Musculoskeletal Research Center

Summer Research Program

2008

Department of Bioengineering

University of Pittsburgh

MSRC

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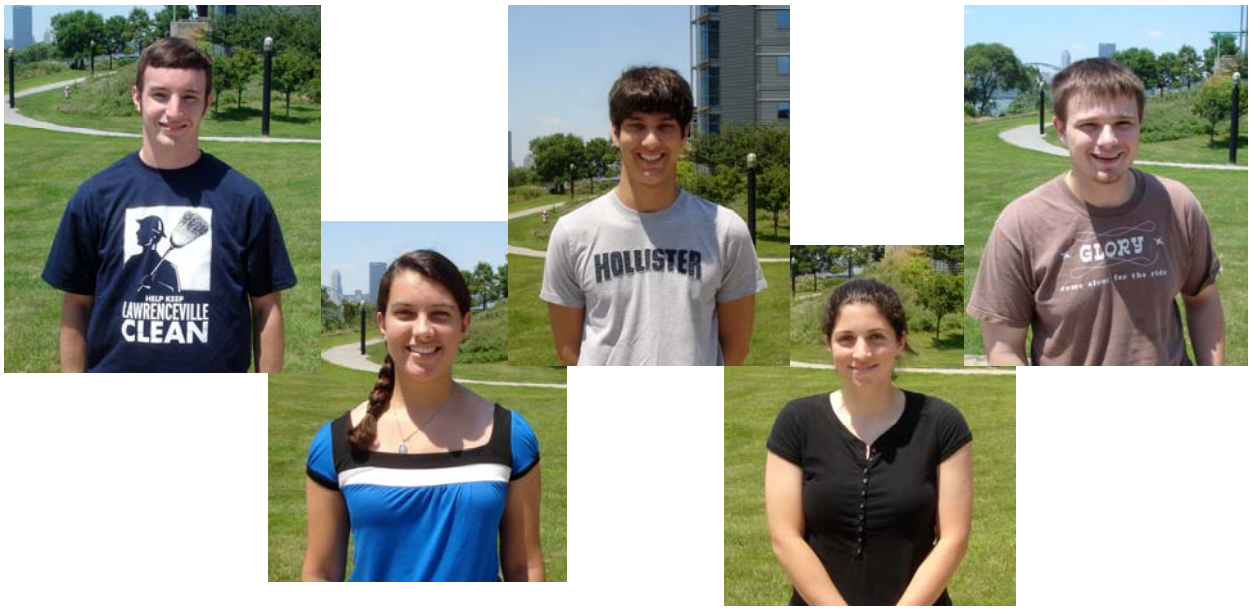
2008 Abstract Book Committee



Tommy Chase, Alex Cirillo, Collin Edington,
Megan Ferderber, Joey Moeller

This book contains the work of every summer student performed over the last few months. However, it does not accurately represent the great amount of knowledge we have attained during the summer. To do that would take a lifetime.

Of course, none of this would have been possible were it not for Dr. Woo's establishment of the MSRC, Dr. Debski's management of the summer research program, and the influence and guidance of the MSRC faculty and mentors.

Dr. Woo has always encouraged us to "think more, ask more, and listen more." This experience has not only given us the opportunity to carry out this philosophy, but it has also allowed for our best qualities to be drawn out and has elicited our best performance as researchers. It is true that we learned more about the process of researching, writing and presenting with regard to our research, but more importantly we have learned the MSRC mantra: the value of commitment, dedication, and pride. The future holds a different path for each of us, but this summer experience will carry over to wherever we find ourselves.

-Abstract Book Committee

2008 Summer Symposium Committee

Andrew Brown, Amy Chaya, Oscar Gonzalez,
Kristen Klingler, Stacy Tokar

This year's Summer Student Symposium was held on July 28, 2007 at the Musculoskeletal Research Center. The symposium drew an audience consisting mainly of faculty and staff of the University of Pittsburgh Bioengineering Department and the MSRC family. Each summer student's presentation was indicative of the hard work that he or she had carried out this summer. The most amazing aspect of the Symposium was seeing the amount of progress, in both research and presentation skills, the summer students had made throughout their stay at the MSRC. It was quite an experience for each and every one of us to give our first presentations at the MSRC and to answer the variety of questions that arose from our audience, many of which we had never considered before. The presenting experience was priceless because it is only through making and giving these scientific presentations that one learns how to improve and perfect the technique. Based on the presentations that were prepared and the responses given to questions, it seems as though all ten summer students really took the challenge of giving a formal presentation seriously and learned a lot in the process.

On behalf of the Symposium Committee, I would like to thank Dr. Woo for giving us such a wonderful opportunity this summer. I would also like to thank Dr. Debski for his guidance throughout the planning process for the Symposium. Finally, I would like to thank all the mentors for assisting the summer students with their summer projects and the entire MSRC community for making the summer such a memorable and fun learning experience.

- Symposium Committee

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I was born in Erie, Pennsylvania and have lived there my entire life. I attended Our Lady of Peace and Cathedral Preparatory School where I was fortunate to have many bright and motivated teachers, particularly in the subjects of science and math, who are responsible for where I am today.

When I started looking at colleges I had no idea where I wanted to go or what I wanted to major in. As I began exploring possible majors I realized that I wanted to study some sort of science or engineering. However, it wasn't until I visited Pitt that I realized bioengineering was the major for me and Pitt was where I wanted to study it. I felt that the bioengineering program at Pitt would provide the foundation and experiences necessary for success...and it clearly has.

I came to the MSRC in March of my sophomore year and began observing experiments and assisting in the research being done in the Shoulder Lab. As the spring semester progressed I became more interested and involved in the work being done and decided to apply to the summer program and was accepted. I was given the opportunity to really focus on a project I could call my own…and I enjoyed my summer and learned so many valuable research skills that I stuck around and decided to do it all over again this year!

I'd like to thank Carrie Voycheck and everyone else at the MSRC for their advice and guidance with my project, as well as for enabling me to have a very fun summer. I'd especially like to thank Dr. Debski, Dr. Woo and Dr. Abramowitch for providing such a great environment for developing the skills and knowledge necessary for becoming a successful researcher and bioengineer.

DEVELOPMENT OF A METHODOLOGY FOR MEASURING IN-VIVO GLENOHUMERAL TRANSLATIONS DURING SIMULATED CLINICAL EXAMS

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INTRODUCTION

The glenohumeral joint is the most dislocated major joint in the body with approximately 2% of the population dislocating between the ages of 18-70.[1] The glenohumeral capsule is a continuous sheet of fibrous tissue that attaches the humerus to the glenoid and is commonly injured during dislocation.[2] Current surgical techniques to repair injured capsular tissue are subjective and inadequate at restoring stability with 23 % of patients still experiencing problems.[4]

The poor surgical outcome of capsular repair procedures may be due to the challenges of accurately diagnosing the capsular pathologies present. Because many pathologies cannot be evaluated using imaging techniques, physical diagnostic exams are most commonly used. These tests are performed by applying a load to the humerus and qualitatively assessing the resulting humeral translations. However, these tests are very subjective because there is no standardized joint position or applied load for performing the test. Additionally, these tests provide no quantitative outputs for diagnoses to be objectively made, which may affect quality of treatment.[5]

The specific aims of this project were to: 1) Design and develop a system capable of quantifying anterior/posterior translations of the humerus, and 2) Quantify the repeatability of the translation measurement protocol. Completion of these objectives will enable the development of standardized test guidelines and specific diagnostic criteria.

MATERIALS AND METHODS

Development of a Motion Tracking Protocol

The first step in developing a complete protocol for quantifying humeral translations was to choose an appropriate motion tracking system. Flock of Birds (Ascension Technology, Burlington, VT) was chosen because its working volume exceeded the 10cm x 10cm x 10cm volume required, reported 3D position measurements in real-time, had an acceptable accuracy of 0.8mm and was non-invasive for the patient and clinician. Flock of Birds is an electromagnetic based tracking system which has the advantage of not needing a direct line of sight between the transmitter and receiver **(Figure 1)**, however it also requires a nonmetal working environment.

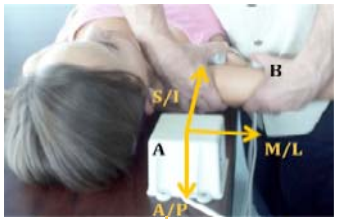

Figure 1. Experimental setup of Flock of Birds motion tracking system. (A) Transmitter, (B) Humeral receiver. Flock of Birds coordinate system and corresponding anatomical axes shown in orange.

The assumption that the global coordinate system of Flock of Birds corresponded to the anatomical axes of the test subjects allowed simplification of translation calculations. **Figure 1** shows the global coordinate system for Flock of Birds, where the x-axis represents the superior-inferior(+) axis, the y-axis represents the medial-lateral(+) axis and the z-axis represents the anterior-posterior(+) axis. The z-displacement of the Flock of Birds receiver was considered to represent anterior-posterior (A/P) humeral translation with respect to the thorax and was the only translation component studied.

Design of Shoulder Positioning Apparatus

A device to stabilize the arm was an important requirement of accurately tracking humeral motion. The major design criteria of the Shoulder Positioning Apparatus (SPAR) were to repeatably position a subject's arm at a variety of joint angles, to stabilize and support various sized arms to allow muscle relaxation and to be metal-free. Given these criteria, PVC pipe was chosen because it is non-metallic, easily adjusted to various rotations and inexpensive, allowing various size pieces to be used for varying arm lengths. The base was constructed using wood and steel reinforcing plates **(Figure 2)**.

Figure 2. Shoulder positioning apparatus used for testing showing (A) Wooden base, (B) Vertical support pipe allowing attachment of (C) Armrest allowing support of the arm at various positions of internal/external rotation and (D) Brace support attachment.

The elbow and armrest portion of the SPAR is completely adjustable allowing for subjects' full ranges of internal/external rotation to be examined, while varying the position and vertical support pipe length of the SPAR allowed different sized arms to be tested. Shoulder fixation could also be provided using wrist braces (Futuro, Wilton, CT) which were tied to the brace support attachment of the SPAR.

Development of a Data Analysis Method

Data analysis of the Flock of Birds output was performed in MatLab. A graphical user interface was developed to enable near real-time display and measurement of A/P humeral translation. This program displayed a graph of A/P translation versus time and allowed the user to define the endpoints of translation for 5 cycles of A/P loading in each trial and then calculated the average translation, which is reported in the results. Intra-observer selection of these translation endpoints was determined to be repeatable to 0.1mm.

Repeatability of Simulated Clinical Exam

Using a constant shoulder position of 90° abduction and neutral rotation and supporting the arm using the brace attachment of the SPAR, a single clinician placed a Flock of Birds receiver on the bicipital groove of the humerus. The receiver was then fully depressed into the skin and 6 cycles of A/P loading were performed. The clinician attempted to limit forces in other directions as this would affect the magnitude of translation measured This procedure was repeated for a total of three trials. A total of four subjects (2 Male/2 Female, Age 23 ± 1.3) were examined. Intra-observer repeatability was determined by calculating the standard deviation of the three reported translations for each subject.

RESULTS

Development of Motion Tracking Protocol

Flock of Birds performed well in the test environment with no noise or unexplained fluctuations in the positions reported. This is further supported by our successful validation of the system's accuracy to 0.8mm. Additionally, the clinician reported no problems with being able to perform the clinical exam naturally with the added presence of the Flock of Birds receiver. The test subjects also reported no adverse effects throughout the clinical exam protocol.

The SPAR was capable of stabilizing the arm in a relaxed position using both the brace attachment and arm-rest. The clinician stated that he preferred the use of the armrest over the brace stabilization because the armrest prevented "bouncing" of the arm associated with rapid changes in the direction of loading. The current design of the SPAR did not allow the height of the apparatus to be easily adjusted. This resulted in the subjects' shoulders being forward flexed when positioned at high angles of external rotation. The armrest also had difficulty maintaining a constant angle with the full weight of the arm placed on it.

Repeatability of Simulated Clinical Exam

The average A/P humeral translations were 18.7mm, 19.0mm, 28.7mm and 19.3mm for Subjects 1-4, respectively. **(Figure 3)** The intra-observer repeatability of translations was found to be 1.6mm, 2.7mm, 1.2mm and 1.3mm for Subjects 1-4, respectively, yielding an average repeatability of 1.7mm.

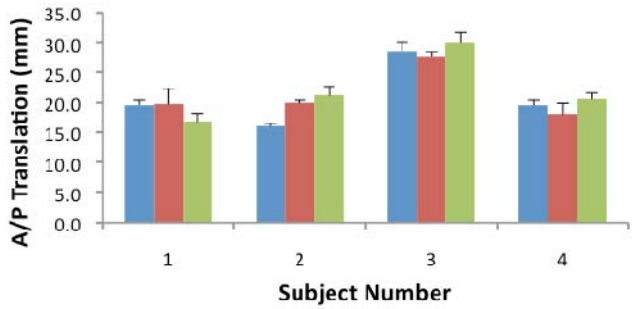

Figure 3. Average anterior-posterior translations (± SD) used to calculate repeatability results for four subjects during Trial 1 (Blue), Trial 2 (Red) and Trial 3 (Green).

DISCUSSION

This study developed a methodology for repeatably measuring A/P translations of the humerus during simulated clinical exams. Flock of Birds was found to be capable of accurately performing in the experimental environment and was non-invasive for both the patient and clinician. A shoulder positioning apparatus was designed to stabilize the arm and performed satisfactorily in preliminary tests. Finally, clinical exams were simulated on four shoulders and it was determined that using the current methodology, the clinical exams were repeatable to under 2mm of anterior-posterior humeral translation.

Averaging translations among all subjects in all trials of the repeatability study, we found average translations of 21.4mm. These data compare well with a study using a similar methodology that found average A/P humeral translations in uninjured shoulders of 23.2mm. Additionally, this study found average translations in injured shoulders of 30.0mm, giving a side-to-side difference of 6.8mm.[6] This side-to-side difference is greater than our repeatability of 1.7mm meaning that we would be able to accurately detect differences between injured and normal shoulders in patients using the current methodology.

While we found that we were displacing the Flock of Birds receiver in a repeatable manner, it is unknown how much of this displacement was truly glenohumeral translation and how much was due to the effects of soft tissue, lack of stabilization of the scapula and differences in the orientations of the glenohumeral A/P and global A/P axes. However, previous cadaveric studies have shown a high correlation between three-dimensional glenohumeral translations obtained cutaneously and intra-cortically, when the scapula is rigidly fixed and a highly controlled load is applied.[7] Our current experimental setup is unable to measure the load applied by the clinician during the simulated exams. Had a more constant load been applied throughout each trial, the observed repeatability may have been lower.

The SPAR had difficulty supporting the arm in some positions due to the lack of fixation at the PVC joints. Additionally, at high angles of external rotation, the shoulder became forward flexed which may affect translations measured. Future directions will include addressing the limitations of the SPAR and examining the effect that factors, such as joint position, gender, age or activity level have on glenohumeral translations. Finally, we hope to use this system to determine the difference between injured and non-injured shoulders in order to create objective diagnostic criteria that will allow surgeons to better detect and treat shoulder pathologies following dislocation.

ACKNOWLEDGEMENTS

I would like to thank my lab mentor Carrie Voycheck and my faculty mentor Dr. Debski for all their guidance throughout my summer project. I would also like to thank Dr. Woo and the rest of the MSRC for their valuable feedback and for enabling me to accomplish so much this summer.

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I was born on June 9, 1987 in Marietta, Ohio. Although I have lived in a small Ohio town all my life, I have been traveling to Pittsburgh for Steelers games since about 1998. This led to a love for the city, as well as my eventual enrollment at the University of Pittsburgh. In the fall I will be entering my junior year in Bioengineering at Pitt, and I look forward to everything that the future has to offer me in this exciting field.

As for my life outside of academics, my main interest is music. Around the age of 15, I received my first guitar lesson and have been playing ever since. Then over the next three years, I taught myself how to play the bass guitar, mandolin, banjo, and harmonica. I also enjoy playing most sports, especially soccer which has been a part of my life since I was five years old. Other than music and sports however, I usually just spend most of my time with my friends and family.

My summer experience at the MSRC has been incredible. To be surrounded each day by such a great group of people is more than I could have ever hoped for, and for that I am truly grateful. The knowledge and experience that I have gained during my stay will help me throughout my entire bioengineering career. For that reason, I would especially like to thank Dr. Woo for giving me this wonderful opportunity, Dr. Almarza for being such a great mentor along the way, and the rest of the MSRC family for making my summer at the MSRC so enjoyable.

SHEAR TESTING OF THE MANDIBULAR CONDYLAR CARTILAGE

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INTRODUCTION

The temporomandibular joint (TMJ) is the articulation of the mandibular condyle with the glenoid fossa and articular eminence of the temporal bone [1]. During jaw movement, the mandibular condyle moves along the fibrocartilaginous TMJ disc, resulting in contact forces. Due to this movement primarily being a sliding motion, the contact forces experienced by the articular surfaces of the TMJ are mainly shear forces. However, abnormal or excessive shear loading can cause the articular surfaces, such as the mandibular condylar cartilage, to deteriorate, resulting in osteoarthritis [2].

Thus, it is clear that shear plays a major role in the functioning of the TMJ, most notably in the mandibular condylar cartilage. By studying the shear properties of mandibular condylar cartilage, a better understanding of how the joint functions can be achieved. In addition, these shear properties can help with the diagnosis of disease in the TMJ, as well as provide some of the necessary design criteria for the tissue engineering of the mandibular condyle.

OBJECTIVE

The objective of this research was to develop a protocol for the linear, oscillatory shear testing of the mandibular condylar cartilage.

MATERIALS AND METHODS

The first step of this research was to assess the rat as an animal model. The rat was chosen for this study because like a human, the rat is omnivorous and has a TMJ that consists of a mandibular condyle and a disc. Therefore, the rat TMJ was at least anatomically a good model for the human TMJ.

For this experiment, the left and right sides of the mandible were dissected from twelve female Long Evans rats, age 3 – 6 months. The mandibular condyle was then isolated from each side of the mandible using a scalpel, leaving as much cartilage and as little bone intact as possible. The mandibular condylar cartilage was then glued to a testing plate in the same upright position as its native orientation on the mandible. Finally, the length and width of the specimen were recorded using a digital caliper and the two values were multiplied together to get the contact area, which was necessary for the calculation of the shear properties of the cartilage.

The oscillatory shear tests were performed on a BOSE ElectroForce LM1 Test Bench using a simple shear sandwich configuration (Figure 1). This device consists of an actuator, which controls the frequency that the top plate oscillates as well as the displacement that it travels, and a load cell, which records the loads experienced by the specimen on the bottom plate. Once the specimen was properly loaded and ready to test, the gap distance, or distance between the plates, was recorded using a digital caliper.

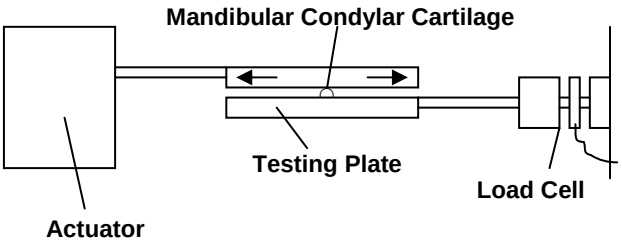

Figure 1. Schematic of a linear, oscillatory shear test using a simple shear sandwich configuration.

The mandibular condylar cartilage was then tested in the anterior-posterior direction at 1.0% shear strain along a frequency range 1 – 90 Hz. During each tested frequency, the displacement and load experienced by the specimen were recorded using the WinTest software. These values, along with the contact area and gap distance, were then used to calculate the viscoelastic shear properties of the mandibular condylar cartilage using Mathematica and Excel.

To calculate the shear properties, the stress and strain data were first fit with two sine curves, assuming the linear viscoelastic theory:

$$\text{Stress} \quad \sigma(t) = y_\sigma + A_\sigma \sin[\omega t + 2\pi\delta_\sigma]$$
$$\text{Strain} \quad \varepsilon(t) = y_\varepsilon + A_\varepsilon \sin[\omega t + 2\pi\delta_\varepsilon]$$

The complex modulus was then calculated, as well as the phase angle delta:

$$\text{Complex Modulus } (G^*) = A_\sigma / A_\varepsilon$$
$$\text{Delta } (\delta) = \delta_\sigma - \delta_\varepsilon$$

Finally, the elastic modulus, viscous modulus, and dynamic viscosity were determined:

$$\text{Elastic Modulus } (G') = G^* \cos(\delta)$$
$$\text{Viscous Modulus } (G'') = G^* \sin(\delta)$$
$$\text{Dynamic Viscosity} = G''/\omega$$

RESULTS

The calculated viscoelastic data for three rat condyles was averaged for each frequency tested. The average dynamic viscosity (Figure 2) and average complex modulus (figure 3) were then plotted, using log-log plots, as functions of frequency from 1 – 90 Hz. As shown in Figure 2, the average

dynamic viscosity, or resistance to flow, decreased from 1.12 – 0.02 MPa*s over the frequency range. The fact that the viscosity of the mandibular condylar cartilage decreased as the frequency increased is good because this trait, commonly called shear thinning, is observed in all biological tissues. In addition, Figure 3 showed that the average complex modulus, or shear stiffness, increased from 28.32 – 41.97 MPa over the frequency range, which is also characteristic of all biological tissues.

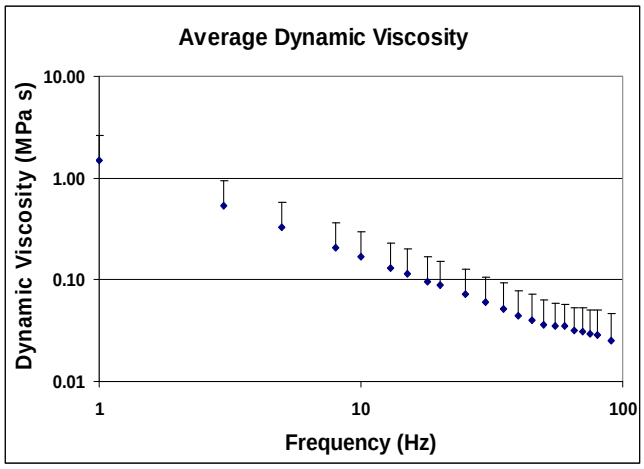

Figure 2. Average dynamic viscosity of rat mandibular condylar cartilage as a function of frequency (n=3).

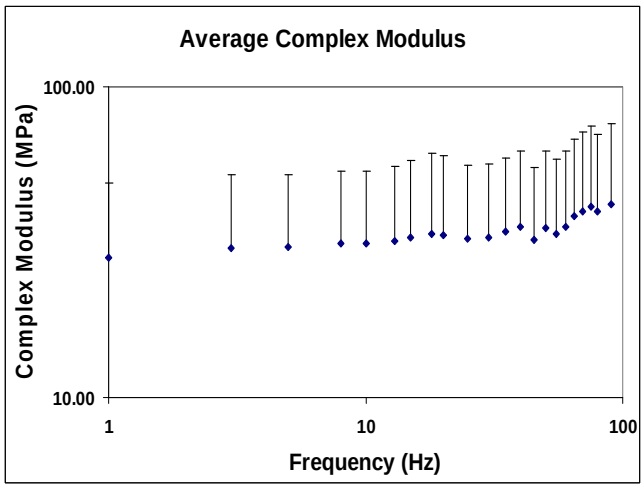

Figure 3. Average complex modulus of rat mandibular condylar cartilage as a function of frequency (n=3).

DISCUSSION AND FUTURE DIRECTIONS

Unfortunately, the data collected in this rat mandibular condylar cartilage experiment did not support previous literature. In 2008, a study was performed on porcine mandibular condylar cartilage. The cartilage was tested in the anterior-posterior direction at 1.0% strain and a frequency range 0.01 – 10 Hz. This experiment produced complex moduli of approximately 0.7 – 2.0 MPa along the frequency range [3]. However, the values for the complex modulus of the rat mandibular condylar cartilage varied from 28.32 – 38.36 MPa along the frequency range 1 – 10 Hz.

There are many reasons for why this discrepancy between the two studies may have occurred. For one, the rat condyle is only a few millimeters long on all sides and is rounded on the superior surface. Thus, it is extremely difficult to glue the specimen onto the plate in an upright position, and even harder to achieve a uniform shear along the top of the condyle. Another reason for this difference could be because the layer of cartilage on the end of the rat mandibular condyle is extremely thin. As a result, it's possible that the underlying bone, as well as the cartilage, was experiencing shear during the tests. If this was the case, the fact that bone is stiffer than cartilage could very well account for the data from the rat mandibular condylar cartilage being so large compared to that of the porcine study.

Other reasons for this disagreement in data could obviously be the fact that the two studies were not using the same animal models. Also, while fourteen condyles were actually tested for this experiment, only three were able to be included in the results because it took eleven condyles to establish the protocol for the shear test. Thus, the rat mandibular condylar cartilage study did not include that many specimens in its results. Finally, there was also some human error in determining the contact areas of the specimens using digital calipers.

Future studies include carrying out this same protocol using porcine mandibular condylar cartilage. The data from that study will then be compared to the porcine data from previous literature, as well as the results from the rat mandibular condylar cartilage study. This could help to clarify whether the error in this experiment stemmed from an error in the protocol, or simply from using the rat as an animal model. Finally, a comparison will be made to human mandibular condylar cartilage data in order to determine how well the rat and porcine animal models truly represent humans for the study of the shear properties of mandibular condylar cartilage.

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I would like to thank Dr. Almarza and Andrew Feola for helping me throughout this entire project. I would also like to thank Dr. Woo and the entire MSRC staff for giving me this wonderful opportunity to work with them this summer.

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My name is Amy Chaya and I was born on April 5th 1987 in Drexel Hill Pennsylvania. In the fall I will be entering my senior year at the University of Pittsburgh studying bioengineering with a concentration in biomechanics. Prior to Pitt, I went to Conestoga High School in Berwyn Pennsylvania, where I spent the majority of my time outside of school taking dance classes and cheerleading.

Although I am unable to continue competitive cheer and dance in college, I participate in a number of different organizations on campus. I am a sister of Phi Sigma Rho, and a member of the Biomedical Engineering Society, Society for Women Engineers, and Campus Women's Organization. I am also a freshmen Peer Advisor on Pitt's Freshmen Engineering Leadership Team. Outside of school, I greatly enjoy spending time with my friends and family, traveling, dancing, working with animals, and of course, working at the MSRC.

My project at the MSRC has been an evaluation of the rheological properties of urethral bulking agents. Under the guidance of Dr. Steven Abramowitch and my graduate mentor Andrew Feola, I have learned a tremendous amount about the biomechanical properties of viscoelastic materials. I have learned not only how to properly collect and process experimental data, but also how to make intelligent interpretations. Overall, my experience at the MSRC has been extremely meaningful and helpful in teaching me how to become a better experimenter. It has introduced me to the field of tissue mechanics and provided me with useful research skills and techniques. With Dr. Woo's particular support, I have been encouraged to think more, ask more, and in turn, learn more.

I give a truly heartfelt thank you to my mentors for their continuous support throughout my research. To Steve for forcing me to remember Biomechanics 1 material during our 2+ hour conversations; and to Andrew for his tolerance of my incessant questions - "what does that mean?", "how do I calculate that?", and "when do I turn the locals on?". In addition, I'd like to thank all of the other MSRC researchers for their knowledge, support, and wonderful personalities, which have made this summer memorable and fun.

An Evaluation of the Rheological properties of urethral bulking agents

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INTRODUCTION

Urinary incontinence (UI), a loss of voluntary bladder control, directly affects 13 million adults in the US alone, which is only a fraction of the 200 million suffering worldwide.[1,2] Although UI affects people of all genders and ages, it primarily targets the elderly, with one in ten adults over the age of 65 directly affected.[3,4] In addition to causing potential embarrassment and daily interruptions, UI can be tedious and expensive to manage. In 1995, adults over 65 years old spent a total of $26.3 billion, approximately $3,565 per person, on UI diagnosis and medication.[5] The primary treatment options for UI include surgery, medication, catheterization, and urethral bulking agent (UBA) injection.[6]

UBAs are injected directly into the urethral muscular wall to increase wall thickness, therefore decreasing the urethral inner diameter. The decreased diameter increases the resistance to urine flow, thus decreasing the flow of urine.[7] UBAs however, serve only as a temporary treatment option and require repeated injections to maintain effectiveness. In addition to potential biodegradation, certain natural shear forces, such as laughing, coughing, and heavy lifting, have been linked to UBA failure.[8] The exact mechanism of failure however, remains unknown, therefore it is important to gather information regarding the biomechanical properties of these gels when subject to varying shear rates. To do so, rheological testing can be used. Rheology, the study of the flow of matter, is commonly used to evaluate the biomechanical properties of gels, however, this type of testing can be highly sensitive to gel hydration and surrounding temperature. Thus, the objective of this study was to evaluate the sensitivity of a common UBA to prolonged air exposure, and to subsequently design a humidity chamber to maintain a physiologically relevant testing environment, which prevents gel dehydration and maintains constant body temperature.

METHODS

To evaluate the gel's biomechanical properties, a shear testing protocol was developed. A 0.15mL sample of the common UBA Durasphere Exp was added to the fixed bottom plate of the parallel plate system as shown in **Figure 1**. An EnduraTEC Electro-Force Mechanical Testing System (Bose, Model 3200) was used to provide sinusoidal uni-axial shearing from 0.1 to 90Hz with 10% applied strain.

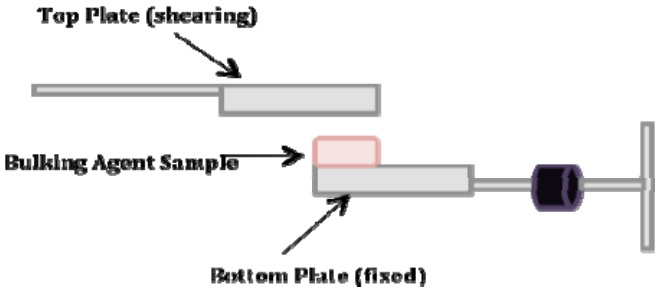

Figure 1. Parallel plate schematic of shear testing equipment

The complex modulus (stiffness) and dynamic viscosity (opposition to flow) were calculated to characterize the gel's biomechanical properties. Preliminary data analysis indicated inconsistent moduli and viscosities due to potential gel dehydration. To evaluate the effect of dehydration, a timed shear protocol was developed to test a 0.15mL sample at 1Hz every 5 minutes for 1 hour. In addition, the temperature at sample level was monitored throughout. The complex modulus was calculated and recorded over the testing hour. A humidity chamber was then designed to prevent potential gel dehydration by maintaining 100% humidity and 37ºC, as show in **Figure 2**. The timed shear test was completed and the complex modulus was calculated as previously described.

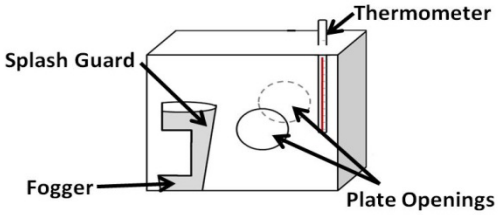

Figure 2. Humidity chamber design

RESULTS

The results of the timed protocol with open air exposure verified the expected increase in gel stiffness. **Figure 3** shows the changes in complex modulus.

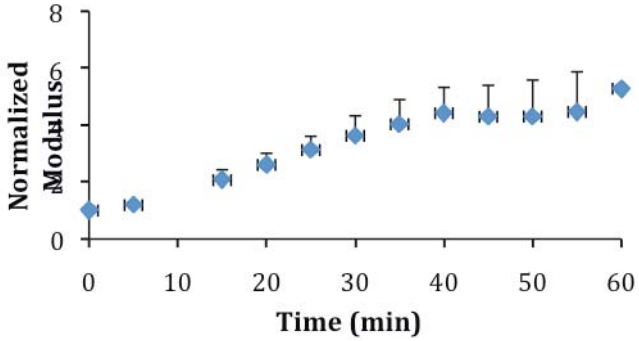

Figure 3. Impact of open air exposure on gel stiffness

As shown, within the first 30 minutes, the complex modulus increased to an average of 3.6 times its original value, indicating substantial gel dehydration.

In order to verify the effectiveness of the humidity chamber, temperature and humidity verification testing is being completed. Two trials have been completed to monitor the temperature within the humidity chamber over 40 and 60 minutes respectively. During the first trial, an average temperature of 35ºC (± 1) was maintained. Adjustments in the water level raised the average temperature of the second trial to 37.5ºC (± 0.5). In addition, one test has been completed to verify the humidity within the chamber. To do so, the timed shear protocol was used with a moistened gum sample, both in open air exposure and within the humidity chamber. **Figure 4** shows the changes in gum stiffness under both conditions.

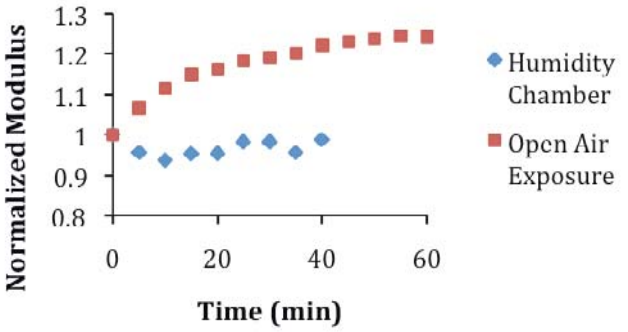

Figure 4. Humidity verification test results

As shown, open air exposure caused an increase in gum stiffness to an average of 1.17 times its initial value, while the humidity chamber limited moduli fluctuations to ± 0.02 times the initial value.

CONCLUSIONS

Preliminary temperature verification results suggest the chamber's ability to maintain a constant temperature which can be controlled by changing the water level. Similarly, preliminary humidity verification results suggest the chamber's ability to decrease sample dehydration, thus providing more accurate force response measurements. Future testing will be completed to further verify the chamber's effectiveness. In addition, other common UBA samples will be tested to further classify and compare their biomechanical properties.

ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Woo and the entire Musculoskeletal Research Center for providing me with the opportunity to complete my research project with them this summer. Specifically, I would like to thank Dr. Steven Abramowitch and Andrew Feola for their continuous help and support throughout my research.

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I was born on September 28, 1988 in Honolulu, Hawaii. With my father in the military, I moved around the country at a young age, but finally came to settle with my family in Maryland. I graduated with the Class of 2006 from Huntingtown High School where I played varsity lacrosse and field hockey and was a member of the student government.

My interest in engineering and how it can be applied to the medical field can be accredited to a combination of my father, my high school physics teacher, and my appreciation for sports. I'm not sure where exactly my interests will lead me, but I am sure that I will enjoy the ride. At Carnegie Mellon University this fall, I will be a Junior, continuing my education in Mechanical Engineering, Biomedical Engineering, and International Relations. I have continued my interest in sports by joining CMU's crew team and rooting for Pittsburgh's sports teams whenever possible. In addition, I am a member of Carnegie Mellon's chapter of the Delta Delta Delta sorority and am deeply involved in the philanthropy work we do for St. Jude Children's Research Hospital.

This summer has been an amazing learning experience for me, and I truly appreciate all the help I've received. I'd like to thank my mentor, Matt Fisher, for his patience, Dr. Debski for organizing this program, and Dr. Woo for his encouragement.

Biomechanical Properties of the Anterior Cruciate Ligament Following Bioscaffold Treatment in the Goat Model

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INTRODUCTION

The anterior cruciate ligament (ACL) connects the femur to the tibia and stabilizes the knee by restricting the tibia from excessive anterior-posterior (AP) translation, internal-external rotation, and varus-valgus rotation [4,6]. Unfortunately, tears of the ACL are very common, particularly in sports, with one in every 3000 people experiencing this injury in the US alone every year [2]. ACL reconstruction using grafts harvested from the patient is usually the most commonly recommended for those who are young and active [4]. While ACL reconstruction does restore initial knee stability, there is still a high incidence of complications associated with the graft donor site and osteoarthritis in the long-term [2,3,4].

In light of these long-term problems, attention has turned to functional tissue engineering (FTE) in search of a viable alternative [5]. When applied to the ACL, FTE approaches using growth factors and bioscaffolds have seen moderate successes. However, in these studies, the biomechanical properties have not been fully restored and the healing tissue remains hypertrophic, indicating tissue of a lesser quality [7].

In our research center, we have attempted to heal ligaments and tendons by applying a porcine small intestinal submucosa (SIS) bioscaffold. SIS can be produced in a sheet or hydrogel form and contains collagen and growth factors. When applied to the medial collateral ligament and patellar tendon, it has shown promise in limiting hypertrophy and improving the biomechanical properties of the tissue [8]. Recently, in collaboration with Revivicor, Inc., SIS has been made available from genetically modified pigs in which the gene that would elicit an immune response in humans is knocked out (KO). Thus, the objective of this study was to incorporate KO SIS sheet and hydrogel into the primary repair treatment of an ACL injury in a goat model to improve healing in the ligament 12 weeks following the primary repair surgery. With this treatment, we hypothesize that it is possible to accelerate the healing response with the SIS hydrogel, while guiding tissue growth and containing the healing response at the injury site with the SIS sheet, all of which will ultimately improve the biomechanical properties of the healing ACL.

METHODS

Six skeletally mature female Saanan breed goats were used in this study. For the primary repair surgery of the ACL, a #1 Ethibond suture was first placed in the proximal and distal thirds of the ACL, and the ACL was fully transected in the middle third. Two bone tunnels were made through both the femur and tibia. The loose ends of the distal and proximal sutures were passed through the opposite tunnels and tied under tension over the bone bridge in order to reapproximate the injured ends of the ACL. The SIS hydrogel was injected onto a Gelfoam sponge at the injury site, and the SIS bioscaffold sheet was wrapped around the ACL. The wound was then closed. After 12 weeks of healing, the goats were euthanized. In preparation for testing, the legs were cut such that approximately five inches of the tibia and femur extended from the knee and the knee was kept intact. The bones were potted in an epoxy compound.

The anterior-posterior tibial translation (APTT) and ACL in-situ force were measured using a robotic/UFS Testing System (Unimate Puma). This system can control and reproduce motion in six degrees of freedom, with a position repeatability of 0.1mm and 0.1° [8]. First, a passive path, which minimized the external forces and moments, was found for the knee as it flexed from full extension to 90°. Then, at 30°, 60°, and 90° of flexion, an AP load of 67N was applied to the tibia, and the resulting kinematics, including the APTT, were recorded. All components of the knee were then subsequently cut away leaving only the ACL connecting the femur and tibia. By replaying the kinematics and measuring the change in forces, the in-situ forces of the ACL could then be found by the principle of superposition.

Following robotic testing, the gross morphology and condition of the ACL was noted. Cross-sectional area of the proximal third of the ACL was measured by a laser micrometer [9]. Next, the goat femur-ACL-tibia complexes (FATCs) underwent tensile testing on an Instron 4502 under a crosshead speed of 10mm/min. Specimens were fixed within custom clamps at 70° of knee flexion. A 2N preload was applied followed by ten preconditioning cycles from 0 to 1mm. Another 2N preload was applied, and the FATC was loaded to failure. Stiffness was calculated from the linear portion of the resulting load-

elongation curve. Ultimate load was defined as the maximum force applied to the specimen before failure.

RESULTS

Gross morphology of the ACLs from the sham-operated knees showed that they were white and opaque with observable collagen fiber alignment. In the SIS-treated healing ACLs, the tissue was reddish in color, and there was no noticeable collagen fiber alignment. Sutures from the primary repair could be seen within the SIS-treated healing tissue. One specimen was discounted from testing and analysis due to an error in the surgical technique and subsequent lack of tissue formation in the SIS-treated healing ACL. For the remaining specimens, the values for cross-sectional area were found to be very similar between the sham-operated and SIS-treated healing ACLs (22.2±4.5mm² vs. 21.4±9.1mm², respectively). Robotic testing showed that APTT in the sham ACLs were lower than that in the SIS-treated healing for 30° (3.6±0.3mm vs. 9.1±2.0mm, respectively), 60° (3.9±0.6mm vs. 11.8±2.4mm, respectively), and 90° of flexion (3.1±0.5mm vs. 10.6±2.2mm, respectively). The in-situ force of the SIS-treated healing ACL was remarkably similar to the sham ACL at 30° and 60° of flexion, and though the in-situ force dropped to approximately 50% of the sham-operated ACL at 90° of flexion, it continued to bear a significant load (Figure 1).

During tensile testing, all of the sham-operated ACLs failed at the femer, while all of the SIS-treated healing ACLs failed in the ligament midsubstance. Stiffness values for the SIS-treated healing ACL reaching almost 42% that of the sham (Figure 2), while the SIS-treated healing ACL had an ultimate load about 12% that of the sham (185.7±85.7 vs. 1601.5±227.1, respectively).

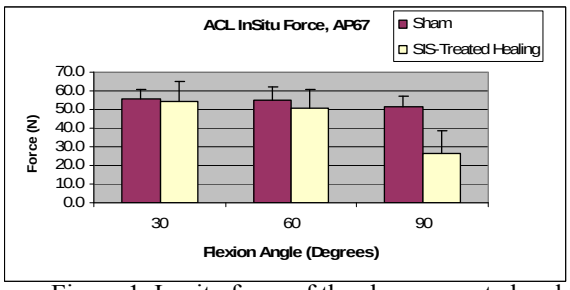

Figure 1. In-situ force of the sham-operated and SIS-treated healing ACL under a 67N anterior-posterior tibial load.

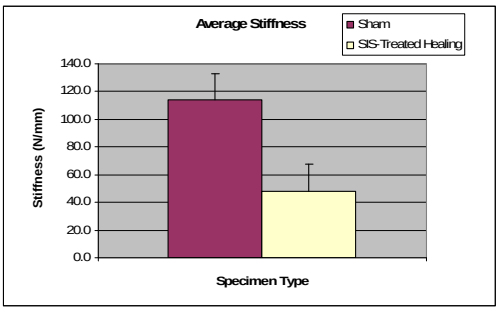

Figure 2. Stiffness of the sham-operated and SIS-treated healing ACLs.

DISCUSSION

Healing of the ACL was analyzed 12 weeks after performing a primary repair surgery supplemented by a KO SIS sheet and hydrogel through robotic and tensile testing. Gross observations and the similarity between the cross-sectional areas of the sham-operated and SIS-treated healing ACL indicate that there was controlled tissue growth and limited hypertrophy in the SIS-treated healing ACLs, thereby supporting our hypothesis. Robotic testing showed that the SIS-treated healing ACL was important to knee function at all flexion angles tested. Tensile testing showed that the SIS-treated healing ACL somewhat restored the structural properties of the sham-operated ACL. Values for APTT, ACL in-situ force, and structural properties of the FATCs are comparable to those found in literature for ACL reconstruction at six weeks of healing and primary repair with a collagen platelet rich plasma bioscaffold in a porcine model at four weeks of healing [1,7].

The results indicate that SIS bioscaffolds used in primary repair did facilitate healing of the ACL. These early results show promise that this technique may be a possible alternative to ACL reconstruction. To further investigate and verify these results, a study of the healing response after a longer time period, standardizing the primary repair surgery, and enhancing cell growth and collagen alignment on the healing ACL would be valuable.

ACKNOWLEDGEMENTS

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I was born on in Pittsburgh on September 22nd, 1987. I went to Upper Saint Clair high school just south of the city. At USC I was involved in many academic activities, I swam varsity for the varsity swim team all four year and was captain during my senior year. I graduated in June of 2006 and am now attending Carnegie Mellon University.

I am beginning my junior year at CMU. My time there so far has been both exciting and challenging. I am double majoring in Materials Science and Engineering and Biomedical Engineering, I am a member of the Kappa Sigma fraternity, and I still swim in my free time. Since my first semester at Carnegie Mellon, I have also been playing waterpolo with the CMU club team and helping to build robots with the robotics club and CMU's Google Lunar-X Prize team.

Working at the MSRC has been an interesting and exciting experience. The opportunity to perform real research as an undergraduate student is incredibly rare and has provided me with invaluable hands-on experience in my future field of work. Everyone has been very friendly and helpful along the way, and I'd like to thank my mentor Antonio Gonzales, as well as Serena Augustine, Matt Fisher, and the rest of the grad students. I would especially like to thank Dr. Abramowitch, Dr. Debski, and Dr. Woo for providing me with this incredible opportunity to learn and grow here at the MSRC.

Characterization of the Structural Properties of the Human Medial Patello-Femoral Ligament

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INTRODUCTION

The Medial Patello-Femoral Ligament has been recognized as the most important stabilizer preventing lateral dislocation of the patella. In athletes, patellar dislocation is not uncommon, and is usually the result of lateral translation of the patella caused by a sudden change in direction, which causes severe twisting of the knee joint.

Reconstruction of the MPFL is often necessary to return stability to the knee, especially if the patient expects to maintain an active lifestyle. The MPFL has the capacity to heal with time and physical therapy, but often does not return to its original strength, as evidenced by the high rates of patellar instability and redislocation (up to 50%).

The structural characteristics of the MPFL are not well documented. The most common replacement graft used is the hamstrings tendon, but the characterization of the Medial Patello-Femoral Ligament would aid in surgical repair procedures and graft selection.

MATERIALS AND METHODS

The MPFL specimens were harvested from human knees of various demographics that had been used in a previous experiment unrelated to the MPFL or patellar dislocation. The MPFL was removed with the entire patella and a segment of the femoral head, cut at 37° from the horizontal of the posterior femoral epicondyles. The ligament was trimmed of excess tissue and then examined for damage or natural defects. The Bone-MPFL-Bone complex was wrapped in gauze, moistened with saline, and frozen until the day of the experiment.

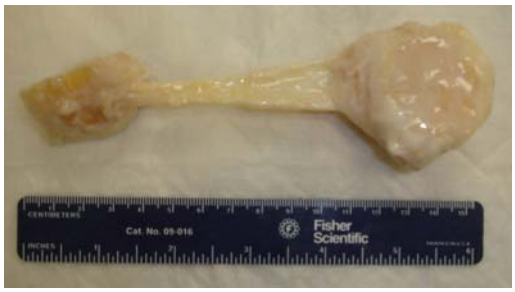

Figure 1: Bone-MPFL-Bone complex

On the day of the experiment, the sample was allowed to thaw to room temperature. The specimen was kept moist during preparation with a saline drip. Measurements were taken using digital calipers [0.1mm]. The length from insertion to insertion was recorded, as well as the patellar, medial, and femoral widths and thicknesses.

The MPFL was secured by potting the femoral end in a metal mold using poly(methyl-methacrylate) resin. The patella was placed in a custom clamp with two screws through the anterior face of the bone.

The cross-sectional areas at the patellar, medial, and femoral ends were taken using a laser micrometer system [0.1mm^2]. The cross sectional area was estimated by take a laser measurement of the diameter at 3° increments until a 180° image had been formed. After measurements were complete, 8-10 black marker spheres (~1mm diam.) were glued to the surface of the ligament to record strain.

Figure 2: MPFL and clamps mounted into the Laser Micrometer scanning system.

The specimen was mechanically tested using an Instron 5565 tensile testing machine and BlueHill2 software package [0.1N]. The clamped MPFL was mounted to the machine in a saline bath and maintained at 37°C using a water heater/circulator.

Upon mounting, the load was balanced and a preload of 2N was applied to establish an initial gauge length. The elongation was tared and the ligament was slacked. After 30 minutes in the bath to ensure full hydration and temperature equilibrium, the specimen was again

loaded to 2N, and any significant change in the gauge length was noted. Next the MPFL was preconditioned with 10 cycles of extension between 0 and 2 millimeters at a rate of 10 mm/min. Finally, a load to failure test was performed at 10 mm/min. Video of the test was recorded using an Adimec 1000m camera system with DMAS Capture software. The DMAS Tracker program was then used to calculate the strain at the point of failure.

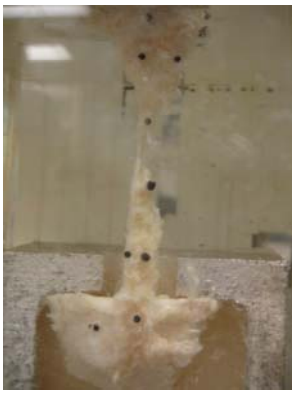

Figure 3: The mid-substance failure of an MPFL. Visible are the black strain markers as well as the aluminum PMMA mold that holds the femoral bone block.

RESULTS AND DISCUSSION

Seven acceptable samples were found and tested. Six samples failed at the femoral insertion site, and one failed mid-substance. Figure 4 contains an example of a typical

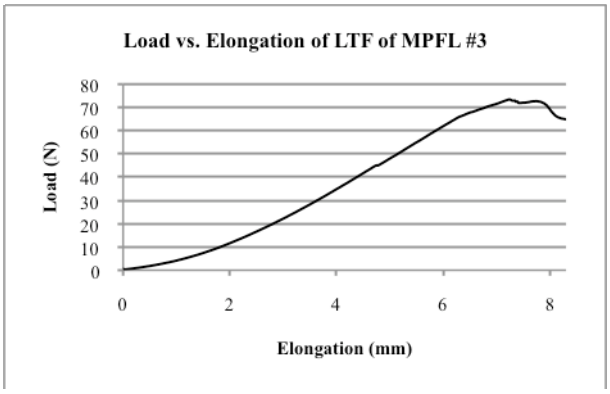

Figure 4: Example load/elongation graph of the LTF test on an MPFL.

load/elongation curve for the MPFL, and Table 1 contains a list of mean structural and mechanical values. The standard deviations of the means varied greatly, with some as low as 5% of the mean, while others could be as much as 95%.

When compared to the findings of Conlan et al. on knee ligament stiffness, our mean stiffness fell within one standard deviation of his mean stiffness for the MPFL. A comparison of our data to Conlan's data is shown in figure 5.

Value	Mean	Std. Dev.
Ult. Load	146.4 N	61.5 N
Peak Stress	3.3 MPa	1.2 MPa
Peak Stiffness	18.7 N/mm	6.9 N/mm
Total Elongation	12.6 mm	4.4 mm
CS Area	48.1 mm^2	22.7 mm^2
Elastic Modulus	13.2 MPa	12.5 MPa

Table 1: Mechanical and structural properties of the MPFL. Means and standard deviations are listed with their respective units.

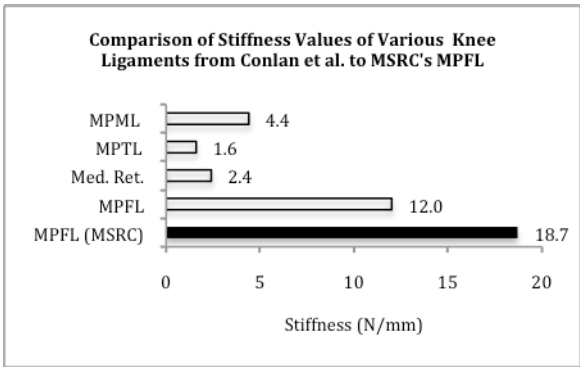

Figure 5: Comparison of stiffness findings from Conlan et al. to our mean MPFL stiffness.

Upon review of our data, it appears to correlate well with what little information has been published about the MPFL. In the future more samples will be tested to increase our sample size and hopefully lower the standard deviation of the mean values.

Other future work might include an examination of age, sex, and health effects on the characteristics of the MPFL. We also plan to apply our findings to suggest surgical procedures and suitable graft ligaments that might be used for MPFL repair.

ACKNOWLEDGEMENTS

Thanks to Antonio for all his help and guidance, the grad students and summer students, Dr. Woo, Dr. Abramowitch, Dr. Debski, and all of the MSRC staff.

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I was born and raised in southeast Alabama, or what southerners refer to as the "Heart of Dixie." I attended high school in Grove City, PA, and then entered the pre-health program at Washington & Jefferson College. I am now entering my senior year, majoring in both cell and molecular biology and Spanish. I'm a 14-year soccer veteran and I still play soccer on the women's college team, even serving as my team's representative to the Student Athletic Advisory Committee and earning a spot on ESPN magazine's Cosida Academic All-District team. I am president of my school's Pre-Health Professions Society and treasurer of Phi Sigma, the biological sciences honorary society. I'm also on my school's payroll as a biology teaching assistant and Spanish tutor. Away from school I love outdoor activities and playing sports or musical instruments, of which mastering the guitar is my most recent endeavor.

My interest in medicine did not develop overnight; it required the influence of close family and friends who had witnessed the global healthcare deficit first-hand, the many injuries sustained by my soccer teammates and myself, the death of a grandfather who was a sports medicine physician, and my own experience working with an international health organization in La Paz, Bolivia. All of these elements fostered my desire to pursue medicine as a profession.

I have greatly enjoyed my time here at the MSRC. I feel that the breadth of knowledge that has been provided to me will considerably assist me in attaining my future goals. I would like to thank Mr. Cook and Merck for funding my experience here, and I am also extremely grateful to my wonderful mentor, Rui Liang, the awesome members of the ACL group, Dr. Abramowitch, Dr. Debski, Dr. Woo, and the entire MSRC family for granting me the opportunity to work here and for providing me with this incredible experience!

BIOACTIVE FACTORS IN GAL-KNOCKOUT ECM BIOSCAFFOLDS

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INTRODUCTION

Biological scaffolds have been utilized to facilitate site-specific healing in vascular, urinary tract, skin, and musculoskeletal applications (2). These are primarily composed of xenogeneic extracellular matrix, comprised of various bioactive factors such as the proteins collagen, fibronectin, and laminin, as well as glucosaminoglycans (GAGs) and growth factors like VEGF, PDGF, and TGF-β1 (2, 3). The effectiveness of bioscaffolds has been attributed to the bioactive factors which, upon host enzymatic degradation of the scaffold following xenotransplantation, are released and available to facilitate healing (2). Porcine-derived small intestine submucosa (SIS) is one scaffold which is commonly used. Previous studies have utilized this porcine SIS in the enhanced healing of the MCL and patellar ligament, where increased tangent modulus, tensile strength, and fibril diameter were demonstrated in the healing MCL and the patellar tendon displayed increased neo-PT tissue formation and better organized tissue matrix (10, 11, 14).

Due to an elicited immune response observed in humans to the α1,3 galactose (α gal) epitope present on mammalian tissues (with the exception of humans and old world primates), genetically-modified pigs (Gal K/O) have been engineered to not express the gene encoding α 1,3 galactosyltransferase (α1,3GT) (1, 12, 13). This enzyme facilitates the glycosylation of N-acetyllactosamine (LacNAc) type oligosaccharides, thereby depositing the α gal epitope that elicits an immune response from humans in xenotransplantation (9). Although α1,3 GT is no longer expressed in Gal K/O pigs, a low level of α gal epitope is still present on cells from Gal K/O pigs, possibly in the form of a lipid, isoglobotrihexosylceramide (IGb3) which is produced by iGb3 synthase (8, 9).

Confirmed bioactive factors of SIS include, but are not limited to, the ECM component fibronectin (Fn) and the growth factor TGF-β1 (5). Fn is a large (440 kDa) protein found in plasma and the ECM and, next to collagen, it is the second most abundant component of the ECM (5, 2). Its importance as a scaffold component is suggested through its role as one of the first proteins deposited in the new ECM in wound healing, in addition to its role in the facilitation of host cell attachment, cell growth and differentiation (5, 2, 3). It withstands SIS disinfection and sterilization procedures, which suggests that it is a good bioactive factor to detect in this study (4).

TGF-β1 is responsible for the regulation of matrix protein transcription and has been cited as serving a pivotal role in tissue remodeling, angiogenesis, and wound healing (6, 7). It may also be the strongest known stimulator of chemotaxis (6). Like other GFs, TGF-β1 is suspected of being sequestered by matrix components, thereby retaining it in spite of sterilization and lyophilization processes (4, 7). Another notable correlation that exists between GFs and ECM components is their mutualism; GFs regulate the presence of ECM components while these components regulate the activity of the GFs" (6).

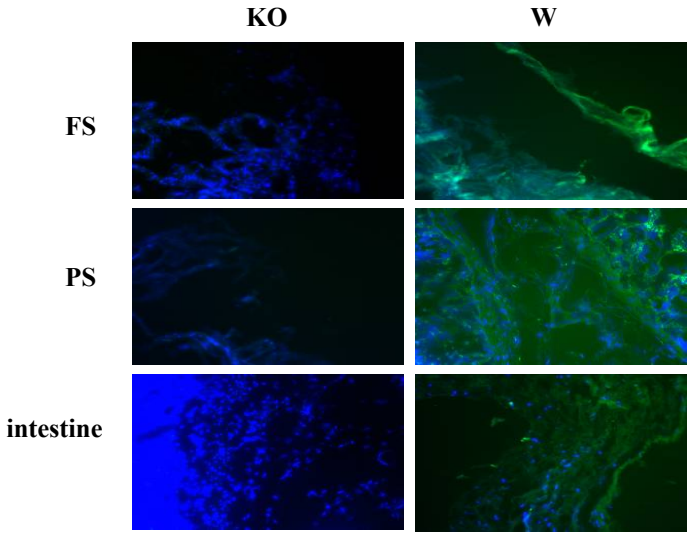

Figure 1. Immunofluorescence of α gal at 200x in SIS and intestine, where blue fluorescence (200 msec exposure) represents the Hoechst staining and green fluorescence (6000 msec exposure)

The development of Gal K/O pigs has led us to question the sustained bioactivity of SIS ECM scaffolds derived from the porcine knockout source in comparison to the bioactivity of SIS ECM scaffolds created from wild type porcine sources. Has the genetic manipulation of the porcine genome (*α1,3GT*) affected the effectiveness of the bioscaffold? Does the α gal knockout porcine SIS demonstrate similar bioactive properties as wild type porcine SIS? We hypothesize that the genetic modification of pigs to knockout the *α1,3GT* gene does not affect the effectiveness of the bioscaffold through the altered expression of certain bioactive factors (fibronectin and TGF beta1) existing in gal-knockout porcine-derived SIS when compared to wild type porcine SIS. To date, no study has yet evaluated the bioactive factors in Gal K/O porcine-derived SIS. Therefore, the objective of this study is to Confirm *α1,3GT* knockout in designated porcine SIS scaffolds through fluorescence, utilize immunofluorescence to determine the presence of an ECM protein, fibronectin, in SIS bioscaffolds, and utilize immunohistochemical analysis to determine the presence of an ECM growth factor, TGF-β1, in SIS bioscaffolds.

MATERIALS AND METHODS

Materials

Antibodies used for immunohistochemistry were anti-Fn (AB1945, Millipore) and TGF-β1 (sc-146, Santa Cruz Biotechnology, INC.). R.T.U. Avidin/Biotin, ABC, and DAB substrate kits (sp-2001; pk-4002; sk-4100) were purchased from Vector Laboratories Inc. (Burlingame, CA). Fn secondary antibody and hoechst (F-2266; 33322, Sigma-Aldrich, St. Louis, MO), along with lectin (Fl-1201, Vector Laboratories Inc.) were additionally obtained. SIS was self-prepared.

Specimen Preparation

Eight SIS scaffolds (fresh wild-type (FS W), fresh knockout (FS KO), processed wild-type (PS W), and processed knockout (PS KO) were utilized. Samples from each respective tissue block were subject to cryosection with a Miocrom HM 550 and frozen to slides. Each sample was cut to a thickness of 8 µm. Wild-type porcine small intestine (519 W intestine) and knockout porcine small intestine (68-3 KO intestine) slide specimens were pre-cut.

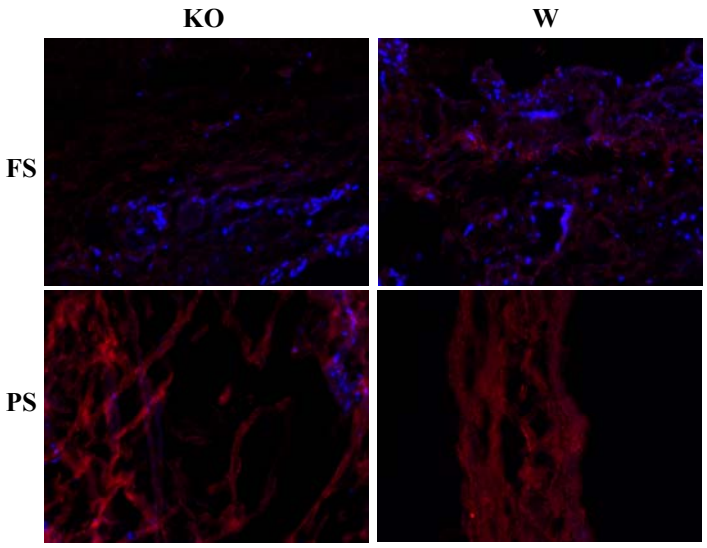

Figure 2. Immunofluorescence of Fn revealed positive results in FS KO, PS KO, FS W, and PS W SIS. Photos were taken at x200 magnification, where blue fluorescence (200 msec exposure) represents hoechst staining and red fluorescence (600 msec exposure) denotes Fn.

α gal Fluorescence

To determine α gal presence, thawed slides were fixed in acetone, washed briefly in TBS solution (1x TBS and 0.05% triton x-100) and then incubated in 1:200 lectin in buffer (1x TBS, 0.05% triton x-100, CaCl2, and sodium azide) for 1 hour at room temperature. Following three additional brief washes (two in TBS solution, and the last in a PBS solution with 0.05% triton x-100), Hoechst was applied (1:3200 stock hoechst (10 mg/ml) to PBS) for two minutes at 37° C. The procedure was completed with three additional washes in PBS.

Fn Immunofluorescence

Following a brief wash in TBS solution, slides were blocked with a serum solution (1 ml abc horse serum and 4 drops Avidin) and incubated at room temperature for one hour. Three additional TBS solution washes were followed by application of the primary Fn antibody (1:100 Fn antibody in abc horse serum plus two drops Avidin) which was allowed to incubate overnight at 20°C. Secondary antibody from the abc kit was applied to slides and incubated for one hour at room temperature following three brief washes in TBS solution. Three additional washes in TBS solution were followed by application of 1:200 Tex Red Avidin D in Hepes solution (10mM Hepes buffer with pH = 8) for one hour at room temperature. Slides were washed twice in TBS buffer, then once in PBS solution. Hoechst was applied for two minutes at 37°C, and then slides were briefly washed three times in PBS.

TGF-β1 Immunohistochemistry

Thawed SIS samples were washed in a TBS solution (1x TBS and 0.05% Triton x-100) and then incubated in 3% H_2O_2 for 15 minutes at room temperature. Following three additional washes in TBS solution, horse serum from the abc kit was applied for 30 minutes at room temperature. Three more TBS solution washes were followed by application of TGF-β1 antibody for 3 hours.

The Avidin/Biotin blocking kit was used. Avidin D solution was applied for 15 minutes. Slides were washed briefly in TBS solution, and then incubated for 15 minutes in biotin solution. Slides were briefly washed twice in TBS solution.

The secondary biotinylated antibody from the abc kit was applied for 30 minutes at room temperature, followed by three brief washes with TBS solution. Abc solution was applied for 15 minutes, followed by three TBS solution washes. DAB solution (5 ml distilled H_2O, 2 drops buffer stock solution, 4 drops DAB, 2 drops H_2O_2) was prepared from the DAB substrate kit and applied for 20 minutes. Slides were rinsed in tap water, and then counterstained using hematoxylin.

All photos for both immunofluorescence and immunohistochemistry assays were taken via SPOT software and camera from Diagnostic Instruments, Inc.

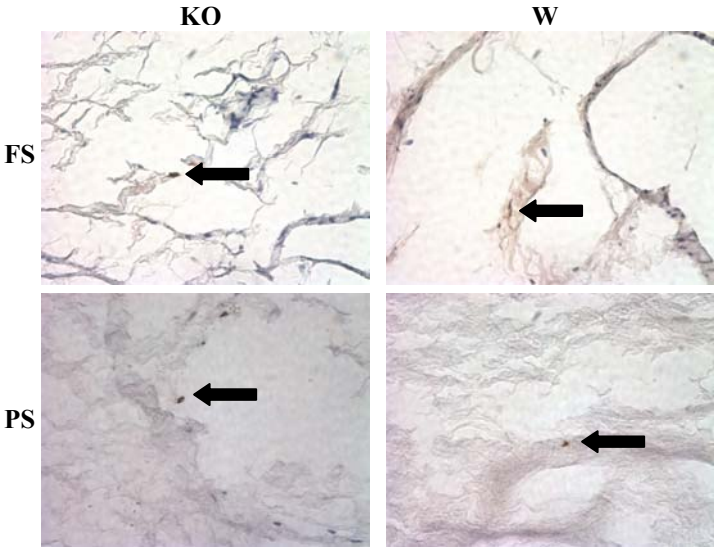

Figure 3. Immunohistochemistry of TGF-β1 revealed positive results in FS KO, PS KO, FS W, and PS W SIS. Photos were taken at x400 magnification.

RESULTS

α gal

The fluorescence of α gal confirmed the gal-knockout in the bioscaffolds and intestine derived from a gal-knockout porcine small intestine (Figure 1). The pronounced presence of α gal in the wild type bioscaffolds and intestine was also displayed.

Fn content

Immunofluorescence of Fn confirmed the presence of this protein in both Gal K/O and wild-type SIS bioscaffolds (Figure 2). The arrangement of Fn appears to closely interact with collagen fibers of the scaffold through the linearity of Fn.

TGF-β1 content

Immunohistochemistry analysis indicated positive results in all SIS samples (Figure 3). However, positive results were very sparse in each SIS sample.

DISCUSSION AND CONCLUSIONS

The identity of Gal knockout and wild type SIS bioscaffolds was confirmed through fluorescence of α gal. These same scaffolds then proceeded to exhibit positive results for the immunofluorescence of fibronectin and the immunohistochemical detection of TGF-β1. According to previous studies, both TGF-β1 and Fn have been noted as being conserved in wild-type bioscaffolds following sterilization procedures (7, 4). Although their presence in gal K/O SIS suggests that the genetic modification of the porcine genome did not affect the expression of these bioactive factors, the next logical step in the analysis of the Gal K/O SIS is to quantify the amounts of bioactive factors. Expansion upon the examined pool of bioactive factors is advisable, as well as the determination of maintained bioactive factor activity.

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I was born on July 23, 1985 in Hampton Virginia. I attended Bethel High School from 1999-2003 lettering in two varsity sports. Playing baseball since I was five, I continued playing varsity through high school. During my sophomore year I was talked into joining the swim team, and enjoyed every minute of it.

In the fall of 2003 I began my freshman year at Longwood University as a Biology student. While at Longwood I worked as an Intramural Sports Programmer, also playing in various sports. As I came into my junior year I began a new interest in the field of Genetics, Microbiology, and Human Anatomy/Physiology. Having a knack for Physics and various math courses, my Physics professor suggested that I take a look at bioengineering. After doing so I realized this was a career path worth pursuing. As such, I decided to apply to Virginia Commonwealth's School of Engineering. In March of 2007 I had been accepted into VCU's School of Engineering. And in May of 2007 I graduated from Longwood University with a Bachelor's of Science in Biology.

As I began my first semester as an engineering student at VCU, I felt that I needed something to keep me in shape and help me meet people. In receiving a VCU e-mail, I saw that the crew team at VCU was looking for new members. Soon after I joined the team and have been "hooked". Approaching the beginning of 2008 I decided to apply for internships. Knowing that I only had 1 year of experience as an engineering student, I had hoped that my degree in Biology would help me to acceptance. In getting accepted I read that I would be working at the Musculoskeletal Research Center along side Dr. Steven Abramowitch and graduate student Andrew Feola.

The experience that this internship has given me is one that cannot be done in just a few words. In working at the MSRC I have been exposed to so many different testing machines, robotics, mechanisms, and protocols. It has also given me a chance to view the advancement in research that is taking place here. Aside from my improvement in scientific terminology, I have gained a great deal of knowledge in the area of Tissue Mechanics. Experiences such as these not only help develop you as a stronger student but also help develop you as a person. These skills will help mold me as a research scientist, and someday propel me in my career.

In closing I would like to thank the Pittsburgh Tissue Engineering Initiative (PTEI) for choosing me to participate in this internship. I would also like to thank Dr. Steven Abramowitch and Andrew Feola for the knowledge they've instilled in me, as well as the patience they had with me as a student. A thanks to Dr. Woo, who is an inspiration of knowledge and dedication to me. Lastly I would like to thank the entire MSRC for taking me in as an intern, and making someone from so far away feel as if he were at home here.

THE IMPACT OF SMALL INTESTINAL SUBMUCOSAL TREATMENT ON THE MECHANICAL PROPERTIES OF THE RAT VAGINA FOLLOWING A SIMULATED BIRTH INJURY

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INTRODUCTION. Pelvic Organ Prolapse (POP) is the protrusion of pelvic organs into or out of the vaginal canal. Women have an 11% chance of undergoing at least one operation for POP or urinary incontinence during their lifetime.[1] The number one risk factor for the development of POP is vaginal childbirth (parity), especially if a vaginal injury, i.e. laceration, occurs during delivery.[2] Recently, soft tissue scaffolds such as small intestinal submucosa (SIS), have been shown to improve the healing response of a number of soft tissues following injury.[3] Histologically, the SIS treatment reduced the ratio of collagen type V/I in the rat vagina following a simulated birth injury.[4] So theoretically, this would allow for an increase in functional tissue and a decrease in scar tissue. The objective of the current study is to perform mechanical testing on these samples to determine if the observed changes in collagen subtypes translate to changes in mechanical properties of the healing tissue. Since SIS reduces the excessive collagen type V produced following injury, We hypothesize that vaginal injuries treated with SIS will heal with mechanical properties that are more similar to uninjured controls compared to those that were injured and untreated.

METHODS. Thirty-five Long Evans rats were utilized in this study. We utilized an established balloon injury protocol to induce a vaginal injury in both virgin and pregnant rats.[4] Thus the following groups were evaluated: virgin controls, virgin ballooned, virgin ballooned + SIS, pregnant controls, pregnant ballooned and pregnant ballooned + SIS. Following euthanasia at 4 weeks after injury, the vaginas were dissected from the rats and length width and thickness measurements were recorded. A laser micrometer, as shown in Figure 1-1, was utilized to measure cross-sectional areas at the proximal, medial, and distal ends. Afterwards, the vaginas were then aligned into a 37°C bath in an Instron™ material testing machine and a preload of 0.1N was applied. The tissue was preconditioned to 7% clamp-to-clamp strain for 10 cycles at a crosshead speed of 10mm/min. This was followed by a load to failure test at the same extension rate. A calibrated Charged-coupled device (CCD) camera with a two-dimensional accuracy of 0.008mm and a resolution of 7.4μm pixel was the optical strain measurement system utilized to measure strain. Parameters describing the mechanical properties of tissue were obtained from the resulting stress-strain curves; of which, tangent modulus and tensile strength are reported. Typical stress strain curves are found in Figures 2, 3.

Figure 1. Laser micrometer

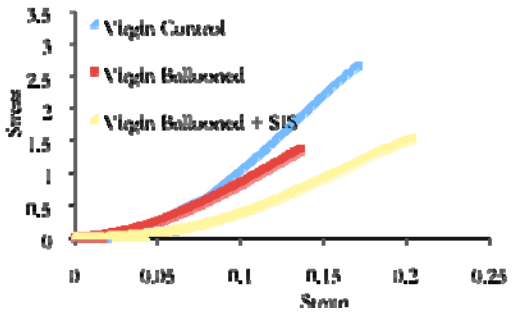

Figure 2. Typical stress/strain curve of rat virgins

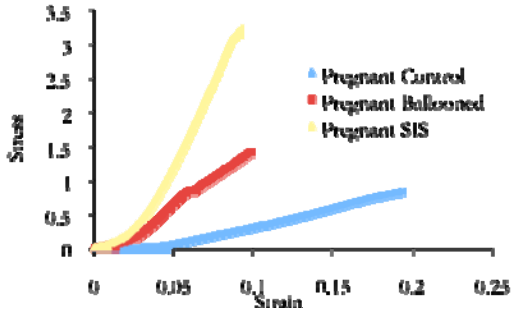

Figure 3. Typical stress/strain curve of pregnant rats

RESULTS. When evaluating the effect of SIS treatment in virgin animals, SIS treatment had no apparent effect on its ability to heal (34%↓ p=0.01-tangent modulus); the untreated injury resulted values similar to the SIS treated with significant values in stress, tangent modulus and strain energy density (45%↓ p=0.01, 39%↓ 0.01, 46%↓ 0.04 respectively). For pregnant animals, the SIS treated sample showed significant increases in stress and tangent modulus (225%↑ p=0.01, 392%↑ 0.03 respectively). The values were increased in the direction of the assessed virgin controls. The untreated sample resulted in stress and strain non-significant differences (134%↑ p=0.05, 28%↓ p=0.08 respectively) but a significant increase in tangent modulus (242%↑ p=0.01).

DISCUSSION. The interesting findings of this study are how the rat vagina is able to recover strength following injury during pregnancy, but it is unable to recover when the animal is not pregnant, as seen in Figure 4, 5. Thus, the extent of injury to pregnant vaginas using this injury model may be minimized by the softening that occurs during pregnancy and/or there may be an inherent reparative/remodeling process during the early post-partum period that allows for recovery. If the latter is true, SIS appears to have an influence on that remodeling process but it is unclear if it is beneficial for the values for tangent modulus and tensile strength to exceed virgin controls.

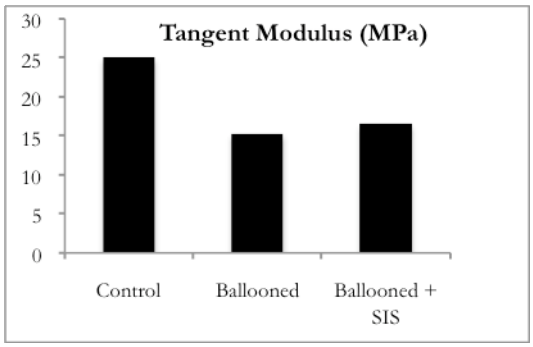

Figure 4. Virgin tangent modulus

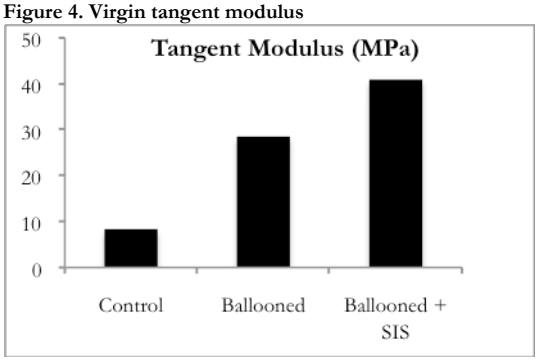

Figure 5. Pregnant tangent modulus

CONCLUSION. Consistent with previous studies in other soft tissues, SIS treatment improves the healing response of vaginal tissue following injury. However, this injury model demonstrated considerable variability in terms of the location and extent of injury to the vagina, which likely limited our ability to delineate the effects of SIS treatment.

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I grew up with my family in Bethel Park, Pennsylvania as the eldest of four siblings. After retiring from competitive gymnastics, I spent my years at Bethel Park Senior High School as a competitive diver on the Varsity Diving team and as an active member of the National Honors Society. Currently, I am looking forward to starting my junior year as a Biopharmaceutical Engineering major at Lehigh University. At Lehigh, I am the President of Alpha Gamma Delta Sorority, a member of Phi Eta Sigma Honors Fraternity, and a Junior Fellow for the P.C. Rossin College of Engineering and Applied Science. I am also involved with the Association of Student Alumni where I work to help plan campus-wide events, maintain relations with alumnae, and give campus tours to prospective students and their families. Outside of the academic world, I enjoy working with children at Gymkhana Gymnastics where I have been a gymnastics instructor for five years.

My experience at the MSRC has provided me the opportunity to learn more about functional tissue engineering and mechanobiology through hands-on experience in the laboratory. I feel privileged to have worked with and learned from some of the most brilliant leading researchers in the field. The MSRC has helped me to further recognize my passion for bioengineering research and the projects I worked on this summer have pushed me to develop both the technical and professional skills necessary for success in this field.

I would like to thank all of the directors, faculty, staff, and students at the MSRC for making this summer internship such a fantastic learning experience. I would especially like to thank my mentor, Serena Augustine, for all of the guidance, encouragement, and insight she provided throughout the summer and for helping me to take my research skills to the next level. I would also like to thank Dr. Woo for challenging me to set my goals high and for providing me with the means to achieve those goals by inviting me into the MSRC community this summer.

THE EFFECT OF CELL SEEDING DENSITY ON THE RELATIVE PROLIFERATION OF FIBROBLASTS SEEDED ONTO SMALL INTESTINE SUBMUCOSA (SIS)

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INTRODUCTION

Annually, between 150,000 and 200,000 sports and work-related injuries result partial or complete rupture of the anterior cruciate ligament (ACL).[1] Approximately 75% of these ruptures are associated with simultaneous damage to the medial collateral ligament (MCL).[1] Since the MCL has been shown to exhibit spontaneous healing, while the ACL possesses little or no healing capacity, current treatment modalities for a combined injury involve conservative treatment of the MCL coupled with ACL reconstruction using autografts such as the bone-patellar tendon-bone complex.[11] Although these methods improve the ability of the knee joint to function following injury, the mechanical properties of the healing MCL remain inferior to those of the intact MCL and donor site morbidity occurs as a result of graft harvesting.[12] Therefore, recent studies have looked into improving ACL and MCL treatments through functional tissue engineering (FTE) to enhance and evoke the healing capabilities of the MCL and ACL, respectively. These FTE approaches often include the use of natural biodegradable scaffolds, such as porcine small intestine submucosa (SIS) which has been shown to increase the structural and mechanical properties of the healing MCL.[4,5]

Additionally, cell seeding and mechanical conditioning are employed in order to increase the matrix deposition of seeded cells as well as to enhance the structural properties of the bioscaffold, which are highly correlated with the structural properties of the healing tissue.[6,7] However, the densities at which cells are seeded onto the scaffolds vary widely throughout literature and range from approximately 0.17 (10^5) – 5.3 (10^5) cells/cm^2.[3-10] Therefore, there is a need to determine an optimal cell seeding density for fibroblasts seeded onto SIS in order to enhance SIS-cell construct performance for future FTE studies that focus on additional factors effecting the regeneration of damaged or diseased tissues, such as mechanical stimulation and the addition of growth factors. The primary aim of this study was to find the density of ACL fibroblasts that provides the most favorable conditions for cell growth. We hypothesized that relative proliferation would increase with increasing cell density until it leveled off at a maximum value which would serve as the optimal cell seeding density for ACL fibroblasts on SIS.

The secondary aim of this study was to compare the growth rates of cells seeded onto a petri dish with cells seeded onto SIS. We hypothesized that the fibroblasts seeded onto SIS would proliferate at a higher rate than the fibroblasts seeded onto the petri dish.

METHODS

The fibroblasts used in this study were isolated by collagenase digestion from the ACL of one Sprague Dawley rat. The ACL was removed, minced, and exposed to 1 mL of a 1 mg/mL collagenase, 0.6 U/mL dispase solution. After one hour, the cells were washed with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine calf serum and 1% penicillin/streptomycin and were centrifuged at 1500 RPMs for 10 minutes. The collagenase solution was aspirated and the cell pellet was plated in 100 mm X 20 mm cell culture plates and stored in a 37°C [5% CO_2] incubator. When the cells reached approximately 95% confluency, they were passaged and replated. Cells used in these experiments were passaged 2-5 times prior to seeding onto either the well surfaces of treated 96-well plates or onto 0.32 cm^2 circular pieces of SIS coating the well surfaces of untreated 96-well plates. The cell densities tested in each experimental group (n=3) ranged from 0.3 (10^5) – 5.0 (10^5) cells/cm^2. In the SIS experiment, the scaffold was hydrated in media for 24 hours prior to seeding. In both experiments, the plates were incubated at 37°C for 24 hours following seeding to allow for cell attachment. In the SIS experiment, the SIS was digested with 1.25 mg/mL collagenase for one hour following the 24 hour cell attachment period.

Measuring Proliferation

For both the cells seeded onto the petri dish and the cells seeded onto SIS, a BrdU assay was used in order to measure proliferation at each seeding density. During the final 4 hours of the cell attachment period, a BrdU label was added to each of the wells to be incorporated into the DNA of proliferating cells. Treatment with a primary antibody (Anti-BrdU) and a secondary antibody (Peroxidase Goat Anti-Mouse IgG HRP) allowed a fluorogenic substrate to mark proliferated cells which had incorporated the BrdU label. The relative fluorescence of each well was quantified using a Fluorometer measuring excitation and emission at 325 and 420 nm, respectively. The relative fluorescence reading for each well was proportional to the amount of proliferation that occurred within the well. Proliferation curves were generated from the fluorescence data of the cells seeded onto both the petri dish and SIS.

Data Analysis

In order to determine the optimal cell seeding density for SIS, the proliferation curve of the cells seeded onto SIS was assessed. A curve was fitted to the data and the model was used to find the cell density at which a local maximum occurred. A one-way ANOVA was performed using Minitab software with a Fisher's LSD post-hoc analysis to determine the effect of cell

seeding density on relative proliferation. Significance was set at $p < 0.05$.

The linear portions of the proliferation curves for cells seeded onto SIS and cells seeded onto the petri dish were used to calculate the growth rates of the fibroblasts seeded onto the corresponding surfaces and the values were compared.

RESULTS

Determining Optimal Cell Seeding Density

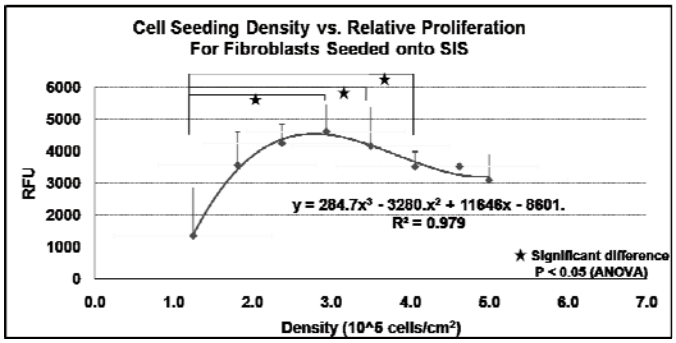

Figure 1: This figure represents the relative proliferation measured by relative fluorescence units (RFUs) for cells seeded onto SIS at increasing cell densities. The ★ symbol indicates a statistically significant difference ($p<0.05$).

Figure 1 represents the relationship between cell seeding density and relative cell proliferation for fibroblasts seeded onto SIS. A 3rd degree polynomial model was fitted to the proliferation data with an R^2 value of 0.979. This model was used to find a local maximum RFU value at 2.8 (10^5) cells/cm^2 which serves as an approximation of optimal ACL cell seeding density on SIS. A one-way ANOVA revealed that cells seeded at 2.9, 3.5 and 4.1 (10^5) cells/cm^2 proliferated significantly more than cells seeded at lower densities. Although all of the aforementioned seeding densities are greater than the calculated optimal seeding density of 2.8 (10^5) cells/cm^2, there were no significant differences between their proliferation values suggesting that leveling-off occurs within this range.

Comparing Growth Rates

Complete proliferation curves, such as the one pictured above, were obtained for the cells seeded onto the petri dish and the cells seeded onto SIS. The linear portion of each curve was fitted to a linear model that could be used to calculate the growth rate of cells seeded onto the corresponding surface. As depicted in Figure 2, the growth rates were calculated as 0.015 and 0.026 RFU/cell for the petri dish and SIS, respectively.

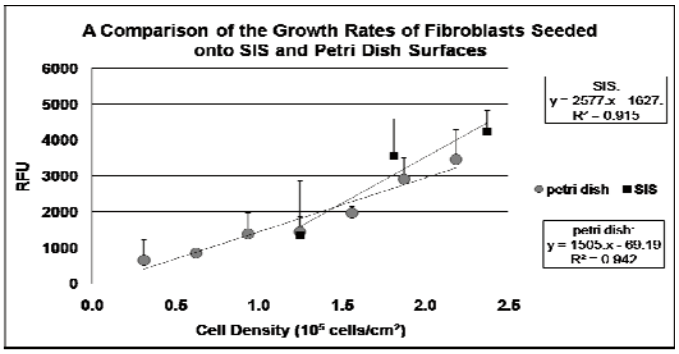

Figure 2: This figure represents the linear portion of the growth curves for cells seeded onto both SIS and a petri dish. These data were used to determine growth rates (RFUs/cell) of cells for both conditions.

DISCUSSION

The approximation of an optimal cell seeding density at 2.8 (10^5) cells/cm^2 was obtained in this study and can be used in future FTE studies in order to create enhanced SIS-cell constructs under optimal fibroblast density conditions. This value was established as an optimal density because it produces a maximum amount of proliferation and is located at the beginning of the density range in which proliferation levels off. It is possible that fewer cells will need to be seeded onto SIS for experiments exceeding 24 hours; therefore, future studies may include longer time periods such as 3 and 5 days.

Further, the higher growth rate of the cells seeded onto SIS indicates that fibroblasts proliferate faster when seeded onto the SIS. This conclusion is important when considering *in vivo* applications of SIS because it is crucial that SIS promotes more rapid proliferation in order to fill the tissue defect with cells that will begin to produce and deposit matrix.

In the future, an analysis of the amount of apoptosis occurring at each of the cell densities tested in the current study will be performed. Proliferation and apoptosis data can be combined in order to determine a density at which programmed cell death reaches a minimum within a density range that maintains cell proliferation.

ACKNOWLEDGEMENTS

I would like to thank my mentor, Serena Augustine, for her continuous guidance and support throughout this project. I would also like to thank Dr. Woo and the rest of the MSRC for making this summer internship program such a fantastic learning experience. Additionally, I would like to acknowledge the PTEI for its dedication to recruiting qualified tissue engineering researchers to the Pittsburgh area.

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I was born on June 6, 1987 and raised in a suburb northeast of Pittsburgh. I attended the North Allegheny School District and took advantage of whatever academic and extra-curricular opportunities I could. In addition to my science-intensive high school schedule, I have taken four years of French, and 14 band and choir classes. I was active in National Honors Society, French club, theater/musical theater, tennis team, marching band, wind ensemble, percussion ensemble, and chamber choir. As a senior I earned the rank of snare captain of the marching band, a highly competitive and coveted position of leadership.

After choosing to attend the University of Pittsburgh, I was considering a dual major in biology and music. My mother insisted that I choose bioengineering instead, and the rigorous engineering program proved it too difficult to keep up both majors, so I chose to express myself in the form of extra-curriculars. Since starting Pitt I have been involved sporadically with the Rugby team, Pitt Men's Choir, volunteering at Kane Nursing Home, volunteering at St. Paul's Vacation Bible School, the College Republicans, and working in a tissue culture laboratory for a company called *Stemnion*.

I came to work with Dr. Abramowitch because of my unexplored interest in biomechanics, and have found it to be interesting and informative. My peers have been very friendly and helpful and I hope to contribute whatever I can to the powerful think-tank that is the MSRC.

I would like to thank Dr. Abramowitch, Andrew Feola, and everyone else in the MSRC who has aided me in the completion of my project.

DESIGN OF A MORE EFFICIENT BIAXIAL TESTING SYSTEM

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INTRODUCTION

Biaxial testing of tissues to determine hysteresis curves is an important part of biomechanical research. Engineers study the mechanical properties of certain tissues in relation to the types of stresses which the tissues undergo in vivo. They can use this data to aid in and to measure their success in their attempts to correct injured or deteriorated tissue. Currently in the MSRC at the University of Pittsburgh, the biaxial testing system consists of clamps to hold the tissue in place, connected to a motor and load cell on each of two axes. The Wintest computer software aids in the reading and calculation of forces, stresses, and strains on tissues. There are several problems with this current system which need to be addressed.

It is a common practice to submerge tissues in a temperature controlled saline bath in order to adequately simulate biological conditions[1]. Keeping the tissue in constant contact with ions at body temperature will prevent the tissue from drying out and prevent its mechanical properties from changing. The current system has no such bath and therefore should be designed to accommodate one.

It has been determined that clamping tissues during mechanical testing produces unwanted "edge effects", which distort the true mechanical properties of the tissue because of the artificial boundary created by the clamp[2]. This is solved by placing hooks or sutures along the outer edge of the tissue to hold it in place while it is loaded. Each of the strings attached to the tissue must carry equal tension or the tissue with exhibit distorted strain values[3]. In order to eliminate edge effects and create an even distribution of tension along all sides, hooks and swivel joints should be included in the design to hold the tissue in place without distortion.

There were several other considerations which were important to note during the design process. The load cells are very sensitive, and may be damaged if forces exceed a maximum value of 22N. Although all forces are generated and controlled by the motors via the Wintest software, it is imperative that no unnecessary moments are created on the load cell, or it could alter data.

Systems which fit all of the criteria could be found in literature[4], and for purchase in industry[5]. Although an estimate of the total cost of the system could not be given, a temperature controlled water bath was estimated between two and four thousand dollars[6]. Both of the testing systems used four hook/suture attachments on each side, swivels and pulleys for distribution of load, and a water bath for simulation of biological conditions. The new design should incorporate all of these aspects, while attempting to be as cost-effective as possible.

MATERIALS

The materials used in the components of the system which do not touch the saline bath were chosen to be constructed of aluminum because of aluminum's low density and ability to easily withstand forces in excess of 22N without undergoing deformation. The components of the system which touch the saline bath must be resistant to corrosion and be able to be removed in order to clean them. Steel screws were chosen for this purpose because of steel's ability to easily withstand forces in excess of 22N without undergoing deformation, and its resistance to corrosion. In a more practical sense, a screw can be inserted and removed easily and while inverted, small pulleys can rest on the screw head.

In order to distribute tension evenly among attachments to the tissue, one string was looped around a small plastic pulley to create half of one side. Ball bearings were used as swivels to evenly distribute forces between the two pulleys which made up one side.

Like most saline baths, the water bath was to be constructed out of acrylic sheet

METHODS

After the design criteria were set, two early designs were developed. Both designs incorporated a temperature controlled water bath, lightweight materials, and even distribution of load along four strands of attachment on each side. As seen in figure 1, design 1 uses metal arm regions which extend over the water bath's walls. These regions need a minimum of 12 mm clearance to account for the maximum displacement of the motor. As seen in figure 2, design 2 uses a system of pulleys to transfer tension from the motor to the load cell, through the tissue.

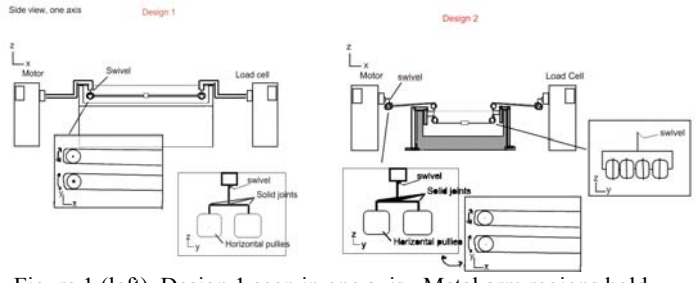

Figure 1 (left). Design 1 seen in one axis. Metal arm regions hold pulleys which carry load from motor to load cell.

Figure 2 (right) Design 2 seen in one axis. Pulleys transfer tension from the tissue to the load cell.

A noted disadvantage to the first design is the small moment generated by the arm region, which deviates from the plane of the tissue. This weight will create a small moment, which may alter measurements. This makes it imperative that the arm region is as lightweight as possible. The second design has a lot of room for

human error, as constructing the design would be much more difficult, expensive, and time consuming. For this reason, design 1 was chosen for construction.

After using various saw and drills in the MSRC machine shop and ordering a few components from McMaster Carr, the first prototype was created (figure 3). Soon thereafter, the angled pieces were replaced by one single bent piece to create the second prototype (figure 4). The second prototype was replicated and used to form a working model (figure 5).

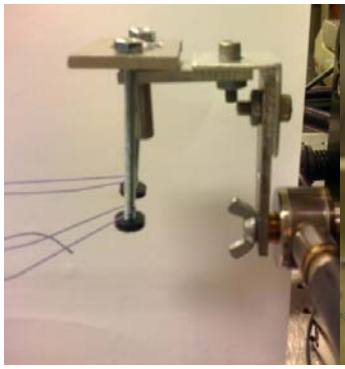

Figure 3. Prototype I.

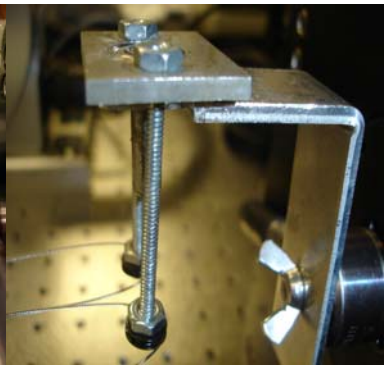

Figure 4. Prototype II.

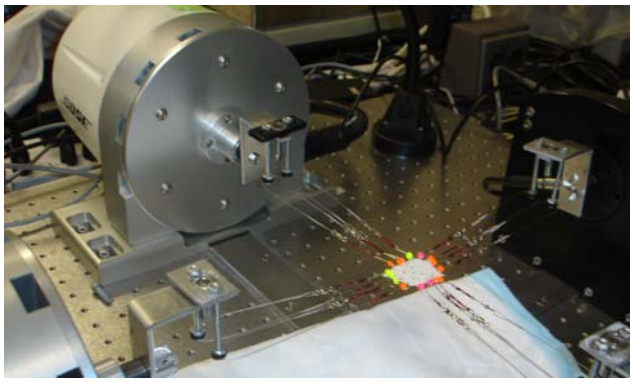

Figure 5. Hooks to tissue designed and built by Stacy Tokar.

Measurements were taken from the working model setup and a design was created (figure 6). This design, along with a set of detailed instructions on assembly were submitted to the University of Pittsburgh machine shop in Benedum Hall on July 18th, 2008.

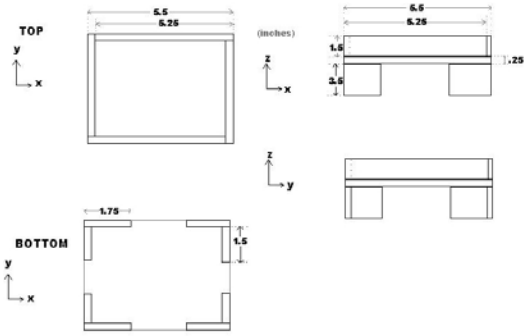

Figure 6. Water bath for Biaxial Testing. Dimensions are in inches at the request of the machine shop.

DISCUSSION

The success of this project is unable to be adequately judged without further testing. The model adhered to the design in an attempt to reduce edge effects. The model was lightweight, constructed out of aluminum pieces and steel screws, generating as little moment on the load cells as possible. From using scrap parts and ordering only the necessities to complete the project, the total cost of the project excluding the water bath was approximately 65$.

During construction, a few of the ball bearings may have been inadvertently compressed which has reduced their ability to freely rotate.

In the future, stress and strain values will be measured using this design and compared to literature values of a particular tissue. If the values are consistent, then the project may be considered a success.

ACKNOWLEDGEMENTS

I would like to thank Dr. Abramowitch, Andrew Feola, Stacy Tokar, and everyone else in the MSRC who have aided me in the completion of my project.

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I was born the 28th day of July in 1986 in a suburb 25 miles east of Pittsburgh, called Jeannette. I grew up alongside my older sister, Crystal, and under the watchful eyes of my wonderful parents Tom and Kathy. I attended Hempfield Area High School in Greensburg, PA where I was a member of many clubs including National Honor Society and French Honor Society. I also played the trumpet in just about every band the high school had to offer including the marching, concert, and stage bands.

After high school, I attended Saint Vincent College in Latrobe to major in Math/Engineering. During my three years there I became a member of Alpha Lambda Delta, Alpha Phi Omega, Orientation Committee, as well as many other small clubs and organizations. Last fall I transferred to the University of Pittsburgh to complete my engineering degree and major in Bioengineering. I am an active member of BMES and was recently inducted into the engineering honor society Tau Beta Pi.

I have thoroughly enjoyed my summer at the MSRC and have made so many new friendships. I will take so much knowledge with me that I have gained in my time here; not only in the subjects of bioengineering and biomechanics, but also knowledge for life in general. I would like to thank Andrew Feola, Steve Abramowich for taking a chance on me, and Dr. Woo for all his wisdom. I would also like to thank my fellow summer students for all their help and the laughs along the way.

Development of Protocol for Biaxial Mechanical Testing of Planar Soft Tissues

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Introduction

Pelvic organ prolapse is the decent of female pelvic organs into the vaginal canal. The extent of prolapse varies from case to case but can be as severe as protrusion outside the body. Prolapse can cause urinary and fecal incontinence as well as pain and social isolation. Risk factors for developing prolapse include age, menopause, obesity, and parity[1].

Motivation for research stems from the dramatic biochemical and biomechanical changes that the vagina undergoes when prolapsed. These changes include the weakening of the pelvic floor muscles, increased collagen content, increased collagen type III, and an increase in active matrix metalloproteinase (MMP)-9[2,3].

Uniaxial testing is not sufficient to create a constitutive model of vaginal tissue because of the anisotropic nature of the tissue[4]. Therefore, biaxial testing should prove to be much more useful in obtaining quantitative data on the biomechanical properties of the vagina. Futhermore, three-dimensional properties can be obtained from two-dimensional tests[5]. This will eventually assist in the prevention and repair of pelvic organ prolapse.

Previously, biaxial studies have been done on many tissue types including skin, blood vessels, heart valves, and pericardium. As with all scientific research, there are difficulties in biaxial testing including small specimen sizes, gripping methods, applying and reading constant forces, and specimen-to-specimen variability[4]. Biaxial testing is commonly done by mounting square specimens (3x3 to 6x6 cm) with four suture loops on each side. The specimen is then immersed in a buffered saline and deformations are tracked using a camera system and markers[4].

In a biaxial testing protocol, the tissue is first subjected to a period of preconditioning. Then a series of protocol ratios are carried out and can be controlled by load, displacement, or most commonly strain.

Objective

To develop a repeatable biaxial testing protocol for use on planar biological soft tissue using the Bose Electroforce LM1 Testbench biaxial testing device. Furthermore, to obtain experimental data using rat skin and compare results to literature.

Methods

Rat skin was obtained from the abdomen area of Long-Evans rats (n=7) and cut into rectangular pieces with about 5 cm on each side. Then 1 cm wings were cut into each side for the attachment of clamps. The specimen and clamps were mounted onto the Bose Electroforce LM1 testbench. Five graphite markers were placed centrally on specimen for use with camera system and to track strain. Testing was carried out using Wintest software.

While the tissue was in a slacked position the load was zeroed. A preload of 0.1N was applied and the tissue was preconditioned for about two minutes at 1 Hz to the set displacement level, usually 3mm depending on tissue stiffness. This was done until the hysteresis reached a steady-state and the tissue was affectively preconditioned. A preload of 0.1N was reapplied to remove slack. Then the protocol was carried out in the order as follows with displacement control: 1:1, 1:0.75, 1:0.50, 1:0.25, 1:1, 0.75:1, 0.50:1, 0.25:1, and 1:1. The 1:1 ratio was done at several points in the protocol to ensure that no damage had occurred and that the tissue had repeatable properties

Results

The data obtained from using the Bose Electroforce LM1 testbench and Wintest software with rat skin was graphed using Matlab.

As seen in Figures 1 and 2 below, skin displays a non-linear stress-strain relationship. Also, there is a hysteresis, or energy dissipation between the loading and unloading curves. Additionally, as the displacement was decreased on one axis, it affected the stress and strain in both directions; therefore, the issue is anisotropic.

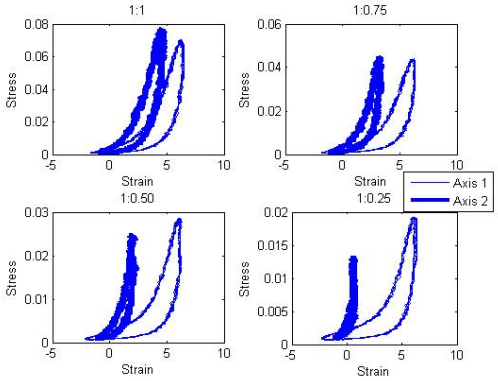

Figure 1: Results from first half of protocol

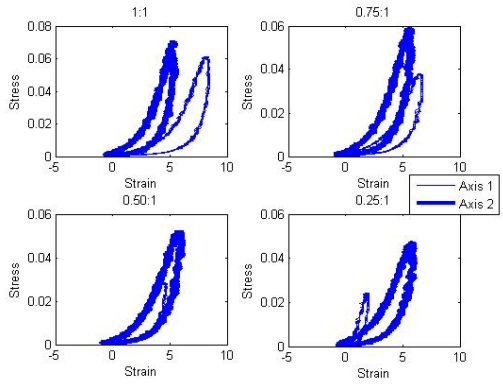

Figure 2: Results from second half of protocol.

An issue arose during testing in which the strain markers needed to be switched from ink to graphite. Ink bled each time the tissue was moistened and could lead to inaccurate strain measurements. As seen in Figure 3, ink markers created excess noise making it difficult to characterize the stress-strain response. However, as seen in Figure 4, graphite markers provided improved strain measurements and minimized the amount of noise in the stress-strain graphs.

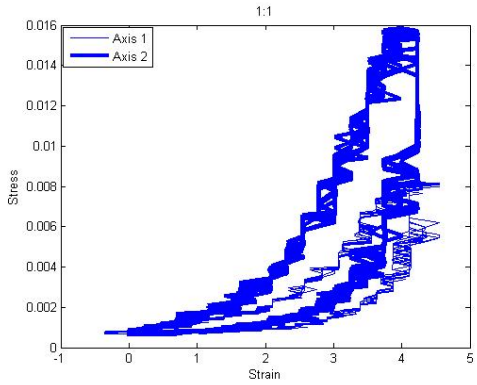

Figure 3: Example using ink markers

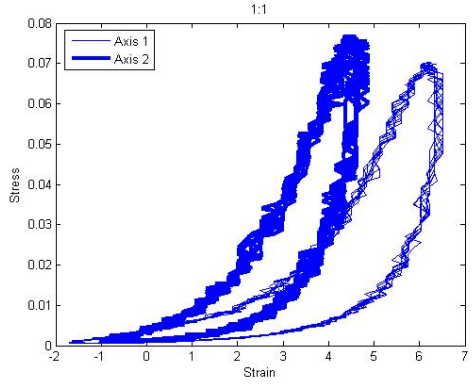

Figure 4: Example using graphite markers

Discussion

Previous studies have shown that skin has a non-linear stress-strain relationship, significant hysteresis between loading and unloading, and specimen-to-specimen variability[5]. As shown in Figures 1-4, this work has successfully shown that skin does in fact have a non-linear stress-strain relationship and hysteresis. Although not illustrated here, variability among the specimens was found especially in thickness and stiffness depending on the site of harvest on the rat.

Overall, the protocol explained above proved to be repeatable. All specimens showed some degree of hysteresis and similar strains were seen throughout. Furthermore, during each protocol the 1:1 ratios gave repeatable results. Additionally, the switch from ink markers to graphite demonstrated to be very helpful in achieving more accurate and precise strain measurements.

Because of the non-ideal setup and lack of environmental controls for this research, future work is needed in order to properly characterize the tissue. Carriages have been developed that will distribute equal loads to all sides of the specimen, as well as hooks to aid in gripping issues of small specimens. Also a temperature controlled saline bath is being developed to simulate the native environment of the tissue by controlling moisture and temperature. Once completed, this biaxial protocol will be used to characterize and model vaginal tissue in an attempt to aid in pelvic organ prolapse research.

Acknowledgements

I would like to thank Andrew Feola and Dr. Steve Abramowich for all they have taught me this summer. I would also like to thank Dr. Woo for creating a great learning environment. Also, thank you to my fellow summer students and the Grad student of the MSRC for all the help and laughs along the way.

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