Musculoskeletal Research Center

Summer Research Program

2006

Department of Bioengineering

University of Pittsburgh

MSRC

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2006 Abstract Book Committee

Sarah Henderson, Noah Lorang, Caressa Watson

The work that you see presented in this book represents the intensive labors of each of the summer students over the last few months. I think I can speak for all the summer students when I say that the work that we put into our research has been some of the most rewarding of our lives, and the amount that we have learned has been incredible.

The research that we conducted would not have been possible without the students, faculty, and staff of the Musculoskeletal Research Center, and to them we are forever indebted. To our mentors, we thank you for your time, guidance, and the education that we've received. I also want to thank Dr. Debski for his hard work in putting together a successful summer program and all of the summer students for their help in compiling this abstract book.

Finally, on behalf of the rest of the summer students, I want to thank Dr. Woo for the opportunity to complete this research, and for your never-ending vision which guides and inspires us all.

Noah Lorang
Editor, Summer Abstract Book

2006 Summer Symposium Committee

Emily Engel, Toby Long, Christopher Carruthers

This year the Summer Student Symposium took place on July 21st, 2006 at the Musculoskeletal Research Center. Each of the seven summer students were given the opportunity to present what they had completed during their 10-12 week internship and answer questions posed from MSRC faculty and staff.

Although butterflies swarmed the stomach of every summer student that day, the presentations were very impressive and the students' ability to answer questions about their projects was outstanding. Not only did the event show how much each student has learned over the summer, but it also gave each student a skill that will be extremely beneficial in their careers. Speaking on behalf of the members of the symposium committee, I would like to thank everyone who helped make this year's symposium a success and to all those who attended. Special thanks to Dr. Woo for the opportunity to conduct and present this research, and to Dr. Debski for his hard work in making the summer program a success.

Toby Long
Chair, Summer Symposium Committee

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I was born in New York City on May 14, 1984. My inclination to enter the field of biomedical engineering was fostered early on through the encouragement of my parents to pursue my dreams, and by the support of excellent teachers in both biology and math. I graduated from Friends Seminary High School, where I played both varsity baseball and basketball.

I am obtaining my B.S. degree at the University of Rochester in biomedical engineering with a concentration in biomechanics. I am vice president of Tau Beta Pi and I serve as an EMT for my campus's Emergency Medical Response Team. Apart from academics, I enjoy informal ultimate frisbee, cycling, basketball, and abundant amounts of snow.

Working at the MSRC has been great. I have gained valuable experience in multi-disciplinary research with the support and guidance of extraordinary researchers. I would like to thank my mentor Rui Liang, and, additionally, Tan Nguyen for their teaching, and encouragement throughout my experience. I would also like to thank Dr. Abramowitch, Dr. Debski, and especially Dr. Woo for establishing such a wonderful program.

COMPARISON OF TWO SCAFFOLDS ON THE HEALING OF THE PATELLAR TENDON: A BIOCHEMICAL AND MORPHOLOGICAL ANALYSIS AT SIX WEEKS

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INTRODUCTION

Anterior cruciate ligament (ACL) injury is the most frequent knee injury with between 150,000-200,000 occurrences and a cost of 2 billion annually in the United States alone. [3,4] The ACL cannot heal by itself. Therefore surgical reconstruction is needed to reacquire the stabilization and function of the knee.

The "gold standard" for ACL repair has been a bone-patellar tendon-bone autograft that has a long clinical history, superior graft strength, and bone to bone healing allowing for better fixation and an earlier return for patients to physical activity.[6,7] However, after harvest of the graft, the patellar tendon (PT) has poor mechanical properties and abnormal histomorphology even years after surgery. [5, 9] The slow healing of the PT defect is closely related to the occurrence of donor site morbidity.

To improve the healing of the PT, functional tissue engineering approaches such as bioscaffolds are promising. [2,8,11] Porcine small intestine submucosa (SIS) is a naturally derived collagen scaffold with bioactive constituents such as growth factors, proteoglycans, etc. which promote cell proliferation and chemotaxis. [10] In the lab, SIS has been shown to enhance tissue ingrowth at the defect area of the PT after harvest of the central third in a rabbit model. The mechanism of why SIS improved the healing is unclear.

Therefore, the research question is whether the healing of the tissue defect is due to SIS acting mainly as a collagen based scaffold, or due to the bioactive constituents of SIS. To answer this question, the specific aim of this project is compare the bioactive scaffold SIS to an alternative collagen based scaffold lacking biological cues (BioMend collagen membrane) on the healing of PT defect. Biochemistry and morphology of the healing tissue is evaluated for a rabbit model at 6 weeks post-injury. It is hypothesized that the biological cues of SIS will increase cell proliferation resulting in greater tissue growth and repair.

METHODS

Ten skeletally mature New Zealand female white rabbits were used for this study. The central third of the patellar tendon was surgically removed from the right hind limb. The PTs of the left limb served as a sham-operated control with incision only on the skin and paratendon (n=9). For the SIS-treated group, one single layer of SIS was sutured anteriorly and posteriorly to the patellar tendon defect (n=3). For the BioMend-treated group, one single layer of BioMend was sutured anteriorly and posteriorly to the PT defect (n=3). The defect was left untreated in the non-treated group (n=3). The rabbits were euthanized at six weeks. (Figure 1)

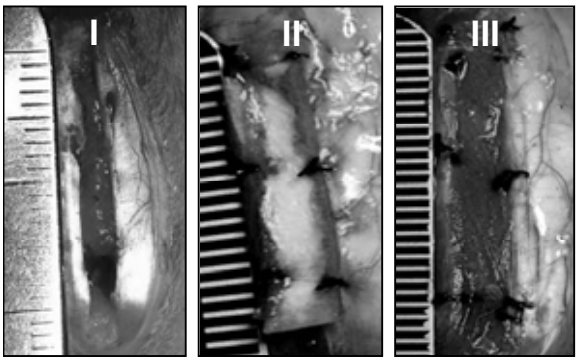

Figure 1: Gross view of rabbit injury model at time of surgery, I. Non-treated, II. Biomend-treated, and III. SIS-treated

For morphology, pictures were taken after the PTs were exposed and dissected. Additionally, cryosections were stained using Hematoxylin-Eosin staining. For biochemistry, collagen content was detected using a hydroxyproline assay; Glycosaminoglycan (GAG) content was detected using a Blyscan assay. The resultant data was normalized via dry weight. An unpaired t-test was used to compare SIS-treated to BioMend treated. A paired t-test was used to compare treatment groups to the sham control. Significance was set at $p < 0.05$.

RESULTS

Gross inspection of the PT defects at six weeks indicate that both the SIS-treated samples & BioMend-treated samples have more tissue ingrowth than the non-treated samples (Figure 2). Additionally, SIS-treated samples have more tissue ingrowth than BioMend treated samples. No inflammation or signs of infection were found at sacrifice. Also, no SIS or BioMend could be found on surface of the defect area.

Hematoxylin-Eosin staining of the tissue ingrowth at the PT defect at six weeks indicates that both BioMend-treated and SIS-treated samples have increased cellularity and fiber alignment compared to the non-treated group with only healing scar tissue (Figure 3).

Collagen content and GAG content detection of the tissue ingrowth at the PT defect at six weeks

indicates that SIS-treated samples have significantly less collagen and GAG content per unit of dry tissue than the BioMend-treated samples (Figures 4, 5), while the tissue in the non-treated group was too limited to analyze.

DISCUSSION

The bioactive scaffold SIS significantly enhanced tissue ingrowth for the PT defect compared to the scaffold BioMend which lacks bioactive constituents, supporting the hypothesis. While SIS has less collagen and GAG content per unit of dry tissue, due to the large healing tissue mass in SIS treated tendon, it is hard to compare the effect of the two scaffolds.

As a result of these findings, a short term study of gene expression is warranted to determine if the quantity of these proteins will change over time. Additionally, a long term study is needed, and the mechanical properties of the healing tissue need to be tested. Ultimately, the goal is to improve both quantity and quality of healing tissue within the PT defect through bioscaffold treatment.

ACKNOWLEDGMENT

Sincere thanks to my mentor Dr. Liang and additionally Dr. Nguyen for their excellent guidance, Dr. Abramowitch, Dr. Debski, and Dr. Woo for providing me this great experience, and NIH Grant AR41820 and the Pittsburgh Tissue Engineering Initiative for their financial support.

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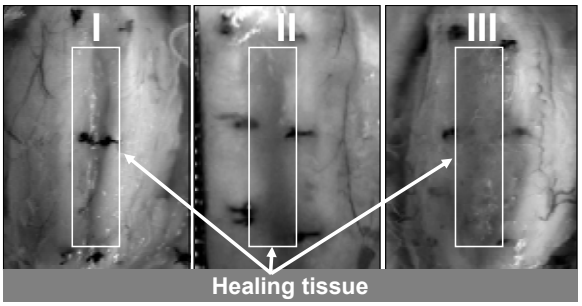

Figure 2: Gross view of healing PT defect at 6 weeks, I. Non-treated, II. BioMend-treated, and III. SIS-treated.

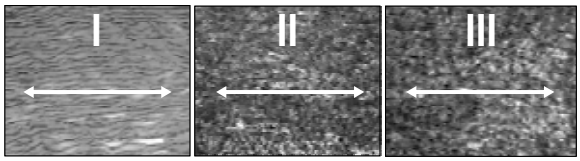

Figure 3: H & E staining of healing PT defect (100 x) at 6 weeks, I. Sham, II. BioMend-treated, and III. SIS-treated. Arrows represent direction of alignment.

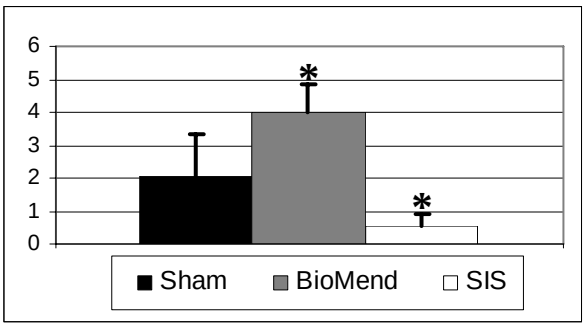

Figure 4: GAG content normalized via dry weight *=significantly different (p<.05)

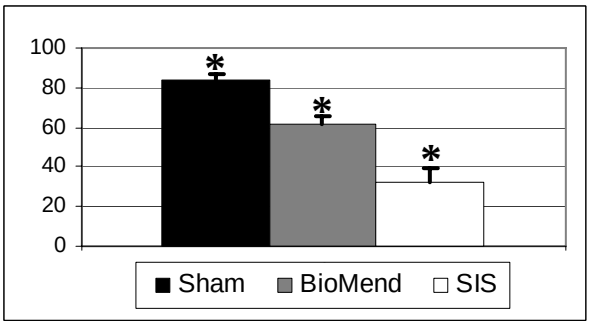

Figure 5: Collagen content normalized via dry weight *=significantly different (p<.05)

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I am originally from Pittsburgh, where I was born on October 2, 1985, and only left two years ago to attend college in Morgantown, WV. I grew up in the South Hills area with my parents and two younger sisters and graduated from Upper St. Clair High School in June of 2004. During high school, I enjoyed participating in a variety of activities including lacrosse, wind ensemble, and the GAPP German Exchange program.

At West Virginia University, where I will be a junior mechanical engineering major in the fall, I've had the opportunity to continue my involvement in a variety of activities while working toward my degree. I am a member of the WVU Club Soccer and WVU Club Lacrosse teams, work on the weekends grooming and caring for show dogs, and am an avid Mountaineer Basketball and Football fan. However, all of this comes second to my passion for engineering and my interest in the medical field. Upon completion of my undergraduate degree, I intend to pursue a career in the field of bioengineering while furthering my education with a higher degree in biomedical engineering.

The opportunity to work at the MSRC this summer has been an invaluable experience. I would like to thank my mentors for all of their support and guidance and the ACL group for adding to such an enjoyable working environment. My sincere thanks go to Dr. Woo, Dr. Abramowitch, Dr. Debski, and everyone at the MSRC for providing such a rewarding opportunity.

REPEATABILITY OF KINEMATICS UNDER APPLIED MUSCLE LOADS IN THE PORCINE KNEE

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INTRODUCTION

Harvesting the central third of the patellar tendon for use in anterior cruciate ligament reconstruction is a common practice among surgeons, particularly because the bone-patellar tendon-bone graft allows for bone to bone union at the insertion sites. [2] However, following surgery there is a high incidence of anterior knee pain, inflammation, and scar formation at the harvest site, which can cause adherence of the remaining patellar tendon to the infra-patellar fat pad. [5] This can alter the normal tracking of the patella, affecting knee kinematics and range of motion**.** [1,6]

At our research center, patellofemoral and tibiofemoral kinematics were compared between intact and patellar tendon adhesion simulated knees using a robotic/universal force-moment sensor (UFS) testing system (position repeatability ±0.2 mm, orientation repeatability ±0.2°)**.** [7] A 400 N external load was applied to the quadriceps tendon to simulate *in vivo* conditions (i.e. patellar movement due to contraction/relaxation of the quadriceps muscles) and 5 degree of freedom (DOF) kinematics of the joint were recorded. However, the kinematics were inconsistent, which was troublesome because this invalidated any significant differences due to the adhesion model.

It was hypothesized that the muscle load was deforming soft tissues in the joint, affecting the kinematics; therefore, it was necessary to analyze the inconsistencies further since the application of muscle loads is essential for patellar tendon adhesion research. Thus, the first objective of this project was to find the largest muscle load for which kinematics were repeatable. The second objective was to determine if a preconditioning method can be developed that will enable the knee to withstand higher muscle loads.

MATERIALS AND METHODS

Seven porcine knees (stored at -20°C) were tested. Prior to testing, specimens were defrosted for 24 hours, all tissue farther than 10 cm from the joint was removed, the fibula was secured, and the femur and tibia were potted in an epoxy compound. The tibia was secured to the end effector of the robotic/UFS testing system and the femur was secured to the base. A pulley system was also attached to apply loads from a nylon strap sutured to the quadriceps tendon of each specimen.

The robotic manipulator (Unimate, PUMA-762) and UFS (JR3, model 4015) that were used in this study can effectively measure and reproduce the moments and forces in 5 DOF of joint motion. [3] (Figure 1) First a path of passive flexion/extension is found, in this case from 30° to 90° of knee flexion, for which all external forces and moments are minimized. [4] After finding the reference path, a 100 N anterior tibial load (ATL) was applied to the knee and the anterior tibial translation was recorded at two specific flexion angles.

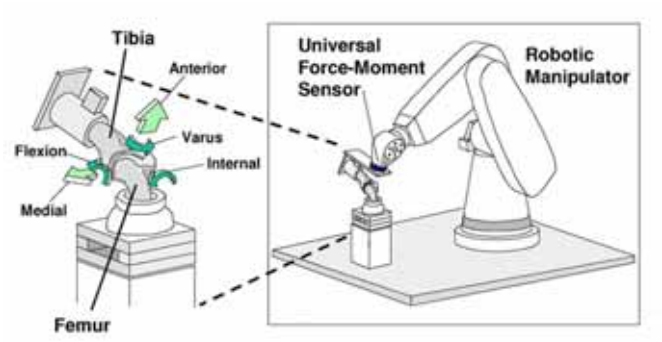

Figure 1. The robotic/UFS testing system with a cadaveric specimen and 5 DOF

Application of muscle loads began at 200 N because no discrepancies had been found with this load at our research center in the past. The load was suspended with the knee positioned at 30° of flexion (Figure 2) and the force-control mode of the robotic manipulator was used to record translation from the reference point in 5 DOF. [7] The load was removed while the knee was repositioned at 60° of flexion, then reapplied to repeat the same process; this was also done at 90° of knee flexion, which completed the first trial. The entire process was repeated two more times so that a total of three trials were recorded.

To determine the largest load for which kinematics were repeatable, consistency was compared at each flexion angle. If the standard deviation between the trials was at or below 0.2 mm, the kinematics were considered repeatable and testing resumed with a higher muscle load (incrementally increased by 50 N) on a new specimen. Also, to determine if a particular load was deforming soft tissues in the joint, comparisons were made between the kinematics recorded in response to the ATL that was applied before and after completion of muscle load testing. Soft tissue deformation was indicated by a significant change (greater than 0.2 mm) between the two sets of kinematics.

Once it was determined that kinematics were no longer repeatable and soft tissue deformation had occurred under the application of a particular muscle load, testing to develop a preconditioning method began. After recording displacements resulting from the ATL, the specimen was preconditioned by applying a 200 N muscle load and cycling it ten times through flexion and extension. The remainder of the load was then applied and the original process was repeated, collecting three trials of kinematics to determine if repeatability had improved.

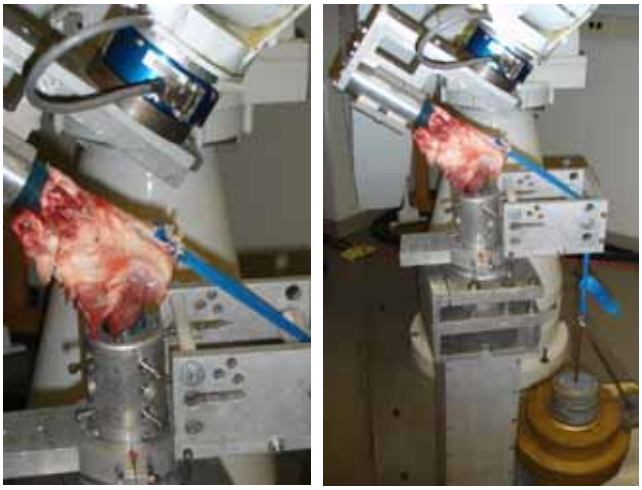

Figure 2. A porcine knee secured to the robotic/UFS testing system with an applied muscle load of 350 N

RESULTS

Of the 5 DOF in which kinematics were measured, anterior tibial translation was primarily analyzed since the tibia is pulled forward by a quadriceps load. With a 200 N muscle load, standard deviation between three trials of anterior tibial translation was at or below 0.2 mm for all three angles of knee flexion; this trend was consistent for loads of 250 N (Table 1) and 300 N as well. From the kinematics obtained under the ATL, it was determined that soft tissue deformation had not occurred.

When 350 N was applied, inconsistencies between the three trials of anterior tibial translation were recorded at all three flexion angles (Table 2). Soft tissue deformation was also indicated in correspondence with this force.

Repeatability of anterior tibial translation improved after preconditioning the knee (Table 3), but deformation of the soft tissues in the joint had still occurred. Without preconditioning the knee, the standard deviation was as high as 1.1 mm, but after preconditioning, the highest standard deviation was 0.4 mm.

Flexion Angle	Trial 1	Trial 2	Trial 3	Standard Deviation
30°	1.5	1.4	1.3	**0.1**
60°	5	4.9	5	**0.1**
90°	3.6	3.7	3.8	**0.1**

Table 1. Repeatability (standard deviation) of anterior tibial translation (mm) under a 250 N muscle load

Flexion Angle	Trial 1	Trial 2	Trial 3	Standard Deviation
30°	8.5	8.6	9.4	**0.5**
60°	11.4	11.5	12	**0.3**
90°	7.1	9	8.9	**1.1**

Table 2. Repeatability (standard deviation) of anterior tibial translation (mm) under a 350 N muscle load

Flexion Angle	Trial 1	Trial 2	Trial 3	Standard Deviation
30°	3.1	3.8	3.8	**0.4**
60°	10.3	10.4	10.7	**0.2**
90°	8.9	9.2	9.3	**0.2**

Table 3. Repeatability (standard deviation) of anterior tibial translation (mm) under a 350 N muscle load on a preconditioned specimen

DISCUSSION

The goal of this project was to establish a standard protocol for testing with muscle loads so that further research can continue on patellar tendon adhesion. After analyzing data collected under various muscle loads, it was determined that 300 N is the maximum for which kinematics are repeatable and deformation of the soft tissue does not occur.

Although improvements in repeatability were recognized when specimens were preconditioned prior to testing, soft tissue deformation was still indicated. Of the two specimens that were tested, only one showed significant improvements in standard deviation. Thus, further development of the preconditioning method must continue before definite conclusions can be drawn as to how well it improves the repeatability of kinematics.

ACKNOWLEDGEMENTS

Dr. Woo, the MRSC, and the Department of Bioengineering are gratefully acknowledged for making this summer research experience possible.

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I was born on September 7, 1980 near Hamburg, Germany and grew up there in the small village of Wohltorf. During my school time I played any kind of sport mostly with my sister and three brothers. In high school time I completely switched from field games to athletics, pole vault and hurdle. I also enjoyed playing my trombone in two big bands performing jazz and blues. I graduated from Hansa-Gymnasium in 2000, and started to become a Reserve Officer. In the next two years I moved nearly every four months to other places and got the chance to learn more about the people and the places in my own country.

Now I am a fourth year student at the University of Technology Dresden and hope that I will graduate in the next year. Prior to coming to the U.S., I also studied at University of Valladolid, Spain, and I received work experience at the X-Ray Department of Philips.

The short time I have spent at the MSRC has been wonderful. The MSRC gave me a very good research perspective for the future and let me feel at home. I'd first like to thank Alexis C. Wickwire for all of her guidance and support. To work in the MSRC, particularly in the Shoulder Lab, will be a memorable time for me. I would like to thank Dr. Abramowitch, Dr. Woo, and Dr. Debski for the opportunity to participate in the summer program.

A SIMPLE FINITE ELEMENT MODEL OF THE KNEE TO ASSESS SENSITIVITY OF INPUT VARIABLES WHEN CALCULATING FORCES

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INTRODUCTION

The meniscus and primary ligaments of the human knee are essential in maintaining stability and are commonly injured. [1] Therefore, the function of these structures, particularly the stresses and forces in the meniscus and ligaments need to be better understood, respectively. This information can provide insight to possible injury prevention and improved treatment techniques. A powerful computational tool to evaluate these parameters is finite element (FE) modeling. FE models can be very complex with multiple input variables that affect the calculated strain, stresses and forces. [2] Using a simplified model, the impact of varying input variables, such as non-linear material properties, ligament attachment location, and contact between the bones, cartilage and meniscus surfaces can be assessed. Therefore, the objective of this study was to develop simplified models of the human knee using non-linear springs and rigid bodies to evaluate the effect of ligament attachment points and line of action on the prediction of ligament forces.

MATERIALS AND METHODS

The two simplified models of the human knee used one-dimensional (1-D), non-linear springs to represent ligaments and rigid shells for the bones. The models consisted of two rigid bodies connected by the following:

1. Model 1: Non-linear spring with five varying attachment points (Figure 1a)
2. Model 2: Series of linear springs with connection points, which prohibit penetration of the line of action into the rigid body (Figure 1b)

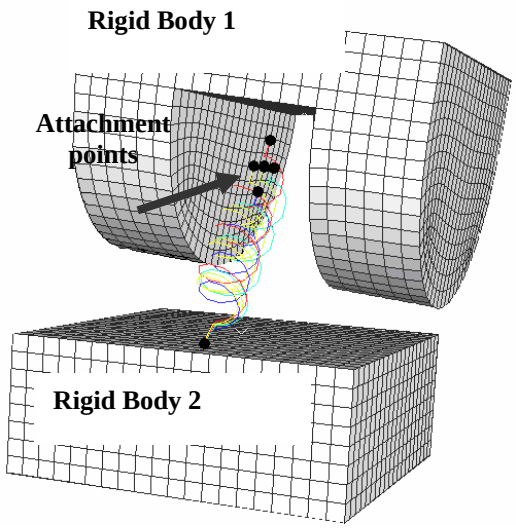


Figure 1a: Rigid Bodies 1 and 2 with springs at five varied attachment points.

Model 1: Non-linear properties (load-elongation relationship) of the anterior cruciate ligament (ACL) were determined previously and used to define the properties of the 1-D non-linear spring. Attachment points of the spring were varied on each of the rigid bodies. The initial attachment point of the spring (x_0) was in the middle of the varied attachment points on Rigid Body 1. These attachment points were varied at 5mm to the left (Spring 1), upwards (Spring 2), right (Spring 3) and downwards (Spring 4) from the initial point (Figure 1a). Variations of 5 mm were based on the size of the ACL insertion side. [3] The reference length of the spring (l_0 = 29.9 mm) was constant. Initially Rigid Body 1 was oriented such that 20° of backwards tilting was simulated and motions were prescribed to Rigid Body 1 that attempted to simulate primary motions of a knee. Calculations performed by the FE software package (ABAQUS) determined the overall force transferred between the two rigid bodies via the springs during these motions. Forces in the springs and their elongation were calculated for all motions.

Model 2: A linear spring (Spring 1) and an equivalent spring comprised of five linear springs in series (Spring 2) were utilized. A single spring can unrealistically penetrate the surface of a rigid body due to its line of action – defined as the line between attachment points. However, the series of springs allowed nodes to be created at the connections between the individual springs such that the nodes could not penetrate the surface of the rigid bodies. Thus, a wrapping effect for the series of springs was created. The stiffness of Spring 1 was estimated as the initial slope of the load-elongation curve between 0 and 200 N and was 47,339 N/m. The stiffness of the five springs that comprised Spring 2 then was 236,733 N/m. Rigid Body 1 was moved through three motions: 1) 48.7° right tilting; 2) 10 mm upwards; and 3) 25 mm left relative to the Rigid Body 2. Forces and elongation along the line of action were collected for each spring.

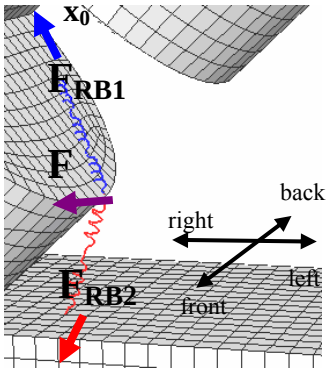


Figure 1b: Two rigid bodies with series of springs (Spring 2).

RESULTS

At the initial joint position for Model 1, each spring had tension or laxity (positive or negative elongation, respectively) compared to l_0 with the total length increasing and decreasing by ±3.7 mm (Δl) and forces up to 150 N. Furthermore, Figure 2 shows that the trend for the elongation curves for each attachment point is similar after the initial joint position for all motions. Spring 1 and Spring 4 (Figure 2) lost nearly all function because of unloading at the initial joint position, meaning that there was no force (negative elongation) in the spring. The backwards tilting and motion in the forward and left directions (Figure 2) were most responsible for unloading in the spring. Backwards, upwards, and right motion are responsible for high tension (positive elongation) in the spring. Throughout the motion, the maximum force experienced for each Spring_x_0, Spring 1, Spring 2, Spring 3, and Spring 4 was 18.64 N, 1.29 N, 258.00 N, 260.82 N, and 5.39 N, respectively. The high forces, particularly for Spring 2 and Spring 3, corresponded with large increases in elongation (Figure 2). Overall, small changes of positive elongation were followed by high forces.

Model 2 demonstrated that substituting a series of springs for a single spring results in the same force if there is no rigid body in line between the two attachment points (Figure 1b). The force on the attachment points in both the single and series of springs are the same. However, during the tilting motion, the force in Spring 1 was 37 N. As Rigid Body 1 came into contact with Spring 2 during this motion, a wrapping effect occurred that created a change in magnitude and direction of forces transmitted between the two bodies via the Spring 2 (F_{RB1} = 176 N, F_{RB2} = 314 N). Additionally, the series of springs applied a force of 183 N to the surface of Rigid Body 1.

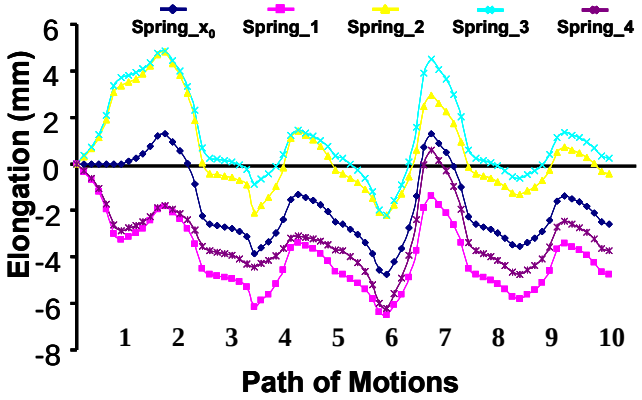
DISCUSSION

The goal of the project was to assess the effect of moving the attachment point (Model 1) and wrapping (Model 2) on the force in each spring. The simulation with Model 1 has shown that springs with a non-linear load-elongation curve are highly sensitive to varying the point of attachment. The step to the initial joint position - to have tension and force in the spring – was critical for the function of the spring during subsequent motions (Figure 2). In addition a high change in force was experienced after a small change in elongation was applied to the model as occurs when varying the attachment point.

Model 2 demonstrated that an approximation with a series of springs is a good solution to avoid penetration of the springs into a rigid body. The attained wrapping effect gave the possibility to look at the applied force to the Rigid Body 1. A mathematical limitation occurs when increasing the number of springs used in the system due to the indeterminacy when determining a solution with springs unloaded.

In the future, a model with two rigid bodies and a deformable body in the middle will be developed to assess the sensitivity to surface contact.

Figure 2: Elongation of the springs due to prescribed motions: 1) initial joint position, 2) 2mm upwards, 3) 20° backwards tilt, 4) 20° left tilt, 5) 10° right tilt, 6) 3mm forwards, 7) 8mm backwards, 8) 5mm forwards, 9) 3mm left, and 10) 6mm right. Each spring represents a different attachment point.



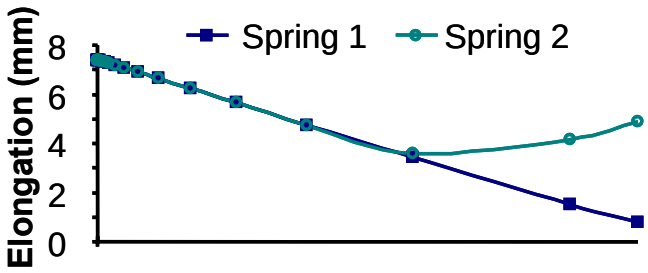


Figure 3: Model 2 - Elongation of the Spring 1 and Spring 2 created by 48.7° of right tilting.

ACKNOWLEDGEMENTS

I would like to thank Alexis Wickwire for all of her assistance throughout this project. I also want to thank my lab advisor Dr. Debski as well as the rest of the Shoulder group. Finally, I want to thank Dr. Woo and the rest of the MSRC for an educational and enjoyable summer and PTEI and IAESTE for having given me the great possibility.

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Being the oldest child and the only girl with three younger brothers has allowed my life growing up to be fun and interesting. I grew up to the east of Pittsburgh in Penn Township, home of the Penn Trafford Warriors. After high school, I decided to attend Bucknell University and become one of the Bison. There, I am majoring in Biomedical Engineering and hoping to minor in German and Math. I am excited to be beginning my junior year this fall.

I love to dance and have been dancing now for sixteen years. I can't imagine life with out dance. On campus, I am involved in many activities including performing in dance shows, swing dancing, directing a small church choir, participating in the RCC Ministry Team, volunteering in the area, TAing a chemistry lab, and working in the biomedical lab, as well as being a member of Alpha Lambda Delta Honor Society. When I'm not busy you'll find me hanging out with my friends and often times listening and singing to music.

At the MSRC, my wealth of knowledge has expanded, and it has been an excellent educational experience. I want to thank everyone at the MSRC, especially the MCL group for enriching my time here. I want to especially thank my mentor, Dr. Alex Almarza, for his time and patience while teaching and helping me this summer. Finally, I thank Dr. Woo, Dr. Abramowitch, and Dr. Debski for making the opportunity available.

THE EFFECT OF CONSTANT ELONGATION OF SMALL INTESTINE SUBMUCOSA

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INTRODUCTION

Each year there are over 95,000 isolated medial collateral ligament (MCL) injuries, and with conservative treatment, these injuries can heal. [6,11] However, only the structural properties of the healing MCL return close to normal. The biochemical, mechanical, and viscoelastic properties remain inferior to the normal MCL. [2,3,10,11] Functional Tissue Engineering (FTE), including bioscaffolds, has been shown to improve ligament and tendon healing.[5] Specifically, in our research center, FTE has been applied to the healing MCL. Studies have applied a bioscaffold, porcine small intestine submucosa (SIS), to the healing MCL to observe the long term effects on the mechanical properties of the MCL. The tangent modulus or slope of a stress versus strain curve, significantly increased between the non-treated and the SIS-treated MCL, signifying enhanced MCL healing. However, the healing of the SIS-treated MCL remained significantly lower than that of the normal or sham tissue.[5]

SIS is a 200μm thick layer located between the mucosa and muscle layers of the porcine small intestine. SIS contains many growth factors and chemoattractants that have been shown to promote healing. Additionally, the collagen fibers of the SIS are preferentially aligned to ±30° from the longitudinal axis. [1,7] Preliminary studies on SIS have shown that constant elongation can improve the collagen fiber alignment to ±8°.

The principle of contact guidance could be applied to bioscaffolds to further improve healing. In previous studies, it has been shown that cells seeded on an aligned substrate will align in the preferential direction through contact guidance.[4,9,12] Interestingly, the extracellular matrix (ECM) produced also aligns in the preferential direction and has been shown to be produced in higher quantities as compared to a non-aligned substrate.[4] Alignment of cells and collagen fibers in the SIS is important because ligaments and tendons are highly organized and aligned.

Thus, the overall objective is to study the principle of contact guidance on elongated SIS to determine the effect highly aligned SIS on cell alignment and gene expression. For gene expression, collagen type I, collagen type III, and GAPDH will be measured because they are the main constituents in ligaments and tendons. It is hypothesized that constantly elongated SIS, with highly aligned collagen fibers, will improve cell alignment and increase the gene expression of collagen, as compared to non-elongated SIS.

METHODS

For the experimental design, the original length of the SIS was 20 mm. The SIS was placed in a stretching chamber and elongated by 15% (3 mm) of its length, giving a new length of 23 mm. The elongated SIS remained clamped at 23 mm throughout the remainder of the procedure. Rabbit bone marrow derived cells (BMDCs) were seeded onto the elongated SIS 24 hours after the elongation (n=3) and remained on the SIS for 5 days. BMDCs were seeded in a similar manner on the non-elongated SIS (n=3), clamped at the original length of 20 mm. BMDCs were also seeded in a petri dish (n=3) as an external control.

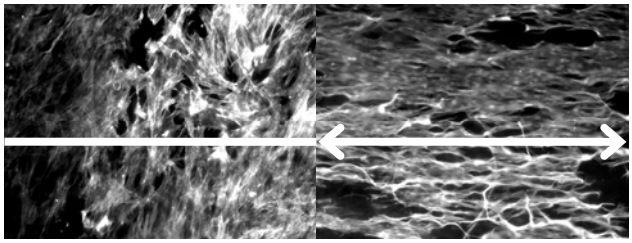

Figure 1. Images of non-elongated SIS (left) and elongated SIS (right) shown as the negative image of a fluorescent actin fiber and nuclei stain. The horizontal line indicates the longitudinal axis of the SIS and the arrows show the direction of the elongation.

To analyze cell alignment, a software program, *Scion Image*®, was used. Fluorescent microscope images, showing the actin filaments and nuclei of the BMDCs on the SIS, were analyzed (Figure 1). A zero degree line placed on the horizontal axis of the image was also the longitudinal axis of the SIS. Vectors were drawn through the major axes of individual cells. The angles between the vectors and the horizontal axis were measured, for an average of 60 cells per sample. The data was analyzed in a semi-quantitative manner.

For gene expression analysis, the RNA was extracted from the BMDCs using Trizol® reagent, following the standard procedure. The RNeasy® MinElute™ Cleanup kit was used to remove any remaining genomic DNA or protein from the RNA. The concentrations of the samples were normalized by measuring absorbance and fluorescence. The gene expression was measured using real time RT-PCR. The RNA was reverse transcribed (RT) to complimentary DNA (cDNA) using SuperScript™ III Reverse Transcriptase, following the manufacture's protocol. The cDNA samples were prepared in triplicate, for real time PCR. In each individual well, 1μl cDNA, 1μl each of forward and reverse primer, 10μl of SYBR® Green Master Mix, and 7μl of RNase free water were combined, to allow the PCR reaction to occur. The PCR amplification process contained 35 cycles of denaturing for 30 sec, annealing for 2 min, and extending for 2 min at 94°C, 55°C, and 72°C respectively.

RESULTS

The reduction in standard deviation of the measured angles was seen in all samples between the non-elongated and elongated SIS (Table 1). The non-elongated SIS showed local areas of cell alignment, but not overall alignment in the longitudinal direction. This trend is illustrated in Figure 2, by the general peak in frequency of cells around -50°. The elongated SIS, shown in Figure 3, showed cell alignment along the longitudinal axis of the SIS by having a large number of cells aligned at 0°. Thus, elongating SIS improved cell alignment as compared to the non-elongated SIS.

	Elongation		No Elongation		Petri Dish	
	Avg	StdDev	Avg	StdDev	Avg	StdDev
Sample 1	5	10	-15	55	10	45
Sample 2	5	15	20	45	0	40
Sample 3	5	10	-5	65	-10	50

Table 1. The average and standard deviation of the angles measured for cell alignment of all samples.

The abundance of collagen type I and collagen type III (Figure 4), relative to the house keeping gene GAPDH, showed no significant difference in the gene expression between the elongated SIS, non-elongated SIS, and petri dish. It seems SIS is limiting the expression of collagen type III as compared to the external control cells.

DISCUSSION

Constantly elongating the SIS improved the BMDC alignment, as compared to the non-elongated SIS supporting part of our hypothesis. However, no significant difference was seen in the gene expression of collagen. It seems for SIS, the alignment and production of ECM are uncoupled. The BMDCs will align through contact guidance, on the aligned SIS scaffold, but do not increase the production of new ECM. The cells were expected to increase ECM production on SIS, since in a study by Lee, cells produced an increased amount of ECM on an aligned nanofiber collagen scaffold.[4] However, several other studies have shown that seeding cells on an aligned substrate was not enough to increase ECM production, but an increase could be obtained if cyclic loading was applied.[9,12] Future studies will look into the effects of cyclic stretching on cell alignment and ECM production of cells seeded on SIS.

Future studies should further look at the effect of SIS on collagen type III gene expression of seeded cells. The gene expression of collagen type V in the elongated and non-elongated SIS will also be determined.

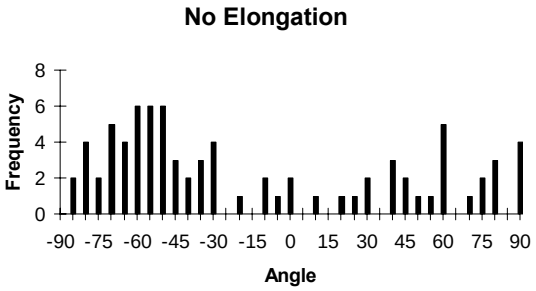

Figure 2. Distribution of cell alignment for sample 1 of non-elongated SIS showing the frequency of cells seen at a particular angle. 60 BMDCs were analyzed.

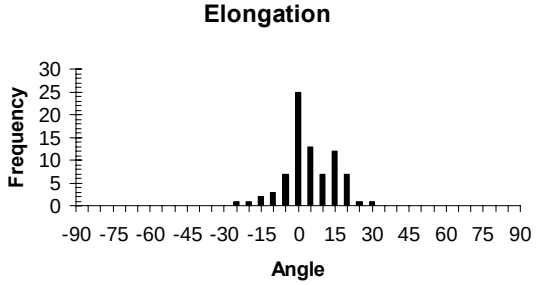

Figure 3. Distribution of cell alignment for sample 1 of elongated SIS showing the frequency of cells seen at a particular angle. 60 BMDCs were analyzed.

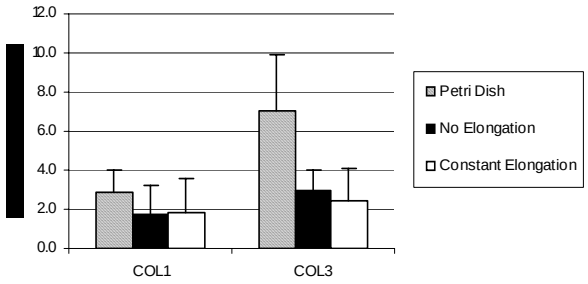

Figure 3. Diagram of test protocol used during testing.

AKNOWLEDGEMENTS

I want to acknowledge and sincerely thank my mentor Dr. Alex Almarza, and addtionally Dr. Debski, Dr. Abramowitch, and Dr. Woo for giving me the opportunity to work and learn at the MSRC.

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I was born in Kingston, PA and moved all over the east coast until I was about 5. We finally settled in Shillington, PA where I attended Governor Mifflin School. I very much enjoyed high school and was active in many activities including cross country, track and field, spring musicals, concert band, and several clubs and leadership groups. Deciding on a college was rather simple for me. I basically applied to and visited Penn State's main campus and got accepted within a month of the start of my senior year and decided to attend. Therefore, while all my peers were scrambling about with SAT II's and other applications, I was just enjoying my decision and the rest of my senior year. Since, I have not once doubted my decision to attend PSU.

I originally went to PSU as a Pre-med major but soon realized it was fairly basic and not very exciting. I then decided on bioengineering with a chemical engineering option because it included the technical side of medicine along with the fairly new field of tissue engineering. I plan on attending medical school after my undergraduate work is complete, but I also hope to continue my research experience as much as possible.

Working at the MSRC has been nothing but a positive experience, and I have truly enjoyed it to its fullest extent. Not only have I learned so much about research, but I have also learned a lot about working in a lab and coordinating efforts with others in order to be efficient and successful. I would like to thank everyone at the MSRC for keeping the lab lively and enjoyable from day to day and especially Dr. Woo, Dr. Steve Abramowitch, and Noah Papas for providing me with this great opportunity and guidance for the future.

THE EFFECTS OF SMALL INTESTINE SUBMUCOSA ON THE BIOMECHANICAL PROPERTIES OF THE MEDIAL COLLATERAL LIGAMENT AFTER A MOP-END TEAR INJURY MODEL: PRELIMINARY STUDIES

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INTRODUCTION

Every year there are about 95,000 isolated medial collateral ligament (MCL) injuries.[1] Although these injuries can heal with conservative treatment their mechanical properties remain inferior long-term and have been associated with a disorganized collagen and extra cellular matrix (ECM) and altered biochemical composition, i.e. increase in type V collagen.[2-6] Recently, functional tissue engineering approaches that employ the use of bioscaffolds, namely small intestine submucosa (SIS), have been shown to promote healing and improve the structural properties and mechanical properties of healing ligaments and tendons.[7, 8] SIS is beneficial to healing tissue because it contains growth factors, resists bacterial infection, promotes revascularization, and incorporates well into the tissue. Previous studies using a 6mm gap injury have shown SIS to be an effective bioscaffold when applied to the healing MCL. However, in this study a mop-end tear injury model was used because of its clinical relevance. The long-term objective of this study is to examine the mechanical properties of SIS healing MCLs at 6 and 12 weeks and compare the values to a non-treated group. As a first step in that research initiative, the focus of my summer project was to perform preliminary testing to evaluate the accuracy and repeatability of our testing protocol relative to historical data.

METHODS

The hind limbs of seven white New Zealand rabbits were utilized for preliminary testing. The femur-MCL-tibia complex (FMTC) was isolated and the cross sectional area (CSA) was measured using a laser micrometer system (Figure 1) [9, 10].

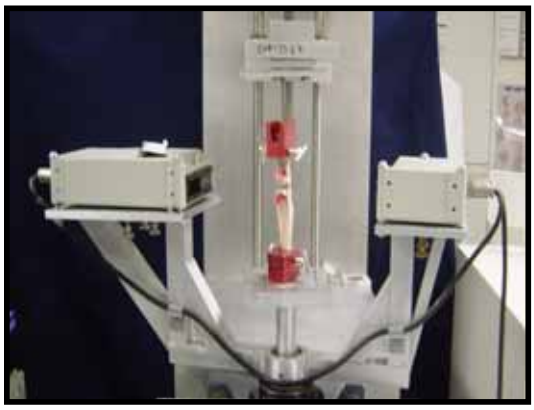


Figure 1. Laser micrometer with FMTC specimen while measuring CSA.

The FMTC was then securely placed into specially designed clamps. Four small black beads were placed along the ligament for strain tracking; 2 at the mid-substance and 2 near the insertion sites. The clamps were then placed in a 37 degree saline bath and fixed to an Instron 5565 materials testing machine (Figure 2).

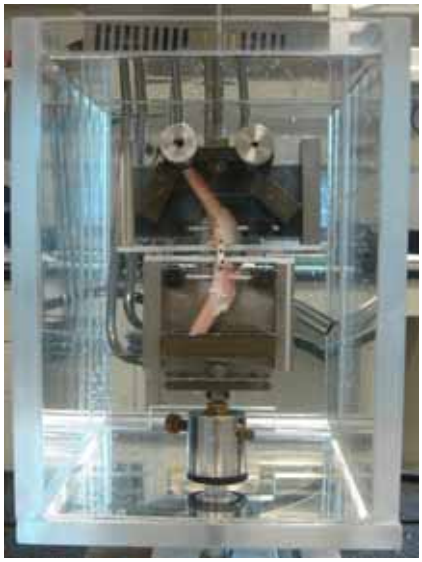
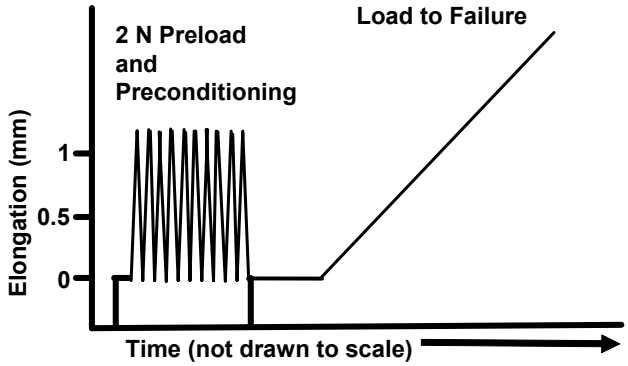


Figure 2. Instron and specimen set up in saline bath.

Using an established test protocol (Figure 3), specimens were first given a 2 N preload to establish a consistent starting point and then 10 cycles of preconditioning to help settle the clamps and obtain consistent strain history. The parameters describing the structural properties of the FMTC and mechanical properties of the tissue substance were determined from the resulting load-elongation and stress-strain curves, respectively.



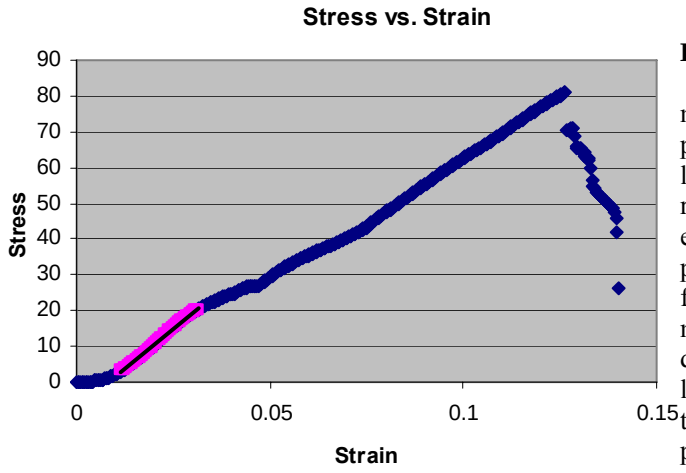


Figure 4. Example of a stress vs. strain curve used to determine mechanical properties (e.g. modulus, strain at failure, and ultimate stress).

RESULTS

A strain rate was determined using a strain vs. time graph from the motion analysis system, and then used to calculate strain with the Instron data. The slope was taken from the load vs. elongation curve in order to determine stiffness and from the stress vs. strain curve (Figure 4) in order to determine a modulus. The resulting structural properties and mechanical properties are listed in Table 1.

Our data is consistent with historical literature in that the values for all of our parameters were within one standard deviation of the mean of those found previously. The coefficient of variation, i.e. SD/mean, was between 0.1124 (modulus) and 0.1503 (ultimate stress) which was also consistent was previous literature.

Table 1: Means and standard deviations (SD) of mechanical and structural properties (n=7) from preliminary testing.

Mechanical Properties of Normal Rabbit MCL				
		CSA (mm^2)	Modulus (Mpa)	Ultimate Stress(Mpa
Means and SD	n=7	4.3 + 0.6	1050 + 118	77.2 + 11.6
Weiss,Woo '91 (	n=7	3.6 + 0.2	1080 + 101	84.4 + 5.2
Mushel '04 (Sha	n=16	4.7 + 1.0	936.3 + 283.6	75.6 + 14.2

Structural Properties of Normal Rabbit MCL				
		CSA	Stiffness	Ultimate Load
Means and SD		4.3 + 0.6	80.5 + 7.2	298.75 + 32.3
Weiss,Woo '91 (	7	3.6 + 0.2	64.2 + 5.2	313 + 17
Mushel '04 (Sha	16	4.7 + 1.0	89.7 + 15.3	332.0 + 50.8

DISCUSSION

This preliminary study revealed that our testing methods are sound as the obtained parameters make physical sense and our data is consistent with historical literature. Thus, the variations in these data can likely result from biological variability rather than experimental error. For example, one biological problem was bone deformation during the load to failure test. To account for this, the stiffness and modulus slopes were taken before the bone deformation occurred. Another problem was a non-linear strain vs. time graph would result from the testing. In order to correct this problem we fit a polynomial to the strain vs. time graph instead of assuming a linear relationship. Therefore, we accounted for the biological variation and obtained an accurate strain rate.

With preliminary testing showing accurate and repeatable results, we will utilize this protocol to determine the effects of SIS treatment on the healing of a mop-end tear injury of the MCL.

ACKNOWLEDGEMENTS

I would like to extend my gratitude to Dr. Woo, Dr. Debski and the entire MSRC. I would also like to specially thank Noah and Dr. Abramowitch for there excellent guidance this summer, and additionally Pittsburgh Tissue Engineering Initiative (PTEI) for there financial support.

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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 intellectual curiosity 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.

My time here at the MSRC has been nothing short of amazing, and I'm excited about the work I've completed and the opportunities that have been opened to me as a result. 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. Thanks also to the ACL group for their guidance and technical support throughout the summer.

REPEATABILITY OF FORCE DIRECTION MEASUREMENTS USING A ROBOTIC/UFS TESTING SYSTEM

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INTRODUCTION

The anterior cruciate ligament (ACL) of the knee plays an essential role in limiting anterior tibial translation and providing rotatory stability.[1] Knee kinematics are dependent on the in-situ force vector of the ACL, which consists of a magnitude, a direction, and a point of application. The in-situ force vector of the ACL in response to an external load can be measured using the robotic/universal force-moment sensor (UFS) testing system developed at this center.[2] This testing system operates in two different modes: force control and position control modes. In force control mode, the robotic/UFS testing system applies forces to the knee and measures the resulting kinematics in multiple degrees of freedom (DOF); in position control mode, the system applies translations or rotations to the knee and measures the resulting forces. By combining these two control modes and using the principle of superposition, the in-situ force vector in the ACL can be determined.

The objective of the present study was to investigate the repeatability of the robotic/UFS testing system in measuring the direction of the in-situ force in the ACL using a porcine knee model. An understanding of the limitations of the testing system in terms of its repeatability in measuring force direction is important in evaluating data collected using the system.

METHODS

The testing system used in this study was one developed at this center using a robotic manipulator (PUMA Model 726, Unimate Corp.) and universal force-moment sensor (Model 4015, JR3 Inc.).[2] The 6-DOF robotic manipulator has a position and orientation repeatability of 0.2 mm and 0.2°, respectively. The UFS is capable of measuring three orthogonal forces and moments. The accuracy of the UFS with a rigid body model has been found to be 0.05 N for force magnitude and 1.7° for force direction.[3]

One fresh frozen porcine knee specimen was tested on the robotic/UFS testing system. The knee was mounted with the femur rigidly fixed into a base pedestal and the tibia fixed to the end effector of the robotic manipulator. A path of passive flexion and extension was found from full extension to 90° of knee flexion.

A 100 N ramped anterior tibial load was applied to the knee at 30°, 60°, and 90° of knee flexion six times at each flexion angle, and the resulting kinematics were recorded in 4 DOF (medial-lateral, proximal-distal, and anterior-posterior translations as well as varus-valgus rotation). The force was applied in nine loading steps to a target anterior tibial load of 100 N. All soft tissue except the ACL was then dissected away, and each of the previously recorded kinematics were replayed six times to find the in-situ force vector of the ACL, which is defined by three orthogonal force components: F_{ML}, F_{PD}, and F_{AP} relative to the tibial coordinate system.

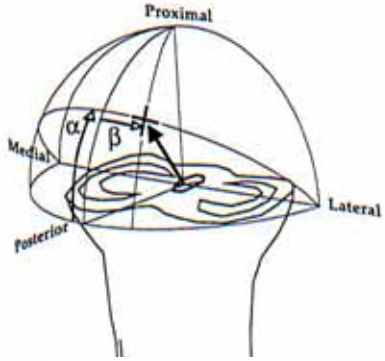

Figure 1: Direction of In-Situ Force in ACL

The direction of the in-situ force of the ACL is described relative to the tibial coordinate system by two angles (Figure 1). The elevation, α, is defined as the angle between the force vector and the tibial plateau; the deviation, β, is defined as the angle between the force and the mid-sagittal plane.

At each loading step, the magnitude and direction of the force were calculated as follows:

$$\left|\boldsymbol{F}_{\boldsymbol{ACL}}\right| = \sqrt{\left(\boldsymbol{F}_{\boldsymbol{ML}}\right)^2 + \left(\boldsymbol{F}_{\boldsymbol{PD}}\right)^2 + \left(\boldsymbol{F}_{\boldsymbol{AP}}\right)^2} \quad (1)$$

$$\alpha = \sin^{-1}\left[\frac{\boldsymbol{F}_{\boldsymbol{PD}}}{\left|\boldsymbol{F}_{\boldsymbol{ACL}}\right|}\right] \quad (2)$$

$$\beta = \tan^{-1}\left[\frac{\boldsymbol{F}_{\boldsymbol{ML}}}{\boldsymbol{F}_{\boldsymbol{AP}}}\right] \quad (3)$$

As conventional methods for statistical analysis do not apply to directional data, a spherical analysis method is used. The mean direction of the in-situ force of the ACL was first calculated from the samples and a cone of 95% confidence surrounding the mean vector was then constructed.[5] To describe the cone of confidence, The radius of the cone of confidence, δ_{95}, is defined as

$$\delta_{95} \approx \frac{140}{\sqrt{\boldsymbol{kn}}} \quad (4)$$

where

$$\boldsymbol{k} \approx \frac{\boldsymbol{n}-2}{\boldsymbol{n}-\boldsymbol{R}} \quad (5)$$

and

$$\boldsymbol{R} = \sqrt{\left(\sum_i \boldsymbol{F}_{\boldsymbol{ML}}\right)^2 + \left(\sum_i \boldsymbol{F}_{\boldsymbol{PD}}\right)^2 + \left(\sum_i \boldsymbol{F}_{\boldsymbol{AP}}\right)^2} \quad (6)$$

RESULTS

The magnitude of the in-situ force in the ACL at each anterior loading step is shown in Figure 2. The maximum in-situ force was approximately 95 N at all knee flexion

angles tested, which is similar to values previous reported.[4] At the full loading condition, elevation of the in-situ force vector in the ACL ranged from 2° at 60° of knee flexion to 11° at 30° of knee flexion. Deviation ranged from 7° at 90° of knee flexion to 13° at 30° of knee flexion. The trend seen is that the in-situ force in the ACL tilted more posterior and lateral under increased flexion angle, which matches physical observations.

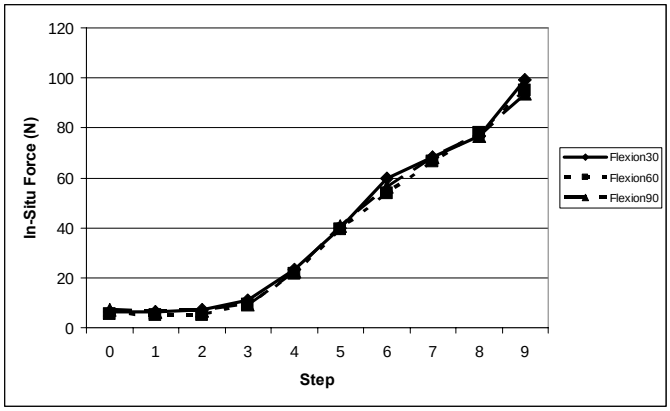

Figure 2: Magnitude of In-Situ Force in ACL

The radius of the cone of confidence, δ_{95}, for each of the anterior loading steps is shown in Figure 3. At the full anterior tibial load, δ_{95} is approximately $0.5°$ for all flexion angles. δ_{95} increases at lower force magnitudes, particularly before loading step 6, which corresponds to an in-situ force of approximately 50 N.

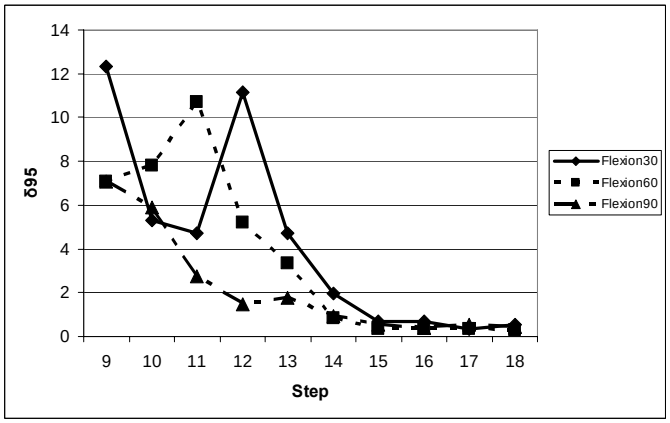

Figure 3: Radius of Cone of Confidence of In-Situ Force

DISCUSSION

At low levels of in-situ force (below 50 N), the repeatability of the robotic/UFS testing system in measuring in-situ force direction is relatively poor, as high as 12°. This degree of repeatability stems from three sources: viscoelastic effects of the soft tissue, the robotic manipulator, and the UFS. In any soft tissue, viscoelastic effects will cause variability in the force required to attain a given displacement. The viscoelastic effects of the knee have been previously shown to cause a variation of approximately 10 N in the magnitude of replay forces. Additionally, due to the finite path and position repeatability of the robotic manipulator, the replay kinematics may not exactly match the recorded kinematics. This discrepancy has a particularly noticeable effect at low levels of in-situ force when the kinematics are closer to the repeatability of the manipulator, thus causing a proportionally larger effect on the replay forces, which explains the increased radius of confidence at lower forces. Finally, the UFS has some inherent error, which accounts for some variation in the forces recorded.

At higher levels of in-situ force (above 50 N), the repeatability of the testing system is within $0.5°$. Repeatability of this level is exceptional, and meets the requirements for in-vitro applications related to measurements of force direction.

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I graduated from Schenley High School in 2004 and have lived in the city of Pittsburgh for the past six years. Pittsburgh has always felt like home, despite several relocations growing up. As I continue my education at the University of Pittsburgh, I am overjoyed to participate in the rich biomedical opportunities available locally that contribute directly to my community.

A short but pivotal trip to Haiti in 2002 set my course and infatuation with the biomedical field. In Haiti I saw the dire need of basic shelter, clean water, food and medical attention. I am now pursuing medical school to be a physician without borders. I will also contribute heavily to the medical practice itself through bioengineering research to better reach those in need with innovative techniques when medical equipment is absent.

Beginning in the summer of 2005, I became a nursing assistant at Shadyside hospital in the short term surgery unit. This and my ongoing internship at the MSRC have been two invaluable experiences! I would like to thank the MSRC with special thanks to my two mentors, Steve and Matt, who have broadened my perspective, knowledge, and increased my passion to pursue it. A final thanks to the ever teaching and guiding Dr. Woo; your drive is an inspiration!

DEVELOPING A PROTOCOL FOR TENSILE TESTING THE PATELLAR TENDON

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INTRODUCTION

With approximately 150,000 to 200,000 Americans suffering from ACL related injuries each year, it is no wonder that the World Health Authority declared that 2000 to 2010 is the "decade of bone and joint engineering".[1] The anterior cruciate ligament (ACL) is located in the knee joint and is a vital component to mobility via rotatory stabilization and preventing anterior tibial translation. Unfortunately, the ACL does not heal spontaneously and requires reconstruction.[1]

The central third bone-patellar tendon-bone complex (BPTB) was considered the "golden standard" autograft for ACL reconstruction for many years. A BPTB graft is created by removing the central portion of the patellar tendon (PT) with bone blocks. The remaining portion (medial and lateral) is left to heal and new connective tissue forms at the defect. The BPTB stabilizes the knee and exhibits quicker bone-to-bone healing better than any other known graft.

Complications such as patella baja, extension loss, swelling and donor site morbidity have reduced the use of BPTB autograft among clinicians. One complication during healing of the PT defect is the significant decrease in mechanical properties of both the remaining (medial and lateral) and neo-tissue, which fills the defect.[2] A study by Tohyama et al. found that not only were the ultimate tensile strength and tangent modulus of neo-PT tissue lower than normal PT tissue, the mechanical properties of the remaining portions of PT were also decreased by 50%.[3]

Therefore, if the quality of the PT postsurgery could be improved, the BPTB graft could regain popularity and improve patient outcome for ACL reconstruction. Functional tissue engineering via the use of small intestine submucosa (SIS) bioscaffolds has been shown to induce cellular organization and tissue formation through chemotaxis, growth factors and contact guidance in the medial cruciate ligament (MCL), while increasing the mechanical properties.[2] Although the MCL heals better than the PT, it is probable that SIS will effect the PT similarly.

Mechanical testing of the BPTB complex (with aspect ratio ~7:1) was previously validated using bone clamps at the Musculoskeletal Research Center. (Aspect ratio = length/width.) The lateral and medial portions of the tendon were removed and stored again at –20°C for later testing.

The overall objective of the current work is to investigate the effects of Functional Tissue Engineering approaches on the mechanical properties of the central and remaining PT. The specific objective is to develop a testing protocol to measure the mechanical properties of the central and remaining PT: using bone clamps for central PT testing and soft tissue clamps for remaining tissue testing. These measured values will then be compared to the BPTB values previously determined in order to validate the soft tissue clamp protocol. A criteria for validity of the soft tissue clamp protocol was based on a study by Yamamotto that showed all portions of the PT exhibit similar mechanical properties. The soft tissue protocol should error within a standard deviation of tangent modulus and ultimate tensile strength (UTS) of ± 60 Mpa and ± 11 Mpa respectively.[5]

METHODS

For this preliminary test, five skeletally mature New Zealand white rabbit legs were obtained and the patellar tendon was harvested from each. The patellar tendon was split into three sections: the central, medial and lateral portions. Each section was tensile tested separately. For biomechanical testing, the tissue was clamped into custom-made soft tissue clamps. The tissue in the clamp was surrounded with gauze to prevent tearing damage done by any sharp edges or ridges on the clamp, which could cause tissue failure at the clamp.

The cross-sectional area of the tissue was measured in three locations along the length of the tissue using a laser micrometer. The PT was then cut into a dogbone shape via sharp dissection to ensure an aspect ratio of ~4:1. The cross-sectional area was measured again and reflective markers were placed on the tissue in the area of constant width. The clamped tissue was then secured into a specially designed saline bath held at a constant temperature of 37ºC on a materials tensile testing machine (Figure 1).

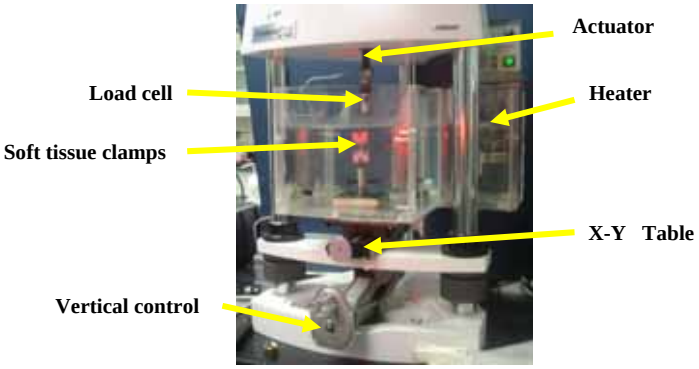

Figure 1 – Tensile testing apparatus for soft tissue clamped specimens on the Enduratec™.

A series of uniaxial tensile tests followed: 0.5 N preconditioning, 10 cycles of preconditioning between 0 and 0.25mm elongation, 0.5 N preload, and a load to failure

(LTF) test. The crosshead speed for all tests was 0.0833 mm/sec with thirty minutes of rest between each test.

The mechanical properties, including tangent modulus and the ultimate tensile strength, were calculated for the soft clamp tissues in the same manner as those for the BPTB complex. Reflective markers were used with video Motion Analysis ™ equipment to determine the strain applied to the tendon and stress was determined from dividing the load by the average cross-sectional area. The tangent modulus was defined as the maximum slope of the stress-strain curve over a 2% interval of strain during the LTF test. The ultimate tensile strength was the maximum stress recorded.

RESULTS

The cross-sectional area (mean ± the standard deviation) for the soft tissue clamp specimens was 0.7 ± 0.3 mm^2 (n=10), while bone clamp specimens was 3.5 ± 0.6 mm^2 (n=16). Their aspect ratios were ~4:1 and ~7:1 respectively. A typical stress versus strain curve for each protocol is pictured below (Figure 2).

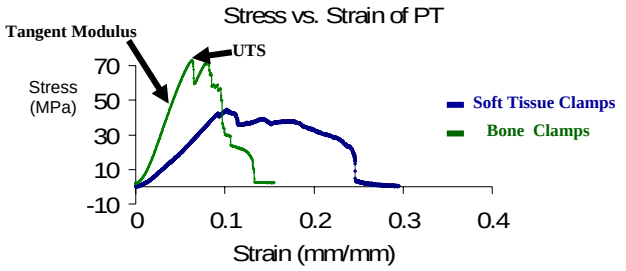

Figure 2 – This stress verses strain graph depicts the typical results of a load to failure tensile test with soft tissueclamps versus bone clamps.

To validate the mechanical properties for soft tissue clamps, the values for tangent modulus and ultimate tensile strength were compared to the values found for the BPTB in Table 1.

Comparing Mechanical Properties of Two Protocols		
Sample	**Tangent Modulus (MPa)**	**UTS (MPa)**
Soft Tissue (n=10)	610 ± 211	46.1 ± 19.9
BPTB (n=16)	1492 ± 538	71.9 ± 9.2

Table 1 Properties of soft tissue clamped PT verses BPTB complex with bone clamps.

DISCUSSION

The specific hypothesis stating that mechanical properties found while using soft tissue clamps should be within the stated range of bone clamp values was not supported dispite evidence of experimental similarity between all portions of the PT. (Range of a standard deviation of tangent modulus and ultimate tensile strength of ± 60 Mpa and ± 11 Mpa respectively.) Therefore, it may be concluded that the protocol designed will not provide accurate results in comparison to the BPTB in later tests with Functional Tissue Engineering applications.

One cause of the huge difference in values could be the difference in aspect ratio. A high aspect ratio ensures that there is uniform, uniaxial strain on the tissue and that there is a mid-substance tear (not at the bone insertion site or clamp). The aspect ratio used in the BPTB complex was approximately 7:1. The soft tissue specimens had only a ~4:1 aspect ratio. Since the soft tissue specimens are so small, it was difficult to obtain a larger aspect ratio. However, the decrease in tissue size should not have caused this inconsistency in values since mechanical properties do not depend on size.

A second possible cause of the discrepancies is the accuracy of cross-sectional area measurements for tissues less than 1 mm^2. The laser micrometer was validated in 1990 for tissues with a mean ± standard deviation of 3.3 ± 0.2 mm^2 with an accuracy of 0.1 mm^2 for rabbit MCL, but had not yet been validated for tissues less than 1 mm^2.[4] Since the soft tissue clamp specimens had a cross-sectional area of 0.7 ± 0.3 mm^2, the laser micrometer has since been found to be accurate within 0.1 mm^2 for dowel rods of $1/32^{nd}$ of an inch in diameter.

The data, however, does not have a consistent magnitude of error between the tangent modulus and the ultimate tensile strength of the two protocols. The tangent modulus is nearly 250% greater with the bone clamps than with the soft tissue clamps while ultimate tensile strength of the bone clamps is only 40% greater. If the cross-sectional area measurement accuracy and the aspect ratio are the only two problems with the designed protocol, then the magnitude of change for UTS and tangent modulus between the two protocols should be similar. Therefore, the strain tracking using the Motional Analysis data collection system was also challenged for accuracy and repeatability using two different types of markers. The system was found to be accurate within ~3% error and highly repeatable with a standard deviation of 0.0001 between trials.

In conclusion, it was later found that the deformation of tissue caused by clamping of the smaller tissues (soft tissue clamp specimens) distorted strain measurements. Currently, alternate soft tissue preparations are being explored in a continued search for an accurate mechanical properties testing protocol for soft tissues. After the completion of this specific goal, Functional Tissue Engineering approaches such as the application of SIS may improve PT healing. By improving knee stability for patients of ACL reconstruction, improved patient outcome may be achieved.

ACKNOWLEDGEMENTS

I would like to thank Matthew Fisher and Dr. Steven Abramowitch who have guided this research from beginning to end. In addition, Dr. Woo, who has created an environment that encourages curiosity in many unique opportunities. I would like to thank the entire MSRC family and summer interns for the constructive criticism provided along the way, without which this research would not be possible! Thank you!

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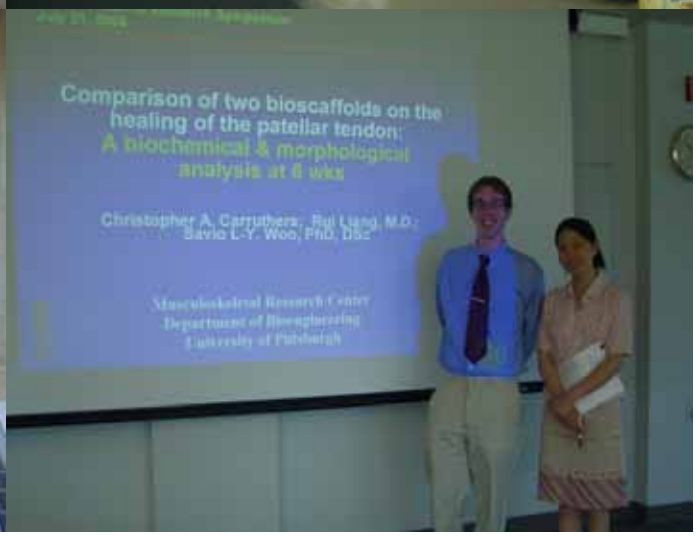

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