and pericytes could lead to improved therapies aimed at manipulating angiogenesis.

Materials and Methods
An angiogenic response was induced in adult immuno-deficient rats by exteriorizing 4 mesenteric windows for 20 minutes. During this time, human green fluorescent protein (GFP) labeled mesenchymal stem cells (MSCs) were injected onto the mesenteric windows. After 1 and 3 days, the rats were sacrificed and the stimulated mesenteric tissues were harvested. The tissue samples were then labeled with NG2 (a pericyte marker), and CD31 (an endothelial marker). Fluorescence microscopy was then used to analyze and image the tissues.

Results
Examination of CD31 and NG2 stained tissue from animals harvested 1 day post surgery showed vascular growth characterized by tortuous vessels, capillary sprouts, and thickening of tissue. Tissue from animals with 3 days of recovery exhibited further vascular growth, characterized by extensive capillary networks spanning multiple focal planes, increased vessel tortuosity and increased tissue thickening.

Bright green nucleated cells were present in all tissues analyzed, which included unstained tissue, tissue that was not exposed to labeled mesenchymal stem cells, and staining controls. These cells were observed randomly throughout the tissue, but were observed at a much higher frequency near vascular vessels. Bright green cells were most dense around pre-capillary arterioles and venules. At this location, they aligned with vessels, and were found on multiple focal planes surrounding post-capillary vessels (Figure 1). Bright green cells were also observed “leading” capillary sprouts, positioned in front of and aligned with developing endothelial sprouts (Figure 2).

Discussion
This study attempts to track the fate of injected GFP labeled MSCs during angiogenesis in adult tissue. While bright green cells were observed to have similar morphogenesis to previously reported GFP bone marrow derived cells in similar remodeling scenarios, the presence of MSCs in our model remains unclear due to auto-fluorescence of similarly shaped cells. Still, the potential GFP+ MSCs were not found wrapping capillary vessels. Instead, these cells were preferentially located near post capillary vessels, indicating MSCs are involved in remodeling of existing vessels in addition to capillary sprouting. The less frequent observation of these cells leading capillary sprouts suggests that MSCs play a paracrine role in angiogenesis.

The identification of the bright green nucleated cells as the injected MSCs is problematic because this type of cell with relatively the same fluorescent intensity was found in control tissues not injected with MSCs. This suggests the presence of morphologically similar auto-fluorescing cells. Since the quantity of green cells in the MSC injected tissue is increased compared to control tissues, we hypothesize that portions of the green cell population are indeed MSCs.

This work will potentially offer valuable perspectives for understanding MSC function during angiogenesis and the efficacy of directly injecting MSCs for cell therapies.

Acknowledgements
This project was made possible by Tulane University in New Orleans, Louisiana.
**Introduction**

The pathology of hypertension is commonly associated with increased microvascular resistance due, in part, to a loss of blood vessels, defined as microvascular rarefaction. Given that peripheral microvascular resistance and rarefaction are associated with an increase in blood pressure, the reversal of this process represents a potential therapeutic option. Current hypertensive therapies include oral delivery drugs, dietary regimens, and relaxation therapies. These have not, however, addressed the microvascular alterations associated with hypertension. For the design of therapies aimed at reversing rarefaction, investigating the ability of hypertensive microvascular networks to undergo angiogenesis, the growth of new vessels from existing vessels, is warranted. Thus, quantification of microvascular remodeling in hypertensive models proves beneficial to the study of hypertension.

The purpose of this study is to quantitatively compare the angiogenic response in adult hypertensive and normotensive animals in response to a wound healing stimulus. To establish a rationale for considering therapies to reverse microvascular rarefaction, an overview of hypertension, microvascular network alterations, angiogenesis in hypertension, and methods for quantifying microvascular growth will be discussed.

**Materials and Methods**

Experiments were conducted according to the regulations of the Tulane University Institutional Animal Care and Use Committee. In order to evaluate the angiogenic capability of hypertensive microvascular networks, the vascular density, vascular area, and the number of capillary sprouts were compared across mesenteric tissues harvested from 4 age-matched experimental groups: 1.) Unstimulated adult Wistar rats (n=4, normotensive control), 2.) Adult Wistar rats (n=4) stimulated by a 3-day wound healing model, 3.) Unstimulated SHR (n=3), and 4.) SHR (n=2) stimulated by a 3-day wound healing model. For the wound healing model, animals were initially anesthetized with intramuscular (IM) injections of ketamine, xylazine, and atropine. Under sterile conditions, a 6-window portion of the mesentery was removed and placed on a plastic stage, which was pre-soaked in 100% ethanol to disinfect and then rinsed with sterile saline. Two central tissue windows were marked with silk sutures, and all windows were exposed for twenty (20) minutes and returned to the abdominal cavity.

Upon post-surgical examination at time points (n=2) of days 0 and 3, he animals were anesthetized and subsequently euthanized via direct, cardiac injection of pentobarbital. The mesenteric windows (6) were surgically located, harvested, and whole-mounted. Microvessels were identified by immunolabeling for PECAM, an endothelial cell marker. Images of the network were taken and examined with a microscope and camera accessory at each time point. A representative area of the montaged network was used to compute the number of sprouts and other metrics to be presented.

**Results**

Surgical examination of the mesenteric windows that underwent wound healing stimuli suggests that the SHR strains exhibit increased angiogenesis. This preliminary observation is also associated with enlarged adipose tissue and prominent vascularization along the edges of the windows. This is indicative of angiogenesis in adult hypertensive animals.

Evidence for reversed angiogenesis in hypertensive tissues is supported by the observation of vascular density within SHR versus normotensive microvascular networks (Figure 1).

**Discussion**

The preliminary results of this study suggest that adult hypertensive microvascular networks are able to undergo angiogenesis. This is in line with previous studies that note the potential for vessel growth in the SHR model. Our findings and the completed quantitative analysis will add to our understanding of the state of the microcirculation during hypertension and lead to future studies regarding the presence of critical angiogenic factors.

If initial inferences are incorrect, there still exists a better understanding of the microvascular remodeling due to angiogenic stimuli in adult, hypertensive rats. This proof of principle study will also provide motivation for examining therapeutic strategies aimed at reversing microvascular rarefaction associated with hypertension.

**References**


**Acknowledgements**

Research was supported by the Department of Biomedical Engineering at Tulane University.
EVALUATION OF LYMPHATIC/BLOOD VESSEL CONNECTIONS AND THE PRESENCE OF PERICYTES ALONG LYMPHATIC SPROUTS DURING MICROVASCULAR REMODELING

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Introduction
Normal functioning lymphatic and blood vascular networks are important to maintain fluid tissue balance in the body. Abnormal function of these networks has been implicated in tumor growth, cancer metastasis and lymphedema. Recently, the current idea that these two networks are distinct has been contested with new evidence of connections at the microvascular level in quiescent adult rat mesenteric tissue (1). Direct cell interactions at the microvascular level are supported by the involvement of common players. One such potential common player is vascular pericytes. Pericytes are periendothelial cells that play a role in blood vessel growth and have recently been suggested to be involved in lymphatic vessel growth. The objective of this study will be to quantify the location and occurrence of lymphatic/blood vessel connections; as well as quantify the association of pericytes along lymphatic capillary sprouts during tissue remodeling in adult mesenteric tissue.

Materials and Methods
For this study, 8 intact adult male wistar rats will be divided into two experimental groups: unstimulated control rats and rats stimulated by a wound healing model.

For the wound healing model, rats were anesthetized and six mesenteric windows were exposed on a sterile stage for twenty minutes as shown in figure 1. This method is known to induce microvascular growth. The animals were then sutured closed and allowed to heal for three days. After this time the animals were euthanized and the stimulated windows were removed and mounted on slides. For the control groups animals were euthanized and six mesenteric windows were immediately harvested.

The tissue specimens were then immunolabeled with PECAM, an endothelial cell marker and NG2, a pericyte marker.

Results
The connections between the blood and lymphatic vessels were quantified by the percentage of blind ended capillaries connecting to a vessel of the other system. The diameter of the vessel where the connection is present will be measured to determine where these connections exist in the vasculature. An example of the connections found at the capillary level in unstimulated tissue is shown in figure 2.

Pericyte presence along lymphatic sprouts will be quantified as the percentage of sprouts invested with NG2 positive cells with characteristics of pericyte morphology.

Discussion
The results of this study will determine the occurrence and location of connections between blood and lymphatic vessels during microvascular remodeling and the association of pericytes within lymphatic vessel growth. Collectively this study will offer novel perspectives for understanding lymphatic vessel function.

References

Acknowledgements
Funding for this research was provided by the Biomedical Engineering Department of Tulane University.
Introduction

The Fontan procedure and Total Cavopulmonary Connection (TCPC) have been the most common surgical procedures implemented to mitigate various congenital heart defects resulting in a non-functional right ventricle. The procedures call for a direct connection between the vena cavae and the pulmonary arteries, effectively routing venous return blood directly into the lungs. Blood movement into the lungs in this system is propelled by the pressure difference created by the pumping left ventricle rather than active pumping by the right ventricle. Since this passivated system suffers from weakened power, optimizing this connection becomes a major concern.

This study analyzes a simple geometric analogue to the four-way vessel connection outcome of a Fontan procedure in an attempt to elucidate how energy losses develop in the system. Computational methods have been used in the past to examine the Fontan system but the inclusion of laminar pulsatile flow, as seen in vivo, has not been rigorously considered. The presented model will also include varying degrees of stenosis in the left pulmonary artery, as these are commonly observed postoperative anomalies in the system that significantly effect performance.

Materials and Methods

COMSOL Multiphysics 3.3 (COMSOL Inc, Burlington MA) was used to create 3D finite element models on which simulations were run. The model consists of 4 tubes (diameters \( \sim 13\text{mm} \)) connected at 90° to each other in the same plane, as modeled in previous studies\(^2\). Stenoses are added to the system as seen in Figure 1B. The superior vena cava is bound with steady pressure while the pressure at the inflow of the inferior vena cava changes sinusoidally with time as an approximation of the experimental findings of Houlind et al\(^1\). The outflows (pulmonary arteries) are bound with a pressure of 0.

The system is governed by the Navier-Stokes equations and the walls are assumed rigid. We assume that the density of the blood fluid is \( \rho = 1060 \text{ kg/m}^3 \), and the dynamic viscosity, \( \mu = 3.5 \times 10^{-3} \text{ Pa}\cdot\text{s} \). The angular frequency at the inferior vena cava, \( \omega = 2\pi \text{ radians/second} \) to correlate with a fast heart rate of 120 bpm.

The Womersley number (\( \alpha \)) is a dimensionless parameter used to approximate the relationship between viscous and inertial interactions in oscillatory and pulsatile flow conditions (Equation 1). At the IVC inflow, we approximate \( \alpha = 9 \). It should be noted that while this flow is unsteady, it is still laminar.

\[
\alpha = \frac{R}{\sqrt{\nu \omega}}
\]  

(1)

Energy over the outflow and inflow boundaries may be evaluated:

\[
E = - \int P \left( \mathbf{u} \cdot \hat{n} \right) \, dA
\]  

(2)

Where \( P \) is the hydrodynamic force per cross sectional area \( A \), \( \mathbf{u} \) is the velocity, and \( \hat{n} \) is the normal vector. Taking the difference of the energies at the inflow and outflow boundaries will determine the total energy lost in the system.

Results

Figure 1. The modeled Fontan system. Arrows indicate flow direction. A) The ideal system B) inclusion of a 50% stenosis in the left pulmonary artery (LPA)

Figure 2 demonstrates how pulsatility can affect transient flow dynamics. When \( \alpha \gg 1 \), inertial forces cause the oscillatory (2A) flow profile to lag out of phase with the steady state solution (2B) due to inertial interactions.

Discussion

Since the reported biological parameters of the Fontan system to be modeled result in \( \alpha = 9 \), it is conjectured that pulsatility will have a significant effect on the transient energy losses in the model. Energy losses will be evaluated over time and compared to steady state solutions. Varying levels of stenosis will also be added to the system, as it is expected that the inclusion of such geometries will lead to further transient flow anomalies and redistribute right/left lung perfusion.

References

\(^1\)Houlind et al. Pulmonary and caval flow dynamics after total cavopulmonary connection. Heart 1999

\(^2\)Pekkan et al. Total Cavopulmonary Connection Flow with Functional Left Pulmonary Artery Stenosis. Circulation 2005

Acknowledgements

COMSOL Multiphysics was provided by the Tulane Department of Biomedical Engineering
Introduction

Regeneration in the central nervous system (CNS) poses many problems that have been investigated in recent years. Glial scarring and the natural inhibition of CNS regeneration have limited the success of axonal regeneration across a transected area of the CNS. The focus of this research is on a specific portion of the CNS, particularly the optic nerve. We chose the optic nerve as a model because it's a classic example of a CNS pathway that does not regenerate following injury.

Recently, researchers have found that regeneration of these retinal neurons via a self-assembling peptide nanofiber scaffold (SAPNS) following severance of the optic tract is not only feasible but is potentially a viable solution to neuron regeneration in the CNS. The SAPNS has been shown to facilitate neural growth due to the absence of contamination, both chemical and biological, and also the resemblance of the resulting nanofiber network to the extracellular matrix (ECM).

The primary goal of the research described in this thesis is to create a system of hydrogels, involving poly(ethylene glycol) (PEG) and the SAPNS, which will guide and facilitate the growth of retinal neurites while creating an aspect of an in vitro model of the optic chiasm. The PEG and the SAPNS have been chosen because they have been shown to be inhibitory and permissive to neurite outgrowth, respectively. The hydrogels will be arranged such that the SAPNS hydrogel will be a 1mm thick cylinder surrounded by photopolymerized PEG hydrogel, in a disk-like fashion (Figure 1).

Materials and Methods

For the SAPNS, we have used PuraMatrix 1% (w/v), which has been shown to facilitate neurite outgrowth. The SAPNS solution is prepared by diluting the solution to .25% (w/v) with distilled water (DI). To trigger self-assembling of the nanofiber matrix, either PBS pH 7.4 or Hank’s Balanced Salt Solution (HBSS) is added to the .25% (w/v) solution of PuraMatrix. The solution is incubated at 37°C for one hour to induce gelling.

We have also used poly(ethylene glycol) 1000 dimethacrylate (PEG), which has been shown to be inhibitory to neurite outgrowth. A solution of 8% PEG is prepared by adding 80mg of PEG to a solution of 1ml of DI or 1ml PBS pH 7.4. Several concentrations of PEG were tested to find the optimal concentration for photopolymerization (PP). In order to induce PP, we added a photoinitiator (PI). We have experimented with both Irgacure 2959 and 2-hydroxy-2-methyl-propiophenone, 97% (HMPP) utilizing different concentrations of each to optimize our PP. The HMPP is mixed with the PEG solution to make a 1% solution of the PI. A solution of 1% Irgacure is prepared by mixing 9ml DI, 1ml of PBS pH 7.4 and 100mg Irgacure. The resulting PEG-PI solution was exposed to UV light with the OmniCure S1000 UV light source. Several exposure times were tested to optimize the final polymerized gels. The solutions were polymerized between two glass slides, and disks were cut from the gel using biopsy punches.

Discussion

While we were able to polymerize the DI-diluted PEG-HMPP solution, the gel completely dissociates in the presence of HBSS or PBS. We hypothesize that this may be due to the osmotic force created by the higher salt concentration of the PBS or HBSS. This is not ideal as in the situation where we will be culturing, the gel will be exposed to culture media and this may compromise the viability of the polymerized scaffold. Since the HMPP is insoluble in PBS, we are abandoning this PI.

We also found that the PuraMatrix was seemingly absorbed into the PEG gel. We believe this is because the PEG gel was not swelled with PBS before gelling PuraMatrix in the center, thus resulting in the PuraMatrix gel being pulled into the PEG.

From now on, we will be using the Irgacure 2959 as our PI. We will be swelling the PBS-dilute PEG disks in PBS and gelling PuraMatrix in the center of the PEG gel. We will also be culturing embryonic rat embryos on this PuraMatrix gel. Histology will be performed to confirm the growth of the retinal neurites into the PuraMatrix gel.

Acknowledgements

The support of the Department of Biomedical Engineering at Tulane University.

Figure 1. A photopolymerized 8% PEG/Irgacure 2959 gel cut into a disk with the center cut out. The bubbles present are air trapped between the gel and the glass slide.
DESIGNING A FUNCTIONAL HYDROGEL WITH EMBEDDED PHOTOLABILE PROTEINS FOR THE GUIDANCE OF RETINAL GANGLION CELL GROWTH

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Introduction

It is well established that the tissue of the central nervous system (CNS) is unable to effectively heal itself after injury. This is due to both the neurons’ lack of regenerative properties and several barriers in the healing process. Unlike naturally regenerating tissues such as skin, scar tissue critically impairs the function of CNS tissue because of the environment formed by the scar tissue. In the process of clearing away debris at the injury site, phagocytes digest the injured nerve cells, creating voids that separate severed axons that could potentially reconnect. Even if the axons are spatially able to reconnect, there is no guarantee that the axons will initiate extension to their severed partners.

An especially critical area in the CNS is the optic chiasm, the vision-processing unit responsible for depth perception in most mammals. Located on the ventral side of the brain, the optic chiasm is the point where the two optic tracts cross and enter the retinas as the optic nerves. These optic tracts are the bundles of retinal ganglion cells (RGCs) that connect to the retina in each eye and are responsible for vision. Since the optic tracts cross, the brain processes information received by the eye with the visual center contralateral to the eye. The RGCs relay the neural signals necessary for visual information from the retina to the brain. As such, a schism in the axons of the RGCs results in blindness in the affected eye.

Since the RGCs are part of the CNS, repairing and directing the growth of RGC axons remain difficult biomedical issues to overcome. To begin the trek toward a viable solution, research has focused on how RGC axons are guided through the optic chiasm. Understanding this guidance pattern and its respective molecules may allow researchers to manipulate it in order to repair the optic nerves. Recent research has shown that modified hydrogels make excellent in vivo scaffolds for tissue repair because of their abilities to mimic the extracellular matrix, promote axonal growth and inhibit scar tissue formation. Since the hydrogels are networks of synthetic peptide sequences, the scaffold can be modified to allow certain proteins to adhere to the scaffold.

This thesis focuses on synthesizing a protected cysteine that can functionalize a self-assembling peptide scaffold. This custom-made cysteine has protective chemical groups that break off upon exposure to ultraviolet (UV) light, revealing active sites for biomolecule binding. Since the cysteine is light-activated, the location and number of active sites can be finely controlled. Known RGC guidance molecules can bind to these active sites and guide axons through the hydrogel.

Materials and Methods

S-2-nitrobenzyl-cysteine (S2NBC) was synthesized using 0.662g of L-cysteine dissolved in 25mL of water and 1.18g of 2-nitrobenzyl bromide dissolved in 25mL of methanol. Both solutions were cooled to 4°C and then mixed together for 2 hours over ice to maintain the 4°C temperature. The flask in which the solutions were mixed was covered in aluminum foil to prevent exposure to UV light. After mixing the solutions for 2 hours, the precipitate was filtered out using a membrane filter and washed three times with cold water.

Discussion

The S2NBC was synthesized in a lab with wide windows blocked only by standard blinders. While care was taken to minimize exposure of UV light to the sample, this experiment should be done in a dark room to ensure minimal exposure to UV light and, thus, maximize the yield of protected cysteine versus deprotected cysteine. After synthesis, S2NBC needs to undergo purity tests in order to verify that the nearly all of the precipitate is the protected cysteine.

Once the protected cysteine has undergone purity tests, it will be sent to a peptide synthesizing company. The company can then synthesize a custom self-assembling peptide scaffold with the protected cysteine embedded within it. This scaffold with the photolabile proteins will ultimately be modified with RGC guidance molecules via attachment to free thiol groups to promote axon growth through the scaffold.

Acknowledgements

I would like to thank the Department of Biomedical Engineering at Tulane University for providing the laboratory and materials necessary to begin and complete this thesis. I would also like to thank the Department of Chemistry at Tulane University for protocol guidance and the Coordinated Instruments Facility for NMR assistance.
GENETIC TRANSFECTION TO TARGET RETINAL GANGLION CELLS

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Introduction
Current work in neuroscience and neuroengineering is dedicated to determining how neural activity in a specific set of neurons contributes to a particular neural computation, behavior, or pathological state. This research is done with the ultimate goal to answer fundamental questions: What is the function of neural synchrony and precise spike timing in circuit pathology? How can neural circuits that have been destroyed by neurological and psychiatric disorders be restored to normal activity? Light-sensitive channels, such as Channelrhodopsin-2 (ChR2) and Halorhodopsin (Halo), have recently been successfully expressed in various neuronal cells and used to stimulate or silence these cells, respectively.

Channelrhodopsin-2, an ion channel that exhibits a nonselective cation flux when exposed to blue-green light, demonstrates high-precision spike initiation. Halorhodopsin, a complementary high-speed hyperpolarizing tool, optically inhibits the neural activity.

Research in our laboratory aims to develop a series of 3D tissue culture models to study how functional biomaterials may influence axon regeneration. The purpose of this study, specifically, is to successfully transfertretinal ganglion cells with ChR2 and Halo in cultured retinas. In the future, activation and inactivation of these engineered neurons will be verified via whole cell patch clamp recordings. This technique will be used to confirm synapse formation between co-cultures and also as an attempt to correlate axon guidance with the structural and molecular stimuli that other researchers will engineer into 3D cultures.

Materials and Methods
Plasmid constructs for ChR-2 and Halo were obtained from AddGene, Inc. (plasmids 15814 and 14750). Plasmids were produced in E. Coli and DNA isolated with a Qiagen DNA Miniprep kit. Lentiviral vector particles were produced in 293T cells, concentrated by ultracentrifugation, and titers were determined by real-time PCR.

The retina was dissected from an adult rat within an hour of the animal being sacrificed. Between one and two hours of the dissection, the tissue was transfected with the purified DNA plasmids containing ChR-2 and Halo. Transfected tissue was then grown in culture overnight in 1.5ml cell media with 37°C and 5% CO2. After 24 hours, the tissue was viewed with fluorescence microscopy. The tissue was then fixed onto a slide with mounting media and imaged.

Results
This study focuses on the lentiviral expression of Channelrhodopsin-2 and Natronomas pharaonis Halorhodopsin (Halo) in retinal ganglion cells. Halo tagged with a fluorescent protein can maintain strong expression weeks after the original infection, and ChR2 sustains stable expression as well (Zhang et al., 2007).

This study expects to achieve successful transfection of ChR2 and Halo in a fully intact retina in vitro. When the whole retina is imaged, the retinal ganglion cell layer will be identified and the fluorescence can be detected to determine successful transfection. Figure 1 identifies individual transfected cells, but for this study the whole tissue will be imaged. After transfection is verified, the study will attempt to activate and inactivate the retinal ganglion cell layer optically, and activation will be verified via patch clamping methods.

Figure 1: Hippocampal neurons demonstrating strong co-expression of NpHR-EFYP and ChR2-mCherry (Zhang et al., 2007)

Discussion
This “functional” neuronal tissue culture model is a combination of several initiatives and the ultimate goal of the lab (see Figure 1). A micropatterned cell-adhesive gel will be placed in a cell-repellent gel. Neurite outgrowth will then only occur within the cell-adhesive hydrogel, allowing for maximum control in axon guidance. Within this cell-adhesive gel, an axon guidance molecule will be immobilized. Neurites expressing receptors for this molecule will cross here, and neurites that do not express receptors for this will remain ipsilateral. A retinal explant expressing the light-sensitive channels, ChR2 and Halo, will be placed on the top surface of the cell-adhesive gel with brain tissue slices on the bottom. A digital micromirror device-patterned light source will project blue and yellow light on the retinal explant, causing activation and inactivation of the engineered retinal ganglion cells, respectively. The depolarizations and hyperpolarizations of the transfected retinal ganglion cells will be measured via patch clamping.

Currently, this study is performed in vitro with live tissue explants. This combined system will eventually be applied to mammalian systems in vivo, linking neural activity patterns with circuit behaviors, and may eventually develop precise optical therapeutic treatments (Zhang et al., 2007). A recent experiment has shown it is possible to genetically alter the inner retinal neurons remaining from a damaged retina, enabling these cells to function in place of lost photoreceptors (Bi, et al. 2006). The introduction of ChR2 was shown to facilitate retinal ganglion cells so they could encode light signals, and it demonstrated the ability to restore visual responses in the visual cortex. These results can be used on our lab as the neuronal tissue culture model is finalized and developed.

References

Acknowledements
Plasmid Constructs were obtained courtesy of Dr. Edward Boyden via AddGene, Inc.
Gene Therapy, LSU Health Sciences Center Vector Core
Tulane Research Enhancement Fund, Phase II Program B
Development of a Multi-Scale Light Based Neurostimulator

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Introduction

The study of neural networks is hindered by the lack of a technique to stimulate multiple neurons simultaneously. In 2007 methods to alter neurons to express a pair of ion channels which respond to light were described in literature. Genetic induction of light-sensitivity to virtually any neuron, including those in tissue and in vivo, suddenly made possible a new method for studying neural networks: a light-based technique where the resolution of stimulation was limited only by the patterning of light onto the culture. A high-resolution light patterning device, coupled with these new light-sensitive neurons, could lead to a new paradigm in neurological research: One in which neurons can be fired or silenced, individually or in groups, anywhere within an entire culture, in milliseconds, in a completely non-destructive way. The goal of this project is to develop a device which patterns light at the micrometer scale, capable of stimulating cultures of genetically altered light sensitive neurons. 

Digital Light Projection technology used in video projectors and lithography systems utilizes a Digital Micromirror Device (DMD) to pattern light. Simply electronically controlled arrays of mirrors, DMDs hold the promise of being able to pattern stimulating blue and yellow light onto cultures of neurons with great control and precision.

Ultimately, computer-controlled patterning of projected light will be adaptable to stimulate many different light sensitive cells in a variety of settings. The stimulating functions will be married to a series of microscopes to observe and verify the stimulation and response of the cells. All of the components of stimulation and observation will be combined into one monolithic device to be used as research equipment in the study of light sensitive neural tissue and networks of cells.

Materials and Methods

Patterning of light is to be done with a DMD. DMDs are available in development kits from Texas Instruments but are also commonly found in video projectors. For the purposes of the construction of the Neurostimulator, utilizing a DMD in a video projector offered a number of benefits including working electronics to control the DMD, ability to display video and images composed on a desktop computer and low cost relative to the development kits. We chose a Mitsubishi SD-105U 600 resolution office video projector.

Producing light of appropriate wavelength to stimulate or silence cells was accomplished by placing band-pass color filters in front of a full-spectrum white light source. We used theatrical gel filter for their light weight, low cost and durability.

Table 1: Ion channels, activating light wavelengths and effect.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Light Wavelength</th>
<th>Effect on Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Channelrhodopsin</td>
<td>~565nm</td>
<td>Depolarization</td>
</tr>
<tr>
<td>Halorhodopsin</td>
<td>~490nm</td>
<td>Hyperpolarization</td>
</tr>
</tbody>
</table>

For projection of patterned light onto the stage and visualization of the entire field of stimulation we used a World Precision Instruments model INV-IQ903 Inverted Microscope. 

World Precision Instruments model INV-IQ903 Inverted Microscope. The INV-IQ903 features fixed optics so the microscope was disassembled and the stage removed. The optics were remounted to the microscope stand body to be vertically adjustable in the Z-direction and the microscope stand body was mounted on a pair of linear slides providing movement in the X and Y directions beneath the stage.

Results

An Optical Neurostimulator was built (Figure 1) which projected patterns of light onto materials placed at the stage. Materials which are semitranslucent can have patterns of light projected upon them. The observation microscope can view these materials and verify that patterned light is being projected in a controlled way.

Light of one wavelength at a time may be projected producing patterns of blue light and no light or yellow light and no light.

Patterns projected through the projecting microscope were crisp at low magnification but blurred when magnification was increased from 1x to 10x.

Discussion

Adapting the video projector to pattern light proved trying. Safety features of the projector made modifications very difficult. Using an inexpensive video projector saved cost and allowed us to prove the concept of light patterning using a DMD but for extended research or commercial production direct control of the DMD would be needed.

The function of the Neurostimulator needs to be verified using transfected cells to determine if the patterned light will correctly stimulate them.

Acknowledgments

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Biomedical Engineering Undergraduate Research and Design

Conference Abstracts
THE EFFECT OF BACTERIAL COLONIZATION ON THE MATERIAL STRENGTH OF BIOLOGICAL TISSUE GRAFTS

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Introduction
Each year there are one million inguinal and nearly twenty-thousand ventral hernia repairs performed in the United States. In recent years the use of an implantable mesh has proven to be the preferred method for repair. The meshes used can be divided into two categories, synthetic and biological tissue meshes. Synthetic meshes have been widely used due to their strength and ease of handling. They have also been linked to bowel adherence and fistula formation as well as a high susceptibility to bacteria and chronic infections.

Biological tissue grafts have shown a high amount of biocompatibility and promotion of neovascularization and tissue in-growth. Due to their resemblance to naturally occurring tissue they result in less of a foreign body reaction. On average six percent of hernia operations result in infection possibly requiring the explantation of the implant. As of yet the effect of bacterial colonization on the material strength of biological tissue grafts has not been quantified. The purpose of this study is to determine the degradation of Surgisis caused by infection of either Methicillindresistant Staphylococcus aureus, MRSA, or by Pseudomonas aeruginosa, PA.

Materials and Methods
Samples of 8-ply Surgisis measuring 1.5 cm by 2.5 cm were used for the tests performed. The procedures were carried out over a two day and a five day time period. Each time frame consisted of twenty-eight samples. Two bacteria were used each at two infection levels as well as a control set.

Stock solutions of MRSA and PA were prepared and allowed to incubate over night. The concentrations of the stock solutions were determined by spectrophotometer and were then subjected to serial dilutions to obtain concentrations of 10^5 and 10^2 colony forming units, cfu’s, for each bacterium.

The samples of Surgisis were placed in 0.9% normal saline solution for approximately twenty minutes to rehydrate. The meshes were then placed on blood agar plates. Each sample was inoculated with 200 µL of either 10^2 cfus of MRSA, 10^5 cfus of PA, 10^2 cfus of MRSA, 10^5 cfus of PA or 0.9% normal saline. Each set of bacteria consisted of six samples while the control set inoculated with saline consisted of four samples. The inoculated plates were then placed in an incubator at 37° C for the defined time period.

After the appropriate amount of time the blood agar plates were removed from the incubator and transferred into a Petri dish containing 25 mL of normal saline to remain hydrated during transportation. The samples were then individually subjected to tensile strength testing utilizing the MTS systems corporation Electromechanical testing system with the ReNew upgrade package 1122 equipped with an Instron Reversible 1000 lb load cell and Testworks 4 Software. The peak load, peak stress and modulus were recorded and compared using a student’s t-test to determine statistical significance.

Results
Following the two day time period each of the samples were subjected to tensile strength testing. Compared to the control only the samples inoculated with 10^5 cfus of PA showed significant degradation, p ≤ 0.05. The samples infected with Pseudomonas were considerably more degraded and in some cases caused the individual ply of the samples to become delaminated.

After the five day time period the samples were again removed from the incubator and subjected to tensile strength testing. The samples that had been inoculated with PA, at both concentration levels, showed significant degradation with p ≤ 0.001 while the 10^5 dose of MRSA did not show significance with a p-value of 0.056. The lower dose of MRSA showed no statistical significance.

Discussion
Pseudomonas demonstrated statistically significant degradation at both the two day and five day time periods. However the samples of MRSA did not show degradation after either time period as expected. The results to this point support our hypothesis that bacterial colonization can lead to significant degradation in the material strength in biological tissue grafts.

On-going research will inoculate samples with elastase as well as a combination of elastase and the bacterium previously used. Additionally the in vitro experiments will be repeated on a variety of other biological meshes and then repeated in an in vivo model.

Acknowledgements
Research was supported by the Tulane University Health Sciences Center and Tulane University.

Figure 1. Results of the average peak stress for the two day and five day time periods with standard deviation error bars.
HYDROPHOBIC / HYDROPHILIC ANALYSIS OF CONTACT LENSES

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Introduction
The comfort of contact lenses has been linked to the degree of wettability or hydrophilicity of the lens surface during usage. The purpose of our investigation is to analyze the effect of lens biofouling by tear fluid components and surface wear on the lens surface structure and overall hydrophobicity. The goal of this study was to devise and implement methodology to determine the degree of hydrophobic variations in the contact lens surface at different phases of use and how those variations may be responsible for the comfort of each lens type.

Specific changes in contact lens surface wettability and polymeric structure were followed using contact angle methods and hydrophobic staining techniques. Contact angles were measured with the air-in-saline captive bubble technique using a Ramé-Hart goniometer. The hydrophobic domains of the lens material were visualized with a hydrophobic dye, Sudan IV, in silicone oil. The results of the hydrophobic staining technique will be used to determine the specific polymeric domain changes that the contact lenses undergo with biofouling and wear.

Materials and Methods
Four types of lenses were analyzed: Acuvue Advance™, Acuvue Oasys™, PureVision™, and O₂ Optix™. Two types of commercial disinfectant solutions, Renu® and Opti-Free®, were used in conjunction with a saline control solution, Unisol-4®. An Artificial Tear Fluid (ATF), formulated with proteins and lipids, was used to simulate in vivo biofouling.

The primary portion of this study used the hydrophobic staining technique to find two different sets of data for each contact lens sample: (1) pictures of the hydrophobic domains of the lens and (2) absorbance readings of the extracted dye. Sudan IV dye solution was made by mixing 96.3 g of silicone oil (PDMS 200) with 2 g of Sudan IV dye powder for 30 minutes. After the solution was centrifuged, the supernatant was diluted with silicone oil until 46.3 g of supernatant was QS’d to 100 ml (stock solution). Dilution curves were taken at $\lambda = 522$nm and, once soaking was completed, the lenses were imaged. Afterwards, the dye was extracted using 1 ml DMSO and sonication. The extract was read using a UV spectrophotometer.

The first set of data collected was to determine the effect of (1) soaking lenses in saline and disinfecting solutions and (2) soaking lenses for different time periods in Sudan IV solution. Five sets of saline and disinfecting solutions were done: (i) 24-hr soak in saline solution, (ii) 24-hr soak in Renu, (iii) 24-hr soak in OptiFree, (iv) 24-hr saline soak followed by 16-hr soak in Renu, (v) 24-hr saline soak followed by 16-hr soak in Optifree. Each of these five sets was followed by 2, 8, and 16-hr soaks in Sudan IV solution.

The second set of data collected was to determine the effect of soaking lenses in ATF. Two sets of data were collected: (i) 24-hr saline soak followed by 2-hr ATF soak and (ii) 24-hr saline soak followed by 16-hr ATF soak. Both sets were followed by a 16-hr soak in Sudan IV solution.

Results
The following figure shows that soaking lenses in disinfecting solution at any phase sharply reduces available hydrophobic areas on the lens. This is apparent because the Sudan IV would naturally bind to open areas of hydrophobicity.

Discussion
The results from our first set of data helped to identify 16-hr Sudan IV soak as the optimal soak time for further experimental studies. Additionally, both studies showed that soaking in disinfectant solutions or ATF yielded less hydrophobic sites than saline solution.

Acknowledgements
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Introduction
Approximately 125 million people worldwide wear contact lenses, more than 35 million in the United States alone. In a survey of contact lens wearers performed by CIBA Vision, Inc. (Guluth, GA) nearly three-fourths preferred a schedule that allows for some type of overnight wear. Thirty-five percent of contact lens wearers preferred a thirty-day continuous wear cycle, 14 percent preferred one to two-week continuous wear, and 20 percent preferred occasional overnight wear. Due to the increase in overnight and extended wear of contact lenses, performance based on durability and sustainability is of increasing importance.

Lengthened periods of contact lens wear present the issue of protein interference and adsorption to the contact lens surface. The protein-rich tear film begins to deposit on the contact lens surface during wear and creates changes in surface topography and roughness. The ability for smooth fluid flow over the contact lens surface is crucial in providing the optimal amount of comfort to the contact lens wearers. This fluid flow can be interrupted by the binding of proteins to the lens surface. Surface analysis will provide roughness measurements for four different contact lens brands, which will then be compared to the concentrations of tear proteins adhered to the lens surface. The purpose of this study is to identify surface changes of various types of contact lenses using a blink-model system through analysis by atomic force microscopy and lab-on-a-chip one-dimensional electrophoresis with the Agilent® Bioanalyzer.

Materials and Methods
Four types of contact lenses were used in this study: Acuvue Advance™, Acuvue Oasys™, Night & Day™ and PureVision™. An artificial tear fluid (ATF) consisting of an aqueous buffer and dissolved proteins was formulated to imitate human tears. A combination of lipids was stored separately and added to the ATF at the time of use. An ex-vivo blinking apparatus consists of 3 identical chambers through which the ATF is repeatedly pumped over the contact lens and then removed to simulate a blinking environment. A circulating water bath maintains constant temperature and an electric syringe pump infuses the ATF into the blinking chambers (Figure 1). This apparatus was designed to mimic blinking action with movement of the ‘tear film’ over the surface of a contact lens at periods of 2, 8 or 16 hours.

Three lenses were biofouled at once in the blinking apparatus. Upon conclusion of the blinking process, the contact lens was removed from the blinking chamber and wiped gently along the non-biofouled edge to absorb any excess ATF. Post-blinking analysis consisted of removal and analysis of deposited surface proteins and surface roughness characteristics. Immediate freezer storage was the most effective way to preserve the post-blinking condition of each lens when analysis occurred at a later time. One lens from each set of three biofouled lenses was immediately prepared after the blinking process and imaged using the Atomic Force Microscope (AFM) to gather surface roughness data. The lenses that were not to be imaged were run through a freeze/thaw technique which freezes and heats the contact lens to break all bonds on the lens surface. Each freeze/thaw process created 300 µL of elution per contact lens and was stored at -70°C until analyzed with the bioanalyzer.

Results
A correlation has been found to exist between the surface roughness of the contact lens when imaged before and after biofouling in the blinking apparatus. The biofouled lenses produced higher surface roughness than the lenses that were not biofouled in the blinking process. A higher surface roughness is believed to correspond to greater adhesion of the lipids and proteins in the ATF to the surface of the exposed lens. The table below shows the molecular weights of each protein found in the elution from the lens surface using the bioanalyzer.

Table 1: Expected and Actual ATF Protein Molecular Weights as Determined by the Agilent® Bioanalyzer Protein 80 Kit

<table>
<thead>
<tr>
<th>Protein</th>
<th>Expected MW kDa</th>
<th>Actual MW kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>58</td>
<td>55.4-56.2</td>
</tr>
<tr>
<td>IgG</td>
<td>26, 59.3-62.3</td>
<td>27.1-27.4, 62.0-62.4</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>80</td>
<td>88.63 &amp; 81.0</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>14.5</td>
<td>14.7-16.0</td>
</tr>
<tr>
<td>α-Acid Glycoprotein</td>
<td>37-40</td>
<td>76.38</td>
</tr>
<tr>
<td>γ-Globulins</td>
<td>20-25 &amp; 55-60</td>
<td>27.3 &amp; 61.58</td>
</tr>
<tr>
<td>Mucin</td>
<td>300+</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>

Discussion
As further research is performed, a greater difference in overall concentration of protein adhesion between the four contact lens types is expected as longer time trials are completed. Data collected to date has supported the original hypothesis that a biofouled lens has greater surface roughness due to adhered proteins to the exposed lens surface. Once data collection is complete, the determination of which lens type performs optimally will be concluded based on the lowest concentration of proteins that adhere to the lens surface, resulting in the lowest surface roughness found using the AFM.

Acknowledgements
This research was supported by LSU Eye Center, Research to Prevent Blindness, Inc. and the National Eye Institute.
DESIGNING A TALAR IMPLANT FOR THE TREATMENT OF OSTEOCHONDROAL DEFECTS

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Introduction
An estimated 850,000 individuals suffer an ankle sprain annually. Approximately six percent of ankle sprains have a concurrent chip-fracture beneath the cartilage on the talar dome1. The fracture may lead to the development of a chronic osteochondral lesion. Surgical intervention is required when the osteochondral defect degenerates through the chondral layers to the subchondral bone.

Ante-retrograde drilling, debriment, and autologous chondrocyte transplantation are surgical treatments in use today. These treatments initiate the growth of fibrocartilage, which is inferior biomechanically and are also expensive, require a long rehabilitation period and in many cases do not fully cover or fill the defect site.

Currently no implants are clinically available for treating osteochondral defects of the talus. The purpose of this project is to develop an implant that will mimic the anatomy of the medial talar ridge, (the site for the highest reported frequency of lesions on the talus), provide cartilaginous articulation, and be able to withstand the biomechanical forces experienced at the ankle.

Materials and Methods
Search keywords “osteochondritis dissecans”, “osteochondral lesions” and “talar dome lesions” were used to locate journal articles reporting statistics for the population distribution of lesion sizes and depths. Literature reporting average lesion area and depth obtained from radiographs and MRI’s were utilized to establish preliminary implant sizing data.

Average geometrical measurements and biomechanical loading parameters for the talus were obtained from the literature, as well. Key parameters included the width of the talar surface, the anterior-posterior radius of curvature of the talar surface, medial-lateral radius of curvature, and the medial angle of orientation of the talus (medial facet angle, Figure 1).

Dissection was performed on a cadaver foot (65 year old female, 4'6”, 139lbs) to understand the limitations posed by the anatomy surrounding the talus, observe the approach used by surgeons in treating these lesions, and to examine the amount of talar exposure obtained through different surgical techniques. A SLA implant was created for talar cadaveric fitting (n=1).

Before the prototype implant was made, surface geometries were measured on the donor talus. Measurements were made by direct measurement and from gross photography with Image-Pro software (Ver. 4.1; Media Cybernetics®) using several digital images of the talus. Photographs were taken using a Nikon D100 digital camera and uploaded into the Image-Pro software for analysis. The arc-fit measurement tool was used measure different surface curvatures on the talus.

Results
The contact pressures on the ankle ranged from 2.6 - 8 MPa, with a mean of 5.5 MPa. The contact pressures become important when considering the type of biomaterial to be used in the development of the final implant.

The 95% confidence interval for medial lesion sizes ranged between 120 - 162 mm² (n = 40), while the mean depth of the medial lesions was 6.3 mm (n = 421)2. The talar surface measurements for the anterior width, middle width, and posterior width were 29.9 ± 2.6 mm, 27.9 ± 3.0 mm and 25.2 ± 3.7 mm, respectively. The radius of curvature for the talar surface was 22 mm for males and 18 mm for females. The medial facet angle was 83.9°, with a range of 70° - 90°.

The measurements obtained during the cadaveric talar fitting were close to the reported measurements. The surface width measured 25.3 mm, 23.4 mm, and 19.4 mm for the anterior, middle and posterior talar surface. The anterior-posterior surface radius was 16.9 mm and the medial-lateral surface radius was 27.6 mm. The medial facet angle was 85°.

Based on measurements obtained from the cadaver study, a box chisel was developed to assist in the excision of the lesion area. The box chisel dimensions will correspond to that of the specific implant being used.

Discussion
The measurements for the dissected talus correlated well to those reported in the literature. Considering that the cadaveric talus belonged to a small female, the measurements were estimated to be smaller relative to the general population.

The anterior-posterior and medial-lateral lengths for the SLA implant were large for the given talus; however the implant fit well into the excised area. The trochlear surface curve and the medial talar ridge curve of the prototype implant also corresponded to the natural curves of the talus. Medial-distal curve was about 1 mm off and a bit proud on the medial edge. A key design issue identified during the prototype trial is that the surface of the talus decreases in width from anterior to posterior. The prototype was not designed with this consideration and as a result the trial implant extended out too far on the posterior edge.

Future design considerations involve further cadaveric trial fittings to finalize a design placing a keel or a wide stem at bottom interior edge of the implant. The stem would be at a 45° angle and will help stabilize the implant.

Acknowledgements
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References
Design and Testing of a Medial Condyle Implant for the Treatment of Focal Chondral and Osteochondral Defects

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Introduction

Over 41,000 surgical procedures are performed annually in the United States to treat cartilage defects in the knee. Cartilage lesions of the medial femoral condyle cause pain and limit motion. Current treatments include microfracture, mosaicplasty, and autologous chondrocyte implantation. Drawbacks to current procedures are that the healing produces fibrocartilage, which has inferior mechanical properties compared to the normal hyaline cartilage found in the knee, and have long recovery periods.

The purpose of this study is to develop the design and mechanical testing criteria for a novel implant to treat focal chondral and osteochondral lesions of the medial femoral condyle (Figure 1a). The implant will restore lost function and decrease the recovery period. The implant design will conform to the geometry of the condyle to reduce the risk of damage to articulating surfaces or the implant itself (Figure 1b).

Materials and Methods

The study was broken into two parts. The first part consisted of developing a scaled-down prototype implant for an in vivo functional animal trial. The second part focused on mechanical testing of the proposed implant to failure in rim loading and off-axis loading to catastrophic device failure replicating worst-case scenarios. Both axisymmetric and asymmetric prototype implants were designed based on an extensive literature search of femoral condyle curvature and defect sizes.

Initial implant sizing trials were placed in 3 post-mortem canine knee joints to ensure that the implants would both fit into the condyle and articulate smoothly. Following exposure of the medial femoral condyle, a defect hole was made. A 6 mm, axisymmetric implant was press-fit into the defect, pictures of the joint were taken (Nikon D100, Tokyo, Japan) and the radius of curvature measured using ImagePro (4.1, Silver Spring, MD).

Specially designed test fixtures were used to find the offset failure load for a worst-case scenario on a 15 mm diameter axisymmetric implant using a MTS Mini-Bionix servo hydraulic test system. Two sets of implants were tested. One set had a polished articulating surface and a thin pyrolytic carbon coating thickness (0.0093”). The other was unpolished with greater coating thickness (0.0165”). The test fixture simulated a scenario where the cap was unsupported and loaded at the rim of the condyle, it is unlikely that the implant will fail in vivo.

Results

Based on curve analysis, it was determined that the canine implant would fit and articulate well with the condyle. The curvature for the canine condyle was 12.05 mm, which was similar to the curvature of the canine implant (11 mm). The implants had the highest mean maximum failure load at 3 mm of offset (1093 ± 287 N & 1172 ± 87.8 N). Implants with the thicker coating also exhibited a greater mean load regardless of offset length than implants with a thin coating (Table 1).

Testing of asymmetric implants showed similar maximum loads regardless of configuration (Table 2). The average load to failure for 20 mm axisymmetric implants was 1343 N and 1629 N for 12.5 mm axisymmetric implants.

Discussion

The average compression force at the knee is 4-4.5 times body weight, with 60-70% of the load distributed to the medial condyles. A person weighing 150 lbs would experience 800 N over the condyle. The maximum load to failure for both asymmetric and axisymmetric implants was higher than the expected load in the knee joint. Since the implant does not cover the condyle, it is unlikely that the implant will fail in vivo.

Acknowledgements

This study was conducted and supported by the Fellowship of Orthopaedic Researchers.
FORCE GENERATION OF HEAT TRANSFORMABLE SHAPE MEMORY (NITINOL) IMPLANTS

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Introduction
There are approximately 6.8 million cases of various bone fractures in the United States every year. Fractures can result from injury or through various medical conditions that weaken the bone. The branch of surgery that deals with fixing fractures and other musculoskeletal disorders is orthopedic surgery. In orthopedic surgery, there are basically two goals: realign the bone pieces and hold them in place while fusion occurs. Plates and screws are commonly used to resolve fractures but staple implants have gained traction as a simpler and potentially cheaper method. There are two varieties of staple implants: static and shape transformable.

Shape Memory Alloys (SMAs) offer the ability to “remember” their pre-deformed shape. There are different versions of SMAs but in this thesis, the focus will be on Nickel-Titanium (Nitinol) Alloys. The objective of this thesis is to measure the forces that are generated by various shape memory Nitinol implant staples. Specifically, the forces generated will be measured in confined compression after the implant is transformed from martensite to austenite. After force generation is completed and various load vs. time graphs obtained, these graphs will be further analyzed for their peak and steady state values. A final conclusion can then be derived.

Materials and Methods
Initially, an environmental chamber was set up on an MTS (Materials Testing System) device with the temperature set to 98°F (37°C). The chamber helps maintain a constant temperature within a few degrees of the desired setting, with little temperature gradient across the specimen. The MTS system was ideally suited for static testing when its’ high-stiffness and precision aligned load frames with integral actuators was coupled with the specially designed grips for consistent loading, and with alignment fixtures for minimizing specimen bending strain. A 1000 lb reversible load cell was inserted on the cross head above the environmental chamber on the MTS device. An MTS device grip was first placed on the bottom of the load cell above the temperature chamber. After this top grip was fixed, it was used to lift the chamber with the crosshead to enable a smaller MTS device grip to be inserted at the bottom.

The next step was to run a calibration check on the device. The template “Simplified Tensile” was run in TestWorks 4. The load channel was zeroed and preloaded to 0.5 lb tension by moving the crosshead up. A load (lbf) vs. time (s) graph was then started by pressing play in the TestWorks 4 software. The electrodes of an Intelifuser (a cordless thermo-activator) were then pressed on each staple for at least 30 seconds to help aid in its deformation. The door of the chamber was then closed and the graph was left on for approximately 150 seconds until its steady state. Thus, various load (lbf) vs. time (s) graphs were obtained for 5 tests for 3 sizes of staples.

Results
Fifteen load vs. time graphs were obtained, with peak and steady state values for each test. Mean steady state values for small, medium, and large implants are tabulated in Table 1.

Table 1. Load Steady State Mean Values and Std. Deviations

<table>
<thead>
<tr>
<th>Size</th>
<th>Cross-section (mm)</th>
<th>Mean Peak Value (lbf)</th>
<th>Mean Steady State Value (lbf)</th>
<th>Steady State Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>1.2 X 1.2</td>
<td>5.91</td>
<td>2.79</td>
<td>0.25</td>
</tr>
<tr>
<td>Medium</td>
<td>1.5 X 1.5</td>
<td>6.18</td>
<td>3.62</td>
<td>0.16</td>
</tr>
<tr>
<td>Large</td>
<td>2 X 3</td>
<td>8.72</td>
<td>7.34</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Discussion
Analyzing the resulting peak and steady state values of load obtained for compression force measurement of each staple, it is observed that as staple size increases, average steady state load value also increase. Steady state loads were on average, 47% of the peak load for small implants, 58.6% of the peak load for medium implants, and 84.2% of the peak load for large implants, respectively.

Acknowledgements
Research was funded by Intelifuse, Inc. New Orleans LA.
Introduction

Reliable in vivo assessment methods of bone surrounding the acetabular component of hip implants are needed to identify osteolytic lesions and evaluate total hip arthroplasty (THA) patients. Microfocus computed tomography (µCT) provides detailed, three dimensional images of bone trabeculae, but is currently constrained to ex vivo use due to specimen size. The presence of a metal implant in a CT procedure also produces streak-like artifacts that obscure the bone-implant interface.

Quantitative analysis of peri-implant tissues to evaluate bone healing and tissue response at the cellular level can be done on ex vivo tissues with histology. Unfortunately, histology is more time-consuming, destructive, and produces a limited number of two dimensional sections. The aim of the present study is to determine the correlation between the percent of mineralized bone measured in a surgically created acetabular defect using histology sections and matched µCT images. Developing an accurate correlation between µCT imaging and histology, as well as µCT and clinical-CT imaging, may impact the use of the clinical-CT to assess peri-implant tissues and degree of bone-implant contact.

Materials and Methods

In a previous study, a defect was created in the acetabulum of twenty canines and then grafted prior to THA utilizing a cementless, porous-coated, acetabular implant. Six weeks after implantation, the tissues were removed, fixed, embedded and µCT scanning of the acetabular component was conducted en bloc. Undecalcified histology sections were then produced and a high resolution microradiograph of each section was obtained. Individual slices of the three dimensional µCT images were matched to the serial histology sections for comparison.

Image-Pro Plus software (Media Cybernetics, Silver Spring, MD) was used to assess the percentage of bone present in the defect region, bone-implant contact, and artifact presence. The percent bone healing was measured in each microradiograph and µCT image using a 4mm x 4mm area of interest (AOI) placed within the defect region (Figure 1). Statistical analysis was conducted using a paired t-test of the mean microradiograph and µCT percent bone present in the AOI (p<0.05). The differences between the percent bone measured in each µCT and matched microradiograph image were calculated and a 95% confidence interval was determined. Qualitative assessment of bone-implant contact was conducted by comparing the amount of bone in direct contact with the implant interface in the µCT images with respect to that seen in the corresponding microradiographs. Artifact presence was analyzed by a visual assessment of the location and quantity of artifact present in µCT images compared to the microradiograph section. Clinical-CT image analysis will be completed in the same manner as the µCT.

Results

The percentage of bone defect fill measured in the µCT images (mean 47.1% ± 15.0% SD) was not statistically different (p=0.32) from that measured in the microradiograph images (mean 44.6% ± 14.1%SD). There was a strong correlation for percent bone measured between the µCT and microradiograph images (R²=0.71, Figure 2). The mean difference between the percent bone defect fill present in the µCT and microradiograph images was 2.5% ± 8.3%SD [95% CI= -2.2-7.2].

The presence of artifact in µCT images occurs most notably in the region extending 2 mm from the surface of the dome and is especially apparent in close proximity to acetabular component fixation spikes. The region directly surrounding the implant dome in the µCT was blurred due the presence of artifact making it appear as though bone-implant contact was made in a significant portion of the defect region. The corresponding microradiograph images, which were not plagued by the presence of artifact, had less bone-implant contact. It was also noted that, relative to the µCT images, the microradiographs showed greater detail of the beaded implant surface and bone trabeculae.

Clinical-CT images are currently being processed and are unavailable for data analysis. The results of a preliminary trial indicated that the clinical-CT data will overestimate the percent bone compared to the µCT data.

Discussion

The µCT imaging technique used in this study has been proven a reliable method of quantitative assessment by accurately determining the amount of bone present in the defect region. The presence of metal artifact in areas of bone-implant contact creates the illusion that bone is present in areas where it is in fact not. This incorrect representation of bone morphology could lead to inaccurate clinical evaluations of THA patients.

Acknowledgements

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Fatigue Effects on Surgical Hand Tremor

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Introduction

Technological advances in surgical equipment in recent years have allowed surgeons to perform new and complex surgeries. Using high-resolution video cameras, microsurgeons have the ability to operate on the smallest of organs, such as the inner ear and eye. While equipment advances now allow for surgeons to operate on the most minute of scales, these advances only increase the precision that is necessary to complete the surgeries without damaging the surrounding parts of the organs. Thus, instrument stability becomes a crucial aspect for achieving a successful surgery.

Hand tremor is a major concern for surgeons. Physiologic tremor is an involuntary muscle oscillation that occurs in every person. Generally, tremor is microscopic and cannot be seen be the unaided eye. However, even minor tremors can have terrible consequences when working on such small and delicate objects. Hand tremor also plays a large role in laparoscopic surgery where long-handled instruments cause the amplification of tremor known as the fulcrum effect.

Even though studies have struggled to show a correlation between caffeine and tremor, many surgeons will avoid coffee consumption before surgery. It is also believed that other factors such as stress, sleep deprivation, and fatigue can affect tremor.

This study will focus on fatigue, which can be a major problem in surgeries that often take several hours and require surgeons to stand and maneuver in uncomfortable positions. In this study, we will measure tremor before and after the fatigue of muscle groups to determine what affects they have on hand tremor. By determining how muscle fatigue contributes to hand tremor, surgeons will be better prepared to reduce tremor during long and arduous surgeries.

Materials and Methods

A goniometer and accelerometer are attached to the back of the hand of the subject. Then a cold sleeve, a sleeve with tubing for cold water sewn into it, is placed over the forearm.

Three initial baseline tremor recordings are taken before any fatiguing test for each subject. The subject depresses a needle with a silicone molded rubber insert on to the stylus of a phonograph cartridge 1 mm and holds for 20 seconds. The phonograph, used as a velocity transducer, transmits the displacement and velocity data to an oscilloscope for immediate viewing and to save the data to a hard disk. After initial tremor is recorded, the subject extends and flexes the wrist maximally with a 5-pound weight in the hand for a few seconds. The goniometer is calibrated to 1 and -1 at the maximum and minimum flexion and extension points, respectively. The graphing window for the goniometer is set to 0.9 to -0.9. After initial tremor measurement the subjects undergoes a fatigue exercise. The subject extends and flexes the wrist maximally with a 5-pound weight in the hand for a few seconds. The goniometer is calibrated to 1 and -1 at the maximum and minimum flexion and extension points, respectively. The graphing window for the goniometer is set to 0.9 to -0.9. The subject then begins the exercise until the subject is unable to reach the 90% flexion or extension three consecutive times (outside of the graphing window); this is the indication that the subject is fatigued. Three more tremor measurements are recorded.

The subject then sits and relaxes in the chair. For treatment A, no water is pumped through the sleeve, leaving the arm at normal room temperature. For treatment B, the sleeve has 4°C C pumped through it for 15 minutes. Thirty minutes after the end of the fatigue exercise, the baseline tests, exercise, and after-fatigue tremor measurements are repeated.

Discussion

Data from this study shows that an exercise that causes forearm muscle fatigue results in a definite increase in hand tremor. Since a surgeon’s tremor is related to performance, this suggests that surgical performance will decline through the course of a long, tiring surgery with this increase in tremor. While the data is inconclusive, it also suggests that tremor will return to baseline in a half an hour or less after fatigue. During long surgeries, a surgeon given a short break for stressing the forearm muscles would likely perform better.

The reduction in the RMS velocity from the first baseline test to the later tests initially seems counterintuitive. However, there may be a few explanations to clarify it. One explanation is that the subject becomes more accustomed to the velocity transducer after a few attempts. Another more interesting conclusion is that the fatigue of the muscle improves muscle control by warming it up, much like an athlete before a competition. If this hypothesis is true, it may be beneficial to complete a slightly fatiguing exercise, or stretching, about half an hour before performing surgery.

Further results from this study will provide surgeons with multiple techniques and strategies to reduce fatigue and tremor, thus improving the overall quality of the surgeon’s work.

Acknowledgement

Research was supported by the Tulane University Department of Biomedical Engineering.

Results

Both velocity and displacement RMS values increase after the fatiguing exercise for both the first and second trials. The data from one subject in Table 1 shows an approximately 25% increase in both tremor measurements after each exercise. There was a slight insignificant increase in the displacement RMS for the second baseline, but also a decrease in velocity RMS suggesting the subject fully recovered from the first exercise. More trials need to be performed before any conclusive data can be obtained.

Results

![Figure 1: A subject records a tremor measurement.](image)

Table 1: RMS Displacement and Velocity for One Subject

<table>
<thead>
<tr>
<th></th>
<th>Displacement (mm)</th>
<th>Velocity (mm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Baseline</td>
<td>0.1477</td>
<td>0.877</td>
</tr>
<tr>
<td>Post 1st Exercise</td>
<td>0.1703</td>
<td>1.020</td>
</tr>
<tr>
<td>2nd Baseline</td>
<td>0.1520</td>
<td>0.650</td>
</tr>
<tr>
<td>Post 2nd Exercise</td>
<td>0.1801</td>
<td>0.832</td>
</tr>
</tbody>
</table>
CHARACTERIZING FATIGUE IN THE SURGEON’S PHYSIOLOGIC TREMOR

Roy Dory, David Rice, Michael Dancisak
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Introduction

Many surgeons express concern about the effect of hand tremors on their surgical performance. Increased sympathetic nervous activity, which is associated with anxiety and fatigue, is known to enhance the physiologic tremor that occurs in all healthy people. These factors affect surgeons during long, delicate operations and can have an adverse effect on their surgical performance. Many surgeons take steps to reduce tremors by refraining from ingesting caffeine, which increases sympathetic nervous activity, or using beta-blocking drugs, which block the effects of catecholamines associated with anxiety1.

The goal of our research is to better understand the influences of muscle fatigue on the physiologic tremor. We examine its effects on the hand tremor over repeated exercises and compare the baseline tremor to the fatigued tremor. We also study the effects of a cooling sleeve on fatigued muscles and examine how it influences the physiologic tremor.

Materials and Methods

A velocity transducer is used to measure the waveforms of the physiologic tremor. The velocity transducer is comprised of a phonograph cartridge, a low pass filter, and an integrator circuit. The phonograph cartridge converts the movements of its stylus tip into an electrical signal that is proportional to its velocity. The low pass filter removes high frequency noise, while the integrator circuit provides displacement data from the velocity signal.

To measure the physiologic tremor, a subject sits in a chair with his dominant arm rested comfortably on foam cushions next to the velocity transducer. The subject holds a pair of locking forceps that grip the head of a syringe with a silicone tip. The subject contacts the silicone tip with the stylus of the phonograph cartridge, depressing it approximately one millimeter. The subject maintains this position, without bumping the sides of the velocity transducer, for 20 seconds, while the data is recorded on an oscilloscope.

To induce fatigue, the subject sits in a chair with the dominant forearm relaxed on its armrest. The subject is equipped with a goniometer, attached to the back of the hand and forearm, to measure the degrees of wrist flexion and extension. Maximum flexion/extension zones are considered areas within 10 percent of the furthest flexion/extension point. A five-pound weight is placed in the subject’s hand with the palm facing the medial direction and the subject’s thumb pointing superiorly. The subject alternates flexing and extending the wrist to the beat of a metronome (frequency = 0.416 Hz). The subject is considered fatigued when he can no longer flex or extend the wrist within the maximum flexion/extension zones while keeping pace with the metronome.

Tremor measurements are taken immediately following the fatiguing exercise. After 30 minutes, a third set of tremor measurements are taken and the fatiguing exercise is repeated. The subject returns the following day to repeat the process with a cooling sleeve. The cooling sleeve is a lightweight garment that fits around the subject’s forearm. For 7 minutes following the fatiguing exercise, cool water (8° C) is run through small tubes sewn into the sleeve. Additional tremor measurements are taken immediately before and after cooling.

Results

The following results compare the frequency spectrums of the baseline physiologic tremor to the tremor following the wrist fatiguing exercise. A negative shift in the frequency spectrum is shown following muscle fatigue.

Table 1: Median Frequency of Baseline Tremor vs. Fatigued Tremor

<table>
<thead>
<tr>
<th>Median Frequency (Hz)</th>
<th>Baseline Tremor</th>
<th>Fatigued Tremor</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.04</td>
<td>9.44</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: The amplitude spectrums of the velocity wave before and after the fatiguing exercise.

Discussion

Our tests indicate that there is very little correlation between the different axes of motion in the physiologic tremor. Therefore, most our analysis is done along a single axis of motion. Results from our pilot study indicate that the physiologic tremor in the hand is influenced by muscle fatigue in the wrist. The results show that the RMS and peak-to-peak values of the velocity and displacement waves significantly increase following the fatiguing exercise. Spectral analysis of the velocity waves shows a negative shift in the frequency spectrum following muscle fatigue. This shift occurs in the 8-12 Hz band of frequencies that is thought to be associated with oscillators in the central nervous system. We hypothesize that the cooling sleeve will decrease the recovery time and allow the subject to perform better on the second fatiguing exercise. If supported, this research will have various applications in the surgical field by reducing the enhanced physiologic tremor associated with muscle fatigue.

References


Acknowledgements

Research was supported and advised by the Department of Biomedical Engineering at Tulane University.
Remotely Monitored Pulse Oximeter

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Introduction
The rapid progression of telecommunications and medical technology paired with the declining costs of electronics has opened up new frontiers in telemedicine. One of the most tangible applications of telemedicine that has yet to be fully explored is the remote monitoring of oxygen saturation. The ability to remotely monitor oxygen saturation is particularly appealing use of telemedicine. One reason for this appeal is the non-invasive nature and ease of use of pulse oximetry. Additionally, many of the most common conditions that require oxygen saturation to be monitored are chronic diseases that require daily attention over long periods of time. Some of these ailments include asthma, cystic fibrosis, chronic obstructive pulmonary disease and many types of cardiovascular problems such as congenital heart disease. In these cases, it is not practical for the patient to remain in the hospital, and it is very inconvenient for the patient to make daily trips to a hospital for monitoring. Therefore, a remote oxygen saturation monitor would be an excellent tool in maintaining both the comfort and health of many of those at risk for hypoxemia.

Materials and Methods
To create a pulse oximeter that can be monitored remotely, the raw data is gathered by a Nonin Medical finger-clip pulse oximeter. That data is then serially inputted into a pre-calibrated Nonin Medical OEM II module interprets the raw data and organizes it into one packet of data containing three bytes. The first byte is the status byte, which contains data on the oximeter’s condition and accuracy as well as the two most significant bits of the heart rate. The second byte contains the 7 least significant bits of the heart rate and the third byte contains the SpO₂.

One packet containing these three bytes of information is sent out serially from the Nonin OEM II module each second. The packet is received by a PIC 16F873A microcontroller via the asynchronous serial input (USART) module that is built into the microcontroller. This data is then sent down several different paths.

First, the data is manipulated into ASCII form and outputted to a Hitachi 44780 LCD screen. Next the SpO₂ data is converted into a pulse width modulated signal with a duty cycle that correlates to the oxygen saturation of the patient. Finally, the data can be sent serially to a computer to eventually be transmitted via the internet.

However, the serial signal outputted from the PIC 16F873A has a 5 V amplitude while a PC only recognizes serial signals with a 9 V amplitude. To step up the voltage of the microcontroller’s serial signal, a max232CPE serial transceiver can be used. Once in the computer, the data can then be transmitted to its final destination in a number of ways.

Results
After limited evaluation, the pulse oximeter seems to be working properly. In a volunteer, the heart rate that the oximeter reported correlated very closely with the heart rate I found manually. Also, the recorded oxygen saturation was usually between 98% and 99% which is average for a healthy individual. After subjecting two volunteers to two minutes of breath deprivation, the SpO₂ reading ranged from 90 to 92%.

Once a more complete prototype is developed, a more thorough evaluation will likely be undertaken.

Discussion
Though the main goal of this project is to simply develop a pulse oximeter that can be remotely monitored, there are many paths that can be taken to this end. Currently, the favored method is to route data serially to a PC for transmittance via the internet. However, a wireless approach is very possible since the code for pulse width modulation has already been written and tested. Also, once the data is on the PC there are numerous methods of transmitting it.

Regardless of which methods are eventually chosen, the largest obstacle to bringing a remotely monitored pulse oximeter is user error or situational problems. Power outages, unreliable ISPs and computer viruses are only a few situational issues that could easily cause huge problems. Additionally, the oximeter’s sensitivity to movement or painted nails could feasibly diminish the reliability of the device. Since the medical field has an understandably small margin for error, these problems all need to be addressed at some point during the oximeter’s development.

Acknowledgements
I would like to thank the Tulane University Department of Biomedical Engineering for the materials, lab and guidance that was provided to me.

References


**Introduction**

Approximately 250,000 Americans have spinal cord injury and among those, 47% are quadriplegic. Out of these men and woman, only 24% are employed 8 years after their injury [1]. The importance of integrating a disabled working class citizen into industry is invaluable, regardless of their disability. With 11,000 new spinal cord injuries reported every year, it is undeniable that further investment in rehabilitative and assistive technology is necessary.

The purpose of this design project is to build a device that allows for a more viable, affordable, and multi-purpose solution to common mobility and control limitations. This device extracts directive data through the use of a rate-sensing gyroscope and dual axis accelerometer. This information is used to translate head movements of the user for use in a wide range of applications. For example, the device can control a computer mouse, wheelchair, or robotic system. This enables a greater freedom of mobility and since of independence for disabled persons in today’s society.

**Materials and Methods**

The design constraints of the device determined the hardware and software features. Affordability, efficiency, and size of the device were principal requirements and to meet these specifications, the Analog Devices ADXL203 accelerometer and ADXRS150 gyroscope were used. The data acquisition by the sensors corresponds to the movement of the transmitter module. The accelerometer measures static acceleration deflection up to +/-1.7g along both the x and y-axis and the gyroscope measures velocity of rotation along the z-axis. This data is sampled continuously using the Microchip dsPIC30F4012 microcontroller, as shown in Figure 1.

![Simplified device diagram](image)

**Figure 1.** Simplified device diagram that indicates flow of data through the transmitter and data acquisition module to the receiver and data processing module.

The raw data is then packed and sent wirelessly to the receiver module with identification and checksum bytes for error removal. Unidirectional wireless communication is sent by the Parallax 433 Mhz wireless receiver and transmitter. The receiver module feeds another microcontroller which processes and calculates the position and velocity of the transmitter module along the x, y, and z axis. The resulting data is packaged and sent using the required protocol for specific applications. The use of a receiver signal processor is necessary because of the error introduced by wireless communication interference.

The software has been designed to allow adequate signal processing and interface to multiple systems. The results and functionality of the device were tested using a serial terminal Windows™ application that displays the processed data from the receiver module. This allows for further development of the device and serves as a platform for debugging and implementation of integral safety features.

**Results**

The initial device prototype presented unanticipated design considerations. The energy efficiency constraint was met with the initial design. The power consumption of the receiver module is not an issue because external power can be applied. The transmitter module consumes 50 mA at 5 V and has a battery pack that is capable of sourcing 2000 mAh. The transmitter module is capable of switching to sleep mode if movement of the module does not occur within 50 sampling cycles. This reduces power consumption by 10%. Upon movement, the device switches back to normal operation. The addition of these features offers a more energy efficient device.

Problems with the software for both the receiver and transmitter modules were found through testing. The consumption of computational resources was equalized by moving the processing of data exclusively to the receiver module, which allows the transmitter module to focus on sampling of the data. The efforts to identify wireless transmissions and data through identification and checksum bytes were necessary because of wireless interference that caused reporting of erroneous data. In addition, a delay was experienced between the actual movement of the device and reporting of the data. The cause of this delay was associated with the serial data input and was rectified using a professional serial terminal. It was also determined that the original sampling rate of 120 Hz was not necessary, and in some cases was detrimental, therefore, it was reduced to 13 Hz. Overall, the device accurately samples and reports data as outlined in the purpose of the device.

**Discussion**

The accuracy and confidence of the data has been secured by error checking. The use of the device by disabled individuals makes safety paramount. The use of redundant communication and signal processing features are the means by which this is accomplished. The filtering of data caused by unanticipated movements is still under development, although a preliminary mode of removal is in place.

**References**


**Acknowledgements**

Research was supported by the Department of Biomedical Engineering at Tulane University.
Introduction

Acoustics to most people is a small set of isolated concepts that have little or no direct impact of their lives. The truth is that acoustics are governed by a small set of physical principles. Through simple demonstrations, these physical principles can be observed.

In teaching young students the fundamental cause of events, rather than blanket explanations that give no real information, true understanding of the commingling of the branches of science can be better understood at an earlier age. For example, telling a twelve year old that a police siren just sounds different when it passes by you because of the Doppler Effect gives the child no actual information. By breaking down concepts into easy to understand terms and ideas, the student gains a much greater understanding on the given topic.

By giving students a deeper understanding of the fundamentals of acoustics, doors open later in education in the form of faster progression through physics classes, or more advanced education in the field of physics. This effect would include an understanding of day to day acoustical occurrences, such as noise canceling headphones or hearing aids for instance. All of this ultimately leads to a better understanding with the world around us and is a step towards progress in the field of scientific education.

The target age group of this project is nine or ten year olds (3rd-4th graders). With the goal of giving an overview of various topics within acoustics, the main points to get across are the concept of waves, pitch, speed of sound, and echo.

Materials and Methods

A lesson plan that uses a series of demonstrations was developed for the purpose of giving a twenty minute lecture to a class of children in the 3rd or 4th grade. The lecture aims at using qualitative demonstrations to show the students first hand acoustical principles.

The initial demonstration uses a simple store bought metal Slinky. With both ends fixed, the various types of waves can be explored. By flicking one side vertically or horizontally, a transverse wave can be shown. By quickly pushing one side of the slinky directly towards the opposite end, a wave is created. The concept of echo can also be shown on the slinky by watching the wave travel back and forth along it.

The second demonstration uses two sound sources, such as two metal rods of differing sizes. By holding the rod in a specific place, a node is induced causing the rod to resonate at a specific frequency. This demonstration illustrates the concept of pitch and is a simple way to show that a vibrating object is also potentially a sound source.

The next demonstration is a tin-can phone approximately fifty feet long. This demonstration will show that sound travels through different materials at different speeds. By using a steel wire, and creating the length long enough, a click on one end of the phone should be heard as two clicks on the other side, because the sound will travel down the wire faster than it will travel through the air. Because the human ear needs between 10 and 20 ms of separation between sounds to be able to identify two distinct sounds, the length of the wire depends on the speed of sound through the medium. Using the equation D = VT, where D is distance, V is velocity, and T is time we can determine the string length needed. Using V_{steel} = 5050 m/s, V_{air} = 343 m/s, and T_{Total} = .02 s, D is calculated to be 7.36 meters, or 24.15 feet. To make sure that the sound difference is audible, double the length will be used to give us a final length of the steel wire to be 50 feet.

The last demonstration will use a homemade washtub bass that is made out of one galvanized steel washtub, two 1 5/8” washers, 1 wooden pole (1” diameter, 4’ long), one large eye screw that fit into the washers, two nuts that fit on the eye screw, and one bolt (1/4” X ½”), 1/4” thick rope. By pulling on the pole and increasing the tautness and plucking the string, different notes can be achieved. This can be used to not only reinforce the concept of pitch that was previously examined using the metal rods, but to potentially illustrate the concept of tension and its affect on pitch.

Results

On April 2, 2008, the lesson plan developed through the course of this project was used in a presentation given to Ms. Mary Ellen Bartkowski’s 3rd and 4th grade class at Audubon Charter School. The students were highly receptive and were eager to ask questions or offer relevant anecdotes. During the twenty minute presentation, Ms. Bartkowski filled out an evaluation form that serves to critique my performance as well as the layout and flow of the presentation itself. The evaluation consisted of a series of questions answer on a scale of one to five (one being the worst response, and five being the best). Of the questions that pertain to my performance or the presentation itself, the average grade was 4.2. In addition, each demonstration was graded on how effective each was on a one to five scale as well (one being not effective, and five being very effective). The results were an average of 4 with the slinky demonstration earning a 5 while the metal rod demonstration earned only a 3. Ms. Bartkowski did say that possibly too much material was covered, and suggested that covering less subject matter more in depth might strengthen the overall effectiveness of the presentation.

Discussion

After viewing these results it is clear what worked in the presentation and what needs to be improved upon. Earning the lowest marks for any demonstration, the metal rods need to be altered. A reduction of subject matter along with an increase of depth behind the remaining subjects would improve the effectiveness of the presentation as a whole, according to Ms. Bartkowski’s suggestions. More practice with then entire lesson, as long as with the demonstrations, particularly the washtub bass, would greatly aid in the effectiveness of the presentation as a whole. The overall response from Ms. Bartkowski was encouraging and it will help in the further development and refinement of the overall lesson plan. The development of further lesson plans in other fields such as optics or magnetism using a similar qualitative demonstration driven approach may be viable in explaining principles to children.

Acknowledgments

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MODELING THE TRANSPORT OF OXYGEN AND GLUCOSE TO OSTEOCYTES

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Introduction

One preeminent mystery in bone is how osteocytes, embedded in calcified bone matrix, obtain oxygen and nutrients and remove metabolic wastes. It is believed that oxygen and glucose mainly diffuse to osteocytes when bone is unloaded, while the transport is enhanced by convection when dynamic loading on bone induces fluid flow in the lacunar-canalicular network. However, the quantitative understanding of these transport mechanisms is very insufficient. Furthermore, osteocyte hypoxia has been hypothesized to play a role in bone mechanotransduction and microcrack-induced remodeling. Thus, it is of great interest to investigate the transport of oxygen and glucose in bone. In this abstract we develop a mathematical model for the transport of oxygen and glucose from capillaries to osteocytes in cortical bone. This model uses the Michaelis-Menten kinetic equation to simulate the osteocyte consumption of oxygen and glucose, respectively, and incorporates the anatomic structure for an osteon in cortical bone.

When diffusion alone is considered, our model shows a critical parameter defined as

\[ \frac{Q_{\text{max}}}{D} \]

which is the dimensionless consumption rate of a single osteocyte and shows the relative magnitude of consumption compared to diffusion. If \( \beta < 0.1 \), diffusion is sufficient to provide nutrients to osteocytes. The metabolic rate of osteocytes in vivo is needed to further assess whether diffusion is a sufficient mechanism to supply oxygen and glucose in bone.

Materials and Method

A model was developed to analyze oxygen and glucose transport and the final equations were solved using Matlab (Mathworks, Inc.). In the model, osteocytes are connected by canaliculi and are asymmetrically arranged in the osteon. The lacunar-canalicular configuration is shown below.

![Model of Haversian Canal-lacunae-canaliculi system](image)

Figure 1. Model of Haversian Canal-lacunae-canaliculi system

This model is described by the following system of dimensionless algebraic differential equations:

\[
\begin{align*}
1) & \quad C_1 - 3C_2 - C_3 = Q_{\text{max}} \left( \frac{C_1 - C_3}{K_m + C_1} \right) \\
2) & \quad C_2^2 - 2C_2 + C_1 = Q_{\text{max}} \left( \frac{C_2}{C_2 + C_3} \right) \\
3) & \quad C_2 - C_3 = Q_{\text{max}} \left( \frac{C_2}{K_m + C_2} \right) \\
4) & \quad Q_{\text{max}} = \frac{Q_{\text{max}}^*}{L} \\
5) & \quad R_{\text{diff}} = \frac{D}{A} \\
6) & \quad Q_{\text{consumption}} = \frac{Q_{\text{max}}^*}{L} 
\end{align*}
\]

Where \( C_1^*, C_2^*, C_3^* \) = dimensionless oxygen concentration in cells 1, 2, and 3 respectively, \( Q_{\text{max}}^* \) = dimensionless rate constant of Michaelis-Menten expression for maximum oxygen consumption, \( K_m^* \) = the dimensionless Michaelis-Menten rate constant \( D \) = diffusion coefficient, \( A \) = crosssectional area of osteon, \( C_0 \) = oxygen concentration in microcirculation, \( L \) = distance between two cells in osteon.

The diffusion and consumption of oxygen and glucose through the lacunae-canaliculi system is represented by the modified Michaelis-Menten kinetic equations (Eqs. 1-3). The dimensionless variables and parameters are defined in equations 5-7.

Results

![Graph 1](image)

Figure 2. Oxygen concentration and oxygen consumption by respective cells when \( Q_{\text{max}}^* = 0.01 \)

![Graph 2](image)

Figure 3. Oxygen concentration and oxygen consumption by respective cells when \( Q_{\text{max}}^* = 0.1 \)

![Graph 3](image)

Figure 4. Oxygen concentration and oxygen consumption by respective cells when \( Q_{\text{max}}^* = 1 \)

Figures 2-4 show that the oxygen concentration and consumption in cells 2 and 3 decrease more drastically as \( Q_{\text{max}}^* \) increases.

Discussion

When \( Q_{\text{max}}^* \) is very small (0.01), we see that each cell in the osteon receives nearly equal amounts of oxygen diffused to them because the metabolic rate of each cell is very low. This allows each cell to obtain adequate amounts of oxygen. However, as we increase the ratio of consumption to diffusion to 0.1 and finally to 1, much more oxygen is depleted by cell 1, leaving less to diffuse through the canaliculi for cells 2 and 3 to metabolize.

Acknowledgements

Matlab was provided by the Department of Biomedical Engineering at Tulane University.
**ISOLATION OF SCHLEMM’S CANAL ENDOTHELIAL CELLS**

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**Introduction**
As of the year 2000, glaucoma had affected approximately 77.8 million people worldwide. Primary open angle glaucoma, the most common type, is typically associated with elevated intraocular pressure attributed to increased outflow resistance of aqueous humor in the area of Schlemm’s Canal. Many studies have attempted to examine the increased resistance of aqueous humor drainage through the outflow pathway.

The aim of this research is to develop an efficient technique to isolate Schlemm’s Canal endothelial cells from nonglaucomatous human eye tissue. Following enzymatic tissue digestion of Schlemm’s Canal endothelial cells and surrounding outflow pathway cells from donor tissue, Schlemm’s Canal endothelial cells can be isolated from the cell mixture based upon their surface expression of platelet endothelial cell adhesion molecule-1 (PECAM-1)/CD31. No other cells in the outflow pathway express PECAM-1, and therefore magnetic microbeads coated with a CD31 specific antibody can be used to isolate Schlemm’s Canal endothelial cells from a mixed population of outflow pathway cells. The goal of this research is to determine whether CD31-positive cells can be extracted from Schlemm’s canal, using an enzyme “flow through” technique that involves flowing trypsin enzyme through the lumen of the canal to release the endothelial cells on the canal wall.

**Materials and Methods**
Previous techniques exist to extract and culture Schlemm’s Canal endothelial cells from the surrounding aqueous humor pathway of an eye, but a more reliable and less time consuming method would be optimal [1].

Cadaveric human eye globes obtained from the National Disease Research Interchange (NDRI) were stored in saline solution until dissection. Using a Feather scalpel No. 11 blade the anterior hemisphere of the globe was separated from the posterior hemisphere. The vitreous humor, retina, and lens were removed from the anterior hemisphere and discarded. The anterior hemisphere was cut into quadrants using the scalpel blade, and they were stored in Dulbecco’s modified Eagle’s medium (DMEM) at 4°C until enzyme treatment, for no more than 24 hours.

Under a dissection microscope, a flexible microneedle was threaded through the canal lumen and a 0.25% trypsin-EDTA solution was dispensed into the full length of the canal. Trypsin is a non-specific protease that liberates cells from their substrate. The trypsin solution was incubated for 5 minutes at 37°C at 7% CO₂ and flushed from the canal with media. The enzyme-cell suspension was collected as it pooled at the end of the canal lumen (DMEM) at 4°C until enzyme treatment, for no more than 24 hours.

To confirm whether the liberated cells were CD31-positive, the cell suspension was fixed in 4% paraformaldehyde and immuno-stained using mouse primary antibodies raised against human CD31 (DAKO, catalog # M0823). Fluorescently-labeled goat anti-mouse secondary antibodies were used to image for the presence of CD31 on a fluorescent microscope. Cells were mounted on slides and counter-stained using fluorescent nuclear dye (DAPI). For positive controls, human umbilical vein endothelial cells (HUVECs; known to express CD31) were stained in the same manner. NIH/3T3 mouse fibroblasts (known not to express CD31) were used as negative controls.

**Results**
Positive controls using HUVECs revealed fluorescent CD31-positive staining, while NIH/3T3 negative controls showed no staining, supporting our staining protocol (Fig. 1). The suspension of liberated cells from Schlemm’s canal showed positive CD31 staining that co-localized with DAPI nuclear staining (Fig. 2).

**Discussion**
Co-localization of nuclear DAPI staining with CD31 positive staining in the extracted cell population supports that some fraction of the extracted cells were indeed CD31-positive, indicating the presence of Schlemm’s canal endothelial cells. This research provides proof-of-principle that Schlemm’s Canal endothelial cells can be extracted by enzymatic “flow through”. Future studies focusing on immuno-isolation and culture of these cells are necessary to leverage this work into a protocol for more efficient isolation of Schlemm’s canal endothelial cells to be used for glaucoma research.

**References**

**Acknowledgements**
Grants from the AHAF and the NEI provided the funding for this research. Tissue samples were received from the NDRI.
Quantifying the Heterogeneity of Aqueous Humor Outflow Patterns in Human Eyes

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Introduction
Glaucoma is the leading cause of preventable blindness and effects approximately 70 million people worldwide (1). In most cases of glaucoma, the elevated IOP is due to obstruction of the aqueous humor outflow, located in the angle of the anterior chamber of the eye within the trabecular meshwork (TM). The molecular nature and precise location of trabecular outflow resistance is still actively being debated and researched; yet, there is increasing evidence that the hydrodynamic patterns of outflow through the trabecular meshwork have a strong influence on the magnitude of outflow resistance generation. The goal of this study was to quantify the hydrodynamic patterns of outflow through the trabecular meshwork of human eyes labeled by fluorescent tracer perfusion through the trabecular meshwork.

In a separate project, six pair of enucleated human eyes were perfused at defined intraocular pressure (7 – 30 mmHg) with mock aqueous humor containing a suspension of fluorescent tracer microparticles (200nm, 0.002 % v/v) to label the hydrodynamic patterns of outflow. Anterior segments of the eye were quadrisectioned into superior, temporal, inferior, and nasal quadrants and imaged in entirety en face at low magnification (1x) with epifluorescence microscopy. The current project focuses on the image processing tools used to quantify these tracer patterns obtained from the separate project.

Materials and Methods
A MATLAB code was developed to quantify and analyze the tracer intensity patterns around the entire circumference of trabecular meshwork for each eye. In the separate project, two colors of fluorescent tracer (red and green) were perfused simultaneously through the trabecular meshwork, and the red and green tracer images from the same quadrant were imported into the MATLAB program.

Results
Tracer patterns exhibited spatial variability both within and between quadrants, with some regions of TM appearing to be preferential sites of outflow. Variability in tracer labeling between quadrants gave a coefficient of variation of 30.0±18.9%, with similar results of 35.2±8.83% within quadrants. The nasal quadrant tended to receive the most tracer.

Discussion
Outflow patterns are spatially heterogeneous with significant inter- and intra-quadrant variability. Continuing work will focus on additionally assessing the microscopic outflow segmentation patterns through re-constructing a 3-dimensional representation of fluorescent tracer patterns in the TM.

References

Acknowledgments
Research was supported by the Department of Biomedical Engineering at Tulane University.
Materials and Methods

Perfusion experiments were previously designed to mimic the in vitro environment of Schlemm’s canal. Monolayers of HUVEC cells were grown to confluence on filters. The cells were stained with Calcein-AM, an indicator of cell viability, and Hoechst, a nucleic-acid stain, and perfused in a basal-to-apical direction. This study focuses on analyzing the behavior of endothelial cells and GVLs by studying image sequences captured during these perfusion experiments.

In order to quantify cellular deformation, it was necessary to identify giant vacuole-like structures that appeared as darkened areas devoid of calcein staining in the monolayer. Several image sequences contained GVLs that grew in response to a constant flow rate. MATLAB was used to quantify the time-dependent deformation of GVLs, the area of these structures and their position with respect to the nucleus as a function of time and perfusion pressure. A MATLAB code was developed to randomize the GVL selection process to eliminate bias in these measurements. A second code calculated the area, perimeter, and centroid of GVLs and nuclei, allowing for the analysis of GVL area and position within the cell.

Results

The HUVEC monolayers demonstrated a cobblestone-like morphology. The nuclei (blue) were fairly evenly distributed with little to no overlap. Darkened areas formed during both high (5-7mmHg) and low (1-2mmHg) pressure perfusion experiments (Figure 1).

It is hypothesized that the gray areas that formed during the perfusion experiments are giant vacuoles. The presence of calcein within the cell throughout the perfusions is evidence that the cell membranes did not rupture. Nuclear deformation was visible as the GVLs grew and pushed the cellular contents outwards. The gray color of the GVLs can be explained by the lack of fluorescent dye in the thinning cytoplasm.

Discussion

HUVEC monolayers perfused in a basal-to-apical direction formed GVLs that exhibited a viscoelastic response to changes in pressure. The pressure dependence of GVLs may enable SCE cells to withstand the forces that are constantly exerted on them by changes in IOP. The unusually high conductivity of Schlemm’s canal in comparison to other types of endothelial monolayers may also be a result of the ability of these structures to respond quickly and viscoelastically to pressure changes. Future studies of giant vacuole formation will provide important insights into the dynamics of transendothelial fluid flow across Schlemm’s canal endothelium.

Acknowledgements

Research was funded by the National Institutes of Health.
Introduction

Glaucoma is a disease of the eye that causes loss of vision over time, often without symptoms or warning until it is too late. Elevated intraocular pressure (IOP) is a principal risk factor for glaucoma, and Schiotz tonometry is currently clinically used to measure IOP by indenting the cornea and determining the pressure from a scale. Although the exact cause of elevated IOP is unknown, it is believed to be linked to an increase in the outflow resistance of the aqueous humor. Tonography focuses on measuring outflow resistance, which may help to understand the underlying root of elevated IOP.

Tonography has the potential to provide insight into the actual cause of glaucoma, but previous methods have been lacking. Initially, tonography was done using a Schiotz tonometer to take IOP measurements over time and the scale measurements were converted using tables to displacement values. This method is not favorable because it involves static measurements and leaves significant room for human error.

Dynamic applanation tonography (DAT) involves the use of a flat applanating surface to depress the cornea over time in an attempt to gather continuous measurements of the time-varying depression of the cornea and contact area of the eye with the applanating surface. These values can be used in conjunction with an existing mathematical model to calculate outflow resistance and stiffness. As the cornea is depressed, the IOP rises, causing the eye to empty as the aqueous humor flows through the outflow pathway. The rate of emptying is related to the stiffness of the eye itself and the resistance that the fluid encounters as it exits the eye.

DAT is more appealing than traditional tonography because it takes dynamic measurements of depression and contact area with minimal human interaction. The model calculates the actual stiffness of the eye, eliminating error from biomechanical variability from patient to patient. Also, the flat surface displacing the cornea does not change the geometry of the eye and cause force vectors in multiple directions.

Previous work has been done by Florine, et. al to establish proof-of-principle for a DAT system using a rubber membrane as a surrogate eye. The goal of this research is to translate the previous work done by Florine into a working DAT prototype designed to measure depression of the cornea and time-varying contact area of a human eye. The design must:

1. Allow for applanation of the cornea, causing depression that is measurable.
2. Allow for camera imaging of the contact surface.
3. Be freely moving with minimal friction.
4. Be both sturdy and as precise as possible.

Materials and Methods

To measure the depression of the cornea, a system was devised consisting of two pulleys at the top of a mounting board with a nylon cable running over them. The mounting board provides stability and precision, while the pulleys and nylon cable minimize friction. On each end of the cable is an 80mm disc. On the front side of the board, the disc contains an optical flat in the center, which makes contact with the cornea and depresses it over time. On the back side of the board, the disc is weighted and in the line of sight of a Laser Displacement Sensor (LDS). Once the back disc is released, the front disc moves downward to applanate the eye, pulling the back disc along with it and allowing the LDS to measure the displacement of the disc, which is equal to the depression of the cornea.

To measure the contact area, a CCD camera is used to image the corneal surface through the optical flat as the applanator descends upon the eye. This required special design of the supporting cable so that the applanating disc could hold the optical flat on the same vertical axis as the camera without the cable contacting the camera. This issue was resolved by adding a ring above the camera. The nylon cable attaches to the ring, then three steel rods connect the ring to the disc and the camera fits in between.

Figure 1. Diagram of Apparatus

Figure 2. Photograph of Apparatus

Discussion

The first prototype for DAT that is compatible with human eyes has been established. If it functions according to design, it should provide a robust and repeatable measure of depression and time-varying contact area necessary for calculation of ocular stiffness and outflow resistance.

Acknowledgments

This work was supported by the Department of Biomedical Engineering at Tulane University.
INVESTIGATION OF THE STRESS DISTRIBUTION AT THE CORNEO-SCLERAL JUNCTION

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Introduction
The shape of the eye, and particularly the curvature of the cornea play an important role in the organ’s functionality. Published literature has shown that the eye is a delicate system, and changes in its shape can have adverse effects on vision. Since the eye is a pressurized system, its shape is directly related to the material properties of the tissues, and how they deform in response to this loading.

The first goal of this study is to investigate how the eye’s geometry, with special attention on the curvature of the cornea, is affected by modifying the stiffness of the sclera and limbus in a pressurized eye. The limbus plays an important role in controlling internal eye pressure. Schlemm’s canal is found in this region and is the site of aqueous fluid outflow. It’s hypothesized that changes to the stress within the limbus affects the shape of this canal and the ability of fluid to flow out through it.

The second goal of this study is to investigate the relationship between the stiffness of the materials of the cornea, sclera, and limbus on stress concentrations along the surface of the eye. This study will evaluate the stress along the limbus, and how it is influenced with varying material stiffness.

Materials and Methods
Values for eye geometry were taken from literature and were used in the model creation. A two dimensional sketch of the eyeball was made, and then revolved 360 degrees to create the model geometry.

The model was then broken into twelve different sections. These section were then defined as one of three tissue types; sclera, cornea, and limbus/transition zone. The tissue was defined as hyperelastic. Hyperelastic coefficients for the cornea, sclera, and limbus were taken from the literature.

The density of the mesh was established via a convergence test with a seed size of 0.3 being used for all investigations. The coordinate system used was spherical and centered at the center of curvature of the cornea. The model was bounded allowing for radial deformation only.

The model was loaded with an outward pressure of 0.002 MPa over the interior surface. This pressure acts as an analog to the intra-ocular pressure of 15 mmHg found inside the human eye.

The creation of the model occurred through iterative mesh design. This was mainly due to limited published literature on the eye’s geometry at zero pressure. For this reason, determining the sketch geometry at zero pressure was achieved through trial and error. When pressurized, the final geometry used in this model accurately reflects the anatomy of the eye.

Results
Increasing the stiffness of the sclera and transition zone/limbus changes the curvature of the cornea after pressurization. Experimental data showed the center of the cornea deformed a greater amount at increased scleral and transition zone stiffness.

Maximum principal stress, von Mises stress, and pressure stress were recorded along the transition zone for each simulation. Plots of each were made for scleral stiffness’s of 1, 1.25, 1.5, and 2 x the original scleral stiffness. Analyzing the maximum principal stress and von Mises stress plots for increasing transition zone stiffness shows a biphasic result.

Figure 1. Contour map: Scleral Stiffness = 1 x Cornea, Transition Zone = 1 x Cornea

Increasing the transition zone stiffness decreases the max principal and von Mises stress at the sclero-limbal transition, however it increases both stress values at the limbo-corneal transition.

Figure 2. Max Principal Stress: Scleral Stiffness – 1 x Cornea

Normalized plots were also created for all three variables. The data showed that the normalized stress values increased as scleral stiffness increased. These plots showed that von Mises stress changed the most with increasing transition zone stiffness. Pressure stress was most unaffected by changes in the stiffness of both the sclera and transition zone.

Discussion
The most important finding of this study is that shape/distortion, von Mises, changes the most with increasing stiffness of material properties. As such, studies of flow through deformed limbal zones could be very important in understanding the effects of increased eye tissue stiffness.

Acknowledgement
Research was supported by the Tulane University Department of Biomedical Engineering.
**Introduction**

In order to develop a better understanding of the transport phenomena involved in droplet trains in microfluidic networks, an accurate mathematical model is critical. The information provided by such a model may be applied to areas such as biomedical devices, physiological systems, or fluidic logic devices.

This model is based on the experimental work of Fuerstman et al. (2007) and we use a particle tracking approach to simulate a train of droplets through the network.

**Materials and Methods**

A simplified model based on the lab set-up of Fuerstman et al. (2007) (Figure 1A) was developed. The viscosity for the bulk fluid and the droplet are $\mu_0 = 0.03$ g/(cms) and $\mu$ = 0.01 g/(cms), respectively and the density of the bulk fluid is $\rho = 0.773$ g/cm$^3$. The interfacial tension between these two liquids is $\gamma = 4$ dyn/cm. The following assumptions were made: The flow is driven by a pressure gradient; inertial forces can be neglected due to a small Reynolds number ($Re = \rho D / \mu = 0.02 < 1$), where $R = 50\mu m$ is the radius and; wall curvature effects are small and can be neglected due to a small Dean number ($De = Re (R/\rho c)^{1/2} = 0.05 < 1$), which is the ratio of the viscous forces acting on a fluid flowing in a curved pipe to the centrifugal force. Since the length of the droplets is much less than the length of channel, the parabolic flow recovers rapidly so that the flow between droplets may be considered parabolic, and we assume that all droplets that enter and exit are conserved. Also, the droplets are modeled as semi-infinite bubbles due to low $\mu_0/\mu$ and assuming the volume of a droplet is greater than $4\pi R^3/3$.

The model, shown Figure 1B, can be represented as four segments: an inlet, a top, a bottom, and an outlet channel. The end of the inlet is connected by a node to the top and bottom branches, which are of unequal length. When a bubble gets to node 1, a comparison of the flow rates in the top and bottom channels determines the path of the bubble. The top and bottom branches reconnect to node 2 at the beginning of the outlet segment. The pressure drop from node 1 to node 2 is the same for both branches and the bubble distance is defined as the distance between one bubble and the next downstream bubble. To determine the flow in the channels, the relationship between droplet pressure and velocity was used ($\Delta P_g \propto U^{2/3}$). The fixed pressure drop used in this model was chosen to reflect the periodicity in the experiment of Fuerstman et al. (2007) so that for $t > 1.04 s$, only one droplet passed through the loop at a time.

**Results and Discussion**

The bubbles were tracked through the network and their behavior was recorded with respect to parameters such as inlet and outlet time intervals.

For a constant time interval between inlet bubbles, a train of bubbles was simulated through the network and the distance spacing between the outlet bubbles was recorded and plotted. This was repeated for a range of inlet time intervals as shown in Figure 2. In Figure 2, large time spacing results in only one bubble going through the loop at a time (region I). As time spacing decreases, the train of bubbles undergo a transition (region T), where they appear as period 3. Smaller time spacing as seen in region II results in a periodicity of 2 while region III exhibits period 5 and 6 behavior and further decrements in time spacing show a transition from lower order to higher order periodicitics.

The model produced qualitatively similar results as those obtained by Fuerstman et al. (2007). In particular, Fuerstman et al. (2007) found that their system exhibited period 3 and 4 behavior for $0.90 < t < 1.04 s$, where $t$ is the interval spacing between the inlet droplets. This computational model shows period 2 and 3 behavior for the same range of $t$.

**References**


**Acknowledgments**

Matlab was provided by the Tulane University Department of Biomedical Engineering.
Introduction
Sequencing the three million base pairs in a human genome currently costs between two and five million dollars. The goal of the National Human Genome Research Institute is to cut the cost of sequencing a human genome down to $1000. According to the Institute, reducing the sequencing costs is vital to advances in biomedical research. Sequencing DNA using a nanopore would be ideal because nanopores are cost effective and have previously been highly accurate in determining single base-pair mutations. When translocating through a nanopore, single strand DNA does not create a base-level, distinguishable current between the electrodes.

In the future, one method of DNA sequencing could involve the introduction of an enzyme that would slice individual nucleotides off of a strand of DNA. The nucleotides would then translocate through the nanopore beside the hairpin DNA molecule.

The purpose of this thesis is to determine if a DNA hairpin inserted into the nanopore can slow the translocation of individual nucleotides through the channel in order to create a distinguishable current.

Materials and Methods
A nanopore detector is a current-detecting instrument built around a membrane containing a tiny pore called an ion channel. The pore is usually just big enough for a strand of DNA to pass through. A voltage is applied across the channel, creating a current that pulls molecules into the pore. When the molecule gets stuck, or passes through the pore, the nanopore detector “reads” the distinctive decrease in the current. The signal is passed onto a computer system that is programmed to detect the differences in molecules.

A lipid bilayer is created when lipids with polar heads and non-polar tails are immersed in an ionic solution. The non-polar tails arrange, while their polar heads create a barrier on either side of the bilayer. The lipid bilayer creates a barrier at the small opening of the cis well to prevent the flow of ions between the two wells of the detector. When a bilayer is formed, the current reading across the two wells drops to zero, or to a very small leakage current.

Results
Currently data has been gathered from all four nucleotides, and preliminary analysis is underway. While each nucleotide is dwarfed by the size of the channel, the presence of 9gC seems to have created a controlled barrier that has a well-documented current blockade signal.

Discussion
Preliminary analysis of data gathered indicates that it is possible to distinguish between the 9GC signal and the signal with a nucleotide added. Further analysis may reveal distinct differences in the current change generated by each nucleotide. It is expected that with additional computational analysis and review, more concrete distinctions will be detected. Further study around nanopore technology and its uses for DNA sequencing is essential in the field of bioengineering.

References

Acknowledgements
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MODELING OF Ca\(^{2+}\) WAVE PROPAGATION IN OSTEOCYTES

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Introduction

Osteocytes are the primary mechanotransducers in bone tissue, directing bone remodeling according to applied stress. Osteocytes communicate via intercellular calcium waves (ICWs), changes in intracellular Ca\(^{2+}\) concentration triggered by ATP released from stimulated cells. It is believed that a breakdown in this communication network is a factor in osteoporosis. ICW propagation begins when a stimulated osteocyte releases ATP. ATP molecules diffuse outward and interact with P2X and P2Y receptors on nearby osteocytes. P2X receptors are ligand-gated ion channels that allow Ca\(^{2+}\) ions to diffuse in from the extracellular space. P2Y receptors trigger the formation of inositol triphosphate (IP3), a second messenger that releases Ca\(^{2+}\) from the endoplasmic reticulum. Together, these processes increase intracellular Ca\(^{2+}\) concentration in nearby osteocytes. Here we develop a model of two-dimensional ICW propagation in a matrix of osteocytes, based on a similar model created by Iacobas et al. (1) for glial cells. This model currently focuses on the interactions between ATP and P2X receptors, and does not account for the effects of IP3.

Materials and Methods

Our ICW propagation model assumes a two-dimensional network of osteocytes spaced at 50\(\mu\)m intervals. A central osteocyte receives mechanical stimulus, triggering a bulk release of ATP, which diffuses outward from the osteocyte in a radially symmetric pattern. This model is consistent both with the Iacobas model and with experiments on osteocytes in vitro. The model currently consists of three equations. The first relates the initial ATP concentration to the magnitude of the stimulus. The second is a partial differential equation describing the diffusion of ATP through the extracellular space. The third describes the change in intracellular Ca\(^{2+}\) concentration in nearby osteocytes, and currently accounts only for Ca\(^{2+}\) influx due to activation of the P2X receptor. The parameters and numerical constants used in these equations are defined in Table 1, below.

\[
[\text{ATP}]_i = \left( \frac{[\text{ATP}]_{\text{max}}}{S_{1/2}/S} \right) \left( \frac{1}{\pi c h} \right) \quad (1.1)
\]

\[
\frac{\partial [\text{ATP}]}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left( rD \frac{\partial [\text{ATP}]}{\partial r} \right) - \delta_s [X]\left([\text{ATP}}(r,t)\right) \quad (1.2)
\]

\[
\frac{\partial [\text{Ca}^{2+}]_{\text{i}}}{\partial t} = \frac{1}{\pi c h} \left( [\text{Ca}^{2+}]_{\text{ext}} + [\text{P2X}] \right) \frac{P_{\text{max}}}{1 + \left( \frac{[\text{ATP}]}{[\text{ATP}]_{1/2}} \right)^{1/\delta_c}} \quad (1.3)
\]

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<td>(Q) Hill slope</td>
<td>*</td>
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Results

![Figure 1: ATP concentration (top) and Ca\(^{2+}\) ion concentration (bottom) vs. time in osteocytes at distances of 50, 100, and 150\(\mu\)m from the initial point of stimulus.](image)

Discussion

Numerical results obtained from this model were consistent with both the results of the Iacobas model and with experimental results obtained by other researchers. Maximum ATP concentration decreased with distance from the stimulated osteocyte, and intracellular Ca\(^{2+}\) in nearby osteocytes increased in proportion to the ATP concentration seen.

Future directions for this model include expanding it to include other cellular systems involved in ICW propagation (such as IP3 triggered release of Ca\(^{2+}\) ions from the endoplasmic reticulum), modifying the equations to model ICW propagation in three dimensions, and creating an in vitro three-dimensional matrix of osteocytes cultured in hydrogel to test the validity of this 3D model.

References


Acknowledgements

Research was supported by the Department of Biomedical Engineering at Tulane University.
ANIONIC EFFECTS ON THE KINETICS OF THE SWEET ALMOND ENZYME: B-GLUCOSIDASE

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Introduction
Characterization of glycosyl hydrolase enzymes is becoming increasingly important in the search for alternative fuel sources such as ethanol. To develop a permissive environment for these enzymes to work, a cheap and effective method for energy production is required. Family 1 glycohydrolases, specifically β-glucosidase, are extremely important in hydrolyzing large biopolymers like cellulose to produce molecules of glucose.

In attempts to streamline the process of microbial ethanol production from cellulose, processes such as Spontaneous Saccharification and Fermentation (SSF) and Ammonia Fiber Expansion (AFEX) have recently been developed. These processes combine stages like cellulose hydrolysis and microbial fermentation. It is important to be able to predict the effect of molecules like metabolic by-products on key enzymes like β-glucosidase, so that the system can be designed to have high yields and be more affordable on the large scale.

Materials and Methods
Initial experiments characterized the uninhibited hydrolysis of para-nitrophenyl-β-D-glucoside (PNPG) by β-glucosidase by measuring initial velocity as a function of initial PNPG concentration. The buffer system used was a 10 mM MES/NaCl solution at pH 6.3. After determination of the parameters, Vₚmax and Kₘ, pseudo-first-order reaction conditions were used to determine the second order rate constant kcat/Kₘ.

A number of anions were tested for inhibition of β-glucosidase activity. To observe their effect on the second order rate constant kcat/Kₘ, a system was used in which the concentration of inhibitor was varied while maintaining a constant pH, substrate, and enzyme concentration.

Results
The uninhibited hydrolysis of PNPG by the enzyme was characterized by a Kₘ = 1.87 mM and a kcat = 43 s⁻¹. This was determined using an enzyme concentration of 0.39 µM.

Five anionic molecules were tested for inhibition of the enzyme, three of which can be trivalent (phosphate, sulfate, and arsenate) and two of which can only be divalent (phosphate, and ethylphosphonic acid). In all, four out of five of the inhibitors studied showed very similar trends to this model. The Kᵢ for sulfate, phosphate, arsenate and phosphite are 23 mM, 28 mM, 31 mM, and 24 mM respectively and each anion showed about 45% inhibition at saturating levels. Ethyl Phosphonic acid, which differs from the previous molecules mainly through its ethyl alkane group on the phosphorous, exhibited a distinctly less potent inhibition (Kᵢ = 380 mM), although it did follow the same model.

Discussion
Anions such as phosphate are common in the cellular environment and have proven to be a simple way to inhibit the impressively efficient catalysis seen with β-glucosidase. From the data collected, it seems that the anions may be binding at a site other than the active site of the enzyme, and that the ethyl group of ethyl phosphonic acid is causing much looser binding. It is thought presently that the divalent species of each molecule is responsible for the inhibition due to the fact that this is the predominate species at ph 6.3. It is clear that the chemical environment of these reactions will have to be highly regulated in order to achieve the efficiency needed for large-scale production. It is true that inorganic phosphate is an absolute requirement of cellular systems in order to harness the energy stored in ATP, so its effect on β-glucosidase must be considered in the combined processes like SSF.

Currently, experiments are being done to characterize the pH dependence of this inhibition. This will allow for a more complete understanding of which protonated form of the inhibitor is binding to which form of the enzyme, and also may give some insight into the mechanism of action and a more complete understanding of the inhibition.

Acknowledgements
Great thanks to the Tulane University chemistry department for providing the equipment for these experiments, and also to the Biomedical Engineering department.
SYNTHESIS OF A STRUCTURAL ANALOGUE OF THE MONONUCLEAR COPPER ELECTRON TRANSFER SITE IN PLASTOCYANIN

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Introduction
Copper metalloenzymes are an important class of enzymes ubiquitous in nature. These enzymes present with interesting electrochemistry in their active sites due both to their structure and ligand coordination. We propose to model the blue copper enzyme plastocyanin by making a synthetic analogue. Plastocyanin is a fairly simple Type I copper protein with 99 residues and a single copper atom. The main difficulty in synthesizing models for Type I copper arises from the reactivity of the copper(II)-thiolate bond. We propose to prevent this by using triptycene as a bulky R group that would inhibit cluster formation or disulfide formation. The synthesis of the active site analogue will begin with 9-anthrene and end with the addition of CuCl bypyridine (Figure 1).

Materials and Methods
A combination of open air and N2 environment reactions were conducted using standard Schlenk technique. Lawesson’s Reagent [([CH3-O-C6H4]2P(S(μ-S)2)], 9-anthrene, sodium borohydride (NaBH4), acetyl chloride, isoamyl nitrite, tetraethyl ammonium hydroxide, 9-bromoanthracene, and copper chloride/bipyridine (CuCl/bpy) as catalyst were purchased from commercial sources and used as received. Silica columns were run in the open air using 60-230 μm silica (Dynamic Absorbants).

Results & Discussion
The crude 9,9'-dithiodianthracene (3) solution contains a large amount of solid waste made of oxidized Lawesson’s Reagent along with unreacted Lawesson’s Reagent and 9-anthrene. The crude solution was filtered with benzene to give the best filtration. However, with new regulations on benzene it has become inconveniently expensive. As a result 1:1 Et3O:Hexanes was used as a substitute solvent. A crystal lattice of 9,9'-dithiodianthracene (3) was obtained and compared with literature values to confirm its synthesis. A melting point is still to be taken to compare against literature values.

During the formation of S-9-anthryl ethanethioate (5) it is important to add acetyl chloride after sodium borohydride. Addition of acetyl chloride first causes the formation of NaCl and acetaldehyde. It should also be noted that this reaction should be done under a nitrogen environment. Otherwise, sodium anthracene-9-thiolate (4) will react with O2 to reform 9,9'-dithiodianthracene (3). X-ray crystallography was used to obtain a crystal lattice structure of S-9-anthryl ethanethioate (5) (Figure 2).

S-9-anthryl ethanethioate (5) will be used to create the acetyl protected triptycyl thiolate through a diels-alder reaction with benzene. The benzyne will be generated in situ using anthranilic acid and isoamyl nitrite. This is the limiting step in the synthesis of the enzyme analogue.

Conclusion
The key step in this reaction is the synthesis of S-9-anthryl ethanethioate (5). From that point, we hypothesize that we can quickly obtain the synthetic analogue through a benzyne reaction that creates a triptycene R group on the sulfur atom. Once the synthetic analogue has been synthesized rigorous electrochemical measurements will be conducted to better understand the redox potentials of the enzyme analogue.

Acknowledgments
Research was supported by the Department of Biomedical Engineering and Department of Chemistry at Tulane University. Funding was provided by the Tulane Lurcy Grant.
Introduction
Tuberculosis is an infectious disease caused by the bacteria *Mycobacterium tuberculosis*. This bacteria infects close to one-third of the human population world wide and causes close to two million people annually. *M. tuberculosis* is characterized by its slow growth and dormancy. This makes the bacteria difficult to control because it can be contained for periods, and reactivated years later.

More successful prevention and treatment of tuberculosis is required to stop it from being a global threat. This requires a better understanding of the biology of *M. tuberculosis*. The 4238 genes of *M. tuberculosis* have been sequenced. Unfortunately, many of the genes have yet to be characterized. Expression studies can have a serious impact on research for drug and vaccine development. Once the function of a gene is determined, it can be observed how the bacteria adapt to their environment. Identifying the genes that regulate transcription in *M. tuberculosis* would be an important clue in understanding the genetics behind the bacterium’s efficacy of infection.

Microarray analysis is an important tool that can be used in order to identify function of genes. It provides a method to observe how an unknown gene interacts and affects the expression of known genes. The purpose of this research is to determine the effects of the expression of two transcription regulators in *M. tuberculosis*.

Materials and Methods
The genes Rv0158 and Rv2745c were inserted into vectors that had constitutive promoter and vectors that had inducible promoters. The bacteria *Mycobacterium smegmatis* was electrotransformed with the vectors and then grown in broth. The *M. smegmatis* that had the constitutive promoter was grown and had RNA isolated. The *M. smegmatis* that had the inducible promoter was grown and then had 0.2% Acetamide added to for the induction. The RNA was then isolated after 1 and 2 hours. A control of untransformed *M. smegmatis* also had its RNA isolated.

For each set of experimental RNA, the following occurred: The experimental RNA and control RNA underwent reverse transcription into cDNA and fluorescent dyes were added; one to the control and one to the experimental cDNA. Both cDNAs are mixed and hybridized onto the microarray chip. Finally, the microarray chip is placed in the microarray machine and analyzed.

Results
The microarray chips for the bacteria containing the Rv0158 and Rv2745c genes that were under inductive and constitutive expression were analyzed by comparison to the control groups. The gene spots on the chip were analyzed for both average signal intensity [A= log2(635/2) + log2(535/2)] and fold change [log2(635, Mean Scale) – log2(532, Mean Scale)]. The gene Rv0158 regulated genes for polyketide synthase and antibiotic resistance. Rv2745c upregulated genes for ATP-dependent Clp protease (Figure 1) and other genes involved in proteolysis and protein turnover (Table 1).

Discussion
This study looked at genes with high intensity and fold changes in expression in order to find the genes that the most definite change in expression caused by the expression of either the Rv0158 or Rv2745c genes.

Rv0158 appeared to be a transcriptional repressor. It regulated a subset of sigF dependent genes. Rv0158 is a sigF dependent transcription factor. SigF is upregulated during *M. tuberculosis* infection of macrophages, stationary growth in broth, and nutrient starvation. SigF controls the transcription of genes involved in cell wall synthesis and fatty acid synthesis.

Rv2745c appeared to be a positive transcription regulator. It upregulated a subset of sigH dependent genes. Rv2745c is a sigH dependent transcription factor. SigH is upregulated during *M. tuberculosis* infection of macrophages, aerosol infection of mouse lungs, heat shock, and oxidative and reductive stress. SigH controls the transcription of genes for the Thioredoxin regulon and α-crystallin regulon.

Acknowledgements
Research was supported by the Tulane National Primate Research Center.