Musculoskeletal Research Center

Summer Research Program

2007

Department of Bioengineering

University of Pittsburgh

MSRC

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2007 Abstract Book Committee

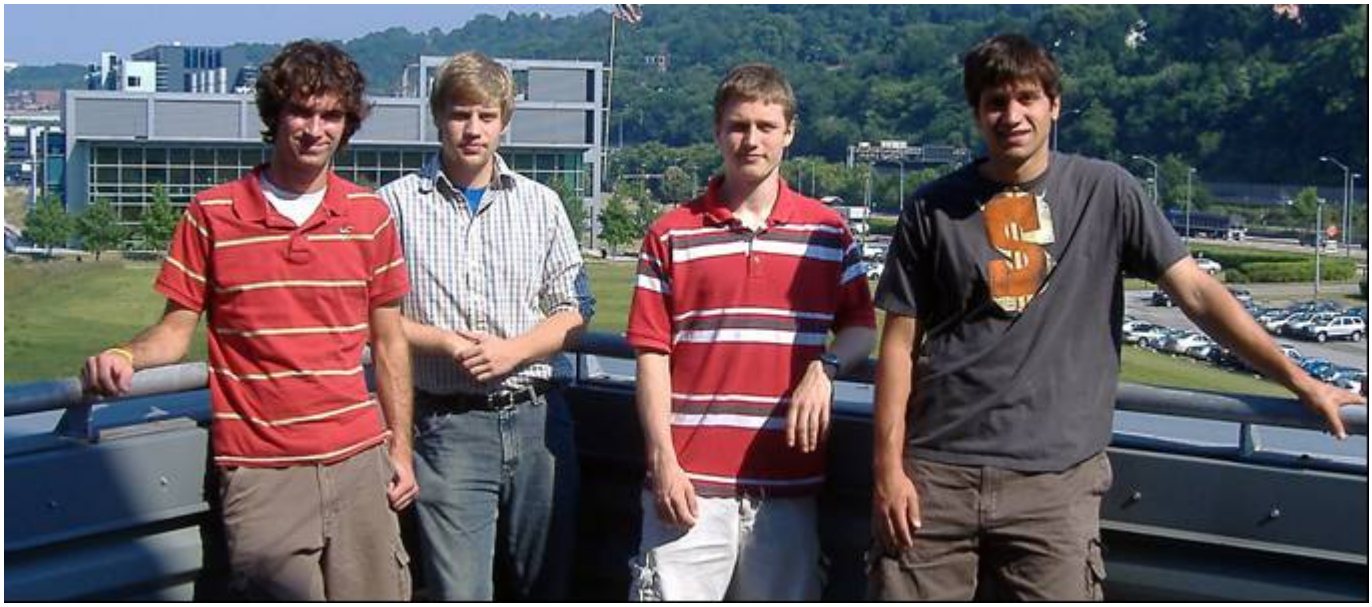
Noah Lorang, Danielle Dukes, Kristin Frawley, Joel Palko

The work presented in this abstract book represents the labors and efforts of the class of 2007 summer interns. I think I can speak for all the summer interns when I say that the last ten weeks have been amazing. We have learned to appreciate the highs and lows of research, improved our writing and presentation skills, and received first class instruction from the best faculty and mentors that the University of Pittsburgh has to offer. Furthermore, we have all worked extremely hard and the fruits of our labor can be seen in these abstracts.

On behalf of my fellow summer interns, I would like to thank the MSRC for providing us with such a wonderful atmosphere to learn and be a part of. In addition, a special thanks goes out to Dr. Woo, Dr. Debski, and Dr. Abramowitch for giving us the opportunity to participate in such an outstanding program and setting the tone for our future successes. The MSRC will always be the foundation of our bright careers in science and research!

-Danielle Dukes, Editor

2007 Summer Symposium Committee



Andrew Brown, David Gladowski, Mitchell Kosowski, Ryan Prantil

This year's Summer Student Symposium was held on August 2, 2007 in the MSRC. The symposium drew an impressive group of spectators including parents, faculty and staff of the bioengineering department, and (of course) the MSRC family. Each presentation was indicative of the hard work that each student has done this summer. The most amazing aspect of the symposium was the amount of progress, in both research and presentation skills, that the summer students made since their first days in the MSRC. I, like most of the other summer students, had dreaded my first presentation at the MSRC. I had seen the carnage at the weekly Monday meetings in which the faculty and students would ask the presenter questions that he or she had not once ever thought about. However, only through making these presentations does one learn how to make the next one better. Based upon the quality of the presentations and the fielding of questions at the symposium, it would appear that all eight of us summer students had taken this lesson to heart.

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 in planning the symposium as well as all the mentors for assisting the students with their projects and the rest of the MSRC.

- Dave Gladowski, Symposium Committee Chairman

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I was born in Erie, Pennsylvania and have lived there my entire life. I attended 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 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 in order to embark on my own project.

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.

NONRECOVERABLE STRAIN IN THE ANTERIOR BAND OF THE GLENOHUMERAL CAPSULE

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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 varies greatly in shape and mechanical properties throughout the population. The capsule is the primary passive restraint in the anatomical positions associated with dislocation, with the anteroinferior region experiencing the highest strains during these traumatic events.[2] Various injuries to the joint and the capsule occur as a result of dislocation, such as permanent deformation of the capsular tissue. Permanent deformation of the capsular tissue results in the development of redundant tissue which contributes to recurrent instability and redislocation.[3] Current surgical techniques to plicate redundant tissue after injury are subjective and inadequate at restoring stability with 23 % of patients still experiencing problems.[4]

The objectives of this project were to: 1) Quantify the magnitudes of the maximum principal strains experienced at the maximum elongation applied to a tissue sample during elongation beyond its elastic limit; 2) Quantify the magnitudes of the resulting maximum principal nonrecoverable strains; and 3) Use the directions of the strains to validate the current methodology. Completion of these objectives could lead to a quantitative evaluation of the amount of redundant tissue developed following injury, enabling clinicians to more objectively perform surgical repairs.

MATERIALS AND METHODS

Four fresh-frozen human cadaveric shoulders (54 ± 9 yrs.) were dissected free of all soft tissue, excluding the intact glenohumeral capsule. The anterior band of the inferior glenohumeral ligament complex (AB-IGHL) was identified and transected and underwent an additional freeze-thaw cycle prior to testing. The AB-IGHL tissue was mounted in soft tissue clamps yielding an approximately 15 mm x 10 mm sample. A 3x3 grid of black delrin markers were attached to the sample using cyanoacrylate to allow for analysis of mid-tissue strain with a non-contact motion analysis software package (DMAS, Spica Technology Co., accuracy = 0.01 mm). The sample was kept hydrated with saline throughout the protocol.

The tissue sample was first subjected to a non-destructive tensile test on a materials testing machine (Thümler, accuracy = 0.01 N). **(Figure 1)** Ten cycles of preconditioning were performed with 1.5 mm of elongation applied. All elongations were applied at a rate of 10 mm/s. A 0.5 N vertical preload was then applied to establish a reference position for measurement of geometry and a 2.75 mm elongation was applied. The tissue sample was then allowed 30 minutes of recovery at zero load.

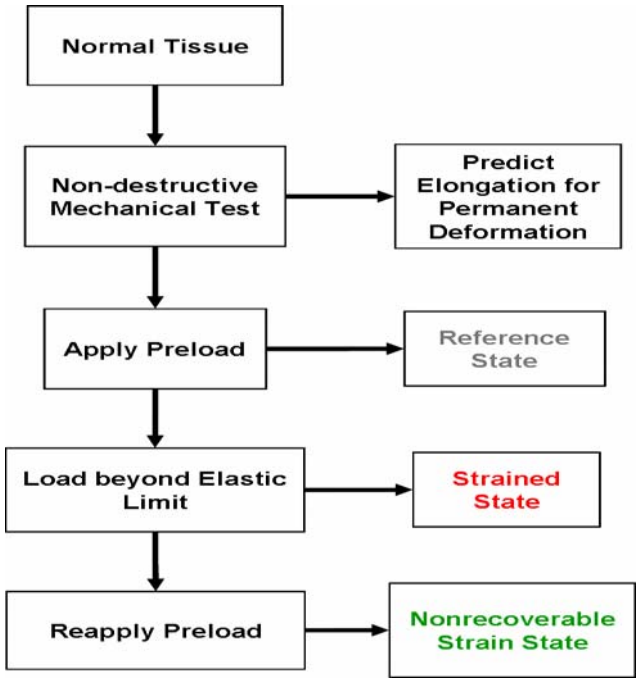

Figure 1. Flow chart of experimental setup.

In order to compensate for variability between specimens, the results of this non-destructive test were used to determine the proper elongation to apply during the subsequent loading for producing permanent deformation. A line was fit from the 2.75 mm elongation end of the resulting stress - percent elongation curve to the start of the linear region ($r^2 < 0.999$) and the percent elongation at that point was recorded.

Following recovery, the tissue sample was loaded in tension beyond its elastic limit in order to produce nonrecoverable strain. To achieve this, the same preconditioning regimen and preload were applied. The positions of the markers at this preloaded state were captured and served as the reference state for later strain calculations. The percent elongation applied to the tissue was twice the percent elongation determined for the start of the linear region of the prior non-destructive test. The positions of the markers were captured at the maximum elongation applied and served as the strained state. The sample was then allowed 30 minutes of recovery at zero load. Following recovery, a 0.5 N preload was again applied and the positions of the markers were captured and served as the nonrecoverable strain state.

The positions of the markers at all three states were digitized using DMAS and reported in an x-y coordinate system. The coordinates of the markers were then inputted into a finite element software package (ABAQUS), to determine the magnitudes and directions of the maximum principal strains in the two states analyzed. Objective (1) was addressed by comparing the strained state coordinates to the reference state coordinates and Objective (2) was addressed by comparing the nonrecoverable strain state

coordinates to the reference state coordinates. ABAQUS was then used to report the average maximum principal strain magnitude for each of the four elements defined in the tissue sample, as well as the associated direction vectors at four integration points within each element. The average maximum principal strains at each element were then averaged and defined as the average maximum principal strain for the sample. The sensitivity of the average maximum principal strains to the repeatability of the camera system was found to be less than 0.25 %.

RESULTS

The average maximum principal strains for the four samples at the strained state were found to be 8.3% , 13.1%, 16.9% and 12.7% for applied elongations of 3.42 mm, 3.57 mm, 3.91 mm, and 3.94 mm, respectively, coupled with average maximum principal nonrecoverable strains of 1.9%, 2.9%, 4.0% and 2.6%. **(Figure 2)** The average maximum principal strain across all four specimens was 11.9 ± 3.1 % and 2.4 ± 0.8 % at the strained state and nonrecoverable strain state, respectively.

Upon qualitative examination, it was found that the directional vectors of the maximum principal strains in the strained state were predominately oriented in the direction of loading. Additionally, the orientations of the vectors in the nonrecoverable strain state were comparable to the vectors in the strained state. **(Figure 3)**

DISCUSSION

Analysis of the strain data found that nonrecoverable strain could be created on the tissue level using a single sub-failure tensile load. Higher strains in the strained state correlated to higher strains in the nonrecoverable strain state. However, greater elongation at the strained state did not necessarily produce higher strains in either the strained or nonrecoverable strain states. The consistency of the directions of the strains between the strained and nonrecoverable state, as well as their orientation with the direction of loading, suggest that this methodology is appropriate for and capable of tracking both large and small strains on the tissue level.

These data compare well with the findings of other injury models. Malicky, et. al. subjected the glenohumeral joint with intact capsule to varying degrees of subluxations. Average maximum principal strains of 16% and 5% in the strained state and nonrecoverable strain state, respectively, were found compared to our averages of 11.9% and 2.4%. [5][6] However, Malicky observed strains throughout the entire anteroinferior region of the intact capsule, while the present study involved a tissue-level injury model. The multi-axial nature of the glenohumeral capsule and the lack of the present model's interactions with surrounding tissue may explain the differences in findings.

The major limitation of this study is the difference in loading conditions of the capsular tissue versus those seen in vivo. The capsule functions multi-axially and should be modeled as a continuous sheet, both conditions that are not addressed in this study. The poor aspect ratio of the tissue sample may contribute to the uneven strain distribution seen in the strain plots, as well as the variability seen between the elongations and resulting strains. However, a large aspect ratio would most likely produce too small a mid-substance area to allow for strain analysis.

Future directions could include studying the differences in mechanical properties and histology resulting from loading a capsular tissue sample beyond its elastic limit, as well as increasing the sample size. Additional joint-level injury models could also be created to observe the capsule's response to other mechanisms of injury, such as excessive rotation. The results of this study could lead to a quantitative evaluation of the proper amount of tissue to be plicated during surgical repair in order to restore stability.

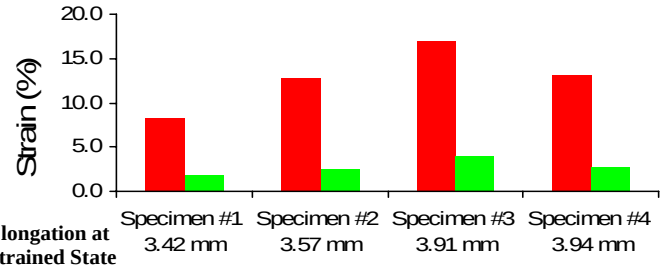

Figure 2. Maximum principal strain in both the strained (red) and nonrecoverable strain (blue) states and the elongation applied for each of the four tissue samples.

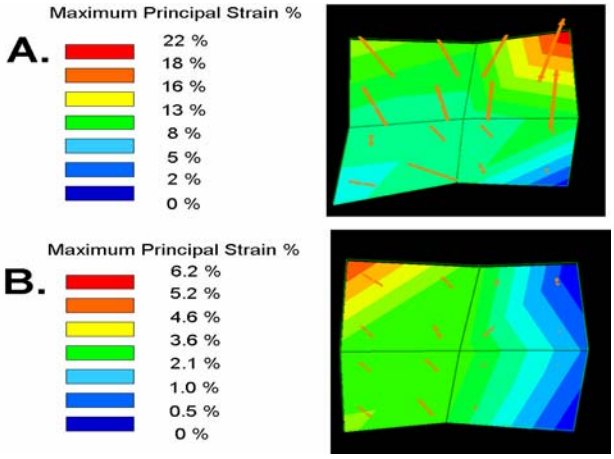

Figure 3. Maximum principal strains for a representative sample at the **A.** strained state and the **B.** nonrecoverable strain state and their associated directional vectors

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I'd like to thank my lab mentor Carrie Voycheck and my faculty mentor Dr. Debski for all their guidance throughout my summer project. I'd 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. Finally, thanks to the Department of Bioengineering for their funding of my summer research experience.

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Born on April 04, 1986 and raised in a fairly small town located in Fairborn, Ohio where I attended Fairborn High School (FHS). At FHS I played soccer and basketball and was involved in many academic activities. In June 2004, I graduated from Fairborn High School and was awarded a basketball scholarship to California University of Pennsylvania (CalU). At CalU I have continued my career path as a college student-athlete and a Pre-Medicine Major. I am scheduled to graduate in May of 2008.

Academically, I have enjoyed my experience at CalU. I am an active member of Sigma Alpha Pi and Beta Beta Beta: the Biological Honor Society. However, being a college athlete not only has taught me how to win and lose gracefully, but it also has challenged me to grow and mature in many ways. When I'm not buried in my schoolbooks, I can be found reading a novel or listening to music. My newly found interest is dog show ring competition and breeding.

Working at the MSRC has been an amazing opportunity. The MSRC has exceeded my expectations for an assignment as a summer research intern. I have gained hands-on laboratory experience in research, broadened my knowledge about the field of tissue engineering, and gained experience in writing and presenting scientific research. I would like to thank my mentor Serena Augustine for always lending a helping hand and explaining EVERYTHING! Also, I would like to extend special thanks out to Alex Almarza, the MechanoBiology Group, and the rest of the MSRC family. Finally, I would like to thank Dr. Woo and Dr. Abramowitch for giving me such a wonderful opportunity to further enhance my foundation in the sciences and gain real-world experience by being a part of their internship program at the MSRC.

CHANGES IN GENE EXPRESSIONOF PASSAGED BONE MARROW DERIVED CELLS IN CULTURE

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INTRODUCTION

Bone marrow derived cells (BMDCs) are an ideal source for functional tissue engineering (FTE) because they are auto-transplantable, easily expandable, and have the ability to differentiate into various lineages[1]. Functional tissue engineering efforts to regenerate musculoskeletal tissues may require large numbers of cells, often obtained through cell passage. While changes in gene expression throughout passage have been documented in chondrocytes[2] and medial collateral ligament (MCL) fibroblasts[3], they have not yet been quantified in BMDCs. Specifically, one study showed a drastic decrease in the RNA expression of the major matrix constituents of articular cartilage (collagen type II and superficial zone protein) by chondrocytes in early passages, suggesting that passage of chondrocytes is not ideal for cartilage FTE.

Results obtained in our research center indicated that rat MCL fibroblasts may be a good source for ligament FTE approaches due to the drastic increase in collagen type I gene expression (*p<0.01, $p<0.05, **Figure 1**) in early passages. In addition, while collagen type III gene expression increased in early passages, the relative abundance (*p<0.05, $p<0.05 **Figure 2**) remained an entire order of magnitude less than that of collagen type I in later passages. This is important because while collagen type III is found in soft tissues such as ligaments and tendons, it has been shown to be upregulated for up to a year following injury[4]. This elevated level of collagen type III has been closely linked to uniformly small collagen fibril diameters in the healing MCL[5], which may contribute to inferior biomechanical properties[6]. Thus, for ligament and tendon tissue engineering, a cell source with high collagen type I gene expression and consistent collagen type III gene expression may be ideal.

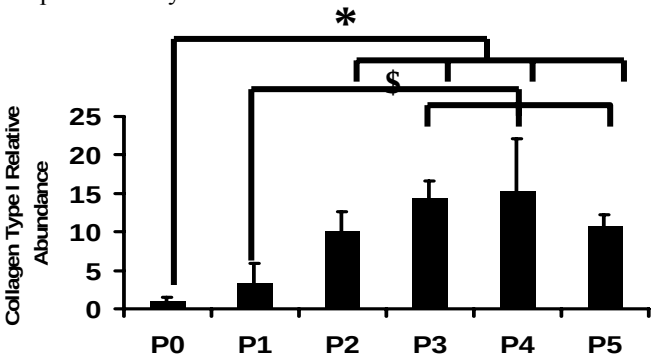

Figure 1. Collagen type I gene expression relative to GAPDH of MCL fibroblasts from P0 to P5. The * symbol indicates statistical significance (p<0.01) and the $ symbol

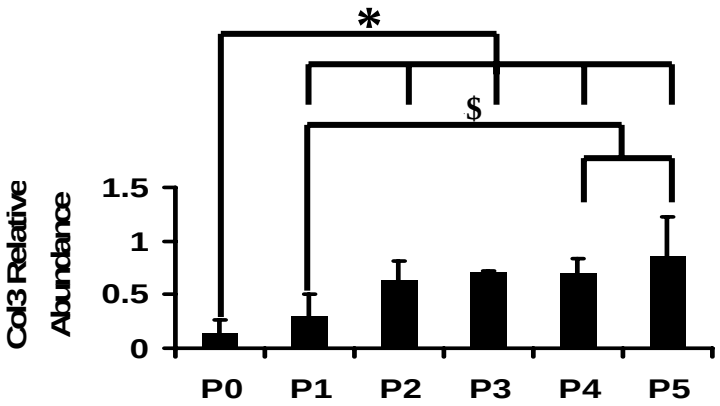

Figure 2. Collagen type III gene expression relative to GAPDH of MCL fibroblasts from P0 to P5. The * symbol and $ symbol both indicate significance at (p<0.05)

Parallel preliminary studies in our research laboratory seemed to suggest that rabbit BMDCs have a fibroblastic phenotype as well; as shown by an elongated cell morphology and high collagen type I gene expression at passage 1. The objective of the current study was to quantify gene expression of collagen type I, collagen type III, and GAPDH through passages in rat BMDCs. Based upon preliminary studies that showed the possible fibroblastic phenotype of rabbit BMDCs and the quantitative increase of rat MCL fibroblast behavior, we hypothesized that rat BMDCs would have a similar high collagen type I gene expression.

METHODS

Bone marrow derived cells (BMDCs) of Long Evans rats (n=3) were isolated from the femur with a syringe and deposited on a petri dish in 10 ml of Dulbecco's Modified Eagle's Medium (DMEM). The cells from the same rats in the MCL study were used, thus allowing a one to one comparison to be made. After 2 days, adherent cells were observed and the media was changed to remove any non-adherent cells. When cells reached confluency, they were trypsinized (P1). From that point on, ascorbic acid (50 mg/ml) was added to the medium and cells were re-plated at 30,000 cells per well in 2 cm^2 wells in 24 well-plates for one more passage. Collagen type I, collagen type III, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene

expressions were quantified by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). After an initial denaturation at 94°C for 15 minutes, the PCR cycle consisted of denaturing at 94°C for 30 seconds and an annealing at 55°C for one minute, with a subsequent extension at 72°C for one minute. During the last point of each cycle the fluorescence was measured for each gene of interest and recorded in real time for a total of 35 cycles. The take off cycle [C(t)] recorded for each gene the primer efficiency (Eff)and was used to calculate the abundance [A] of the gene of interest (Equation 1)[7].

$$\textbf{(1)} \quad A = \frac{1}{(1+\mathrm{Eff})^{C(t)}}$$

Results for collagen type I gene expression and collagen type III gene expression were both normalized to GAPDH abundance. A one-way ANOVA to compare collagen type I and collagen type III gene expression across passage was performed with significance set at α=0.05.

RESULTS

Results for the relative abundance of collagen type I revealed that gene expression significantly increased at P2 compared to native tissue (P0) or P1 cells (*p<0.05, **Figure 3**). Furthermore, observations showed that there is no significant difference from P0 to P1.

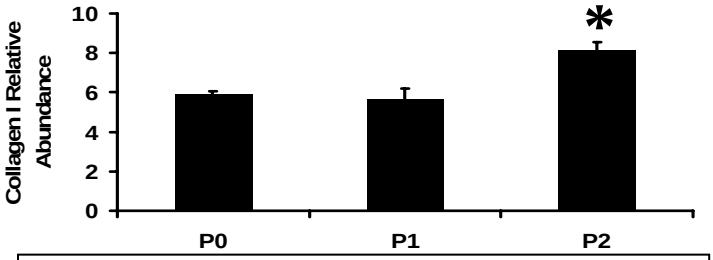

Figure 3. Collagen type I gene expression relative to GAPDH of BMDCs from P0 to P2. The * symbol indicates statistical significance (p<0.05).

Results for the relative abundance of collagen type III showed that gene expression significantly increased at P2 when compared to native tissue (P0) or P1 cells (*p<0.05, **Figure 4**). Furthermore, observations showed that the magnitude of collagen type III gene expression is much greater than that of collagen type I gene expression in later passages (**Figure 4**).

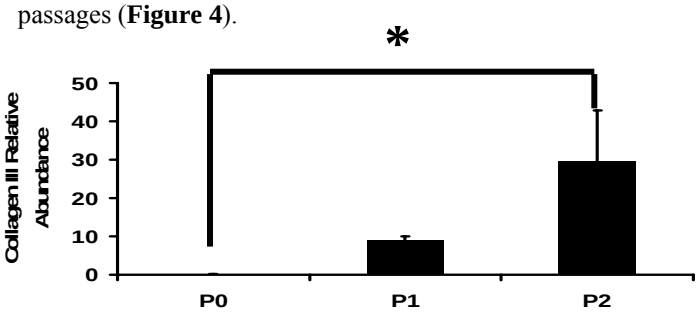

Figure 2. Collagen type III gene expression relative to GAPDH of BMDCs from P0 to P2. The * symbol indicates statistical significance

DISCUSSION

The relative abundance of collagen type I from P0 to P2 in rat BMDCs is comparable to that of MCL fibroblast. Observations show that although there is a slight difference between MCL fibroblasts and BMDCs at native tissue (P0), P1 and P2 are very similar. However, the relative abundance of collagen type III gene expression in BMDCs was much greater than that of MCL fibroblasts at similar time points. Therefore, despite the increase of collagen type I gene expression, the drastic increase of collagen type III indicates that BMDCs may not be an ideal cell source for FTE approaches to regenerate tendons and ligaments. The upregulation of collagen type III is not ideal because it has been linked to small collagen fibril diameter[5] which may contribute to inferior biomechanical properties of the healing tissue[6]. Future work may include quantifying collagen type I and collagen type III gene expressions from P2 to P5 because more passages are commonly used to obtain appropriate cell number for FTE approaches. Also, gene expression of other important molecules such as TGF-β1, fibronectin, MMP-13, and TIMP1 must be measured and evaluated to further assess if BMDCs may be an appropriate cell source for FTE approaches.

ACKNOWLEDGEMENTS

I would like to acknowledge PTEI and the NSF-REU for their financial support. I wish to thank Dr. Savio L-Y. Woo and the entire MSRC staff. In particular, I wish to thank my mentor Serena Augustine and Dr. Alejandro Almarza for their help and guidance.

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I was born in Annapolis Naval Hospital in Maryland and grew up in Pittsburgh. When my husband joined the Air Force, I returned once again to Maryland. Two kids, a dog, and a cat later and we are all back in Pittsburgh.

I left school to follow my husband and back his military career, I have been a wife and a mother and now I decided to try student again. I am entering my sophomore year at CCAC in Biotechnology. When I am not studying, I enjoy spending time with my family and friends.

The MSRC has been an incredible experience. I was worried walking into the tissue mechanics lab that I had little background and of course I am a tiny bit older than most interns. Everyone here is amazing to work with. I am more thankful than I can ever express to Dr. Woo and Dr. Abramowitch for this opportunity. I am most thankful to my mentor Matt Fisher for his knowledge and constant patience.

THE EFFECTS OF CLAMPING ON DETERMINING THE MECHANICAL PROPERTIES OF THE MEDIAL COLLATERAL LIGAMENT AND THE PATELLAR TENDON DURING TENSILE TESTING: A PRELIMINARY STUDY

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INTRODUCTION

Tensile testing is commonly performed to determine the mechanical properties of ligaments and tenons[1]. Mechanical properties describe the quality of the tissue and should be independent of small changes in size or clamping method. When tensile testing small tissue it may be necessary to use clamps, such as when testing functional tissue engineered scaffolds, tendon insertion into muscle, as well as when studies investigate different portions of the same ligament or tendon.

The rabbit medial collateral ligament (MCL) and patellar tendon (PT) have been commonly used to study ligament and tendon biomechanics through uniaxial tensile testing. Often the bones (which the MCL or PT connect) are clamped during testing; however these tissues are large enough in some animals to cut into a dogbone shape and measure mechanical properties using soft tissue clamps. Previous studies in our research center have shown that the PT displayed a significant decrease in mechanical properties with soft tissue clamping compared to bone-to-bone clamping methods.[2] One explanation may be a change in collagen fiber alignment when clamping due to thickness of the PT tissue (1-2mm). Thus we hypothesized that the measured mechanical properties for the MCL, which possesses a highly aligned collagen fiber structure and thinner insertion sites (<1mm), should be independent of the clamping method, regardless of whether bone-to-bone or soft tissue clamping methods were used. The objective of this preliminary study was to 1) evaluate the effects of soft tissue clamping methods on the mechanical properties of the MCL by comparing the results to historical bone-to-bone clamping method data and 2) compare the changes in the MCL due to clamping to those of the PT mentioned earlier.

METHODS

To test this hypothesis, MCLs (n=5) were taken from 3 rabbits and the dimensions were measured with calipers before and after clamping and after cutting the tissue into a dog-bone shape (aspect ratio ~ 7mm). The cross-sectional area (CSA) was measured using a laser micrometer system.[3] Markers were placed on the tissue's midsubstance to track strain using a Dynamic Motion Analysis System™. An Instron ™ materials testing machine was used for uniaxial tensile testing. A 0.5 N preload and preconditioning were performed followed by a load to failure test at a crosshead speed of 10mm/min. To determine the mechanical properties of the MCL, stress (load/CSA) and strain $((l- l_o)/ l_o)$ were calculated to create a stress-strain curve (Figure 1). From the stress-strain curve, the mechanical properties of the tissue, i.e. tangent modulus (slope of the linear portion of the curve) and ultimate tensile strength (UTS) (maximum stress), could be determined. An unpaired t-test was used to compare tissue dimensions for the MCL and PT before and after soft tissue clamping. An unpaired t-test compared the mechanical properties of the MCL using current soft tissue clamping to historical bone-to-bone clamping data with significance set to $p<0.05$. The same comparison was made for historical PT soft tissue and bone-to-bone clamping data as well.

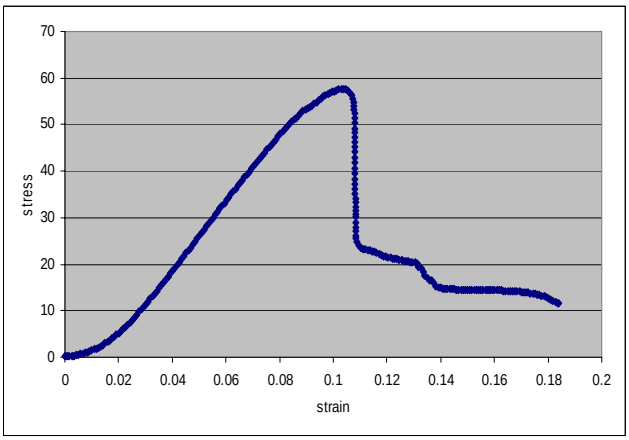

Figure 1. Typical stress-strain curve (from MCL#3)

RESULTS

Gross observation of the MCL and PT tissue before and after soft tissue clamping revealed little change in the MCL's shape, compared with the significant change of the PT. (The cross sectional area of the MCL and PT samples ($0.6 \pm 0.3mm^2$ vs. $0.7 \pm 0.2\ mm^2$, respectively) and aspect ratio (MCL ~7 and PT~6) following cutting the specimen into a dogbone shape were very similar.) The PT displayed a 21% decrease in tissue thickness between before and after clamping (1.4 ± 0.4mm vs. 1.1 ± 0.1mm, respectively, $p<0.05$); and a 46% increase in the tissue width before and after soft tissue clamping (3.7mm ± 0.8 vs. 5.14 ± 0.1mm, respectively, $p<0.05$). There were no significant differences between the width (4.1mm ± 0.5 vs. 4.2 ± 0.7mm, respectively, $p<0.05$), and thickness (1.2mm ± 0.2 vs. 1.1 ± 0.3mm, respectively,

$p<0.05$) before and after soft tissue clamping of the MCL. (Table 1)

When comparing soft tissue clamping (n=13) to that of bone-to-bone clamping (n=16) for the PT, a 60% decrease in the tangent modulus (58 ±1 99 MPa vs. 1,506 ±5 23 MPa, respectively, $p<0.05$) and a 35% decrease in UTS (47.1 ± 19.4 MPa vs. 71.9±9.2 MPa, respectively, $p<0.05$) was observed.[2]

For the MCL, no differences in the tangent modulus could be detected between the soft tissue (n=5) and the bone-to-bone (n=16) clamping methods (762 ± 136 MPa vs. 936 ± 284 MPa, respectively, $p< 0.05$).[4] The first two MCL specimens failed at or near the soft tissue clamps, the UTS values could only be compared for the remaining three MCLs. When comparing the UTS of the MCL soft tissue (n=3) to that of the MCL bone-to-bone (n=16), no significant decrease was detected (63.6 ± 6.7 vs. 82.3±18.4, respectively, $p<0.05$)[4] (Table 2)

Tissue Dimensions				
	(mm)	Before Clamping	After Clamping	After dogboning
PT	length	19.0 ± 1.1	8.7 ± 1.2	5.7 ± 0.8
	width	3.7 ± 0.8	5.4 ± 1.0*	0.9 ± 0.2
	thickness	1.4 ± 0.4	1.1 ± 0.1*	0.7 ± 0.1
MCL	length	21.2 ± 2.4	10.9 ± 2.0	6.8 ± 1.2
	width	4.1 ± 0.5	4.2 ± 0.7	1.0 ± 0.2
	thickness	1.2 ± 0.2	1.1 ± 0.3	0.8 ± 0.2

Table 1. Tissue dimensions for the MCL and PT
*Significantly different from before clamping ($p<0.05$)

Mechanical Properties				
		Tangent Modulus (MPa)	Ultimate Tensile Strength (MPa)	n =
PT	Bone-to-Bone	1,506 ± 523	71.9 ± 9.2	16
	Soft Tissue	581 ± 199*	47.1 ± 19.4*	13
MCL	Bone-to-Bone	936 ± 284	82.3 ± 18.4	16
	Soft Tissue	762 ± 136	63.6 ± 6.7 (n=3)	5

Table 2. Mechanical properties of the PT and MCL
*Significantly different from bone-to-bone clamping ($p<0.05$)

DISCUSSION

Clamping of the soft tissue did not alter the width or thickness of the MCL. No difference in the mechanical properties for the MCL could be detected between clamping methods, although we were only able to determine the UTS of three of the five MCLs we tested, since the first two MCLs failed in the clamps. Based on the results of this preliminary study, there appear to be some differences between the MCL and PT. The PT demonstrates changes in tissue dimensions after clamping and changes in mechanical properties due to soft tissue clamping. Variables that may complicate obtaining reliable tangent modulus and UTS results when using soft tissue vs. bone-to-bone clamping methods with the PT include the tissue thickness at the insertion site and the collagen fiber alignment after it is clamped.

Since the MCL has a smaller tissue thickness at the insertion site (1mm) and more highly aligned collagen fibers it has the ability to retain shape during clamping of the soft tissue. This was noted visually when clamping, and may be linked to the smaller changes in mechanical properties. The data from this preliminary study suggests a controlled study describing the changes in mechanical properties due to clamping methods should be performed. Contralateral limbs can be utilized to test the PT and MCL bone-to-bone and soft tissue clamping methods and should provide more accurate results since the mechanical properties of these limbs should be similar. Thus, allowing us to more conclusively test the effects of clamping on the mechanical properties of the MCL and PT.

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I would like to thank my mentor, Matthew Fisher, for his help and guidance throughout this summer program and also Dr. Abramowitch and Dr. Woo for giving me the opportunity to work at the MSRC.

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I was born on October 01, 1986 and, like both my parents, have lived in the Pittsburgh area my whole life. While growing up, I had always paid a little more attention in my science classes and a little less in everything else. In my final year at Fox Chapel Area High School, I once described myself as a "math and science kind of guy" to my English teacher. She vowed that by the end of the year I would be a "math and science and English kind of guy". Boy was she wrong. I maintained that simply nothing was more valuable than a new scientific breakthrough. After graduation, I headed off to Johns Hopkins University (a beacon of knowledge) in Baltimore (an otherwise scary place). Don't worry, I am still a huge Steelers fan and still hate the Ravens as much as ever. In my first year and a half at Hopkins I majored in biomedical engineering (which helped immensely in developing my quantitative skills), but eventually settled on biology. In my biology classes, I have learned about many of the techniques researchers use, but had never used them myself. This summer, the MSRC has given me the chance to do so.

When I am not too busy with schoolwork, I enjoy participating in casual sports matches, fishing and hunting (though college has sent me on a temporary hiatus from the latter), rock climbing, weight training, and watching movies. At Hopkins, I am a member of Sigma Phi Epsilon social fraternity and Alpha Phi Omega service fraternity. In the future, I intend to either head off to medical school or enter a MD/PhD program.

I would like to thank Guoguang Yang for mentoring me this summer as well as Dr. Almarza for his constant guidance and Dr. Woo for inviting me into his lab. My summer here at the MSRC has given me some much-needed experience in the field of research and has inspired me to continue researching at Hopkins this fall.

USE OF A BIOSCAFFOLD TO IMPROVE HEALING OF A PATELLAR TENDON DEFECT FOLLOWING GRAFT HARVEST FOR ACL RECONSTRUCTION: A BIOCHEMICAL ANALYSIS

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INTRODUCTION

Bone-patellar tendon-bone autografts are often used for anterior cruciate ligament reconstruction. However, the untreated healing patellar tendon (PT) is structurally and mechanically inferior to the native tissue. Furthermore, adhesions to the underlying fat pad are often observed. Functional tissue engineering methods are being employed to aid the healing response, such as the use of bioscaffolds. Of particular interest, porcine small intestine submucosa (SIS) is an extracellular matrix derived bioscaffold that consists primarily of collagen type I, but also contains other types of collagens, fibronectin, glycosaminoglycans, and growth factors. SIS also degrades rapidly in-vivo and prevents cell infiltration from its luminal side (which is useful for reducing adhesions to the fat pad), while its abluminal side promotes cell proliferation. Recent research has shown that when SIS is applied to a medial collateral ligament (MCL) defect, the healing MCL exhibits better structural and biochemical properties as well as larger fibril diameters and lower collagen type V content when compared to the non-treated group[1]. Elevated levels of collagen type V have been shown to limit collagen fibril diameter and cause poor mechanical properties in ligaments[1]. Collagen type III is also found in elevated concentrations in healing ligamentous tissue and is thought to limit fibril diameter, but collagen type III concentrations gradually decrease over time to values found in native tissue. These encouraging results led to the application of SIS to a PT defect[2].

In that study, the central third of the PT was removed from the right hind knee (not including bone blocks) of 20 New Zeland White rabbits[2]. The rabbits were divided into two groups of ten with one group receiving SIS treatment while the other was non-treated. The SIS-treated group had one layer of SIS sutured onto both the anterior and posterior sides of the defect with the luminal side of both pieces of the SIS facing the outside of the defect. In both groups, the left hind leg served as a sham control. After 12 weeks, gross morphological results showed that the SIS-treated group had fully filled in the defect and adhesions to the fat pad were greatly reduced while the non-treated group showed a concavity in the defect and multiple adhesions to the fat pad. The SIS-treated group also had a 68% greater cross-sectional area (Fig. 1) than the non-treated group. Structurally, the SIS-treated group exhibited a 98% greater stiffness (Fig 2) and a 113% greater ultimate load when compared to the non-treated group ($p<0.05$).

The objective of this preliminary study is to determine the effects of SIS treatment on the biochemical properties of the neo-PT tissue after 12 weeks on the same samples used for mechanical testing. Specifically, this study is focused on determining the effect of SIS treatment on the ratio of collagen type V to I and collagen type III to I in the healing tissue. It will also be determined if the total collagen concentration can be determined after tissue is prepared for collagen typing. A one-way ANOVA to compare the ratios of collagen type V to I and type III to I of the SIS-treated to non-treated will be performed.

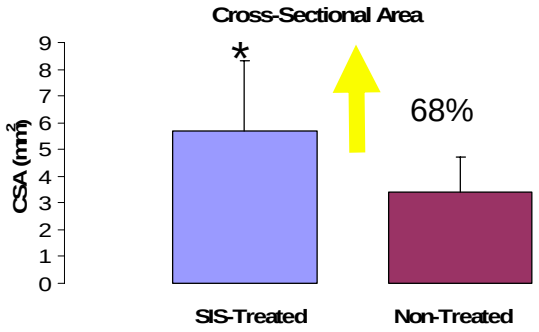

Figure 1: Cross-sectional area of neo-patellar tendon tissue. (*) denotes significant difference at $p<0.05$

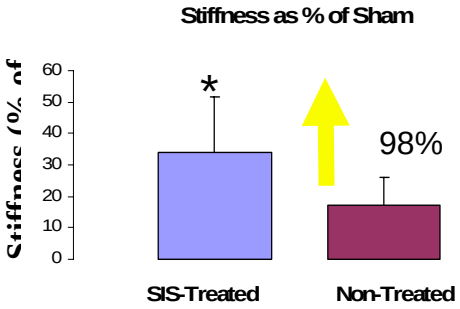

Figure 2: Stiffness of neo-patellar tendon tissue as compared to sham control. (*) denotes significant difference at $p<0.05$

METHODS

The study had three groups: SIS-treated (n=3), non-treated (n=3), and sham control (n=3). The tissue was first

dried and weighed. The tissue was then degraded using 500μL of 1M acetic acid per mg of dry tissue and pepsin at a 30:1 dry weight to pepsin ratio. The solution was then given 48 hours to digest. SDS-PAGE was performed to compare the ratios of collagen type V to I and collagen type III to I. A collagen type I, type III, and type V standard was run alongside the samples to show the placement of the respective bands. SDS unfolds proteins and uniformly places negative charges along those proteins. The proteins then travel through a gel at a velocity that is inversely proportional to the protein's molecular weight. Bands form on the gel, with each band corresponding to a single molecular weight. ImageJ software will be used to analyze the bands for their relative concentrations. A Sircol assay was performed on the pepsin digest to determine total soluble collagen concentration. A small portion of the pepsin digest (1-2 mL) was further digested with a corresponding equal amount of 100mM papain solution. The papain solution was prepared at room temperature and was then added to the sample. The papain/tissue solution was then heated to 65ºC and allowed to digest overnight. From the papain digest, a hydroxyproline assay was performed as another means of determining total collagen concentration using both hydroxyproline and collagen standards. A ratio of 8:1 collagen to hydroxyproline was used to normalize the hydroxyproline standard. The collagen standard was used to validate the collagen to hydroxyproline ratio.

RESULTS

The Sircol assay revealed that 67.2% ± 16.6% of the dry weight of the sham control was soluble collagen. The hydroxyproline assay showed similar amounts with a value of 64.8% ± 5.9% using the hydroxyproline standard and 64.7% ± 5.2% using the collagen standard.

DISCUSSION

The SDS gel could not be analyzed due to the poor quality of the bands. The first three gels that were run showed well defined bands of collagen strands, but the bands were not dark enough to analyze. A fourth gel was run after the samples were partially dehydrated, effectively increasing the concentrations of proteins in solution. However, some of the sham control bands could not be distinguished from other bands in their same respective lanes. Also, the collagen type I, III, and V bands were not well defined. The collagen standards showed poorly defined bands because the standards themselves were too old. We believe that the poorly defined bands for the sham control in the fourth gel can be addressed by simply running another gel with less concentrated solution. As for the total collagen concentrations, prior studies state that approximately 86% of dry PT tissue is collagen[3]. Such a large discrepancy is directly related to the study design. The main purpose of this study was to determine the ratios of collagen type V to I and collagen type III to I. Digestion with pepsin permits the use of SDS-PAGE. However, in order to get the best results for total collagen concentration, the entire sample would need to be digested with papain. Papain, unlike pepsin, cleaves at glycine residues. Since collagen strands would be cleaved at every gly-x-y repeat, SDS-PAGE cannot be performed. In the future, after a successful SDS gel is run, we will be able to run a power analysis to determine the number of rabbits needed for a full study. If that study reveals favorable results, then the study will be performed on a more clinically relevant model, such as goat knees.

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I would foremost like to thank Dr. Savio L-Y. Woo for inviting me into his lab and making this summer research experience possible. I would also like to acknowledge Dr. Alejandro Almarza for his constant guidance, Guoguang Yang for teaching me how to perform the tests I have used, and the rest of the MSRC for introducing me to the basics of bioengineering.

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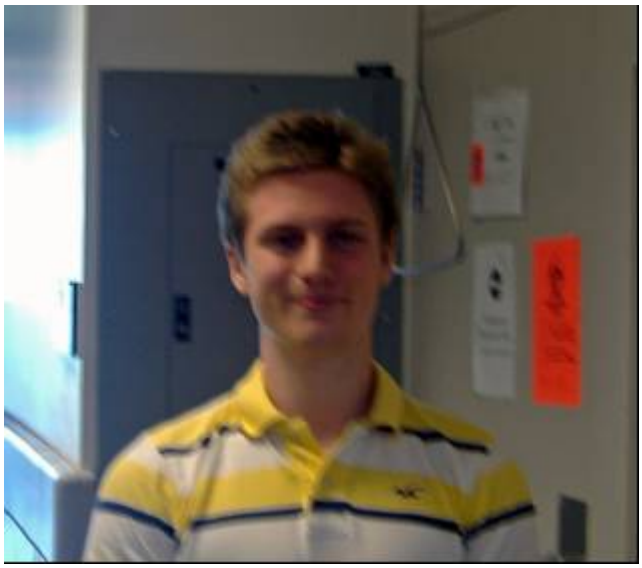

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I was born in Houston, Texas and moved around to many places before coming to my current residence in Wheeling, West Virginia. I went to two high schools; in Grand Rapids, Michigan, I was the starting fullback for our Division II state champion rugby team – I don't remember much of that year due to repeated head injuries. In Wheeling, I was chosen to be a WV representative for the 2005 Presidential Scholars. You can pick me out in the photos because I was the only guy wearing a tan suit.

I enrolled at Carnegie Mellon in the fall of 2005 as a materials and biomedical engineer. Originally, I wanted to be a doctor but after following one around in the Preceptorship program at Shadyside hospital, I decided that people are gross and money is beautiful. I now plan on entering the financial field after I graduate.

Here at the MSRC, I've learned so much in a short amount of time. It truly is astounding the amount and caliber of research that goes on here and I hope that I have done my small part in it. I would like to thank Dr. Debski, Dr. Wu, and Dr. Woo so much for guiding my research and Hilda Diamond at CMU for providing the necessary funding. I would especially like to think Noah for his eternal patience and help at every step of the way.

THE DEVELOPMENT OF A KNEE JOINT STIFFNESS MATRIX A PRELIMINARY STUDY

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INTRODUCTION

In the human knee, the ACL acts as the primary restraint to anterior motion of the tibia, among other functions. [1] There are 200,000 ACL injuries with 100,000 reconstructions in the US alone. [2] These surgical reconstructions are required for the joint to heal, but are not perfect due to incomplete understanding of the joint. Additionally, there were approximately 19 million visits made to physicians' offices due to knee problems in 2003. [3]

Obviously, the knee joint is a joint of great interest both to the research community and general population. This led to the development of a robotic/UFS testing system to study the kinematics of the joint. [4] The robotic UFS/ testing system used for this study was a 6-axis articulated robotic manipulator that provided 6 DOF motion with a 2100 N payload and .1 mm position repeatability. The universal force-moment sensor (UFS) attached to the end-effector measured forces and moments, while robot allowed for application of multidirectional physiological loading conditions. The testing system does not directly contact tissues of the joint, which allows for accurate measurement of joint function. The objective of this study was to utilize this testing system to develop a stiffness matrix of the porcine knee.

MATERIALS AND METHODS

An example stiffness matrix is given in Figure 1. In the clinical setting, a doctor applies forces and moments on a patient's knee (the left side of the equation). In turn, the patient's knee translates and rotates (the far right side of the equation). A stiffness matrix related the doctor's application of forces and moments to the patient's knee's displacements and rotations. It is important to remember that terms in the matrix are not constant and that each term is a function of three displacements and three rotations. [6]

Motion at each step of the experiment was constrained to 1 DOF. If a medial-lateral force is applied, a medial-lateral displacement will result. The medial-lateral force divided by the medial-lateral displacement gives the top term in the first column of the stiffness matrix. In this experiment, motion was constrained to 1 DOF, so there would only be displacement in the medial-lateral direction for this part of the protocol. However, there will be coupled forces and moments developing in other directions and rotations. These coupled forces and moments divided by the medial-lateral displacement give the other terms that fill in the first column of the stiffness matrix. The experiment is repeated to fill in the remaining columns.

The method used to determine these stiffness terms in this study was pioneered by Hull. It entails separating the load vs. displacement curves generated by the robotic/UFS testing system into two regions, first a low stiffness region that develops into a high stiffness region with increasing load. [5]

$$\begin{bmatrix} \Delta F_{ML} \\ \Delta F_{AP} \\ \Delta F_{PD} \\ \Delta M_{FE} \\ \Delta M_{VV} \\ \Delta M_{IE} \end{bmatrix} = \begin{bmatrix} \frac{\partial F_{ML}}{\partial d_{ML}} & \frac{\partial F_{ML}}{\partial d_{AP}} & \frac{\partial F_{ML}}{\partial d_{PD}} & \frac{\partial F_{ML}}{\partial \theta_{FE}} & \frac{\partial F_{ML}}{\partial \theta_{VV}} & \frac{\partial F_{ML}}{\partial \theta_{IE}} \\ \frac{\partial F_{AP}}{\partial d_{ML}} & \frac{\partial F_{AP}}{\partial d_{AP}} & \frac{\partial F_{AP}}{\partial d_{PD}} & \frac{\partial F_{AP}}{\partial \theta_{FE}} & \frac{\partial F_{AP}}{\partial \theta_{VV}} & \frac{\partial F_{AP}}{\partial \theta_{IE}} \\ \frac{\partial F_{PD}}{\partial d_{ML}} & \frac{\partial F_{PD}}{\partial d_{AP}} & \frac{\partial F_{PD}}{\partial d_{PD}} & \frac{\partial F_{PD}}{\partial \theta_{FE}} & \frac{\partial F_{PD}}{\partial \theta_{VV}} & \frac{\partial F_{PD}}{\partial \theta_{IE}} \\ \frac{\partial M_{FE}}{\partial d_{ML}} & \frac{\partial M_{FE}}{\partial d_{AP}} & \frac{\partial M_{FE}}{\partial d_{PD}} & \frac{\partial M_{FE}}{\partial \theta_{FE}} & \frac{\partial M_{FE}}{\partial \theta_{VV}} & \frac{\partial M_{FE}}{\partial \theta_{IE}} \\ \frac{\partial M_{VV}}{\partial d_{ML}} & \frac{\partial M_{VV}}{\partial d_{AP}} & \frac{\partial M_{VV}}{\partial d_{PD}} & \frac{\partial M_{VV}}{\partial \theta_{FE}} & \frac{\partial M_{VV}}{\partial \theta_{VV}} & \frac{\partial M_{VV}}{\partial \theta_{IE}} \\ \frac{\partial M_{IE}}{\partial d_{ML}} & \frac{\partial M_{IE}}{\partial d_{AP}} & \frac{\partial M_{IE}}{\partial d_{PD}} & \frac{\partial M_{IE}}{\partial \theta_{FE}} & \frac{\partial M_{IE}}{\partial \theta_{VV}} & \frac{\partial M_{IE}}{\partial \theta_{IE}} \end{bmatrix} \begin{bmatrix} \Delta d_{ML} \\ \Delta d_{AP} \\ \Delta d_{PD} \\ \Delta \theta_{FE} \\ \Delta \theta_{VV} \\ \Delta \theta_{IE} \end{bmatrix}$$

Figure 1: An exemplar stiffness matrix. The columns for flexion-extension and internal-external were ignored because flexion-extension is fixed throughout experimentation and porcine knees are too lax in internal-external rotation.

Two fresh-frozen porcine knees harvested from two-year-old pigs were used. The porcine model was chosen to minimize the variability associated with activity level and age. Knees were stored at -20 °C and thawed 24 hours before use. All soft tissue was removed approximately 10 cm away from the joint line on the femur and tibia. The femur and tibia were secured by using an epoxy compound and then tibia was mounted to the end-effector of the robot through the UFS while the femur was rigidly fixed relative to the base of the robotic manipulator. After the knee was mounted, the joint coordinate system was determined with a digitizer.

The path of flexion/extension was then determined by minimizing the forces and moments associated with moving the knee throughout its range of flexion. Then a loaded path was performed constrained to 1 DOF (100N medial/lateral, 300N anterior/posterior, 15Nm varus/valgus, 150N distal, 300N proximal). Tests were performed at 30, 60, and 90 degrees of flexion.

These loaded paths gave load vs. displacement curves for data analysis. First, the primary curves (for example, medial-lateral force vs. medial-lateral direction) were separated into low stiffness and high stiffness regions based on force limits (45N for anterior/posterior, 30N for medial/lateral, 3Nm for varus/valgus, 30N for proximal/distal). The displacement achieved at the force limit was then recorded for use in the coupled curves (for example, anterior-posterior force vs. medial-lateral displacement). The coupled curves were then separated into low stiffness and high stiffness regions based on the displacement at the force limit of the primary curve. Examples can be seen in Figure 2.a. and 2.b. This method was repeated to fill in the stiffness matrix, a portion of which can be seen in Figure 3.

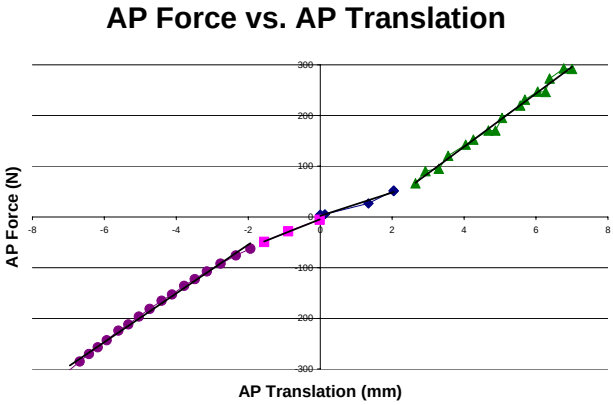

Figure 2.a.: Example of a primary curve

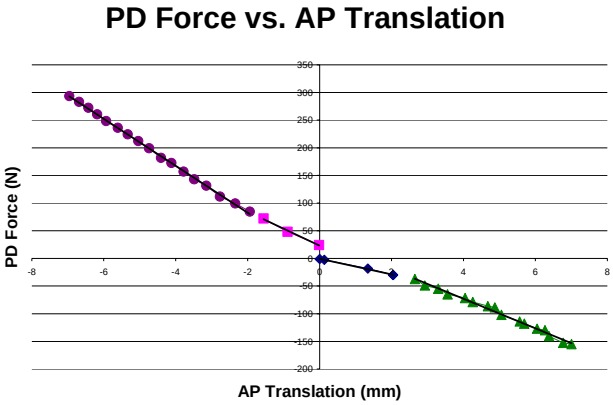

Figure 2.b.: Example of a secondary curve

RESULTS

	Low		High	
	LAT	MED	LAT	MED
ML	**29.0**	**16.4**	**37.1**	**41.1**
AP	3.5	-8.5	6.2	-12.7
PD	-38.2	21.6	-51.5	34.9
FE	0.0	0.0	0.0	0.0
VV	1.2	0.5	1.4	1.1
IE	0.0	0.0	0.0	0.5

Length of Low Stiffness Region

LAT	MED
-0.9	1.8

Figure 2: For displacements up until and including 1.8mm, a 16.4N/mm stiffness is measured in the medial direction. Above 1.8mm, a stiffness of 41.1N/mm is measured. (First knee tested at 60 degrees of flexion)

Stiffness matrices were developed for both knees at 30, 60, and 90 degrees of flexion. The highest primary stiffness values were recorded in the proximal direction followed by the distal direction. The repeatability of the robotic/UFS testing system is 3.5N and .35Nm, while the average low stiffness region was ~ 1mm. Because we want to discern between stiffness regions, directional stiffness values less than 3.5N/mm and rotational stiffness values less than .35Nm/mm were deemed insignificant and set to zero. Because of this, most of the values in the flexion-extension and internal-external rows were zero.

DISCUSSION

The goal of the project was to develop a porcine stiffness matrix. From these two knees, it seems that a porcine stiffness matrix is not subject-specific, and a characteristic porcine stiffness matrix can be developed.

There truly is a wealth of data from this experiment. High stiffness values were approximately 1.5-2 times low stiffness values. The length of the low stiffness region was different between both knees (with the second knee about twice as lax), but primary high stiffness values for both were very similar. In addition, both primary and coupled high stiffness values were similar between knees. Proximal stiffness dropped as flexion angle was increased with other stiffness values remaining relatively constant.

In the future, more porcine knees should be run to develop a characteristic porcine stiffness matrix. In addition, I would encourage testing of populations of human knees (such as young female athletes) to see if a characteristic human knee matrix could be developed. If such a matrix could be developed, doctors could move from using the more qualitative changes in motion to diagnose injury (such as the anterior tibial drawer test) to the more quantitative changes in stiffness to diagnose injury.

ACKNOWLEDGEMENTS

I would like to thank Noah Lorang for his constant help throughout this project, from running experiments to answering questions. I also want to thank Dr. Debski and Dr. Wu for valuable insight and discussions. Finally, I want to thank Dr. Woo and the rest of the MSRC for an educational and enjoyable summer and Hilda Diamond for offering the funding necessary for it.

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I was born the youngest of four children in Ann Arbor, Michigan, where I spent most of my childhood. Like most adolescents, I had no idea what I wanted to do with the rest of my life, but becoming a doctor, firefighter, and airline pilot all crossed my mind. Just before high school, I moved to Chapel Hill, North Carolina, and there my development really took off. Through introductory and then advanced science courses, I realized that my long term interests lay in some form of engineering. With this in mind, I enrolled at Carnegie Mellon University in the fall of 2004.

My family has a long history of mechanical engineering. My father studied mechanical engineering in college, and his father worked with the U.S. Navy for over two decades in engineering and maintenance. Despite all this, I originally thought electrical engineering would be my field of study. Once I discovered what mechanical engineering was all about (compared to what electrical engineering was), I realized that mechanical engineering was far more enjoyable to me. As my studies progressed in mechanical engineering, I found that I was seeking a more focused and applied discipline, so I began studying biomedical engineering in addition to mechanical engineering.

The time that I've spent at the MSRC has been invaluable, and I've learned more than I could imagine, and had the opportunity to accomplish more than I believed possible. Thanks to Dr. Woo and Dr. Debski for making the opportunity available for me to work here this summer and to Hilda Diamond from Carnegie Mellon for providing the funding to make it possible. Additionally, I owe extensive gratitude to Dr. Changfu Wu, who has guided my research at the MSRC and provided me with extensive skills as I move through my academic career. Finally, my thanks to Dr. Giovanni Zamarra for his clinical assistance with this project, and to Dr. Steve Abramowitch for his ongoing supply of insight and ideas.

TECHNIQUES FOR SIMULATION OF IN-VIVO ACTIVITES WITH A ROBOTIC/UFS TESTING SYSTEM

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INTRODUCTION

In order to study the mechanisms of non-contact ACL injuries, as well as to improve functional outcomes after anterior cruciate ligament (ACL) reconstruction, a thorough understanding of ACL function is necessary. Robotic technology has been in use for the last decade for the study of knee joint biomechanics, which provides a highly accurate method for applying six degree-of-freedom (DOF) forces or tibiofemoral kinematics to a knee and measuring the resulting joint kinematics or external joint forces, as well as the in-situ force in structures within the knee joint [1, 2]. Such testing systems typically combine a six degree-of-freedom (DOF) robotic manipulator with a universal/force moment sensor (UFS) to apply loads or kinematics to the joint with excellent repeatability and accuracy [2].

To apply robotic technology to the study of in-vivo loading of the knee joint, one approach is to first collect tibiofemoral kinematics from one set of knees in-vivo and then reproduce the kinematics on another set of knees in-vitro using a robotic/UFS testing system in order to estimate the external and in-situ forces. Previously, it has been shown that simply replaying average tibiofemoral kinematics relative to passive flexion will result in inaccurate estimations of external and in-situ forces, with differences between estimated and true values reported of up to 100% [3]. In order to improve the accuracy and precision of such measurements, we propose to apply a preload to the joint so as to remove the effects of variability in laxity between specimens.

METHODS

To validate the proposed methodology, a prescribed external force was first applied to a set of source knees and the resulting tibiofemoral kinematics and the in situ force in the ACL were measured. Then, the kinematics measured on the source knees were used to calculate the tibiofemoral kinematics to be reproduced on a set of target knees. Finally, such tibiofemoral kinematics were applied to the target knees and the resulting external and in situ forces were determined, which were then compared with those of the source knees.

A total of n = 12 (6 as source and 6 as target) fresh-frozen cadaveric porcine knees were tested on a recently developed robotic/UFS testing system (Robot model: KR210, KUKA Robotics; UFS model: Theta, ATI Industrial Automation), which is capable of applying loads of up to 2100 N with position and orientation repeatability of 0.1 mm and 0.1°, and has been shown to repeat applied loads within 10% [4].

For all specimens, a path of passive flexion/extension was determined from full extension through 90° of knee flexion by flexing the knee in 1° increments while minimizing external forces and moments. For the source knees, a 100 N anterior tibial load was applied through an iterative algorithm in 10 N incremental steps at 30°, 60°, and 90° of knee flexion, and the resulting kinematics relative to the passive flexion path were recorded. The ACL was then transected and the previously recorded kinematics were replayed while recording the applied force, yielding the in-situ force via the principle of superposition. This provided known external and in-situ forces for the source knees.

The incremental kinematics relative to a position of 0, 10, 20, and 30 N of anterior load were then calculated by subtracting the positions corresponding to that preload from the kinematics of all successive incremental load steps for each of the source knees and were averaged to create average source kinematics, simulating in-vivo motion. These kinematics were then replayed on 6 separate target knees by adding the average incremental source kinematics to the target knee positions found for the anterior preload, and the force required to reach these positions was measured, providing an estimation of the originally applied external force. The ACL was then transected in this group of target knees and the kinematics were again replayed, yielding an estimate of in-situ force.

Statistical analysis of measurements of external and in-situ force was conducted using an F-test to compare the precision of measurements with various amounts of preload, as well as with one-way ANOVA to compare the resultant discrepancy between measurements of external and in-situ force.

RESULTS

The average resultant discrepancy between the source external force and the estimation from replay of average kinematics on the set of target knees is shown in table 1. The resultant discrepancy ranged from a minimum of 15.1 N for replay from a 30 N preload to 56.0 N for replay from no preload. Significant improvements were seen for estimations of external force from any amount of preload compared to no preload ($p < 0.05$).

	Preload			
Flexion Angle	0 N	10 N	20 N	30 N
30°	51.9 ± 23.0 N	26.1 ± 14.9 N *	25.0 ± 13.8 N *	21.3 ± 12.2 N *
60°	38.4 ± 25.1 N	29.8 ± 22.0 N *	24.9 ± 18.7 N *	15.1 ± 11.3 N *
90°	56.0 ± 37.5 N	28.1 ± 17.1 N *	25.4 ± 18.3 N *	17.7 ± 13.8 N *

Table 1- Average resultant discrepancy between true and estimated external force for increasing preload (* $p < 0.05$)

The average discrepancy in ACL in-situ force magnitude between the actual measurements made on the set of source knees and the estimation made by replaying of kinematics on target knees is shown in table 2. The average magnitude difference was as low as 5.8 N for a 30 N preload, and ranged to a maximum of 21.9 N for no preload. There were significant decreases in the average difference for any amount of preload compared to no preload ($p < 0.05$).

	Preload			
Flexion Angle	0 N	10 N	20 N	30 N
30°	21.9 ± 19.1 N	13.5 ± 11.5 N *	14.0 ± 10.2 N *	12.4 ± 8.9 N *
60°	9.4 ± 11.6 N	7.7 ± 9.9 N	8.3 ± 9.0 N	6.5 ± 5.8 N
90°	11.3 ±10.2 N	7.1 ± 4.6 N *	6.0 ± 5.5 N *	5.8 ± 5.4 N *

Table 2- Average magnitude discrepancy between true and estimated in-situ force for increasing preload (* $p < 0.05$)

For measurements of external load, measurement from a 30 N preload was consistently more precise as evaluated with an F-test than measurement from no preload, while measurement from a 10 or 20 N preload was more precise in 50% and 87% of cases, respectively. For measurements of in-situ force, measurements using a 10, 20, or 30 N preload were more precise than no preload in 23%, 17%, and 40% of cases, respectively.

DISCUSSION

The results clearly show that the application of a preload to determine in-vivo joint kinematics significantly improves both the accuracy and precision of measurements of external and in-situ force. The use of a 30 N preload resulted in up to a three-fold decrease in error in measurements of external and in-situ force, and the same preload resulted in a significant improvement in precision of measurements when compared to no preload. The use of this method for determining and replaying in-vivo joint kinematics captured on one set of knee joints on a separate set of joints provides a method to determine forces at the joint to an accuracy of within 20%, and can be applied to provide the forces experienced at the joint or carried within a structure during in-vivo activities.

ACKNOWLEDGMENTS

The technical assistance of Giovanni Zamarra and the past work of Shon Darcy is gratefully acknowledged. The support of NIH Grant AR39683, the Musculoskeletal Research Center, and the Department of Biomedical Engineering at Carnegie Mellon University is also acknowledged.

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Tissue Mechanics
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Thomas W. Gilbert, PhD

I was born in Latrobe, Pennsylvania on July 20, 1986. A natural love for math and science at an early age encouraged me to pursue engineering. With the help of some excellent teachers at Greater Latrobe High School, I soon discovered that my interests lied within the human body. I am also deeply involved with athletics, participating in varsity football, basketball, and track during my high school. With a truly last minute decision I enrolled into Carnegie Mellon University's Institute of Technology.

I am obtaining my BS in Chemical and Biomedical Engineering with the goal of attending medical school once I have finished my undergraduate work. I participate in varsity track and field at Carnegie Mellon, where we recently won our conference title. I am also a member of the Lambda Sigma Honor Society, Doctors of Carnegie, and the local Best Buddies program. With the little free time that I have, I usually read and play intramural sports such as basketball, football, and hockey.

Working at the MSRC has been a fantastic experience. I have learned so much about the attitude and environment of a successful research institute. The positive teaching atmosphere of the MSRC has opened my eyes to the exciting possibilities in biomedical engineering. I would like to thank Dr. Abramowitch and Dr. Gilbert for giving me this opportunity and patiently guiding me with my project. The knowledge I have gained from the students and staff of the MSRC has motivated me even more to pursue my passion in engineering and medicine.

AN EXPERIMENTAL METHODOLOGY FOR THE DETERMINATION OF THE VISCOELASTIC PROPERTIES OF THE VOCAL FOLD AT HUMAN PHONATION FREQUENCIES

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I. INTRODUCTION

The vocal folds are subjected to some of the largest magnitudes and frequencies of deformation of any tissue in the human body during normal voice production. Vocal fold scarring creates a functional deficit in this highly specific tissue that compromises the integrity of an individual's voice. Scarring is the leading cause of dysphonia which often leads to a decreased quality of life, especially to those whose voice is an integral part of their profession.

Shear tests are utilized to better understand the vocal fold and its biomechanical properties. Due to testing system limitations, torsional shear tests apply inhomogeneous strain distributions with magnitudes more than ten times less than physiological deformations[1]. Alternatively, a shearing methodology which allows a uniaxial, uniform strain with magnitudes approaching the lower limit of that seen *in vivo* (approximetaly 50%) would provide more physiologically relevant biomechanical data. Thus, the objective of this research was to establish an accurate and repeatable linear, single lap shear test that will be used to obtain the biomechanical properties of the vocal fold. The optimal sample strain amplitudes and plate gap size were determined using a derived Newtonian force equation and gap limitations determined previously by Schrag[2] (1977), respectively. The present linear rheological methodology was then validated by comparing experimental results to that of previously published data[3] on the viscoelastic properties of the vocal fold bulking agent Radiesse. Radiesse is a homogeneous material consisting of calcium hydroxylapatite suspended in a water and glycerin gel.

II. MATERIALS AND METHODS

A. Apparatus

The single lap, sinusoidal, oscillatory shear test was modified from Xu et al.[3] (2007) using an EnduraTEC Electro-Force Mechanical Testing System (Model ELF 3200, Bose, Minnetonka, MN) which is capable of producing strain rates up to approximately 200 Hz. The mechanical apparatus used to perform the oscillating shear test consisted of a stable, stationary base plate and an oscillating upper plate. The sample was mounted between these plates (Figure 1). A Sensotec Miniature Load Cell (Model 31, Honeywell, Columbus, Ohio) with a resolution of 1.0 grams was attached to the stationary end of the testing machine via an adjustable X-Y table. The top plate was attached to the actuator of the testing machine and was used to apply a controlled displacement. The resulting shear strain and stress data (Force/Area) was obtained and utilized to calculate viscoelastic shear properties consisting of the complex shear modulus, elastic modulus, dynamic viscosity, and phase lag within a frequency range from 0.1 Hz to 100 Hz assuming the linear theory of viscoelasticity.

B. Gap Limitations

When applying a dynamic shear deformation to a material, care must be taken regarding the shear wave that originates at the oscillating plate and propagates through the sample. According to Schrag (1977), the gap distance between the plates should be sufficiently small enough that the shear wave moving away from the oscillating plate is immediately reflected. This ensures that the strain throughout the tissue is in phase with that of the oscillating plate, preventing phase lag errors. The limit is determined by the ratio of the shear wavelength (λ) to gap distance (d).

$$\frac{\lambda}{d} \geq 40 \tag{1}$$

This inequality must be satisfied in order to ignore sample inertia. The shear wavelength as determined by Schrag can be determined by

$$\lambda = \frac{\sqrt{\frac{G^*}{\rho}}}{f \cdot \cos\frac{\delta}{2}} \tag{2}$$

where λ, G^*,ρ, f, and δ represent shear wavelength, complex shear modulus, density, frequency, and phase shift, respectively. The gap distance was set to approximately 1.0 mm with metal spacers. The G^* and δ data was taken from preliminary experimental results while the density was assumed to be 1100 kg/m^3. Setting the gap distance resulted in a maximum frequency in which sample inertia could be ignored for individual samples.

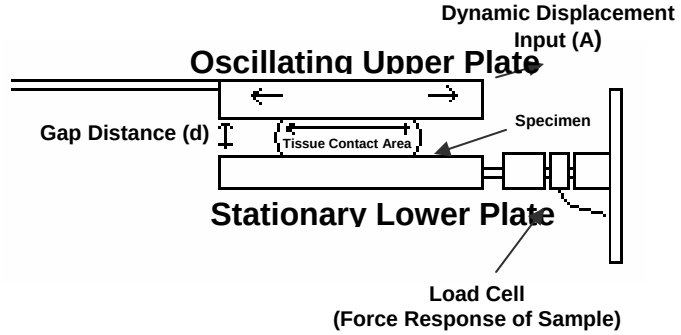

Figure 1. Schematic of linear controlled rheometer (not to scale).

C. Optimal Displacement Amplitude

Due to the limitations with respect to the resolution of the load cell (1.0 g), the applied displacement had to be large enough to generate a force greater than the load cell's resolution. A parametric study based on a Newtonian force equation was used to determine the maximum force for a

given displacement and frequency. The basic force equation,

$$F = kx + c\frac{dx}{dt} \quad (3)$$

where *k*, *c*, and *x* represent a spring constant, damping coefficient, and displacement of the oscillating plate, respectively, was used to derive the specific force modeling equation,

$$F = \frac{G'}{d}S[A\sin(\omega t)] + \frac{\eta}{d}S[A\cos(\omega t)]\cdot\omega \quad (4)$$

where G', η, A, S, d, ω, and t represent the elastic modulus, dynamic viscosity, displacement amplitude, tissue contact area, gap distance, angular frequency, and time, respectively. The input parameters, elastic modulus and dynamic viscosity were assumed values taken from literature[3]. The specimen contact area was calculated using the set gap size (1.0 mm) and the volume of sample used (approximately 0.1 mL^3). The maximum force was then calculated for increasing displacement amplitudes. A displacement amplitude of 0.10 mm was determined to provide force measurements more than an order of magnitude greater than the resolution of the load cell. Based on the gap distance, this displacement corresponds to a 10% shear strain which approaches the lower range of vocal fold displacement during voice production.

III. RESULTS

The calculated viscoelastic data for six Radiesse injectable samples was found to correlate well with previously published data[4], following the same trends up to 100 Hz. Data over 100 Hz did not satisfy the gap limitation and was excluded due to expected sample inertial errors. The dynamic viscosity (Figure 1) and elastic modulus (Figure 2) was plotted as a function of frequency. The mean dynamic viscosity at 0.1 Hz was 5700 Pa·s and decreased monotonically to 7.0 Pa·s at 100 Hz. This shear thinning phenomena as well as approximate ranges for the dynamic viscosity was observed previously for Radiesse[4]. The mean elastic modulus increased from 3000 Pa at 0.1 Hz to 8500 Pa at 100 Hz, demonstrating a stiffening of the material at increasing shear rates which was also noted in previous research[4].

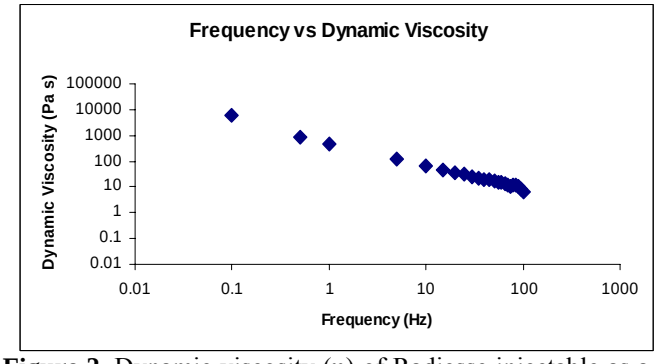

Figure 2. Dynamic viscosity (η) of Radiesse injectable as a function of frequency

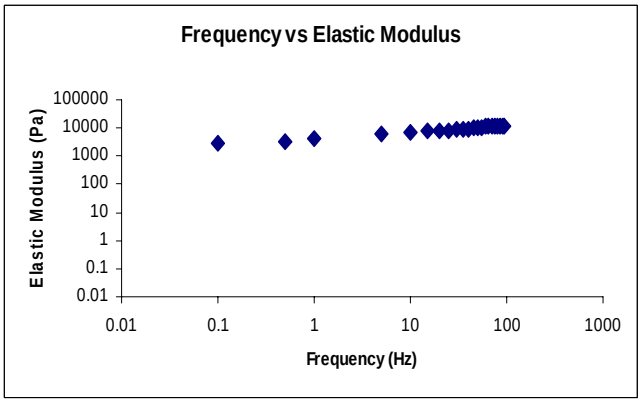

Figure 3. Elastic Modulus (G') of Radiesse injectable as a function of frequency

IV. DISCUSSION AND FUTURE STUDIES

In this study, we developed a linear shear testing methodology for biomechanical testing of homogeneous materials. The test provided repeatable and accurate viscoelastic data when compared to the results of a torsional rheometric method testing the Radiesse bulking agent[4]. The advantages of the linear single lap shear test are that it will allow applied strains to tissue specimens to be more uniform and have magnitudes more similar to those experiments in vivo. This is an improvement over torsional rheometry in which different strain magnitudes are applied throughout the tissue depending on the tissue's location with respect to the radius of the oscillating plate. In addition, a decrease in inertial error was observed using the present shearing method due to the use of only a single, lightweight moving plate.

The linear shear test has proven to be capable of accurately shear testing samples up to a frequency range of 100 Hz. Further studies must incorporate the sensitivity of the viscoelastic data to changing strain amplitudes. A strain sweep performed at a fixed frequency will establish the dependence of the viscoelastic properties on the percent strain applied. This is necessary because the vocal folds vibrate at strain magnitudes of approximately 50%, depending on the volume of voice, about an order of magnitude higher than the strains applied in current rheological testing.

V. ACKNOWLEDGMENTS

The support of the Musculoskeletal Research Center, the Department of Otolaryngology, and the Department of Biomedical Engineering at Carnegie Mellon University is acknowledged.

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Faculty Advisor: Savio L-Y. Woo, PhD, DSc
Changfu Wu, PhD

Born in Steubenville, Ohio on February 17, 1985, I grew up and followed my brother around and watched him play basketball. Eventually, he went to Syracuse University while I moved to West Virginia with my family. There, I attended Brooke High School; where I played basketball and football. Although I was not very good, I learned how to motivate myself. I earned excellent grades and recognized my love for math and science. I also had good role models; my older siblings all studied the engineering curriculum. Soon, I enrolled at Syracuse University and majored in Biomedical Engineering.

When my passion for basketball fizzled to a hobby, I became suddenly infatuated with mechanical and electrical courses. However, I still enjoyed playing basketball and football in intramurals; these activities helped me to develop some longstanding friends and to stay healthy. With three years of college and no research, I was unable to find my niche in Bioengineering. Fortunately, the MSRC gave me a great opp opportunity to do research with them this summer. I love the possibility of exploring my options.

Although I do not know if I will stay in the mechanics portion of Bioengineering, I have definitely learned about the experimental process in the robotics laboratory. I really appreciate the information I acquired from my valuable experience. Furthermore, I would like to thank Dr. Changfu Wu for helping me with my presentation and advising me about the analysis of my experiment. Finally, I would also like to express my gratitude to Dr. Woo and to Dr. Debski for their insight and especially for giving me this opportunity to acquire knowledge about Bioengineering research.

The Conventional ACL Reconstruction versus the All-Inside Technique: A Preliminary Study

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INTRODUCTION

Annually, there is an estimated 200,000 injuries contributed to the ACL, and 100,000 of those are reconstructed. The Anterior Cruciate Ligament is located between the Femur and the Tibia. It is known to stabilize anterior tibial translation, varus-valgus rotation, and internal and external rotation. Athletes, (especially involved with basketball and football), are susceptible to tearing their ACL due to the fact that these sports involve movements of cutting or sudden decelerations.

In order to improve ACL reconstruction for patients, six variables are considered in research. Those variables include: graft choice, tunnel placement, graft tension, graft fixation, tunnel motion, and graft healing. [3] Improvement of those factors leads to a better restoration of intact ACL function.[3] In single bundle reconstruction, two types of procedures are used: traditional single bundle ACL-R and the all-inside technique. These two types differ in that the traditional version uses two different hamstring tendons for the graft while the all inside technique uses only one hamstring for the replacement graft.

The all inside technique is classified in that half tunnels are drilled from inside the joint cavity away from the joint line inside the tibia and the femur. This allows for a larger diameter graft to be placed. Usually, the femoral side of the graft is fixed with an Endobutton while the tibial fixation varies with two types: cortical screw fixation and interference screw fixation.

The following study will utilize the all inside technique in order to determine the optimal method for fixation for the tibial side. The goal of this study is to determine which method of tibial graft fixation better restores the kinematics and ACL in-situ force to that of an intact joint. It is hypothesized that interference fixation positioned closer to the joint line will decrease tunnel motion. Thus, interference screw fixation must be the closest to model intact function.

METHODS

To verify the hypothesis, a robotics/UFS testing system was used since it is repeatable, accurate, and can manipulate 6-DOF. However, before such testing could take place, the specimen had to be prepared. First, the specimen which was porcine was taken out the night before dissection to be thawed. Next, the dissection consisted of the tibia and the femur being cut 20 cm as well as flesh being removed 10 cm from the joint line. After, both bones were fixed to two steel cylinders with an epoxy compound which permitted the knee to be placed on the KUKA robotics machinery.

In total, three porcine specimens were prepared and tested. A typical testing protocol consisted of the following: passive path, force controls 1 and 2, and replay kinematics. For the passive path, the point at each degree of flexion angle where minimal forces and moments existed was found from full extension to 90 degrees of flexion. Thereafter, in force control 1, an applied force of 100 N was directed in the anterior and posterior direction and the kinematics was taken at flexion angles 30, 60, and 90 degrees. Next, in force control 2, a moment of 10 Nm in the valus direction was applied incrementally, and the kinematics was also taken only at 30 degrees of flexion angle. Afterwards, the ACL was transected and the kinematics controls were replayed. Therefore, with the law of superposition upheld, the in situ force of the ACL was obtained. Finally, the aforementioned force controls were run for the ACL-deficient state, and again, the kinematics was obtained.

At this point, the all-inside technique was performed by medial arthrotemy. The femoral side of the graft was fixed by endobutton. Next, the tibial side of the graft was fixed by first a cortical screw and washer and, then, an interference screw. Testing resumed after each type of tibial fixation, and the in situ force as well as the kinematics was measured by the same outlined procedure done for the ACL.

After gathering much data, focus was placed upon the kinematics and the in situ forces which were analyzed for differences by ways of

a repeated measures ANOVA. The groups analyzed for kinematics were intact-ACL, deficient-ACL, graft 1 fixed by cortical screw, and graft 2 fixed by interference screw. Significance between states was set at $p < 0.05$.

RESULTS

The average anterior-tibial translations of ACL-intact, ACL-deficient, graft 1, and graft 2 in response to an anterior load of 100 N are outlined in figure 1. Significant differences were apparent at the flexion angles of 60° and 90°. At 60°, those differences were found between intact and deficient states as well as intact and graft 1 state. However, a post hoc analysis showed that there were no significant differences between any states at 90°. For the valus 10 Nm at 30°, significance was found to be equal to .759 for the measured anterior-tibial translation. Therefore, there were no significant differences.

Similarly, the in situ forces of the two grafts and the ACL are pictured in figure 2 in response to an anterior load of 100 N at flexion angles 30°, 60°, and 90°. There were no significant differences found at either flexion angle. Likewise, the in situ force measured in response to a 10 Nm load was also found to be insignificant amongst comparisons with the three constraints.

Finally, the valus-varus rotation in response to a valus 10 Nm load was also deemed to be insignificant in all four groups. Although not pictured, its p-value came out to be .402.

DISCUSSION

Looking at graphs 1 and 2, you can see an increase in anterior-tibial translation and a decrease in forces with an increasing flexion angle when comparing the ACL-intact state between the two grafts. This is due to the surgeon's femoral placement of the grafts at 10 o'clock which is where the femoral insertion site of the ACL is found.[4] This further verification existed with the increase not only in rotatory stability but also in forces of the constraints in response to a rotatory load.[4]

In the quest to prove legitimacy, the only types of studies that I could find were ones dealing with unidirectional cyclic loading and monotonic loading to failure.[2] Thus, this study could shed some enlightening evidence on tibial fixation methods. Also, possible differences would become evident in the human knee because the porcine proximal tibia is not a good model for interference screw fixation.[1] This is due to the fact that the proximal tibia's cancellous bone for the porcine knee was found to be almost twice the bone mineral density of the cancellous bone in a cadaver's proximal tibia.[1]

In conclusion, I could only find significant differences existing between the ACL-intact state and deficient state as well as the ACL-intact state and graft 1 state at 60°. Thus, there is not enough validation for my assumption that interference fixation would replicate intact-ACL function best because of the reduction in tunnel motion. Therefore, more data is needed in order to see if a difference does exist between the two fixation methods.

Figures:

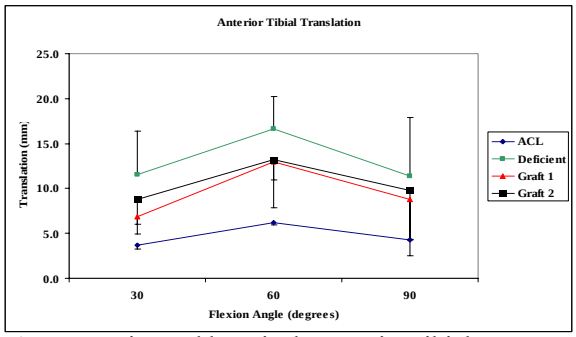

Figure 1: Pictured here is the anterior-tibial translation in response to an anterior load of 100 N.

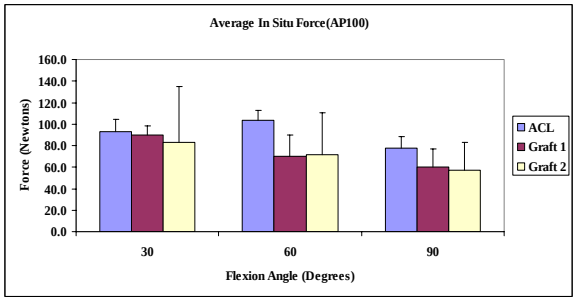

Figure 2: Pictured here is the in situ forces in response to an anterior load of 100 N for the ACL, graft 1, and graft 2.

ACKNOWLEDGEMENTS

Thanks to my mentors Giovanni Zamarra, M.D. and Changfu Wu, PhD, as well as Noah Lorang for all their help and guidance. Thanks to Dr. Woo and Dr. Debski for this opportunity. Thanks to the entire ACL group and MSRC for their support.

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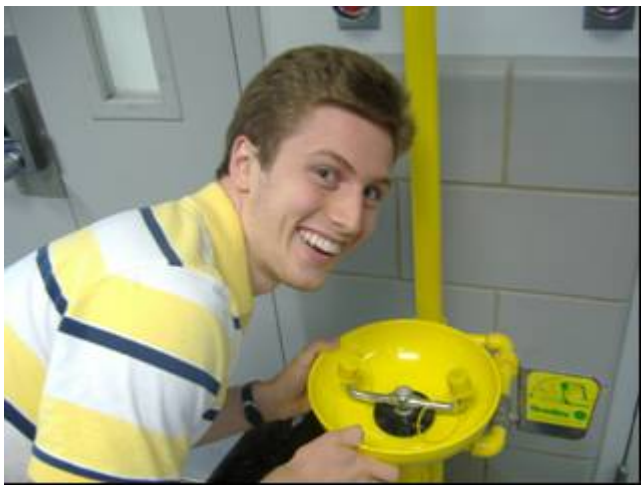
2007 Summer SnapShots

Danielle hard at work

Mitchell, Ryan, Kristin, and Dave

Tan-tastic Monday!!!



Who Knows?

Steve's still got it

Dave wishes he had it

Ryan playing frisbee

Free Food!!!

Abstract Book vs. Symposium

2007 Summer Student Symposium

Noah, Carrie, and Andrew

Joel and Dr. Woo

**Summer Students, Dr. Debski, Dr. Woo,
and Dr. Abramowitch**

Musculoskeletal Research Center
Summer 2007

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