Musculoskeletal Research Center

Summer Research Program

MSRC

Department of Bioengineering

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2005

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2005 Abstract Book Committee

Caitlyn Mooney, Shawn Burton, David Shin, Matthew Fisher

I think I can speak for the other seven summer interns when I say that this has been the fastest summer of our lives. It seems like June was a couple days ago. Yet, I feel that all of us have gained an immense amount of knowledge from our mentors, fellow lab members, and faculty in a short period of time. We all worked extremely hard during the summer, and I believe this is best displayed by the quality of the work in these abstracts.

On behalf of all the summer students, I would like to thank the MSRC for providing us with helpful advisors and a setting to learn how to perform research correctly. The "MSRC family" has been very kind to us, and we'd like to thank Dr. Woo, Dr. Debski, and Dr. Abramowitch for inviting us to work at the MSRC and providing valuable advice on just about everything along the way. Certainly, our experience at the MSRC will strongly influence all of our future work.

-Matt Fisher, Editor.

2005 Summer Symposium Committee

Amanda Roof, Dana Irrer, Mike Anderson, Niki Bailey

Prior to the completion of the research displayed in this book, it was presented at the Musculoskeletal Research Center's Summer Student Symposium on July 26, 2005. This event allowed this summer's eight students to display the research they had completed during their 10-12 week internship and field questions from the faculty and staff of the University of Pittsburgh's Bioengineering Department.

The enthusiasm with which the research was discussed was displayed in the attentiveness of the audience, whom had not had their morning fix of coffee due to its late arrival to the symposium. Due to the previous planning, the remainder of the breakfast and symposium progressed smoothly. Speaking on behalf of the members of the symposium, I would like to say thank you to everyone who helped make this year's symposium a success and to all those who attended.

-Amanda Roof, Chairperson.

The MSRC Faculty

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Foreword

It is a great pleasure to "sing the praises" for this year's students in our summer research program. We have a very unique group of dedicated undergraduate students who "camped out" at the Musculoskeletal Research Center. Together they have really made a difference in many of our research projects. More importantly, all eight students have plans to continue to work or collaborate with us in the coming school year.

Mr. Matt Fisher is now a graduate student in bioengineering at the MSRC. Mr. Mike Anderson, Ms. Nikki Bailey and Mr. Shawn Burton are from Pitt, and will continue to work at the MSRC on projects which they have started. Mr. Dana Irrer and Mr. David Shin, from Carnegie Mellon University, will also do likewise and work at the MSRC. Ms. Amanda Roof will commute from Bucknell University to continue her project here. Ms. Caitlyn Mooney, of Notre Dame, also wishes to return to the MSRC from time to time during the holidays to finish her research. (NOTE: Whether she returns to Pitt after we trounce Notre Dame on 9/3/05 remains a question!) So, now the "switches" for research have been turned on in their minds. All of these students are a tribute to their mentors, Mr. Dan Moon (who is now attending medical school in Philadelphia), Dr. Jens Stehle (who has just returned to Germany to continue his orthopedic residency), Ms. Susan Moore, Mr. Eric Rainis, Dr. Tan Nguyen, Dr. Rui Liang, Dr. Kazu Miura, Ms. Sabrina Noorani, Mr. Guoguang Yang, Dr. Ozgur Dede and Dr. Yin-Chih Fu (who has just returned to Taiwan to continue his orthopedic practice). To all of you mentors, my "collective hats go off" to you. Thank you most sincerely for teaching these young investigators "the right stuff" and for guiding them in the right direction on a daily basis.

I especially enjoyed the annual symposium that was held on July 26, 2005. For the students, this symposium represented the crescendo of working in the MSRC summer program. It is easy to visualize that you have learned well. Your presentations were not only polished, but the content was complete and sound. Your rationale, research questions, hypotheses, methodologies and results as well as future directions were convincing. Moreover, you also carried yourselves exceedingly well in addressing all of the questions that were "thrown at you"--- an impressive performance by all. I trust that the readers of this volume of abstracts will agree with me 100%.

Finally, I think we should give "three cheers" to Dr. Richard Debski, who directs our summer research program. Under his guidance this program has really blossomed and matured. Our faculty mentor, Dr. Steve Abramowitch, also deserves special recognition. Credit must also be given to Ms. Hilda Diamond of Carnegie-Mellon University, not only for helping us with financial support, but also for sending us high quality students on a regular basis. In addition, we are grateful to the Pittsburgh Tissue Engineering Institute for giving us Mr. Matt Fisher.

It is a distinct pleasure to acknowledge Mr. Mike Anderson for receiving the Beckman Scholarship, and Mr. Matt Fisher who has won the BiRM scholarship. We are happy to continue to guide these incredibly bright students.

Even though it sounds like a cliché, the faculty and I really feel this year's summer students were the most outstanding group that we have hosted. That is really saying a lot because we have always had wonderful and incredibly bright students. I thank all of the students for making the MSRC part of your family, and I look forward to your presence and predict that with your participation great things will continue to happen at the MSRC!

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January 22, 1984. Super Bowl Sunday. It was during a huge blizzard on this fateful day that I was born. I grew up in the small settlement of Jennerstown, PA. As a child, my life revolved around sports. From sunrise to long after sunset, I would play anything and everything. In high school, I played football, basketball and baseball. I also learned how to play guitar and spent my high school days playing in a number of bands with my friends. When it came time to decide on a college, I knew I wanted to experience "city life". That fact, combined with the fact that Pitt has an outstanding engineering department led me to shack up in Oakland for the next 4 years. Coming to Pitt has been an awesome experience. Aside from schoolwork, I am active in intramural football, basketball, and volleyball and I play just about everything recreationally.

This has been my second summer at the MSRC, and the experience continues to be great. I'd like to thank Dr. Woo, Dr. Debski, and Dr. Abramowitch for putting together such a worthwhile program. I also would like to thank my mentor Jens for his support and friendship over the last two years. By the time this book is printed, his stint here in Pittsburgh will be up and he'll be back in Germany. The MSRC will truly not be the same place without him.

GLENOHUMERAL JOINT CONSTRAINT ANGLE: EFFECT ON ROTATOR CUFF MUSCLE FORCES AND SHOULDER STABILITY

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INTRODUCTION

The glenohumeral joint of the shoulder is the articulation between the humeral head and the concave surface of the glenoid, a part of the scapula. This joint has the widest range of motion of all joints in the human body. However, it is also the most often dislocated major joint. Cooperation among multiple muscles at the shoulder is required for joint mobility and stability. Muscle force vector analysis can be used to quantify the stability of the glenohumeral joint.[1] In order to prevent dislocation of the humeral head from the glenoid cavity, the direction of the resultant reaction force vector across the glenohumeral joint must be constrained in some way. The glenohumeral joint constraint angle is defined as the angle of a cone around the direction defined by the normal to the midpoint of the glenoid cavity.[2,4] (Figure 1) For joint stability, the direction of the resultant force vector must be constrained to remain within this angle. This angle represents the effective area into which the resultant reaction force vector can be directed in order to provide compression of the humeral head into the concave surface of the glenoid, and thus provide joint stability. A larger constraint angle means that the glenoid provides more bony stability to the joint and a smaller constraint angle means that the glenoid provides less bony stability to the joint. The objective of this study was to examine the effect of variations in the value of the glenohumeral joint constraint angle on muscle forces at the shoulder using a three-dimensional computational model.

METHODS

A three-dimensional computational model of the shoulder ("Shoulder Model v3.0") developed by Högfors, Peterson, Makhsous, and Siemienski was used to obtain muscle force outputs of 38 muscles or muscle parts based on input parameters of upper extremity position, muscle condition, glenohumeral joint constraint angle, anthropometric data, and external load. The muscles included in the model satisfy the criteria of having attachments on the humerus, the scapula, or the clavicle. In this model, the bones are considered as rigid bodies and the muscles are considered as stretched strings that follow the shortest path between attachment points. The maximum force output of a muscle can be manipulated by altering the physiological cross-sectional area (PSCA) of that muscle. External loads can be applied in any direction at any point along the arm. All loads in this study were applied in a downward direction at the hand to simulate holding a small object, as would be experienced during an activity of daily living. The model also allows for custom choices of anthropometric data. For this study, adult male anthropometric data derived from cadaveric dissections was used in all tests.[5]

As the model treats the bones as rigid bodies, the number of muscle forces exceeds the number of degrees of freedom, and the system is statically indeterminate. Thus, muscle forces are calculated by solving 19 equilibrium equations using a quadratic objective function that is minimized subject to various constraints, including upper and lower limits of muscle forces and the glenohumeral joint constraint angle.[3] This model assumes that the glenohumeral joint is a pure ball-and-socket joint and that scapular motion is included with arm elevation. The output of this model has been validated by extensive comparison to EMG data.[3,4]

Three constraint angles were examined with various arm positions: 1) 22°, 2) 32° (average value for an adult male as described in the model), and 3) 42°. Muscle forces were examined from 0° to 90° of abduction in the coronal plane in 15° increments for each of the three constraint angles. The muscle forces examined in this study include the supraspinatus, upper infraspinatus, lower infraspinatus, and subscapularis muscles of the rotator cuff, and the anterior and middle portions of the deltoid which are important to joint mobility. The average value of each muscle's force output throughout the abduction range was calculated. The magnitude and percent change in average muscle force between the three constraint angles were examined. All analyses were performed with the elbow in full extension using adult male anthropometric data and a hand load of 1 kg.

RESULTS

The absolute value of each muscle's average force output for each of the three constraint angles can be seen in Table 1. From 0º to 90° of abduction, as the glenohumeral joint constraint angle was increased from the average value of 32º to 42º, the average muscle force decreased in all muscles examined except the anterior portion of the deltoid. Figure 2 shows the percent decreases in average muscle force for this change in glenohumeral joint constraint angle for the muscles examined. As can be seen in this figure, the rotator cuff muscles are affected by changes in the glenohumeral joint constraint angle to a greater degree than the deltoid muscle. As the constraint angle is increased from 32º to 42º, the average forces in the supraspinatus, upper infraspinatus, lower infraspinatus, and subscapularis decreased by 62%, 38%, 45%, and 33%, respectively, corresponding to changes of 17N, 14N, 3N, and 17N. The forces in the anterior and middle portions of the deltoid changed by only 2% and 4%, respectively, corresponding to changes of 1.5N and 4N.

As the constraint angle was decreased from 32º to 22º, the average muscle force increased in all muscles examined except the anterior portion of the deltoid. The percent changes in force output can be seen in Figure 2. Again, the rotator cuff muscles are affected by changes in the glenohumeral joint constraint angle to a greater degree than the deltoid muscle. As the constraint angle is decreased from 32º to 22º, the average forces in the supraspinatus, upper infraspinatus, lower infraspinatus, and subscapularis increased by 110%, 48%, 68%, and 61%, respectively, corresponding to changes of 31N, 18N, 5N, and 32N. The forces in the anterior and middle portions of the deltoid changed by only 1% and 15%, respectively, corresponding to changes of 0.5N and 14N.

DISCUSSION

From 0º to 90º of humeral abduction, muscle force output was found to be inversely correlated with changes in the value of the glenohumeral joint constraint angle for all muscles examined except the anterior portion of the deltoid. This suggests that as the constraint placed on the resultant force vector across the glenohumeral joint is lessened (larger value of the constraint angle, more area into which the reaction force can be directed to provide joint stability) the muscles providing stability to the shoulder must exert less force to satisfy this constraint. Similarly, as the constraint placed on the resultant force vector across the glenohumeral joint is tightened (smaller value of the constraint angle, less area into which the reaction force can be directed to provide joint stability) the muscles providing stability to the shoulder must exert more force to satisfy this constraint. These findings are comparable with the work of Nieminen et al., who found that predicted force levels were inversely correlated with changes in the glenohumeral joint constraint angle during submaximal contractions with a related shoulder model.[2] The authors of this study suggest that more simultaneous contraction of the muscles is needed to accurately stabilize the contact force direction within a small angle, thus muscle forces are higher for smaller values of the constraint angle and lower for larger values of the constraint angle.

The muscles of the rotator cuff were more affected by changes in the value of the glenohumeral joint constraint angle than the anterior and medial portions of the deltoid. That is, the percent changes in force output were larger for these muscles for a given change in the value of the constraint angle. This supports the findings of previous studies that have shown that the rotator cuff muscles play a more active role in the stabilization of the glenohumeral joint than the deltoid, which contributes more to the mobility of the shoulder rather than to the stability.[6,7]

ACKNOWLEDGEMENTS

The support of the NIH, the Beckman Foundation, and the MSRC are gratefully acknowledged.

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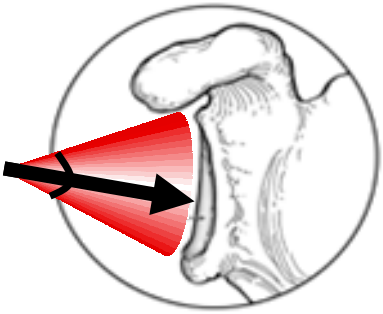

Figure 1. Glenohumeral joint constraint angle. For joint stability, the resultant force vector across the shoulder (black arrow) must be constrained to remain within the constraint angle (cone).

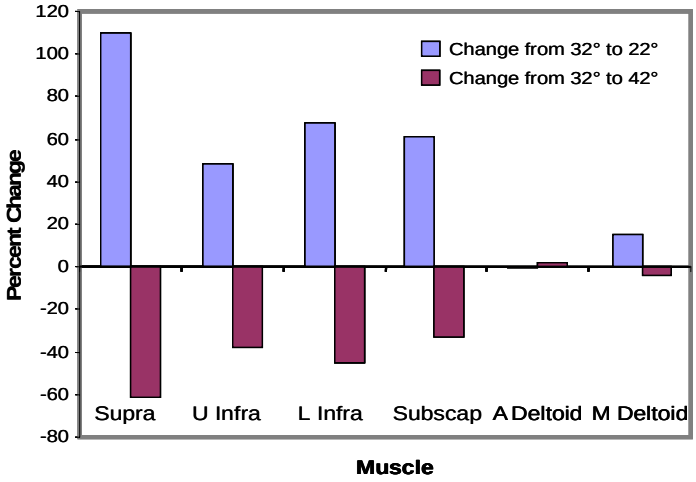

Figure 2. Percent change throughout abduction in average muscle force between constraint angles for six shoulder muscles or portions of muscles in an intact shoulder.

	Supra	U Infra	L Infra	Subscap	A Delt	M Delt
22°	59	54	12	85	77	112
32°	28	37	7	53	78	97
42°	11	23	4	35	79	93

Table 1. Average muscle forces from 0º to 90º of abduction for six shoulder muscles or portions of muscles for three different values of the glenohumeral joint constraint angle.

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I was born in Massachusetts and moved four times along the east coast before I was 5. We settled in Landenberg, PA where I grew up eldest in a family of 4 children. Brianna a '05 grad will be joining me at Pitt this September, leaving my brother, Michael (16) and sister, Samantha (10) to hold down the fort.

Kennett High School was good to me. I was president of National Honors Society and Latin Club. I played soccer, ran the 100m hurdles and 800m track events, and even met my high school sweetheart (now fiance) Peter McPherson. We are getting married next May. My parents were always very supportive of everything I did and (being engineers themselves) eagerly supported my decision to become a Bioengineer. I love Pitt and have remained active participating in SWE, BMES, and Newman Club of which I am vice president.

Working at the MSRC has been an excellent experience! I am extremely enthused over my future in bioengineering. I have learned so much mostly thanks to my fabulous mentors Eric Rainis and Susan Moore. Jens, thanks for everything. I'll miss you and your family. Special thanks to Dr. Debski, Dr. Woo and the REU program for this opportunity. I would like to thank everyone at the MSRC especially the shoulder group for being so friendly and helpful.

EVALUATING THE REPEATABILITY OF THE METHODOLOGY TO DETERMINE THE STRAIN DISTRIBUTION ACROSS THE GLENOHUMERAL CAPSULE

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INTRODUCTION

Researchers are seeking to improve patient outcome after shoulder injury by determining the strain distribution across the glenohumeral capsule. Malicky and coworkers developed a method to determine the 2-D strain on the anterior-inferior region of the glenohumeral capsule [1]. A grid of markers was fixed to the capsule and stereoradiogrammetry was used to find the marker locations and calculate the strain. A novel method to define a reference strain configuration was developed and the study showed that the capsule should be treated as a continuum as opposed to multiple thickenings [1].

Our laboratory has designed a method to find the strain on the inferior glenohumeral capsule (IGHC) in clinically relevant joint positions using a non-contact tracking system [3]. The methodology is similar to the previously described study [1] except a robotic testing system is used to move the joint in 3 degrees of freedom. This method is very complicated, involving many steps of unknown accuracy or repeatability. Thus, our objective was to evaluate the repeatability of the overall method.

METHODS

One shoulder specimen was dissected free of all skin and musculature leaving only the glenohumeral capsule and coracoacromial ligament. A grid of plastic markers (7x11, 1.6mm diam.) spaced 5mm apart and 1 cm from all insertions was affixed with cyanoacrylate to the inferior glenohumeral ligament beginning on the anterior band.

The specimen was then mounted in a plastic jig with 6 degrees of freedom at 60° of glenohumeral abduction and neutral horizontal abduction. The specimen was rotated from 15° of internal rotation to 15° of external rotation in 5° increments. At each joint orientatioin the capsule was inflated to 0.7 and 4.8kpa [1]. The marker locations were recorded with a custom built camera system (Spicatek) at each pressure. The accuracy and repeatabiliy were determined in pilot studies to be ±0.07mm and ±0.08, respectively. The location of the markers were found by manually digitizing their centroids using 3D tracking software. Then the reference strain configuration was defined as the joint position with the least amount of marker motion between the low and high pressures. The capsule was then inflated to 1 kpa at this joint orientation and the location of the markers at this pressure became the reference strain configuration. This method was repeated 3 times to obtain 3 trials for the reference strain configuration. Between trials the camera configuration and calibration, and the specimen alignment were re-established. Thus the marker locations of the 77 markers were obtained for each trial at the reference strain configuration.

The specimen was then mounted in a robotic/universal force-moment sensor testing system, at approximately 0° of glenohumeral abduction and 0° external rotation [2]. The robotic testing system moved the shoulder to clinically relevant joint positions (0° & 60° external rotation) while applying a 50N anterior load. At each joint position the marker locations were captured with the camera system. These marker locations defined the strained configuration and they were obtained in three trials by manually digitizing the centriods of the markers using a 3D tracking system. In between trials the camera and robot systems were reconfigured and recalibrated. The specimen was also realigned in the testing system.

The effects of the observer on the marker location were also investigated. The intra-observer repeatability was determined by having 1 observer digitize the same data set 3 separate times. The inter-observer repeatability was determined by having 3 different observers digitize the same data set one time.

The variability of the location of each marker during the inter- and inter-observer was determined by fitting a sphere around the 3 marker locations (one location from each trial). The centroid of the sphere was calculated from the three marker locations and then the radius, which was defined to be the largest distance between the centroid and the marker locations, was used to compare the repeatability of the marker locations. This parameter was also calculated for the location of the markers during the three trials of the reference strain and strained configurations.

Then the 3 trials from the reference strain configuration were randomly matched with the 3 trials from the strained configuration. With 77 markers the max principal strain was calculated at the centroid of 60 elements (defined by 4 adjacent markers) by imputing the marker locations of the reference strain configuration and the strained configuration into ABAQUS.

The overall repeatability of the entire methodology was calculated by averaging the standard deviations describing the variability of the strain within each element.

RESULTS

The variability of the marker locations for the intra- and inter-observer were 0.09mm and 0.12mm, respectively. The max variability for a marker location for the intra-observer was 0.18mm. The max variability for a marker location for the inter-observer was 0.34mm.

The variability of marker locations for the reference strain configuration was 1.53mm while the variability of the marker location in the strained configuration at 0° & 60° external rotation was 0.94mm and 0.98mm, respectively. The max variability for a marker location of the reference strain configuration, strained configuration at 0° external rotation, and the strained configuration at 60° external rotation were 2.54mm, 1.85mm and 1.76mm, respectively.

The strain distributions across the IGHL had the same trends between all three trials (Figure 1). The highest strains were consistently in the anterior glenoid corner of the IGHL and the lowest strains occurred across the center of the anterior half of the IGHL stretching from the middle of the axillary pouch near the glenoid to the anterior side near the humerus. The average strains at 0° and 60° external rotation ranged from 9.2-12.5% and 9.9-13.7%, respectively. The max strain occured in trial 3 for both 0° and 60° external rotation (31.2% and 33.7%, respectively). The repeatability of the entire methodology was found to be ±3.0% at 0° external rotation and ±3.5% at 60° external rotation.

		Trial 1	Trial 2	Trial 3
0°ER	**Avg (%)**	9.2 ± 5.9	12.5±5.4	10.6±6.0
	Max (%)	23.7	23.3	31.2
60°ER	**Avg (%)**	9.9±5.4	13.7±5.8	11.6±6.5
	Max (%)	22.0	25.6	33.7

Table 1. Average and Max Strain across the IGHL at 0° and 60° external rotation(ER) for each of the 3 matched trials (avg±sd).

DISCUSSION

In this study the repeatability of the methodology to determine the strain on the inferior glenohumeral ligament complex were evaluated.

The marker locations in the reference strain configuration varied approximately 1–2mm between trials. The marker locations in the strained configuration varied less than 1.3mm between trials. The observer had a smaller influence on the marker locations. One observer could vary marker locations up to 0.17mm and between multiple observers the marker locations could vary 0.13mm. The strain distribution between trials was consistent (Figure 1).

The overall repeatability of the methodology was found to be ±3.0% at 0° external rotation and ±3.5% at 60° external rotation. This repeatability is acceptable because it is an order of magnitude less than the strain at which the inferior glenohumeral ligament fails. Research shows the IGHL tends to fail around 30% and specimens can vary up to 10% [1,3].

This repeatability takes into account many factors throughout the entire experimental protocol. Between trials in the reference strain and strained configurations the cameras were removed from the testing environment and repositioned. The working volume of the camera system was recalibrated. The robotic testing system (which has a repeatability of 0.2mm for position and 0.2° for orientation) was restarted and the path of passive ab/adduction was redefined [2]. The specimen was removed from the testing environment and realigned. The initial locations of the markers could be different due to varied capsule inflation for each trial. The observer also influenced error when they selected and labeled the centroids of the markers.

Yet, despite all these factors our repeatability of ±3.0% at 0° external rotation remains low and only slightly higher than Malicky and coworker's repeatability (±2.8%) for the reference strain configuration at 0° external rotation [1]. Our repeatability may be slightly higher since our repeatability includes our strained and reference strain configurations, whereas Malicky and coworker's repeatability only accounts for his reference strain configuration.

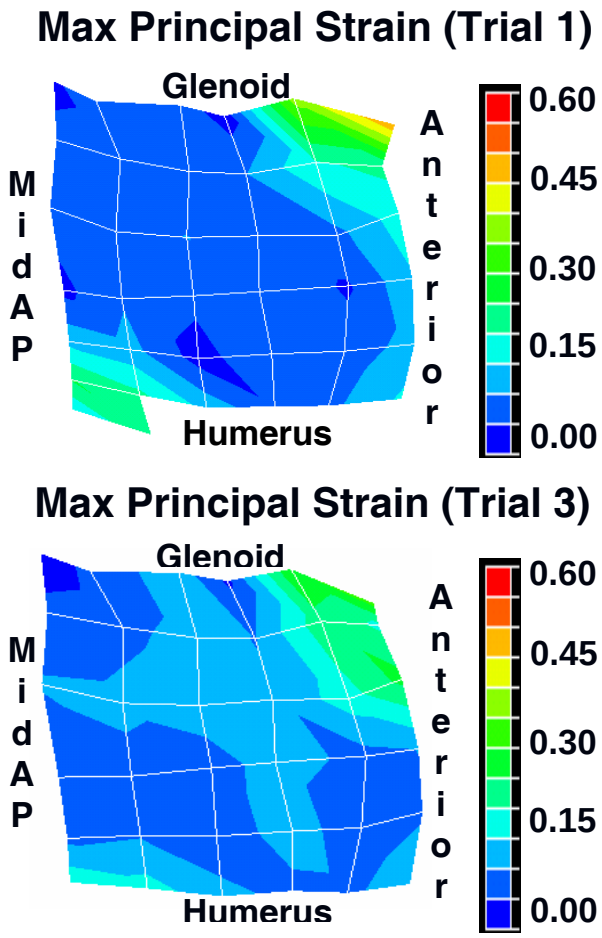

Figure 1. Fringe Plots displaying the Maximum Principal Strains across the IGHL at 0°ER in Trial 1 and Trial 3. MidAP is the Middle of the Axillary Pouch.

There were a few artifacts that may have influenced the repeatability of this study. Due to the small geometry of the specimen many of our markers were not visible to our camera system. The testing was performed over a 2 day period and so there may be changes in the properties of the tissues that influenced the repeatability. Also, all of the data used to calculate the strain was digitized by a single observer and thus the inter-observer effect of on the strain was not investigated.

In the future, this methodology can be used to obtain strain distributions to validate a finite element model and aid clinicians in their diagnosis, treatment, and rehabilitation of patients.

ACKNOWLEDGEMENTS

I would like to thank the Musculoskeletal Research Center, the Department of Bioengineering and the National Institute of Health for all their support.

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I hail from the quaint town of Grove City, Ohio where I spent the entirety of my pre-college existence. Defined by my diehard support and love of the Syracuse Orangemen, the decision to attend the University of Pittsburgh in the fall of 2003 was a difficult one. Having recently completed my sophomore year as a bioengineering/microbiology student and a member of the NSCS and the Sigma Alpha Lambda Honor Society though, I am eager to enter into my junior year.

When not hidden away in a library corner pinned under stacks of textbooks, I immerse myself in music and enjoy an eclectic selection of activities. I recreationally pursue the sports that occupied my high school career (football, track) as well as the occasional basketball, snowboarding, and frisbee contest. During my coffee breaks one can generally find me attached to a Star Wars novel or Spiderman comic, and in between classes a guitar is never far from my reach.

Coming to the MSRC from the Pittsburgh Bacteriophage Institute drastically broadened my research perspective from the level of viruses to functional tissue engineering. The MSRC also exposed me to biomechanical testing and analysis, histology, tissue and *in vivo* application, and techniques and approaches to bioimaging. I am extremely thankful to have received the opportunity to partake in all of these experiences, and I owe the utmost gratitude to my mentors Drs. Tan Nguyen and Rui Liang, as well as Dr. Stephen Abramowitch, Dr. Savio L-Y. Woo, and the whole MSRC family.

QUANTIFYING THE IMPROVEMENT OF COLLAGEN FIBER ALIGNMENT OF CELL-SEEDED SMALL INTESTINAL SUBMUCOSA AFTER MECHANICAL CONDITIONING

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INTRODUCTION

Rupture of the medial collateral ligament (MCL) is a prevalent and substantial injury, occurring frequently during athletic competition and daily activity [5]. The MCL is capable of spontaneous healing, yet the healing tissue exhibits disorganized extracellular matrix and inferior mechanical properties compared to normal MCL [4,6,11]. Musahl et al. and Liang et al. showed that application of a porcine small intestinal submucosa (SIS) bioscaffold to MCL injuries significantly improved collagen fiber alignment and mechanical properties [4,6]. Tangent modulus and stress at failure of SIS-treated groups were still considerably lower than normal MCL values, however.

Sacks and Chuong correlated mechanical properties to the overall fiber alignment of a collagenous tissue [9]. We speculate that through contact guidance, the SIS collagen fiber alignment determines the healing MCL fiber alignment. To improve the fiber orientation and mechanical response of a healing MCL, functional tissue engineering (FTE) can potentially enhance fiber alignment of SIS through in vitro cell-seeding and cyclic stretching. SIS naturally displays a preferred fiber orientation (±28°) [7] and shows improvement in collagen fiber alignment upon cyclic uniaxial tension [1]. Fibroblasts adhering to an aligned surface demonstrate cytoskeletal alignment and heightened collagen production parallel to the preferred surface fiber direction [2,10]. Additionally, cyclic stretching of fibroblasts induces increased levels of collagen production [2,10,12].

We thus hypothesize that seeding fibroblasts onto the SIS scaffold coupled with subsequent cyclic uniaxial stretching will improve the overall collagen fiber alignment of the FTE SIS. Specifically, we will use small angle light scattering (SALS) to quantify collagen fiber alignment improvement.

METHODS

Eight SIS samples were examined in this study. Each sample was hydrated and cut into a standard testing shape (2 cm^2 midsubstance). Samples were then scanned with a SALS device to quantify initial fiber alignment. Consistent with previous studies, glycerol was used as a scanning medium [7,9]. A final SALS scan followed experimentation.

SALS operates on the principle of single-slit light diffraction; a 4 mW HeNe laser (λ=632.8 nm, within an order of magnitude of collagen fiber diameter) is passed through the tissue, scattering light perpendicular to the fiber axis orientation (Φ). A single collagen fiber thus diffracts light Φ+90°, and a collection of fibers within the beam envelope distributes a pattern of light intensity I(Φ+90°), demarcating the greatest fiber alignment in the direction of maximum intensity of I(Φ) [8].

SALS centroid (Φ_C) and orientation index (OI) values were used to quantify collagen fiber alignment. The centroid of a scattered light intensity distribution I(Φ) vs. Φ defines the maximal light intensity and preferred fiber alignment at a test point. The orientation index is a normalized measure of fiber deviation ranging from 0% for randomly oriented tissue to 100% for perfectly oriented tissue. For all calculations, 1000 test points were selected from the midsubstance of the specimen. Centroid values are reported with respect to the meridian axis corresponding to 0°, with positive counter-clockwise rotation and a range of -90° to 90°.

Seeded Stretched (n=2)
SIS was seeded with 3.0 x 10^5 P_1-P_3 rabbit MCL fibroblasts for 1 day. After seeding, samples were cyclically stretched (stretch 2 hrs, rest 2 hrs, stretch 2 hrs, rest 18 hrs) (elongation: 10%, frequency: 0.5 Hz) along the meridian axis for 5 days.

Seeded Non-stretched (n=2)
SIS was seeded without cyclic stretching.

Non-seeded Stretched (n=2)
SIS was cyclically stretched without seeding.

Non-seeded Non-stretched (n=2)
SIS was neither seeded nor stretched to determine the effect of time and media conditions on collagen fiber alignment.

RESULTS

Table 1 summarizes all SALS measurements.

(n=2)	Average Centroid		Average Percent Increase of Tissue with OI>50%
	Before	After	
Non-seeded Non-stretched	-9.0°± 27.0°	4.8°± 15.4°	1.2% ± 4.1%
Non-seeded Stretched	9.5°± 23.6°	8.6°± 15.8°	25.9% ± 15.1%
Seeded Non-stretched	17.5°± 47.1°	-3.7°± 19.3°	48.7% ± 7.0% *
Seeded Stretched	-17.6°± 47.6°	-7.8°± 10.5°	59.4% ± 14.5% *

Table 1. Average Φ_C and OI% improvement.
*p<0.05, significant OI improvement (1-factor ANOVA)

Figure 1 shows the collective ΦC distribution for non-seeded non-stretched negative control samples.

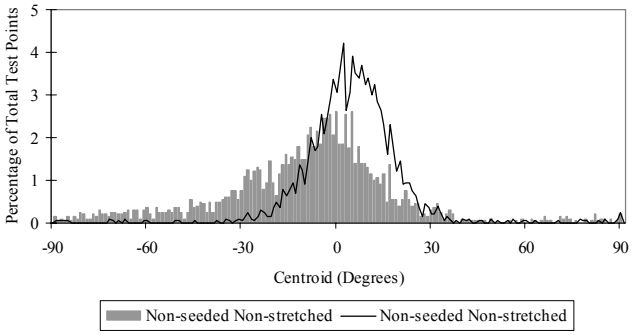

Figure 1. *Non-seeded Non-stretched* Φ_C distribution (n=2). Figure 2 shows the collective Φ_C distribution for non-seeded stretched samples before and after stretching.

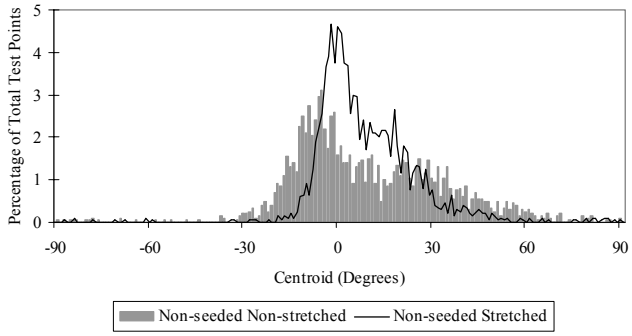

Figure 2. *Non-seeded Stretched* Φ_C distribution (n=2).

Figure 3 shows the collective Φ_C distribution for seeded non-stretched samples before and after seeding.

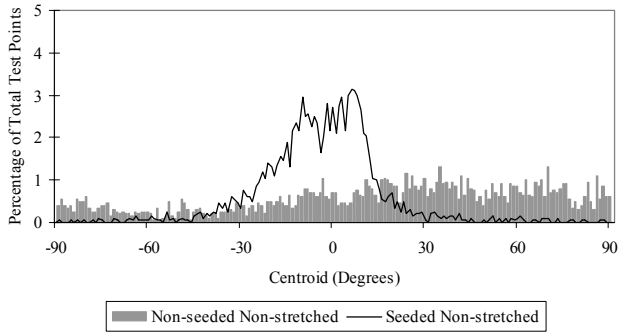

Figure 3. *Seeded Non-stretched* Φ_C distribution (n=2).

Figure 4 shows the collective Φ_C distribution for seeded stretched samples before and after seeding and stretching.

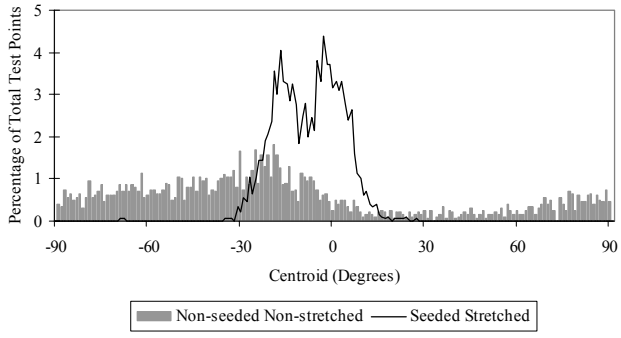

Figure 4. *Seeded Stretched* Φ_C distribution for (n=2).

Figure 5 summarizes the average increase in the percent of tissue with an OI>50% for each experimental group.

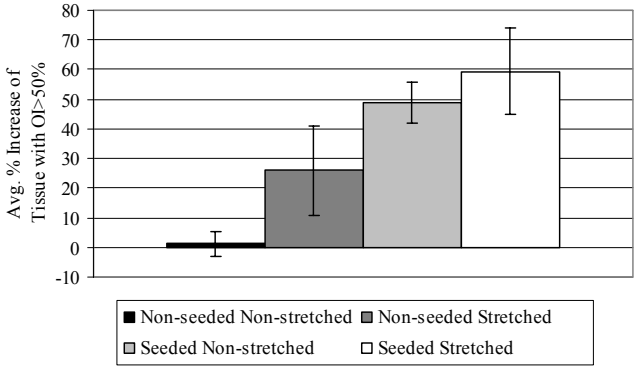

Figure 5. Average OI improvement.

DISCUSSION

The effects of cell-seeding and cyclic stretching on SIS collagen fiber alignment were evaluated in this study. All FTE approaches used showed average centroids significantly closer to 0° (the direction of stretch). Additionally, both cell-seeding and cell-seeding with stretching significantly increased the percentage of tissue with an OI>50% from initial states. We thus conclude that techniques of cell-seeding and cell-seeding with stretching significantly improve the SIS collagen fiber alignment. It is important to note that one sample showed 79.5% more tissue with an OI>50% following cyclic stretching with elongation of 10-15%, demonstrating the potential of optimization in this study.

Various factors could contribute to the improvement of fiber orientation observed in FTE SIS, including cellular and SIS fiber realignment, de novo collagen production, cytoskeletal contraction, and scaffold remodeling. Protocol optimization and increase in sample population could enhance the trends and statistical significance presented in this preliminary study. In future projects we plan to elucidate the role of fibroblasts and de novo collagen produced using histological analysis and quantification of collagen production. A subsequent multidisciplinary study applying FTE SIS to a MCL gap-injury in a rabbit model could also allow comparison between SIS and FTE SIS healing.

ACKNOWLEDGEMENTS

Lab and faculty mentors, Fengyan Jia, Guoguang Yang, Steven Abramowitch, David Merryman, and the MSRC are gratefully acknowledged.

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I was born on December 6, 1982, in the suburban South Hills of Pittsburgh, PA. I have four siblings, a brother and three sisters, and therefore, had a very loud house growing up. I graduated from Baldwin High School in 2001 and played four years of varsity tennis.

After graduating, I departed for Columbia University in New York City. I majored in Biomedical Engineering and met some of my best friends along the way. I started researching cartilage tissue engineering my sophomore year and still haven't looked back. There's always something different to do in New York City, so I often found myself roaming around the city. I also loved playing in many informal, but competitive, basketball, football, and tennis matches against friends.

The short time I have spent at the MSRC has been wonderful. I can only hope it remains this way because I will be working in the lab throughout graduate school, which starts in the fall. I'd first like to thank Dan Moon for all of his guidance. The MSRC, particularly the Tissue Mechanics Lab, will not be the same without him. Dr. Karaoglu also deserves thanks for working side-by-side with me to complete this project. I'd also like to thank Dr. Abramowitch, Dr. Debski, and especially Dr. Woo for allowing me to participate in the summer program.

THE EFFECTS OF BIOSCAFFOLD TREATMENT ON THE BIOMECHANICAL PROPERTIES OF HEALING PATELLAR TENDON AFTER REMOVAL OF ITS CENTRAL PORTION

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INTRODUCTION

The anterior cruciate ligament (ACL) is the most frequently injured ligament in the knee, and ruptures often require surgical reconstruction.[1] Central third bone-patellar tendon-bone (BPTB) autografts are commonly used for ACL reconstructions. Despite many advantages of the BPTB graft, harvesting of the graft often results in donor site morbidity including patellar baja and patellofemoral pain.[2] Furthermore, the resulting defect in the patellar tendon (PT) has been observed to remain for years.[3] Functional tissue engineering approaches have shown the potential to promote healing within the PT defect.[4] Our research center has demonstrated a bioscaffold, porcine small intestinal submucosa (SIS), enhanced healing of an injured medial collateral ligament with a gap injury in a rabbit model.[5] The research question of this study is whether SIS could also enhance healing of a central PT defect. Thus, the objective of this study was to investigate the effect of SIS on the PT tissue dimensions, structural properties of the healing central BPTB complex, and mechanical properties of the healing PT tissue at 12 weeks after creation of a central third defect in a rabbit model. Since SIS is an inductive bioscaffold,[6] the hypothesis was that SIS will promote the growth of new healing tissue within the defect, resulting in an increased cross-sectional area (CSA) of the healing tissue and improved structural properties of the healing central BPTB complex.

METHODS

12 skeletally mature female New Zealand white rabbits were used for this study. A central third defect (3-4 mm) was created in the right PT, while left knees served as sham controls (**Fig. 1**). In the SIS-treated group (n=6), one strip of SIS was attached to both the anterior and posterior sides of the PT defect. The SIS was sutured with one stitch on each of the four corners of the SIS and one stitch on the middle of the remaining PT on either side of the defect. Similarly, sutures were placed on the non-treated (NT) specimens (n=6), but the defect was left open. All samples for each treatment group (12 in total) were used for CSA measurement and biomechanical testing at 12 weeks.

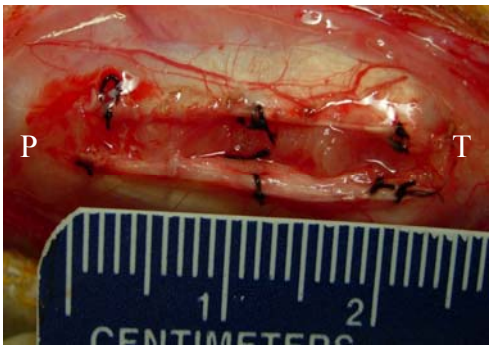

Figure 1. Central defect in the rabbit PT (P=patella, T=tibia)

At 12 weeks post-surgery, the animals were sacrificed and prepared for tensile testing by isolating the healing tissue via sharp dissection using the sutures to define the width. A standard width of 2 mm was used to dissect sham tissues. The CSA of the PT was measured before and after isolation of the healing tissue using a laser micrometer system.[7] Reflective markers were placed on the PT midsubstance for strain measurements (**Fig. 2**), which were recorded using a Motion Analysis™ video system. The healing central BPTB complexes were fixed to an Instron™ in custom-made clamps (**Fig. 2**), preloaded to 2N, preconditioned for 10 cycles from 0 to 1 mm, and loaded to failure. An elongation rate of 10 mm/min was used for all procedures. Structural properties of the healing central BPTB complex (stiffness and ultimate load) and mechanical properties of the healing PT tissue (tangent modulus and ultimate tensile strength) were obtained from the resulting load-elongation and stress-strain curves, respectively. The maximum slope over a 1 mm interval of elongation was used to find stiffness. A 2% interval of strain was utilized to determine tangent modulus. An unpaired t-test was used to compare treatment groups. A paired t-test was utilized to compare treatment groups to their respective sham controls. A Bonferroni adjustment was utilized to correct for multiple comparisons. As a result, significance was set at $p < 0.03$.

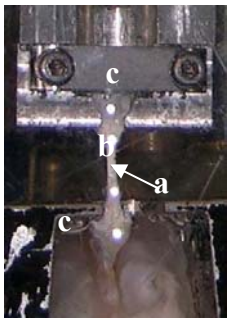

Figure 2. BPTB complex (a) with reflective markers (b) within clamps (c) for tensile test in saline bath on Instron™.

RESULTS

Gross observations revealed the NT tissues displayed concave defects, while the SIS-treated tissues possessed defects with no concavity and a larger width at 12 weeks.

SIS treatment did not alter the whole PT CSA compared the NT specimens (31.9 ± 12.4 mm^2 vs. 28.3 ± 10.4 mm^2, respectively, $p > 0.03$), but both treatment groups were significantly greater than sham controls (17.7 ± 2.7 mm^2, $p < 0.03$). CSA measurements of the healing tissue measured 56% greater in the SIS-treated group compared to those in the NT group (5.0 ± 2.2 mm^2 vs. 3.2 ± 1.3 mm^2, respectively), but this result was not significant ($p > 0.03$).

During load to failure tests, all specimens failed in the midsubstance, except for one sham specimen for each group, which failed at the patellar insertion. SIS-treatment showed trends of increasing stiffness and ultimate load of the healing central BPTB complex by 40% and 55%, respectively ($p > 0.03$, **Fig. 3, Table 1**). Tangent modulus and ultimate tensile strength were similar between healing PT tissues of the SIS-treated and NT groups ($p > 0.03$, **Table 1**). All parameters representing the structural properties of the healing central BPTB complex and mechanical properties of the healing PT for each treatment group were significantly lower compared to their respective sham controls ($p<0.03$, **Table 1**).

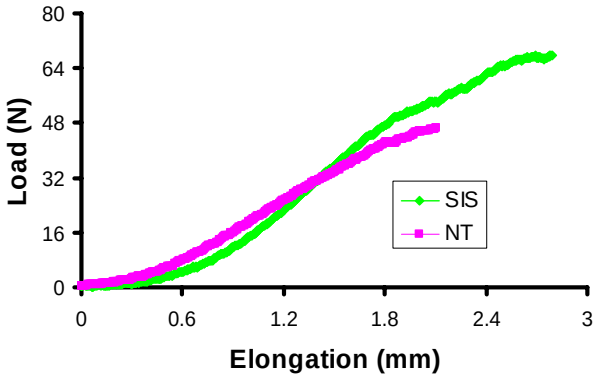

Figure 3. Representative load-elongation curves describing the healing central BPTB complex for each treatment group.

Structural Properties of the Healing Central BPTB Complex.			
	Sham	**SIS-treated**	**NT**
Stiffness (N/mm)	151.6 ± 21.9[a]	33.9 ± 14.0	24.3 ± 14.9
Ultimate Load (N)	250.3 ± 69.0[a]	67.7 ± 25.8	43.8 ± 27.4
Mechanical Properties of the Healing PT Tissue.			
	Sham	**SIS-treated**	**NT**
Tangent Modulus (MPa)	1435.6 ± 568.8[a]	238.1 ± 88.0	213.4 ± 99.7
Ultimate Tensile Strength (MPa)	71.9 ± 11.2[a]	14.0 ± 4.5	14.1 ± 9.0

Table 1. Parameters representing biomechanical properties for sham (n=12), SIS-treated (n=6), and NT (n=6) groups at 12 weeks (mean ± std). a= significantly different from treated groups ($p<0.03$).

DISCUSSION

Based on the results of this study, SIS-treatment shows the potential to increase the quantity of healing tissue and structural properties of the healing central BPTB complex after a surgically created central third PT defect, supporting our hypothesis. Compared to other studies that have performed biomechanical testing of healing tissue within a PT defect, the results obtained for the sham and NT groups compared favorably.[5] Since the results suggest that SIS has positively changed the healing response of the PT, further investigation is warranted for this application. To this end, longer-term studies focusing on PT healing with SIS-treatment are underway as well as experiments aimed at enhancing the healing potential of the scaffold prior to implantation via cell seeding and mechanical conditioning. Ultimately, the goal is to improve both the quantity and quality of the healing tissue within the PT defect in an effort to reduce donor site morbidity following BPTB graft harvest.

ACKNOWLEDGEMENTS

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I grew up in Okemos, Michigan, where I have lived my whole life up until coming out to Pittsburgh for college. I am currently attending Carnegie Mellon University where I study Mechanical and Biomedical Engineering and run competitively and am involved in several service organizations on campus. This past year I achieved All-American status by placing 22nd in the country in cross country and hope to better that this upcoming fall.

My choice to enter into the engineering field was fueled, in part, by an older brother working to design parts for an automotive supplier in Michigan. Eventually my desire to help others and an interest in the mechanics of the human body led me to specialize in biomedical engineering and eventually find a place here at the MSRC. From here I hope to continue on to medical school.

I cannot thank Dr. Miura, the ACL group and the rest of the MSRC enough for the experience that they have given me. The atmosphere of this lab is like nothing I have experienced before with collaboration between researchers, overall academic pursuit and in general, a fun place to work. I would also like to thank Drs. Woo and Debski for opening up a world class facility to students like me interested in pursuing a career in the biotechnical field.

FORCE DIRECTION OF THE ANTERIOR CRUCIATE LIGAMENT GRAFT WITH DOUBLE BUNDLE PROCEDURE

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INTRODUCTION

Although anterior cruciate ligament (ACL) reconstruction procedures reconstructing only an anteromedial (AM) bundle have been commonly performed in order to restore knee stability after injury, an inappropriately large number of patients still experience less than satisfactory post-surgical results.[4] Recently, there has been an increase in using the double-bundle anterior cruciate ligament reconstruction (DB-ACLR) to reproduce both the anatomy and kinematics of the intact ACL, specifically in respect to both the AM and posterolateral (PL) portions at different angles of knee flexion.[2] It has been demonstrated that a DB-ACLR that places separate grafts into two femoral tunnels located at the anatomical insertions of the two bundles of the ACL on the femur has biomechanical advantages over procedures that use a single graft at either the AM or PL femoral insertions.[7] Since the anatomical configuration of each bundle is reproduced in DB-ACLR, it is necessary to determine whether the force orientation vectors of the intact ACL, AM and PL bundles are also restored after the procedure. We hypothesized that different protocols for knee flexion angle of graft fixation of the DB-ACLR will show similar *in situ* force orientations for both the overall reconstructions and within each graft.

Human cadaveric knees were tested using a robotic/universal force-moment sensor (UFS) testing system. Both anterior tibial and rotatory loads were applied between full extension and 90° of knee flexion and the 5-degree of freedom (DOF) kinematics, magnitude and direction of the in situ forces of the two bundles of the intact ACL as well as those of the two grafts in DB-ACLR were measured and compared.

METHODS

Ten human cadaveric knee specimens (43.3 ± 8.1 yrs) were tested using a robotic/universal force-moment sensor (UFS) testing system.[1] (figure 1) The following external loading conditions were first applied to the intact knee: 1) a 134 N anterior tibial load at knee flexion angles of full extension (FE), 15°, 30° , 60° and 90° and 2) rotatory loads of 10 N-m valgus and 5 N-m internal tibial torque at knee flexion angles of 15° and 30°. The resulting 5 DOF knee kinematics in response to each loading condition were recorded and the AM or PL bundle was transected. The robotic manipulator then repeated the previously recorded path of motion so that the *in situ* forces in the ACL as well as AM and PL bundles and could be determined.[3]

In all cases, DB-ACLR was performed using the specimen's semitendinosus and gracilis tendons for the AM and PL grafts respectively. The grafts were fixed using an EndoButton® CL, spiked washer and screw. Two tunnels were drilled in the femur in the anatomic AM and PL insertions and one tunnel was drilled in the tibia through the AM footprint in the surface.

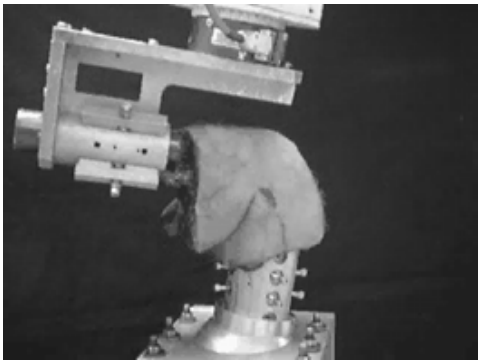

Figure 1. Human cadaver knee mounted on 6 DOF Robotic arm and UFS testing system.

In order to determine the effect of different angles of knee flexion for graft fixation, two separate fixation protocols were used. In the first (R1), the grafts were both simultaneously fixed at 30º of knee flexion and for the second protocol (R2), the PL graft was first fixed with the knee fully extended followed by the AM graft being fixed at a knee flexion angle of 60º. The kinematics of the ACL-reconstructed knee and *in situ* force in the ACL grafts were determined following the same method as for the intact knee. The data obtained included the *in situ* force of the intact, ACL-deficient, and ACL-reconstructed knee as well as intact AM and PL bundles and AM and PL grafts.

The force orientation was described using elevation and deviation angles. The elevation angle (α) was defined as the angle between the projection of the force vector in sagittal plane and the tibia plateau, and the deviation angle (β) was defined as the angle between the projection of the force vector on tibial plateau and the sagittal plane. The orientations were analyzed by averaging the elevation and deviation angles across the specimens for the intact ACL, the ACL reconstructions and each bundle. Confidence intervals (CI) were used to compare orientation between the intact knee and the grafts and were described as the area mapped onto a unit sphere where it is 95% certain that the force orientation vector lies.[5] The separate fixation protocols were further analyzed with respect to each other by comparing the angles that each reconstruction and graft force orientation vector made with the intact ACL and AM and PL bundles

force orientation vectors. This angle was determined using the dot product of each set of vectors as follows:

$$\Theta = \cos^{-1} \frac{A \bullet B}{|A| \cdot |B|}$$

A and B are the 3-dimensional force orientation vectors of the intact ACL or its bundles and the ACL reconstruction and its grafts respectively. Θ is the angle formed between them. A repeated measures ANOVA test was used to determine significance between these values, defined as $p < 0.05$.

RESULTS

Elevation and Deviation

Under the 134N load, both reconstructions compared rather favorably to the elevation and deviation of the intact ACL. At 30° of knee flexion the intact ACL showed an elevation of 20.4±5.8° and deviation of 0.7±5.2° while R1 showed α = 23.1±3.8° and β = 3.4± 6.0° and R2 provided α = 21.5± 6.1° and β = 0.6± 12.5°. Towards both FE and 90° of knee flexion, the reconstructions did not do quite as well at mimicking the intact ACL. R1 provided an elevation angle about 5° degrees lower than that of the intact ACL at both FE and 90° flexion and a deviation over 9° higher at FE and 4° higher at 90°. R2 was further off with an elevation and deviation reaching 9° greater than intact force orientation although these values still lie with in their respective standard deviations.

The intact AM bundle ranged from an elevation angle of 26.0±9.8° and a deviation of -1.3± 9.7° at full extension to an elevation of 8.1±4.2° and deviation of -0.2±6.5° at 90° of flexion. Both AM grafts stayed in a similar range, with R1 varying from α = 20.8±12.0° and β = 15.4±18.2° at FE to α = 16.3±5.1° and β = 4.1±6.0° at 90° knee flexion while R2 ranged from α = 31.0±9.5° and β = 19.6±17.4° at FE to α = 16.2±5.6° and β = 8.7±7.8° at 90° knee flexion.

Meanwhile, the intact PL bundle and its grafts showed very little difference in orientation at low angles (FE to 30°) of knee flexion despite different bundle fixation protocols. For the intact PL at 30° of knee flexion, α = 23.9±8.9° and β = 1.5±3.8°. This is quite comparable to both α = 18.7±5.0° and β = 1.5±3.8° for R1 and α = 17.5±8.9° and β = 4.7±11.5° for R2. At 60° of knee flexion and above, however, both PL grafts show rather high elevation and deviation angles with large standard deviations. This is seen in that the intact PL bundle shows and elevation of 31.6±24.6° and a deviation of 9.8±31.5° at 90° of knee flexion while at the same angle, R1 provides α = 18.1±26.6° and β = 7.9±16.2° and for R2, α = 3.4±51.0° and β = 6.8±41.7°.

Confidence Intervals

The CI areas of the force orientation vector were compared by mapping their area onto a unit sphere around a tibial plateau (figure 2). An overall trend is seen that the CI areas for the intact and ACL reconstructions are centered (average value of the force orientation vector) in between the CI areas of both the AM and PL bundles and the AM and PL grafts respectively with the AM CI areas centered more superior and medial compared to the average ACL orientation and the PL CI areas centered more inferior and lateral. Comparing the reconstructions to each other and the intact ACL showed that at all angles of knee flexion, the CI areas of the intact ACL, including those of both the bundles, significantly overlapped the CI areas of both reconstructions and their individual grafts. The CI areas of the force orientation vector for each reconstruction, and each AM and PL graft CI overlapped each other as well.

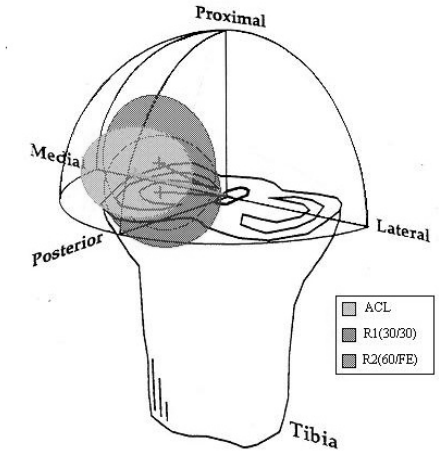

Figure 2. *In situ* force direction and confidence interval area of intact ACL and two different Fixation protocols for DB-ACLR at full knee extension and under a 134N anterior tibial load.

The CI areas for the intact ACL and its AM and PL bundles are also found to be noticeably smaller than the CI areas for the ACL reconstructions and individual grafts, with neither reconstruction having a consistently larger CI area than the other. The area of the CI for the intact PL bundle and the PL grafts become larger at higher angles of knee flexion, as the area of a CI is largely affected by the standard deviation. The overlapping of the CI areas shows that a difference is not seen between these reconstructions attempting to reproduce the intact force orientation of the ACL.

Angle Deviated from Intact

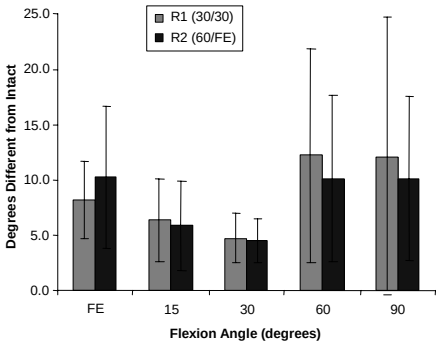

Figure 3. Degrees of deviation from intact ACL *In situ* force orientation vector of two different fixation protocols DB-ACLR under a 134N anterior tibial load.

It was seen that the angles formed between the *in situ* force orientation vector of the intact ACL and each reconstruction were similar regardless of fixation protocol. At 30° of knee flexion and under a 134N anterior tibial load, the resulting direction of the force of R1 deviated from the intact ACL direction by 4.8±2.2° while R2 similarly deviated by 4.5±2.0°. The greatest difference in orientation angle occurred at 90° of knee flexion for both reconstructions, with the difference in R1 being 12.2±12.5° and R2 being 10.2±7.4°. No significant difference was found between each reconstruction trying to mimic the intact ACL force orientation angle.

Similarly, the AM and PL grafts showed no significant difference between the separate fixation protocols. These angles ranged from 13.7±9.1° for the AM graft of R1 and 18.2±12° for the same graft in R2 with the knee at full extension to an angle of 12.9±10.5° and 11.6±6.0° for the AM grafts of R1 and R2 respectively at 90° of knee flexion. The PL grafts' force orientation did show a large direction difference from that on the intact PL bundle at high angles of knee flexion. At 90° of flexion, The PL graft of R1 gave a force direction difference of 94.7±40.3° while R2 provided a difference of 87.2±37.9°. The large deviation between the PL graft and the intact PL bundle force direction is accompanied by a large standard deviation, and still provides no significant difference between the two fixation protocols.

DISCUSSION

The importance of research into graft force orientation is that a reconstruction that improperly mimics the intact ACL may place undue constraint on a knee. The consequences of a reconstruction force angle that is too high are an ACL graft that is less efficient in resisting anterior tibial load and a possible increase in cartilage contact stress in the ACL reconstructed knee. A deviation angle of a graft that is higher than that of the ACL might explain an over-constrained internal tibial rotation observed after ACL reconstruction. Failure to recreate intact ACL function may contribute to a biomechanical environment that may predispose the knee to early degenerative changes after surgical reconstruction.

We have seen that in terms of *in situ* force orientation, no significant differences exist between using a fixation protocol for DB-ACLR where both grafts are fixed at 30° of knee flexion and one where the AM graft is fixed with the knee at 60° of flexion and the PL graft at full knee extension. Both reconstructions and their respective grafts deviated by similar amounts from the force orientation of the ACL and its individual bundles. Although this method of analysis alone does not give any consideration to the direction that each reconstruction and graft deviated from intact, when it is considered with the overlapping confidence intervals it can be determined that any difference in the direction with which the reconstructed force orientations deviated from intact are not found to be significantly different. This would suggest that angles for knee flexion at graft fixation in DB-ACLR have little effect on the direction of the *in situ* force for the reconstructed ACL and the AM and PL grafts. The importance that proper force orientation of the ACL reconstruction plays in the kinematics and healing of the knee is still in question and it still may be a factor that affects knee kinematics after ACL reconstruction. We have speculated that choice of insertion site for the graft tunnels on the femur and tibia may have a larger effect on the direction of the *in situ* force vector in DB-ACLR, although differences in tibial insertion site have not been shown to have a significant effect on reconstructed knee kinematics.

The large variance in the PL graft for both reconstructions may be considered unimportant because the PL is found to be under little or no load under high angles of knee flexion[6] when a large difference in orientation angle can have little effect. It also explains a large CI area for the PL grafts at high angles of knee flexion because bundles acted upon by smaller forces will have large CI's.[5]

A larger sampling of specimens and testing of different protocols of knee flexion for graft fixation is still required to determine the feasibility of the grafts to reproduce the force orientations of the intact ACL and its individual bundles. Eventually, force data from *in vivo* studies will provide more accurate *in situ* constraints to better test the ability of the DB-ACLR to reproduce the force orientation vector of the intact ACL.

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I was born on March 15, 1984 in Pittsburgh and was an only child for approximately 11 minutes of my life at which point my twin sister Kara was born. I graduated from Baldwin High School, which is in the South Hills of Pittsburgh in 2002. While in high school I was on the varsity track, indoor track, and cross country teams. I also rode horses and did competitive dance for many years.

In August of 2002 I traveled westward across many miles of cornfields to South Bend, Indiana, which is where I have spent the majority of the past three years. In South Bend I attend the University of Notre Dame where I have a preprofessional major and a science, technology, and values minor. At Notre Dame I am a member of the Premedical Honor Society and I participate in the premedical clubs. Additionally, I have been the Women's Running Club president since spring of my freshman year. I finished my first Chicago Marathon last fall and am hoping to finish more in the future.

Working at the MSRC this summer has been an excellent educational opportunity for me. I would like to thank Dr. Woo, Dr. Debski, and everyone at the MSRC and University of Pittsburgh Bioengineering Department for making this summer program possible. I would especially like to thank Michael Fu M.D. and Steve Abramowitch Ph.D. for their guidance and patience throughout the summer. Finally, I would like to thank all the doctors in the MSRC for looking at all the summer students' injuries.

EFFECT OF BIOSCAFFOLD TREATMENT ON COLLAGEN CONTENT IN A HEALING PATELLAR TENDON DEFECT

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INTRODUCTION

The anterior cruciate ligament (ACL) acts as a critical knee stabilizer for anterior and rotational motion of the tibia with respect to the femur. ACL injuries are prevalent and occur at a rate of about 150,000-200,000 in the US per year [1,2]. Another issue of concern is the ACL's poor healing potential when ruptured [3]. Conservative methods are often unsuccessful, thus, ACL reconstruction using an autograft is usually required [4].

The central third of the patellar tendon is a commonly used autograft because it has structural properties that are comparable to the ACL and allows for rigid fixation and bone to bone healing[5,6]. The defect does not heal well even years later and thus donor site morbidity is a common problem with the procedure and occurs in 40-60% of patients [7]. Complications include: anterior knee pain, disturbances in anterior knee sensitivity, inability to kneel, decreased quadriceps strength, decreased range of motion, patellar fracture, patellar tendonitis, patellar baja, and arthrofibrosis [5,7,9]. The repair tissue of the patellar tendon defect has been found to be not comparable to normal patellar tendon in structural, mechanical, histological, and ultrastructural properties [8] and the resulting change in the morphology and mechanical properties may cause associated problems [5].

Functional tissue engineering methods have been utilized to enhance the properties of healing tissue through the use of growth factors, gene transfer, cell therapy, scaffolding, and mechanical factors. It has been shown that the use of bioscaffold can promote cell migration to enhance the revascularization and repair. It has been shown that the bioscaffold porcine small intestinal submucosa (SIS) has resulted in improved mechanical, morphological, and biochemical composition, in addition to denser, more organized collagen fibrils in the medial collateral ligament [10].

Preliminary histology has shown a greater amount of tissue and collagen staining in the SIS-treated than in the non-treated at both 6 and 12 week time points. Additionally, the collagen matrix is more organized and there is the appearance of spindle cells in the SIS-treated tissues. The non-treated tissue is less dense and less aligned.

OBJECTIVES AND AIMS

The primary aim of this study is to quantify the collagen content in patellar tendon defects treated with SIS in the rabbit model at 6 and 12 weeks. It is hypothesized that the SIS-treated patellar tendon defect will have a higher total collagen concentration than the non-treated group.

MATERIALS AND METHODS

Twelve skeletally mature New Zealand White rabbits were used for this study. Four were used in each group with the three groups being: SIS-treated 6 weeks, SIS-treated 12 weeks, and non-treated 12 weeks. A mid-line incision about 3 cm long was made in both hind legs. An incision was made in the peritenon over top of the patellar tendon in the left leg, which served as the sham-operated control. In the right leg, the central third of the patellar tendon was removed without bone plugs. Two strips of SIS (one layer over the fat pad side and one layer over surface) were sutured in the animals that served as the SIS-treated group. The defect was left untreated in the non-treated group. The rabbits were euthanized at 6 and 12 weeks, at which point gross observations and dimensional data was recorded.

The hydroxyproline assay indirectly measures the amount of collagen in the sample by measuring the amount of hydroxyproline, an amino acid found primarily in collagen [11]. Samples were dried in a thermosavant for 8 hours. The samples were digested in 2 mL of papain in a water bath overnight at 60°C. 200 μL of 4N NaOH was added to aliquots of the samples, and the samples were autoclaved for 45 minutes. Upon removal 200 μL of 4N HCl was added followed by 1.2 mL of Choloramine-T reagent (1.41 g Chloramine T, 20.7 mL distilled water, 26 mL isopropanol, and 53.3 mL stock buffer) and left to incubate at room temperature for twenty minutes. 1.2 mL of Ehrlich's reagent (15 g Ehrlich's, 60 mL isopropanol, and 26 mL perchloric acid) was added and the samples were placed in the water bath at 65°C for twenty minutes. The absorbance was measured at 550 nm. The amount of hydroxyproline was calculated by means of a standard curve using a collagen standard. The total error in the assay was experimentally determined to be +/- 9%.

RESULTS

Upon gross analysis of the tissue, the non-treated defect still had a concave appearance at 6 weeks. Additionally, there was not enough tissue in the 6 week non-treated samples to perform collagen analysis. The SIS-treated group had a denser, less concave appearance at both time points in comparison with the non-treated. The width of the defect tissue was significantly greater ($p<0.05$) in the SIS-treated at both time points, while tissue shrinkage was observed in the non-treated samples. The length of the defect tissue is also larger in the SIS-treated tissue, however there is no significant difference ($p>0.05$) for either time point.

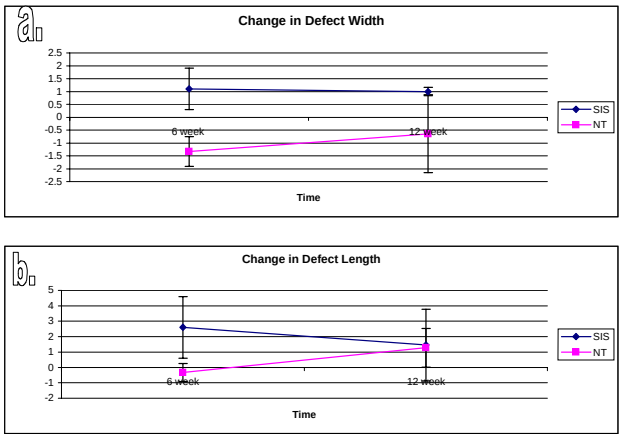

Figure 1. Defect Dimensions. a. The width of the defect tissue in the SIS-treated tissue and the non-treated tissue relative to time zero. b. The length of the defect tissue in the SIS-treated and non-treated relative to time zero.

The collagen concentration of the normal tissue and sham-operated control were the greatest at 95+/-10% and 90+/-10% respectively. The 12 week non-treated and SIS-treated had the next highest concentrations at 65 +/- 20% and 60 +/- 15% respectively. The SIS-treated 6 week tissues had the lowest concentration at 40 +/- 15%. The non-treated 6 week samples did not have enough tissue to test. There is no significant difference between the non-treated and the SIS-treated at 12 weeks ($p=0.38$). There is a non-statistical increase between SIS-treated 6 weeks and SIS-treated 12 weeks ($p=0.25$). However there is a significant difference in collagen content between the sham and all of the treated groups ($p<0.05$).

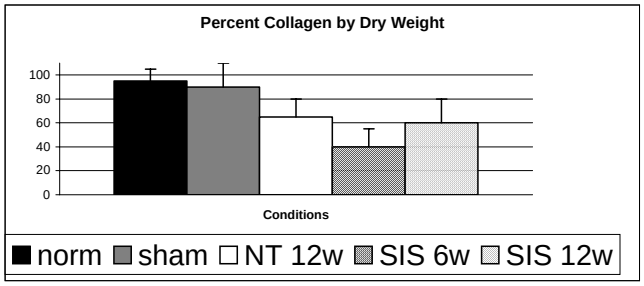

Figure 1. Collagen Content Normalized to Dry Weight.

DISCUSSION

The collagen content demonstrated that both treated groups had significantly lower collagen concentration from the sham tissue, demonstrating that the defect tissue is not comparable to the native tendon tissue in either treated group. This observation was consistent with gross analysis and histology. Our results with the hydroxyproline assay for the normal tissue (95+/-10%) were consistent with previously published data for the patellar tendon (86.72 +/-8.9%) [12]. The hydroxyproline assay was consistent, but was not sensitive enough to measure the small differences between the treated groups accurately. Additionally, since the tissue did not all come from the same portion of the tendon, the results could have been effected by the origin of the tissue tested.

Of particular interest, the results of this study suggest that while there is a greater amount of tissue in the healing defect of the SIS-treated vs. the non-treated as demonstrated by the histology, dimensions data, and gross analysis, the collagen composition of the tissue does not appear to be significantly different between the two treated groups. Therefore, although the SIS treatment does not increase the collagen concentration to a composition closer to that of the native tissue as hypothesized. However, it does produce a greater quantity of tissue of a similar quality to the non-treated tissue, thus obtaining a greater total amount of collagen. The increase in quantity of the healing tissue could improve the function of the healing tendon.

Future studies will aim to determine methods to enhance the healing and to further elucidate the biochemical and mechanical properties of the healing tissue in order to determine ways to enhance the quality of the tissue. Studies will also aim to enhance the SIS scaffold, through methods such as mechanical stretching and invitro conditioning of the scaffold to improve its healing qualities. These further studies will be done in order to reduce the occurrence of donor site morbidity caused by the patellar tendon defect.

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I would like to thank my mentor, Dr. Yin-Chih Fu for his guidance. I additionally would like to thank Dr. Woo and Dr. Abramowitch for giving me this opportunity to work at the MSRC this summer. I additionally would like to thank all members of the MCL group, the MSRC, and the University of Pittsburgh Bioengineering Department for making this opportunity such a great and educational experience.

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Before Punxsutawney Phil awoke to predict the weather on February 2, 1984, I was born as the first of three girls. I spent most of my childhood near Greensburg, PA and attended Penn-Trafford High School, graduating in 2002. While there, the experiences I had in a sports medicine class and as a student athletic trainer aide confirmed that a career in the field of medicine was for me.

I'll complete the first step on my way to becoming a doctor/medical researcher in May 2006 when I graduate from Bucknell University. Academically, I have been published as a result of research in polymer chemistry, am completing a minor in psychology, and am a member of the Order of Omega. Also at Bucknell, I have developed an interest in biomedical engineering and pursued it by serving as Bucknell BMES Chapter's secretary for 3 years. Outside of academic work, I am a sister in Alpha Chi Omega Sorority and am a member of the Bucknell Women's Rowing Team. In 2004, after my team placed 9th in the U.S., we were given the honor of representing Bucknell at the Women's Henley Regatta in England.

My time at the MSRC has strengthened my curiosity for biomedical engineering and my desire to enter the medical field, especially due to the interdisciplinary work occurring here. I am very grateful to have been invited to work in biomechanics and experience another genre of medical research; thank you Dr. Woo and Dr. Debski. A huge thank you to my mentors, the ACL group, and Susan Moore for their guidance and patience during my learning/research experience. Thanks MSRC!

THE REPEATABILITY OF A REGISTRATION BLOCK IN THE MEASUREMENT OF PATELLAR MOTION

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INTRODUCTION

As a sesamoid bone, the patella's path of motion varies due to the influence of the soft tissue restraints, bone geometry, and quadriceps angle. Apart from obvious odd positions of the patella, subtle abnormalities are difficult to diagnose since a normal patellar path has yet to be determined [1]. Clinically useful x-ray systems were first used to track the motion of the patella [2], however, other more precise systems have been developed: electromagnetic [3], video [4], MRI, and computed tomography [5] systems. In our research facility, future studies may use a robotic manipulator in order to increase repeatability and accuracy of the movement of the knee joint as compared to previous joint manipulators. Thus, a system that can be used in conjunction with the robot must be developed. The system presented here utilizes a registration block to create a local coordinate system; this methodology has been useful in the kinematics measurements of other joints, with accuracies of less than ±0.1mm and ±0.1° [6,7].

OBJECTIVES AND HYPOTHESES

This study explored the (1) intra-observer repeatability associated with the measurement of discrete kinematics, by digitizing a registration block, of a sesamoid bone and (2) the intra-observer repeatability of the entire methodology, a combination of the digitizing and robotics system, both as functions of the tension in soft tissue restraints.

The greatest repeatability is hypothesized to occur upon the application of larger quadriceps load at the highest knee flexion angles, since research has shown tracking to be most variable at low flexion angles [5].

METHODS

Preparation and Femoral anatomic system: Porcine knees were stored at -20°C. After thawing at room temperature for 24 hours and securing the fibula, the soft tissue more than ~10cm from the knee was removed. A plexiglass registration block was rigidly attached to the patella using a screw and a nylon strap was sutured to the distal part of the quadriceps tendon in order to apply an external quadriceps load. The tibia and femur were embedded in epoxy and attached to the testing device, a robotic manipulator (KUKA KR210 Robotics Corp., Stearling Heights, MI) (position, orientation repeatability = ±0.1mm, ±0.1°). The femur was rigidly fixed to a grounded pedestal while the tibia was attached to the end-effector of the robotic/UFS manipulator. After a clinician marked the insertion sites of the MCL and LCL, these points, as well as 4 points proximally and distally around the femur, were digitized with a Microscribe-3DX spatial digitizer (position accuracy = ±0.23mm, Immersion Corp., San Jose, CA, USA). These digitized anatomic landmarks were used to create the femoral coordinate system (Figure 1); the method is described in previous work [8]. The positive directions of the x, y, and z axes of a right knee are defined as medial, superior, and anterior respectively. The x axis is positive in the lateral direction in a left knee.

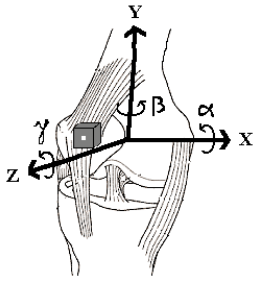

Figure 1. Coordinate systems of femur on right knee (X=medial, Y=proximal, Z=anterior, α=flexion/extension, β=medial/lateral tilt, γ=medial/lateral rotation) and general placement of the registration block

A path of passive flexion/extension was found. A high load, 200N, and low load, 10N were applied to the quadriceps strap through a pulley system to create tension in the soft tissue restraints, simulating muscle loading of the knee [9] and muscle tonus. Dehydration of the tissues during testing was prevented with 0.9% saline solution.

Intra-observer repeatability of registration-block-measured discrete kinematics of patella as a function of soft tissue restraints: In one specimen, the repeatability of the use of a registration block to digitize the position of the patella with the microscribe was evaluated. At each measured flexion angle (30°, 45°, 60°, 90°), 10N was applied to the quadriceps tendon and the equilibrium position (where forces in the knee are minimized) was found by the robot. As the robot held the knee in this equilibrium position, three orthogonal faces of the patella registration block were digitized ten times each. The process of loading the knee, finding the equilibrium position, and digitizing was repeated at each flexion angle. This methodology was also performed with the application of a 200N external load, allowing evaluation of the intra-observer repeatability with different soft tissue tension.

Intra-observer and inter-specimen repeatability of digitizing and robotics methodology as a function of soft tissue restraints: To evaluate the repeatability of the entire system, the equilibrium position of the knee was found under the tension created by 10N at 30°. At this position, the block was digitized as described, but only once. Upon releasing the soft-tissue tension, this was repeated at the other flexion angles. With the positions established, the knee was randomly cycled, loaded, and block digitized through a total of ten sets of the four flexion angles.

Calculations: Local coordinate systems, in the microscribe global reference frame, were created on the patellar registration block, following the method described previously [6]. Since the position and orientation of the femoral anatomic system was fixed in respect to the microscribe global system throughout the test, the transformation from the femoral anatomic system to the patellar registration block at each angle and condition could be determined. This transformation provides the relative motion of the patella since the patellar block's rigid fixation allowed it to move as the patella moved. The rotation sequence used to find the orientation of the

patellar block system in the femoral anatomic system was as follows: rotation about the femoral X-axis, rotation about the Y-axis, and rotation about the Z-axis. After decomposition of the rotation matrix, the position and orientation values were compared to the data of replicated conditions by computing the standard deviation between the repeated sets (i.e. when evaluating objective #2, the standard deviation among all of the positions and orientations at 30 degrees was calculated). Vector sums of the position deviations were also calculated (Tables 1-5).

RESULTS

The intra-observer repeatability of the registration block kinematics of patella position and orientation during the 200N condition were ±0.3 mm and ±3.7 degrees, respectively (Table 1), while in the 10N condition, they were ±0.5 mm and ±5.4 degrees (Table 2). Similarly, the repeatability was worse in the 10N condition of the digitizing and robotics methodology. Large inter-specimen variations were also observed. Specimen 1 showed a repeatability of ±0.8 mm and ±7.7 degrees (Table 4) while specimen 2 displayed values of ±4.8 mm and ±13.1 degrees (Table 5). Both specimens showed repeatability around ±0.6 mm and ±7.5 degrees in the 200N condition (Table 3).

Flexion angle	Vector Sum	α	β	γ
30°	0.3	2.2	0.3	2.0
45°	0.2	3.7	0.2	3.7
60°	0.1	1.1	0.1	1.2
90°	0.3	1.1	0.4	1.1

Table 1. Intra-observer repeatability (standard deviations [stdev.]: mm, degrees) of registration block kinematics with 200N quad. load

Flexion angle	Vector Sum	α	β	γ
30°	0.2	1.7	0.3	1.8
45°	0.1	3.7	0.1	3.8
60°	0.5	5.4	0.3	5.4
90°	0.2	1.4	0.3	1.3

Table 2. Intra-observer repeatability (stdev.: mm, degrees) of registration block kinematics with 10N quad. load

Flexion angle	Vector Sum	A	β	γ
30°	0.6	4.8	0.5	4.6
45°	0.5	7.5	0.3	7.5
60°	0.4	1.5	0.3	1.4
90°	0.5	0.8	0.2	0.8

Table 3. Intra-observer repeatability (stdev: mm, degrees) of digitizing and robotics methodology with 200N quad. load

Flexion angle	Vector Sum	A	β	γ
30°	0.8	3.9	0.4	3.8
45°	0.7	5.3	0.5	5.3
60°	0.5	5.3	0.5	5.4
90°	0.5	3.3	7.7	3.4

Table 4. Intra-observer repeatability (stdev.: mm, degrees) of digitizing and robotics methodology with 10N quad. load – Specimen 1

Flexion angle	Vector Sum	A	β	γ
30°	4.6	2.9	0.7	3.0
45°	4.5	8.4	0.6	9.2
60°	4.8	13.0	0.3	13.1
90°	4.7	2.8	0.5	2.9

Table 5. Intra-observer repeatability (stdev.: mm and degrees) of digitizing and robotics methodology with 10N quadriceps load –Specimen 2

DISCUSSION

This research was designed to determine the repeatability of the digitation of the registration block on the patella and the repeatability the digitizing and robotics methodology combined, both as a function of soft tissue tension. The repeatability of the digitizing and robotics methodology accounts for the position and orientation repeatability characteristics of the robot, while the repeatability of the registration block kinematics does not. Each methodology reflects the repeatability of the application of the load, intra-observer digitizing, digitizing stylus, and accuracy of the angle establishment by the robotic manipulator.

As expected, the more the patella falls into the trochlear groove of the femur (increased soft-tissue tensions and flexion angle), the more repeatable its position, supporting the convention of measuring the patella while loaded.

When two specimens were tested in the digitizing and robotic methodology, large inter-specimen variability under tension caused by 10N was observed. Unlike the 200N condition, the patella kinematics at lower soft-tissue tensions can vary greatly between specimens.

The registration block kinematic repeatabilities are close to prior results; yet, the values of the intra-observer repeatability in the digitizing and robotic methodology are higher than previous research reports. This is attributed to accounting for the repeatability of the entire testing system instead of just the digitizing system. Error may be caused by movement of the quadriceps strap in the pulley system. This could be minimized by the creation of a new load application system to prevent the quadriceps strap from moving between testing situations. Another source of inaccuracy may be the varying amount of pressure applied to the registration block as the researcher digitized; this error in repeatability may be improved by replacing the microscribe with a non-contact digitizer, i.e. a video system.

Thus depending on the level of repeatability desired, the system presented here can be used to provide an easy, relatively inexpensive method to measure patellar motion. Planned extensions of this project include establishing the repeatability of the creation of a patellar coordinate system to combine with the registration block method and then eventually determine the absolute position of the patella in relation to the femur.

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Born in Los Angeles on August 21, 1984, I grew up, quite literally, next to the stars of Hollywood (the stars cemented in the sidewalk on Hollywood Blvd). I lived there for the first 18 years of my life with my parents and older sister. I then moved to Pittsburgh, where I have learned and experienced so much over the last three years.

At CMU, my initial focus was chemical engineering; however, following the culmination of my first year, I realized that I was also interested in biomedical engineering. When not studying, I spend my time actively involved in a myriad of cultural and engineering organizations. I was vice president of the Taiwanese Students Association (even though I am Korean), as well as, social chair of AIChE to name a couple. I also stay active, participating in various intramural sports such as soccer, football, tennis, basketball, and volleyball. Off campus, I hang out with my friends, attend church regularly and lead the "Ninja Turtles" as Leonardo in a bowling league.

Working at MSRC was one of the greatest experiences of my life as it has reinforced my passion for the field. I would like to thank my mentors, Guoguang Yang and Rui Liang, for all their help, advice, guidance, and patience throughout this experience. I would also like to thank Dr. Woo, Dr. Debski, and Hilda Diamond for giving me this opportunity. Thank you to the doctors of MSRC, especially Ozgur for diagnosing my injury, as well as to the rest of the MSRC for making this a memorable experience.

ALIGNMENT OF RABBIT MEDIAL COLLATERAL LIGAMENT FIBROBLASTS SEEDED ON SMALL INTESTINAL SUBMUCOSA

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INTRODUCTION

Porcine small intestinal submucosa (SIS) is a biological scaffold that has been successfully used in the replacement or repair of large and small arteries, veins, urinary bladder defects, skin, achilles' tendon, cranial dura mater, and abdominal wall.[1, 2, 3, 5, 6] This bioscaffold is primarily composed of type I collagen with a preferred fiber alignment along the longitudinal axis[5]; that varies by approximately ±30° from the preferred alignment.[6] Although SIS has been shown to induce more collagen production[1], as well as, improve the mechanical properties of the MCL healing tissue by producing a more organized extracellular matrix (ECM)[4], the collagen fibrils and the ECM was still disorganized and the mechanical properties was still inferior compared to normal ligaments.

Previous studies have shown that the orientation of the cells affects the organization of the collagenous matrix produced by the cells.[8] It has also been established that fibroblasts seeded on a microgroove surface had alignment and an elongated shape, even without cyclic stretching.[9] Such phenomenon where cells lie in the same direction as the surface grooves is known as contact guidance.[7] This demonstrates that the cell environment influences the orientation of the cell. Therefore our research question was whether or not SIS with tension can bring about more collagen fiber alignment and thus more cell alignment.[2] The hypothesis of this study is that the rabbit MCL fibroblasts (RMCLFs) seeded on SIS, subjected to pretension, will align more uniformly along the elongated direction.

OBJECTIVES

To evaluate the alignment of rabbit MCL fibroblasts seeded on SIS subjected to mechanical pretension and to determine collagen synthesis of RMCLFs seeded on pretension SIS.

METHODS

Normal rabbit MCL fibroblasts P_3–P_5 were cultured in growth medium (DMEM, 10% FBS, and 1% P/S). Two experimental groups were tested: SIS with and without pretension. For the pretension group, before seeding, a 10mm^2 piece of hydrated SIS was elongated to 20% on a custom made apparatus. It was incubated and held in the stretched position overnight. For the non-pretension group, SIS dealt with the same conditions except there was no tension applied. To make sure no cells fell off the SIS during cell seeding, a custom well was made (Figure 1). Both treated and non-treated SIS was wrapped over the tube edge to form a well.

After harvest, 5x10^4 cells were put into custom made wells using the same medium as before. The wells were placed in a good culturing incubator set at 37°C, 90% relative humidity, and 5% CO_2.

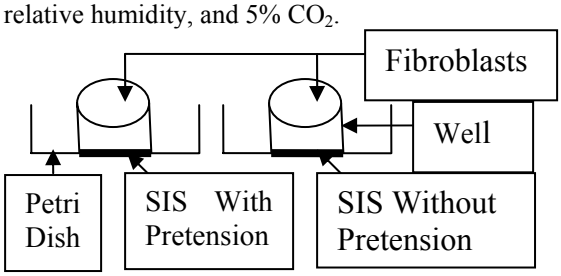

Figure 1: Custom Made Wells. SIS wrapped to bottom of custom made well and the cells, mixed in medium were seeded inside the well

Four days after seeding, the actin filaments and nuclei were stained. Briefly, the cell layers were washed with PBS and then fixed with 3.7% paraformaldehyde for 15 minutes. Then 0.25% of Triton-X 100 was used for 10 minutes to permeabilize the cells. The actin filament was stained using 0.66μM rhodamine phallodine for one hour. The nucleus was labeled using 5μM Heochst 33342 for 5 minutes. To enhance picture quality, SIS was cut from the well and placed on a microscope slide. Vectashield Mounting Medium was used to produce a better signal. Pictures were taken using Spot Advance software.

The collagen synthesis was measured using a collagen assay kit (Sircol). The supernatant from each well was collected and put in separate Ependorf tubes; then dried for ~2-4hours. To solute the sample, 0.5M acetic acid was added to each. Next, 1000μL of a dye reagent was placed in each sample and placed on a mechanical shaker for 30mins to allow the dye to bind to the soluble collagens. The tubes were then placed in a microcentrifuge and spun at >10,000 x g for 10mins. In order to drain the unbound dye solution, the tube was carefully inverted and gently tapped. Then 1000μL of Alkali reagent was placed in each sample and mixed thoroughly using a vortex mixer. Once cells were extracted, 200μL of each sample (in duplicates) was taken and put in a 96 well plate where a spectrophotometer called SpectraMax 190 was used to read the samples. Standards of 0, 10, 25, 50, and 100 were used. The absorbability was set at 540nm. Software called SOFTmax Pro was used to record the data.

RESULTS

The actin staining, as seen in Figure 2a and 2b showed SIS with pretension having more fiber alignment than SIS without. The figure also shows the cell nuclei following the direction of the fibrils with pretension.

The collagen assay showed that the SIS with and without pretension had a higher concentration of collagen than the control dish as seen in Figure 3.

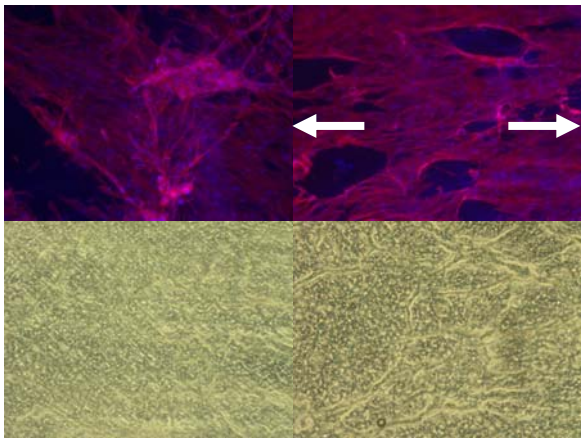

Figure 2a: Staining Results in SIS Without and With Pretension 100x. These are the pictures of the actin filaments and nuclei stained under the fluorescence microscope with the corresponding RGB pictures to see the fibrils of the scaffold. The arrows show the pretension direction.

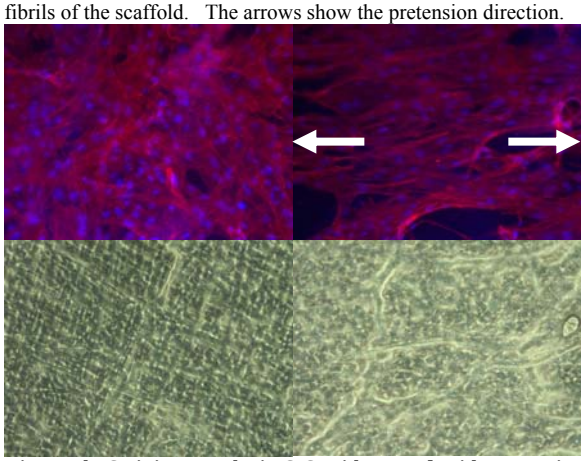

Figure 2b: Staining Results in SIS Without and With Pretension 200x. This is a zoomed in picture of 2a. The arrows show the pretension direction. When zoomed in, it is clearer to see the cell nuclei's follow the direction of the fibrils.

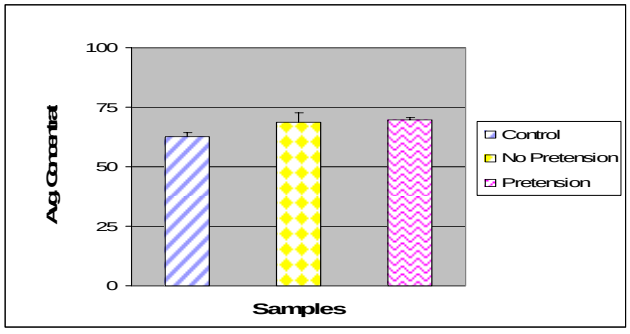

Figure 3: Collagen Assay Results. This collagen assay diagram shows that the average concentration of collagen in SIS with and without pretension was more than the control.

DISCUSSION

Unlike the RMCLFs seeded on SIS without pretension, those seeded on SIS with pretension showed more uniform alignment, which comply with the concept of contact guidance. This also provides further evidence that fibrils are more aligned under pretension.

Previous studies have compared SIS (without pretension) versus non-treated MCL injured sites *in vivo*; these studies have reported that SIS can improve the mechanical properties by 33%; however, the strength of the healing tissue was still much lower compared to normal ligament.[4] Therefore, the fibrils were aligned *in vitro*, through various functional tissue engineering methods; this can potentially improve the effects of the scaffold when applied *in vivo*. Our pretension study indicated the significance and the possible outcome of remodeling the SIS bioscaffold through mechanical stimulation, i.e. stretching.

From the collagen assay results, SIS can induce more collagen production when under tension; however, there are some factors that might influence these results. In our experiment, we used 10% FBS in the medium; however, to get significant data, it is recommended that less than 5% FBS be used. Experiments were conducted by our group to understand the influence of 10% FBS; the results are shown in Figure 4. When taking the ratio of pretension SIS over 10% FBS, it is relatively small; however, if a lower percentage of FBS was used, this ratio can potentially be much higher, which would show more significance in the assay of collagen production. However, based on our findings, we could conclude that SIS with tension did not suppress the production of collagen with fibroblasts seeded on it.

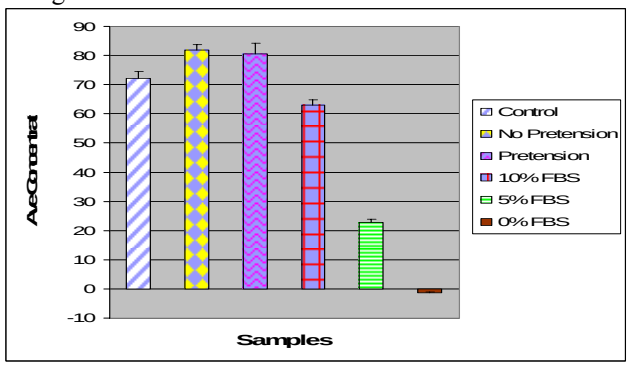

Figure 4: Influence of FBS on Collagen Assay. 10% FBS has a major influence on the results.

Future studies will need to test the affects of cyclically stretching SIS with cells seeding. Also, other cells, i.e. stem cells, would be applied to see whether the scaffold, under tension, is suitable for various cell types.

ACKNOWLEDGEMENTS

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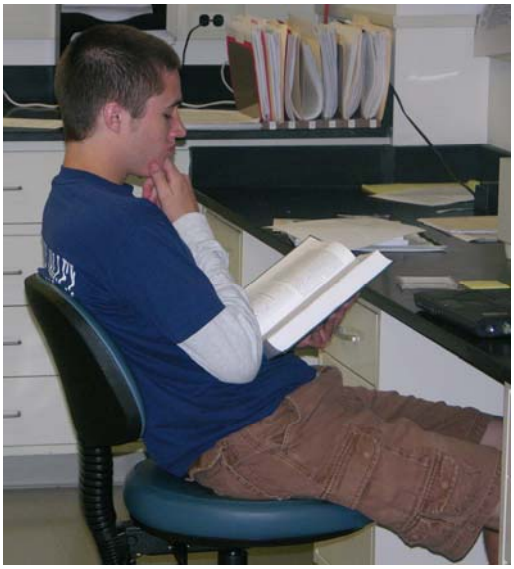
SUMMER 2005 SNAPSHOTS

Dana, Jason and Amanda

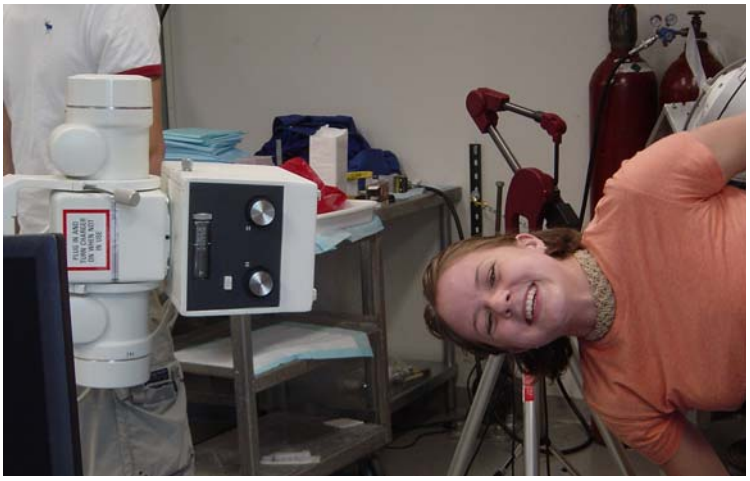
Caitlyn hard at work

Dana cookin' up something good



Mike sleeping or reading…we'll never know

Enjoying the food and entertainment at Fox Chapel Racquet Club



Whoa, Amanda is parallel to the floor

Mentors after half a day with mentee

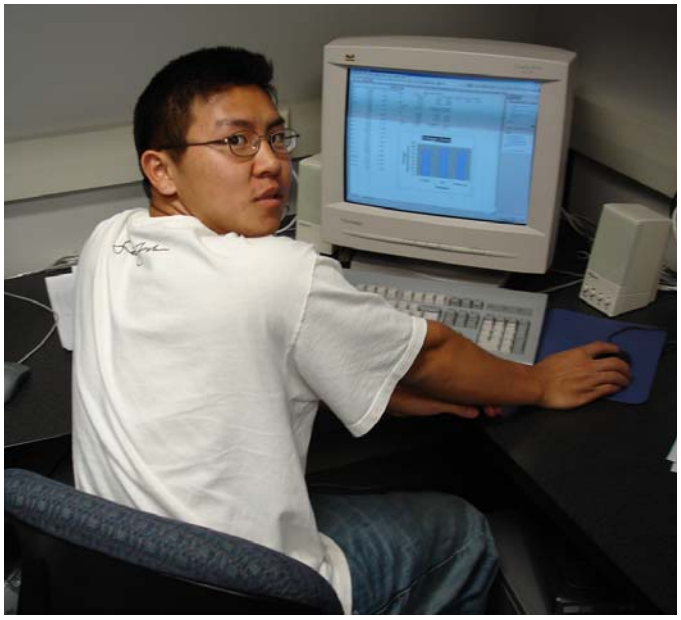
Shawn working hard…like a plumber

David and his big, deformed arm

Fabio finding out about cookie bouquets for the first time from Dana

MSRC having fun

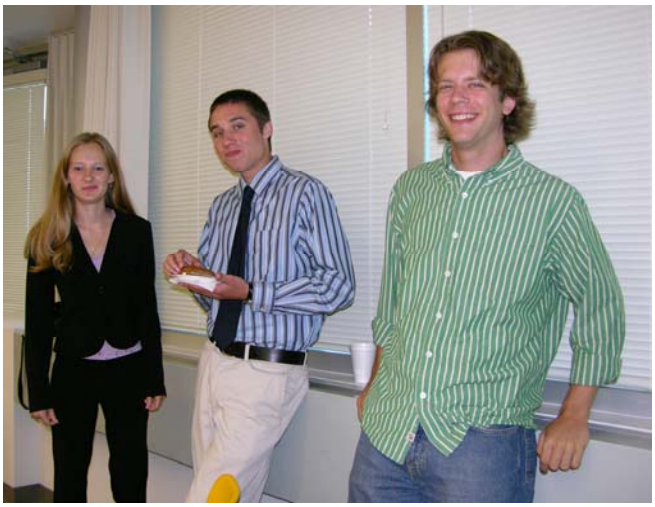
Niki and Eric having fun around the computer



David's having fun around the computer too

Amanda washing up after hard days work

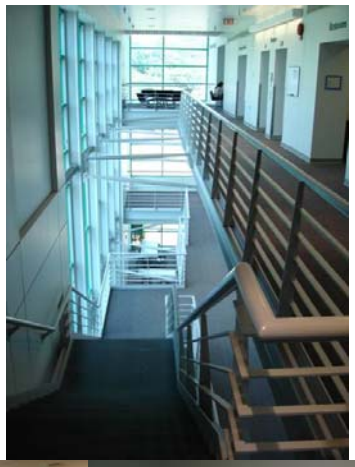
Mischievous Sabrina operating robot…
Ozgur and Amanda unaware

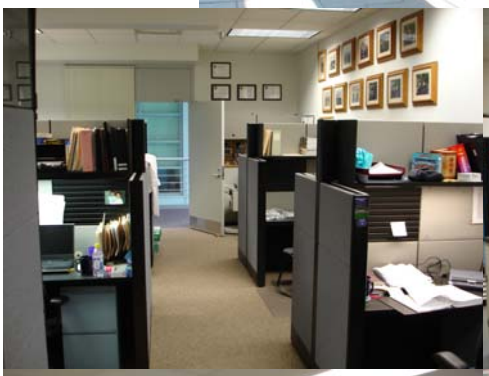


Niki, Mike and Mark

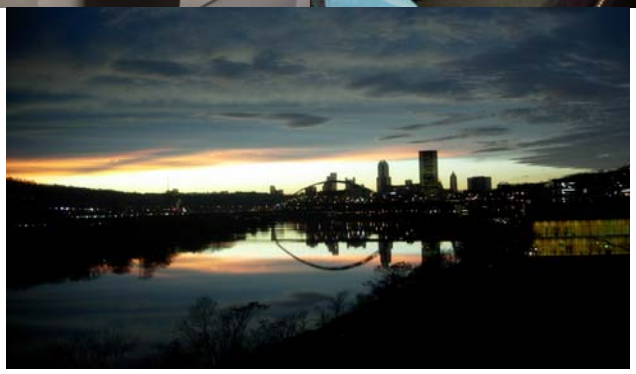
Everyone at MSRC

The new center…





THANK YOU MSRC!!!



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