Musculoskeletal Research Center

Summer Research Program

MSRC

2003

University of Pittsburgh

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2003 EDITORS

Editors: Katie Dillon and Hilarie Stern

If we had to sum up this year's group of summer students in one word, we would have to choose the word "diverse". We vary in our educational backgrounds as well as our hometowns, with our homes spanning from Saudi Arabia to California to Pennsylvania and several places in between. However, our diversity allowed us to fit neatly into the MSRC, where we had the opportunity to interact with people from all around the world.

While learning about the demanding world of research, we learned more about our own strengths and weaknesses as well as the importance of teamwork. The lessons we have learned while working at the MSRC are more valuable than any instruction in a classroom, and we will carry these lessons with us throughout our lives.

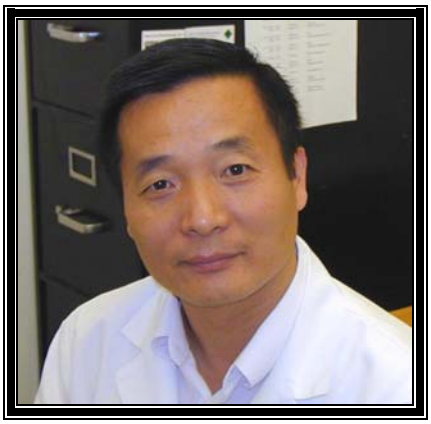
We would like to thank our mentors and advisors for their help and patience throughout the summer. We would also like to thank Dr. Debski for making the summer research program a great experience. We would finally like to thank Dr. Woo for giving us the chance to learn about research firsthand this summer. We will always remember the words of wisdom he imparted on us and the level of excellence he inspired in us this summer.

The MSRC Faculty

Savio L-Y. Woo, Ph.D., D.Sc.
Ferguson Professor & Director

Patrick McMahon, M.D.
Associate Director

Richard E. Debski, Ph.D.
Director, Summer Research Program



James H.C. Wang, Ph.D.
Faculty Mentor

Zong-Ming Li, Ph.D.
Faculty Mentor

Foreword

Under the leadership of Dr. Richard Debski, our Summer Undergraduate Research Program continues to blossom. A really great bunch of undergraduate students with diverse backgrounds in neuroscience, biology, chemistry, exercise science, bioengineering, and mechanical engineering joined us. They came from as close by as Pittsburgh and from as far away as Saudi Arabia. In a brief period of 12 weeks, these students have learned. Their words on "deep respect for the research process and scientific matters" were spoken clearly – all music to my ears.

During the Annual Symposium on July 29, 2003, all thirteen (13) students gave impressive presentations. It was clear to everyone who attended how sophisticated they are. Their ability to disseminate knowledge with clarity and poise, and more importantly, answering all questions from the audience with confidence stand out clearly in my mind. I recall specifics such as understanding matrix transformations, appreciating literature, developing specific software on complex geometries, learning the theory, detecting specific problems and finding appropriate solutions, error analysis, taking the pain to go through step-by-step examination of the repeatability of measurements, looking into the complexity of molecular biology, and specialized analytical techniques to quantify histological and biochemical analyses, and so on. Meanwhile, others learned statistics, complex viscoelastic analysis of tissues, and the use of modern technology for studying joint motion and tissue strength. They all told me their most impressive and beautiful stories of accomplishment.

Our program continues to have a strong collaboration with Carnegie Mellon University, through Ms. Hilda Diamond, as well as with the Pittsburgh Tissue Engineering Institute and the Department of Bioengineering at the University of Pittsburgh. We thank them for providing financial support for some of our students. The financial support by faculty advisors is also gratefully acknowledged. Even more importantly, the mentors for our students deserve special thanks as they are the reason for the success. Thus, to Mr. Jesse Fisk, Dr. Xinguo Ning, Mr. Daniel Moon, Ms. Maribeth Thomas, Mr. Shon Darcy, Ms. Charu Agarwal, Dr. Fengyan Jia, Dr. Yoshiyuki Takakura, Ms. Sarah Brown, Mr. Souchen Dun, Mr. Steve Abramowitch, Dr. Chris Ugobulue, Ms. Susan Moore and Mr. Danny Harkness, my heartfelt gratitude for their tremendous efforts and dedication. The staff of MSRC also deserves special recognition for their significant contribution to this program. My hat's off to Dr. Richard Debski, Dr. Zong-Ming Li, Dr. James Wang, and Dr. Patrick McMahon for their leadership. As our summer students head to medical school and dental school, or switch to the new field of biomechanics (this is a new one!), or return to their undergraduate studies in bioengineering, mechanical engineering, chemical engineering, or material science, we know that they are now much more mature investigators than they were only three months ago. I would like to personally invite each and every one to come back to the MSRC to be a part of our research family. My hope is that many of them will be working with us in future years. Indeed, the Summer Undergraduate Research Program continues to be my favorite program at the MSRC!

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I was born at Magee-Women's Hospital in Pittsburgh, PA on May 11, 1983, and have lived in Belle Vernon, PA all of my life. I attended Belle Vernon Area High School, where I was very involved in athletics and various clubs and organizations. I played and lettered in varsity soccer for two years, varsity basketball for four years and in varsity softball where I was the starting pitcher for all four years.

I am currently a junior bioengineering major at the University of Pittsburgh. I am a member of The National Society of Collegiate Scholars, Phi Eta Sigma National Scholastic Honors Society, The Society of Women Engineers, The Biomedical Engineering Society, and the Freshman Engineering Leadership Team (FELT), where I tutor freshman engineers in the areas of calculus, physics, and chemistry.

In my spare time, I enjoy working out at the gym, being outdoors, going to concerts, skiing, and hanging out with my friends. I am also a PIAA and ASA softball umpire.

I would like to thank my mentor, Dan Moon, for all of his encouragement and guidance this summer. In addition, I would like to thank Dr. Woo and the rest of the MSRC staff for all of their direction and support. My summer research at the MSRC has helped me to enhance my educational experience at the University of Pittsburgh. I will always be grateful and never forget this rewarding opportunity.

EVALUATION OF THE CURRENT LASER MICROMETER SYSTEM TO MEASURE THE CROSS-SECTIONAL AREA OF SOFT TISSUES

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INTRODUCTION

The accuracy of determining cross-sectional area (CSA) plays an important role in evaluating the stress-strain relationships of soft tissues, most importantly in the calculation of ultimate tensile strength and tangent modulus. Some methods of determining CSA are with measurements by rulers or calipers [1], the constant-pressure area-micrometer [2] and the molding technique [3]. However, due to direct contact with the specimen, these methods may yield inaccurate results because of specimen distortion or user subjectivity.

In our laboratory, the laser micrometer system was developed as an accurate non-contact method of determining the CSA of soft tissues [6]. In this system, a specimen is rotated in the field of a collimated laser beam while diameter and reference distance are measured and recorded. Using these parameters, an algorithm is then used to reconstruct the cross-sectional shape (CSS) and calculate CSA.

Recently, the original system was replaced with a modified version in which the laser rotates around a stationary specimen. The advantages of the new system are that it eliminates specimen vibration and takes faster measurements, minimizing the specimen's exposure to air and dehydration. The new system was found to have an accuracy for CSA determination of 0.5% for a circle, 1.8% for a square and 10.7% for a triangle [4].

It was discovered this past year that the new laser micrometer system was not reconstructing CSS correctly and calculating CSA accurately. The objective of this project was to determine the source of this error and correct it. In order to accomplish this goal, the theory, algorithm and raw data files obtained from both laser micrometer systems were investigated.

METHODS

The new laser micrometer system uses a stepper motor (Stepper Motor HT23-401, Applied Motion Products, Inc) to rotate a laser transmitter and receiver around a stationary specimen. The system utilizes the shadow method, where a specimen's obstruction of the laser beam produces a shadow on the receiving end of the laser [5]. The shadow gives rise to two parameters, reference distance and profile width (Figure 1), which can be used to reconstruct the CSS of the tissue.

Several programs written in Visual Basic (Visual Basic 5.0, Microsoft) allow the user to send commands to the motor driver, collect data from the laser (LS-3060, Keyence Corp.) and determine the CSA and CSS from the data collected.

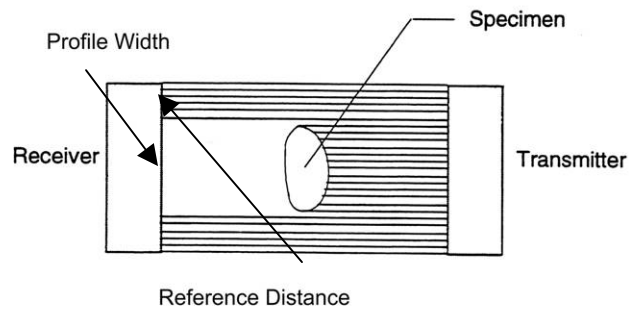

Figure 1: Laser transmitter and receiver with parameters profile width (shadow width) and reference distance [4].

Since the accuracy of the new system appeared to deteriorate as the specimen was moved off center within the laser field, it was originally suspected that the source of error was within the algorithm itself. Specifically it was postulated that the algorithm for the old system did not apply to the new system. Therefore, investigation of the theory behind the image reconstruction, specifically the calculation of the COR, R1 and R2, were the first steps taken in analyzing the system. Correlation of the theory, algorithm, program code, hand calculations of parameters, and reconstruction of CSS in Microsoft Excel was done in an attempt to locate any error within the algorithm. Identical raw data files were also entered into both the old and new systems to ensure that the algorithm had not changed.

The investigation of the system's theory and algorithm showed that the old system's algorithm does in fact apply to the new system, no transfer error had occurred and that centering within the laser field should not affect the new system's accuracy.

In order to determine if the error was due to the method in which the new laser collected data, the CSA was determined of a 0.5-inch wide square using both the original and new systems. A square was chosen because the maximum and minimum profile widths should be 45-degrees apart from each other when profile width is graphed verses the increment angle. These graphs were extrapolated from the raw data files and comparisons of the two systems were made (Figure 3).

When the graphs were extrapolated from raw data files obtained from the two systems and superimposed onto one another, the new system's data did not match what should be seen for a square CSS (Figure 2).

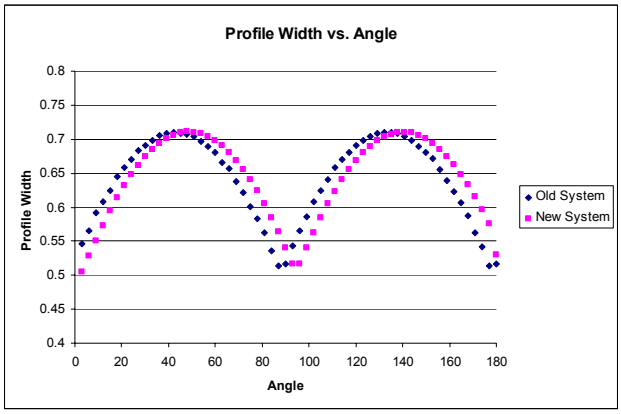

Figure 2: Graph of profile width vs. angle for both the old and new systems.

More detailed inspection of these graphs revealed that the new laser micrometer system was not rotating in increments of 3 degrees because 45-degrees did not separate the minimum and maximum profile widths. Upon further investigation, the laser was found to be rotating in 2.7-degree increments to give a total 162 degrees of rotation, instead of the 180 degrees that the algorithm assumes.

Investigation of the Quick Start program, a program that controls the motor, led to the evaluation of several parameters within the motor program including: motor resolution, step size and number of steps taken for each increment. By taking the motor gear ratio (4:1) into account, calculations were made to determine that the correct amount of steps the motor should move per increment is -6.66 steps. The step increment was then increased within the motor subprogram from -6 steps to -6.66 steps per increment.

To test the accuracy of the system before and after changes were made, geometric shapes of known CSS and CSA were placed at various points within the field of the laser. The three geometric shapes used were a 126.68 mm^2 circle, a 162.2 mm^2 square, and a 212.78 mm^2 triangle. The percent error between the experimental areas and actual areas were then calculated and compared both before and after changes to the motor program were made. The accuracy of the system with specimens centered and off centered was also assessed to see if specimen orientation within the system had any effect on the accuracy. These values are shown in Table 1.

A femur-medial collateral-ligament complex (FMTC) was dissected out and its CSA measured in order to evaluate the performance of the new system with biological specimens. Ten measurements were taken at the midsubstance of the medial collateral ligament (MCL) at various orientations within the field of the laser.

The average CSA given by the new laser micrometer system for the rabbit MCL was 3.42 ± 0.07 mm^2. The typical result for CSS of a rabbit MCL is shown in Figure 3.

	% Error (Before)	% Error (After)	% Error (Before)	% Error (After)
	Center		Off Center	
Circle	0.247 %	0.145 %	1.182 %	0.434 %
Square	2.57 %	0.966 %	2.081 %	1.361 %
Triangle	17.88 %	6.54 %	14.46 %	6.29 %

Table 1: Percent errors of CSA measurements of circle, square and triangle positioned in the center and off center before and after the motor program change.

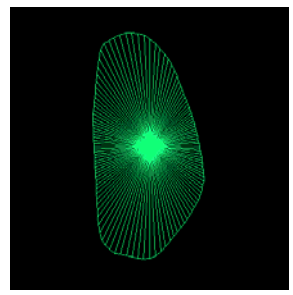

Figure 3: A typical rabbit MCL CSS given by the new laser micrometer after the motor program change.

DISCUSSION

The accuracy of CSS reconstruction and CSA calculation were successfully restored by changing the number of steps the laser moves per increment in the Quick Start motor program. After the program change, the accuracy of the CSS and CSA determination increased for all three shapes. The new laser micrometer's calculated CSA of the rabbit MCL reflects previously reported historical data and the reconstructed CSS also correlates with those shown in histological cross-sections of MCL tissue [7]. It is suspected that the error within the motor program arose when the computer that ran the system crashed. At this time, all parameters had to be reprogrammed, and the step size had been entered incorrectly.

As previous studies have already mentioned, the main limitation of the laser micrometer system is its inability to detect concavities and accurately reconstruct the CSS of shapes with sharp corners and angles, such as triangles. However, most biological tissues tend to be rounded with few concavities, hence making the laser micrometer system well suited for reconstructing CSS and determining CSA of biological tissues [4, 6, 7].

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I was born August 15, 1983 in Somerset, NJ. Since then my family has moved around quite a bit, but finally came to settle in Macungie, PA, a small town southwest of Allentown. My family includes my mom, dad, two younger sisters, Karen, 15, and Megan, 11, and our husky/lab mix, Cheyenne. I spent my secondary school years at Emmaus High School, the highlight of which was a senior year trip to Spain. We visited the cities of Madrid, Cordoba, Seville, Granada, and Malaga in a whirlwind 9- day trip. After graduation in 2001, I came to Pitt specifically for the Bioengineering program, and have found it to be very rewarding. Outside of class, I keep busy with several extracurricular activities. I am an active member of BMES, SWE, and the National Society of Collegiate Scholars, and will be serving as business manager of Tau Beta Pi, the engineering honor society, in the fall. In my free time I enjoy listening to bands you've probably never heard of. I'm also an avid rollercoaster enthusiast, and like to travel, ski and play billiards, Frisbee, softball, and basketball.

My summer research experience in the MSRC has sparked my enthusiasm for the challenging field of biomechanics. I'm now considering higher-level degrees in bioengineering or possibly medical school for orthopedic surgery. I'd like to thank Shon Darcy for his endless patience and guidance, all my test subjects for enduring my horrendously long trial runs, the entire ACL group for all their support, and Drs. Debski and Woo for making this incredible summer experience possible.

EVALUATING REPEATABILITY OF ESTABLISHING KNEE COORDINATE SYSTEMS USING TWO DIFFERENT METHODOLOGIES

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INTRODUCTION

The accuracy and repeatability of recorded knee motion is limited by the precision and repeatability with which the anatomical coordinate systems of the tibia and femur are determined. Several methods have been developed, but no universal methodology for determining the locations and orientations of knee coordinate systems has been established [1-6]. Differences in the position and orientation of anatomical coordinate systems can introduce increased variability in recorded kinematics, decreasing statistical power. Studies on the effect of a change in location or orientation of knee coordinate systems on recorded kinematics have shown that knee kinematics in all 6 degrees-of-freedom (DOF) can be affected by variations in the location and orientation of anatomical coordinate systems [1, 7, 8]. These variations are especially apparent when the location of the anatomical coordinate system is used to apply loads to the knee.

In this study the inter- and intra- observer repeatability of two different methods will be used to evaluate the effect of differences in the locations and orientations of the knee coordinate systems. Observers with different levels of familiarity at detecting anatomical landmarks will provide insight into how the interpretation of anatomical landmarks affects the position and orientation of the knee. Also this study will compare knee kinematics recorded by two methodologies.

RESEARCH OBJECTIVES

- To evaluate the inter- and intra- observer repeatability of a digitizing methodology and its effect on knee motion
- To compare the recorded knee motion from the digitizing methodology (Method 1) to that recorded by the standard methodology (Method 2) during passive path and loading

METHOD

Coordinate systems are established with the digitizing methodology (Method 1) using a digitizing arm (Microscribe- 3DX®) to determine the relationship between the tibia and femur. Four points digitized at the perimeter of the proximal and distal (PD) ends of the tibial shafts are averaged to form the tibial Y- axis and the most prominent medial and lateral (ML) points on the femur are digitized to form the tibial X- axis (Figure 1). The third axis (Z- axis) is the result of the cross product of the first two axes. The tibial X- axis is redefined to ensure orthogonality. The femoral coordinate system is established in a similar manner with the exception that the femoral Y- axis is redefined instead of the X- axis. This establishes 2 orthogonal coordinate systems. The origin of the femur and tibia is at the midpoint between the femoral ML points at full extension.

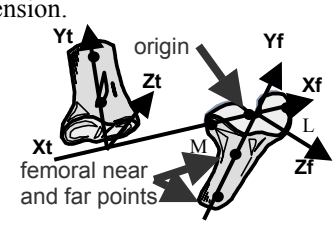

Figure 1. Knee coordinate systems using digitizing methodology

The standard methodology (Method 2) establishes the knee coordinate systems by redefining the origin of the tool coordinate system to the midpoint between the femoral ML points at full extension. The femoral coordinate system is set coincident with the tibial coordinate system at the reference position, the position of the knee at full extension in which no external forces or moments are applied [9].

To determine intra- and inter- observer repeatability, a cadaveric knee was digitized by six different observers 10 times. Each observer also measured initial FE (flexion-extension) and VV (varus-valgus) angles and the origin of the coordinate systems once using Method 2. To achieve the second objective, the digitizing process was applied at 0°, 15°, and 30° degrees of flexion during passive path and for anterior- posterior (AP) and VV loads at full extension. The differences in recorded motion of the knee between Method 1 and Method 2 were determined.

RESULTS

The most inter- observer variability in the orientation of the knee for Method 1 occurred in the VV angle (±6.5º). Method 1 introduces higher inter- observer variability than using Method 2 (±1.2°) in determining the VV angle. Due to the way Method 1 calculates the direction of the axes, internal-external (IE) rotation between the bones is essentially zero at full extension, and the only significant rotations are VV and FE. IE stiffness is greatest at full extension; therefore it is valid to assume IE rotations at full extension are zero [10]. The position of the origin varied the most in the PD direction (±4.7mm), and the least in the ML direction (±1.2mm) when using Method 1 on a cadaveric knee. Method 1 is comparable to Method 2 when measuring the position of the origin of the anatomical coordinate system. The ML position is more repeatable than other positions due to symmetry of the bone. Figure 2 shows the inter- observer repeatability in the location of the origin of the anatomical coordinate system and orientation of the knee using Method 1 and Method 2.

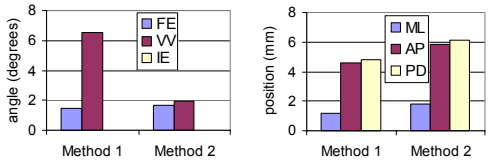

Figure 2. Inter-observer repeatability of knee orientation and position of the origin for a cadaveric knee

While the inter-observer variation in measured VV angle in the cadaveric knee is high (±6.5°), the observers with the most familiarity with interpreting the anatomical landmarks of the knee could determine the VV angle to within ±1.4° while those observers least familiar at interpreting anatomical landmarks could only achieve a precision of ±6.5°. Figure 3 shows the intra- observer repeatability of measuring FE and VV angles using the digitizing methodology.

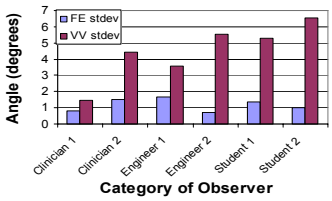

Figure 3. Intra- observer repeatability of knee orientation for a cadaveric knee

To determine the cause of the large intra-observer variation in VV angle when using Method 1, a sensitivity analysis was performed for a set of one subject's trials. First, all tibial and femoral points in the set were set equal and ML points differed. The resulting standard deviation of the calculated flexion angle and VV angle were ±0.19° and ±3.2°, respectively. Second, all ML points in the set were set equal and tibial and femoral points differed. The resulting standard deviation of the calculated flexion and VV angle was ±1.65° and ±0.67°, respectively.

The difference in position of the origin in space between Method 1 and Method 2 was 2mm in the X- direction, 6mm in the Y- direction, and 6mm in the Z- direction. The difference in orientations of the tibial axes between the two methods was 1.2° about the X- axis, 1.8° about the Z- axis, and 1.3° about the Y- axis.

When comparing kinematics determined using the two methodologies, anterior translation differed by 0.7mm under an anterior load, posterior translation differed by 0.9mm under a posterior load, varus rotation differed by 0.01° under a varus load, and valgus rotations differed by 0.1° under a valgus load. Kinematics during passive path from 0° to 30° were comparable between the two methods in all 6 DOF. Differences in translations ranged from 0 to 3.3mm, and differences in rotations ranged from 0 to 3.2°.

DISCUSSION

The objective of this study was to evaluate the inter- and intra- observer repeatability of a digitizing methodology (Method 1) and to determine how the digitizing methodology compares to the standard methodology (Method 2) when recording kinematics. The worst inter-observer repeatability was seen when measuring VV angle using Method 1. The shift to the clinical center is defined as a function of the natural valgus angle [7]. Applying loads to the clinical center more accurately reproduces the loading technique used by clinicians during examinations. A change in recorded valgus angle will result in a change in the point of application of the load. The intra- observer variation in the measured VV angle for the most experienced observer would result in a shift of up to 2.5mm to the clinical center, while the intra-observer variation of the measured VV angle for the least experienced observer would result in a shift of up to 9 mm to the clinical center. A shift of 7mm in the point of application of an anterior load has been shown to cause significant differences in IE rotation and minimal change in AP translation [7]. As shown by a sensitivity analysis, a change in ML points cause the largest change in measured VV angle. Therefore, when using Method 1 it is imperative that the user be adept at defining the rotational axis of the knee, which is determined using the ML points. In the future when using Method 1, a goniometer should be used to verify the VV angle.

When comparing kinematics of the knee under AP and VV loads using Method 1 and Method 2, the methods are comparable. In the primary DOF for applied loads, differences in AP translations under AP loads and VV rotations under VV loads were within the accuracy of the Microscribe (±0.2mm) and the robotic manipulator. Minimal variability was seen in recorded kinematics at other DOF. This variability was similar to that found in a study by Pennock and Clark [1] that compared calculated kinematics resulting from the transformation between coordinate systems that were located using different anatomical landmarks. In their study, the change in kinematics calculated using these transformations was up to 3° in VV rotation, 5° in IE rotation, 1mm in ML translation, 4mm in AP translation, and 9mm in PD translation. The difference in kinematics seen in this study can be accounted for by the difference in orientation of the coordinate systems. Other differences in kinematics may be due to error in digitizing. In the future, Method 1 will be used with a high-payload robotic/UFS testing system to record joint forces and kinematics.

ACKNOWLEDGEMENTS

Thank you to Shon Darcy for his endless guidance, patience, and enthusiasm, and also to Robert Kilger and Mary Zettl for assistance in running experiments. Finally, thank you to Dr. Woo and Dr. Debski for making this summer research experience possible, and the support of NIH Grant AR 39683.

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Tendinitis Group

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Born on February 3, 1982, I spent most of my childhood in Kingwood, TX with my brother and sister. In the fall of 1993, my family followed my father to Dhahran, Saudi Arabia, where my dad works as a Senior Engineering Consultant for the Arabian American Oil Company and my mom works as a preschool teacher. Although the initial move was a bit of a shock, which involved both changes in culture and lifestyle, it has afforded opportunities in international travel, education, and athletics.

After spending three years at a boarding school a few miles south of Santa Barbara, I came to the city of Pittsburgh to attend Carnegie Mellon University, where I continue to pursue engineering and science interests. When I'm not juggling classes or working, I enjoy hanging out with friends, relaxing and catching a game. I am the social chair of the biomedical engineering society and a member of Carnegie Mellon's baseball team.

Although I usually coach baseball during the summers, I am thankful for the opportunity Ms. Agarwal, Dr. Wang, and the MSRC has offered me. I plan to use the research experience gained in the pursuit of a graduate degree in drug delivery and development.

DIFFERENTIAL α-SMOOTH MUSCLE ACTIN EXPRESSION IN HEALING AND NORMAL FIBROBLASTS

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INTRODUCTION

The primary purpose of fibroblasts in soft tissues such as the medial collateral ligament (MCL) is to maintain the extracellular matrix in healthy tissues and repair and remodel injured tissues [5]. Therefore, fibroblasts in injured and healthy soft tissues should exhibit different phenotypic expressions. The purpose of this study was to test whether fibroblasts isolated from healing MCLs express higher levels of α-SMA than their normal counterparts.

MATERIALS AND METHODS

Cell Culture

In the right knee of three skeletally mature rats, a 2 mm gap model injury was created in the MCL and the contralateral leg was used as a sham control. After ten days, the rats were sacrificed, and fibroblasts from both healing and control MCLs were harvested. Healing and normal fibroblasts were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) (Invitrogen), in an atmosphere of 5% CO_2 and 100% humidity at 37ºC. Cells sub-cultured up to four times were used for all experiments.

Immunostaining

Immunostaining was performed to detect expression of α-SMA in both healing and normal fibroblasts. MCL fibroblasts at a cell number of $8x10^4$ were plated overnight in a 6-well plate. The cells were then fixed with the addition of methanol and α-SMA was stained using a mouse monoclonal anti-actin α-SM antibody (Sigma Aldrich Corp.) followed by a FITC-conjugated goat anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories Inc.). Fluoresecent microscopy and digital photography were used to record the α-SMA expressed in the samples.

Western Blot

Western blotting was performed to measure α-SMA expression levels. Briefly, equal amounts of total protein were run on a 10% Tris-HCl gel using SDS-PAGE and then transferred to a nitrocellulose membrane using a standard protocol. A 5% milk solution was used to block non-specific binding, after which α-SMA was detected using a mouse monoclonal anti-actin α-SM antibody followed by a peroxidase conjugate goat anti-mouse antibody (Jackson ImmunoResearch Laboratories Inc.). An ECL detection kit (Amersham Biosciences UK Limited) was used to expose the α-SMA bands.

RESULTS

Immunostaining of the cells showed that both healing and normal rat MCL fibroblasts expressed α-SMA. The α-SMA stress fibers were observed in both experimental groups (**Fig 1**). Furthermore, Western blot analysis demonstrated that α-SMA expression level of healing MCL fibroblasts was markedly higher than that fibroblasts from the sham-operated MCL (**Fig 2**). In three separate experiments, healing fibroblasts expressed α-SMA 1.68 fold that of normal MCL fibroblasts **(Fig 3)**.

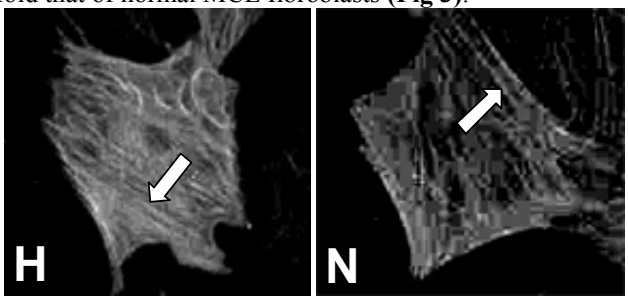

Figure 1. Both healing (H) and normal (N) rat MCL fibroblasts exhibit α-SMA stress fibers.

Figure 2. Healing rat MCL fibroblasts (H) express markedly higher levels of α-SMA than normal rat MCL fibroblasts (N).

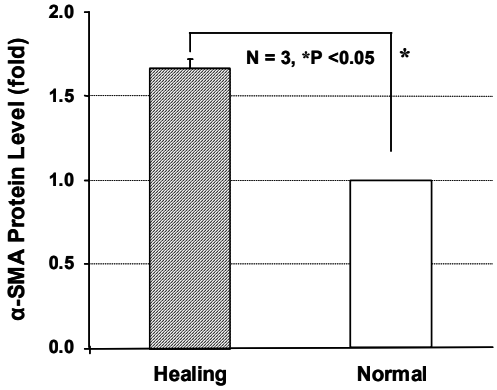

Figure 3. Fibroblasts harvested from the healing rat MCL had 1.68±0.05 ($p < .05$) fold greater expression of α-SMA than that of their normal (N) counterparts. Three separate experiments were performed and consistent results were observed.

DISCUSSION

This study shows that healing fibroblasts express a higher level of α-SMA than normal fibroblasts, confirming our hypothesis. The greater expression levels of α-SMA in healing fibroblasts compared with that of normal fibroblasts may account for the differential contraction seen in previous studies [3]. Increased α-SMA expression indicates that healing fibroblasts may behave more like myofibroblasts [1], which are known to produce greater contractile forces in healing tissue [2,4], exhibiting not only a phenotypic difference, but also a difference in functionality. These results suggest that down-regulating, but not inhibiting, the α-SMA expression levels of healing fibroblasts in wound sites may be an effective approach to maximizing the mechanical properties of the healing MCL. Moreover, since healing fibroblasts express different levels of α-SMA than normal fibroblasts, this study has illustrated that it may be necessary to use healing fibroblasts instead of normal fibroblasts when studying the cellular and molecular mechanisms of ligament healing. Further studies should focus on the possibility of differential production of collagen by healing and normal fibroblasts as well as characterizing the relationship between proliferation, α-SMA expression, and contraction.

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I was born on December 30, 1982 in Manhattan, New York. Until I was five years old, I was raised in Matawan, New Jersey. Then, my family and I moved to the neighboring town of Marlboro. My parents, two younger brothers, and younger sister still live in Marlboro, NJ. I attended high school at the Science and Engineering Learning Center at Manalapan High School. My high school experience furthered my interest in engineering.

At CMU, I am double majoring in Mechanical Engineering and Biomedical Engineering. When I am not busy studying, I enjoy being an active member of my fraternity, Alpha Epsilon Pi, and editor of the school yearbook. In my free time, I love to go skiing, watch football, and build model railroads.

I was first introduced to the MSRC when I went on a tour with my biomedical engineering seminar. Ever since, I have been fascinated by the work of the lab.

After finishing my undergraduate studies, I plan to attend medical school. This summer at the MSRC has been a valuable experience that has shown me how closely clinical medicine and biomedical research work together. I would especially like to thank my mentor, Chris, and my advisor, Dr. Li, for all their guidance, patience, and advice throughout the summer. Thank you to Dr. Woo for giving me this great opportunity to work in his lab. Lastly, I would like to thank all of the faculty, staff, and students at the MSRC for making this past summer so educational and enjoyable.

VISCOELASTIC PROPERTIES OF THE TRANSVERSE CARPAL LIGAMENT

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INTRODUCTION

Carpal tunnel syndrome (CTS) is a very prevalent health disorder, which causes the highest number of days away from work of all non-fatal occupational injuries or illnesses [1]. It results from the compression of the median nerve, which can be caused by an increase of pressure within the carpal tunnel [2]. This buildup of pressure can be relieved through non-surgical methods (such as anti-inflammatory medications, splints, exercise, and alternative therapies) and by surgery [2,3].

Beneath the skin of the palmar surface of the wrist lies a moderate amount of fat. Beneath this fatty layer and above the transverse carpal ligament, several blood vessels and muscles are found [4]. The transverse carpal ligament (TCL) arches over the depression in the carpal bones forming the carpal tunnel [5]. The TCL is attached medially to the pisiform and the hook of the hamate and is attached laterally to the tubercle of the scaphoid and the trapezium [5]. The extrinsic finger flexor tendons and median nerve pass through the carpal tunnel. According to previous research by Armstrong and Chaffin, flexor tendons are displaced against one of the walls of the carpal tunnel when the wrist is not in the neutral position [6]. The carpal bones support the flexor tendons when the wrist is extended, while the flexor tendons are supported by the TCL when the wrist is flexed [6]. In a previous study, Sucher found that the TCL exhibits viscoelastic properties in the distal and proximal directions [9].

During surgery, the TCL is divided to enlarge the carpal tunnel [3]. Surgery usually permanently relieves the symptoms of carpal tunnel syndrome. However, hand strength may be reduced as a side effect of the TCL being divided since the flexor tendons are no longer supported by the TCL [2]. Netscher studied this effect and found that patients who had TCL reconstruction had grip and pinch strengths greater than those patients who just had the ligament divided [7]. Another complication of the division of the TCL is the bowstringing of tendons or the prolapsing and anterior migration of flexor tendons [8]. In order to find better treatments for carpal tunnel syndrome, it is important that we better understand the biomechanical properties of the transverse carpal ligament.

The objective of this study was to determine the viscoelastic properties of the transverse carpal ligament under loading perpendicular to the palmar surface.

MATERIALS AND METHODS

Specimen Preparation. One fresh-frozen cadaveric right limb was obtained from an 81 year old male with no record of CTS. The limb was thawed and attached to an aluminum board using straps to prevent movement. The fingers were taped down to further restrict movement.

Indenter Location. In order to identify the indenter location, the TCL was found and marked by the following method:

a) The pisiform, scaphoid, hook of hamate, and trapezium were palpated and marked.
b) The positions of these bones were confirmed by radiography (Figure 1).
c) The indenter was centered between these bones.

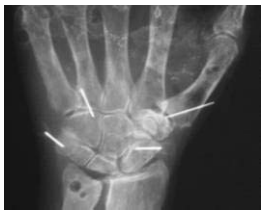

Figure 1. Indenter Location

Testing Protocol. An EnduraTEC ELF 3200 (controlled by the WinTest Control System) with a 10 mm diameter flat point indenter was used to displace the TCL.

Part 1 – Preconditioning: The limb was preconditioned by cyclic strain from 0 mm to 6 mm for 5 minutes at 2 Hz.

Part 2 – Load Ramping and Relaxation Test: A ramp indentation to 8 mm at a rate of 2.00 mm/sec was applied to the palmar surface above the TCL and held for 30 minutes. Force, displacement, and time values were recorded at a sampling frequency of 1 Hz. The test was repeated for a total of 3 trials (with preconditioning before each test). One-hour was allowed between trials for the TCL and soft tissue to recoil.

Part 3 – Cyclic Load Relaxation Test: The palmar surface above the TCL was cyclically indented from 0 mm to 8 mm at 2 Hz for 10 minutes. Force, displacement, and time values were recorded at a sampling frequency of 200 Hz. The test was repeated for a total of 3 trials. One-hour was allowed between trials for the TCL and soft tissue to recoil.

Part 4 – Dissection of Tissue Above the TCL (TCL-exposed): The superficial tissue above the TCL was dissected, exposing the TCL and leaving the carpal tunnel contents intact. Tests outlined in the above procedure were then repeated directly on the TCL.

Part 5 – Removal of Carpal Tunnel Contents (TCL-only): Finally, all of the carpal tunnel contents including the flexor tendons and median nerve were removed. Tests outlined in the above procedure were then repeated directly on the TCL.

RESULTS

During load ramping and relaxation, the stiffness, maximum force, and minimum force values for trial 1 were consistently different than the values for trials 2 and 3 (Table 1). However, the values for trials 2 and 3 were relatively comparable. For example, stiffness values during ramp indentation of the TCL-only decreased from 10.1 N/mm for the first trial to 7.4 N/mm for the second trial corresponding to a 26.7% change. The stiffness values for the second and third trials were nearly identical (7.4 N/mm

vs. 7.5 N/mm). Therefore, only the results of the second trial will be discussed.

During ramp indentation, the force-displacement curves of all three conditions exhibited a nonlinear "toe" region followed by a more linear region (Figure 2). The stiffness is calculated from this relationship. The stiffness for the TCL-exposed condition is more than three times the stiffness for the palmar surface condition and TCL-only condition. The maximum forces occurred at the end of ramp indentation. Under the TCL-exposed condition, the maximum forces were 72.7% greater than the palmar surface condition and 70.5% greater than the TCL-only condition. Under all three conditions, it took between 20-30 minutes to reach load relaxation equilibrium (Figure 3). The equilibrium loads were 23.5%, 16.8%, and 29.9% of the maximum forces for the palmar surface, TCL-exposed, and TCL-only conditions, respectively.

During cyclic relaxation tests, the peak load decreased over an increasing number of cycles. At the end of 1200 cycles, the average equilibrium loads were 81.6%, 63.1%, and 63.1% of the maximum peak forces for the palmar surface, TCL-exposed, and TCL-only conditions, respectively (Table 2). Again, the palmar surface condition and TCL-only condition have more similar viscoelastic properties than the TCL-exposed condition.

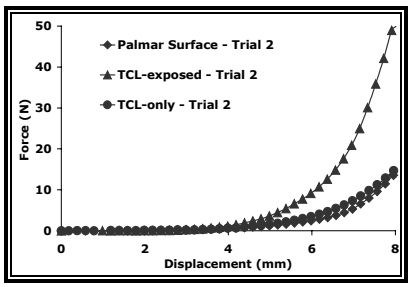

Figure 2. Force-displacement relationship for ramp indentation

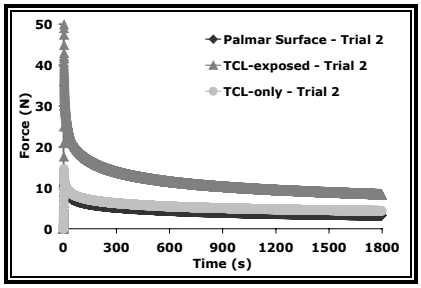

Figure 3. Load relaxation

Condition	Trial	Stiffness (N/mm)	Maximum Force (N)	Minimum Force (N)
Palmar Surface	1	15.6	27.1	3.9
	2	8.2	13.6	3.2
	3	6.8	11.3	3.1
TCL-exposed	1	46.0	80.5	9.6
	2	29.8	49.9	8.4
	3	26.4	43.6	7.8
TCL-only	1	10.1	21.7	4.5
	2	7.4	14.7	4.4
	3	7.5	14.5	4.3

Table 1. Summary of Load Ramping and Relaxation

Condition	Trial	Maximum Peak Force (N)	Minimum Peak Force (N)	Max Area Hysteresis (N-mm)	Min Area Hysteresis (N-mm)
Palmar Surface	1	12.5	10.6	15.1	5.4
	2	12.8	10.1	13.4	5.5
	3	13.3	10.8	13.9	5.7
TCL-exposed	1	40.6	26.6	27.7	20.3
	2	43.8	27.1	29.3	21.8
	3	44.8	27.8	28.1	20.4
TCL-only	1	15.9	11.3	7.9	5.0
	2	16.9	10.3	8.3	4.8
	3	17.9	10.4	9.1	5.2

Table 2. Summary of cyclic indentation

DISCUSSION

In this cadaveric study, load relaxation tests and cyclic indentation tests were performed to determine the viscoelastic properties of the transverse carpal ligament under loading perpendicular to the palmar surface.

During load relaxation, the TCL-exposed condition exhibited higher forces than the palmar surface condition or the TCL-only condition. This is most likely due to the tendons and median nerve supporting the TCL. The palmar surface condition exhibited similar forces to the TCL-only condition due to the superficial tissue above the ligament.

In all of our testing, the values for all trials were different. This can be caused by a combination of factors since viscoelastic properties are both time- and history-dependent. Therefore, several limitations existed using the developed protocol. Levels of temperature and hydration must be controlled. In addition, the TCL must be able to fully recover from one trial to the next. Finally, it is possible that the hand was not rigidly fixed and thus moved slightly upon initial indentation for each condition. Addressing these limitations will allow for more accurate determination of the viscoelastic properties of the transverse carpal ligament.

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I was born on January 16, 1983 in Pittsburgh. I have lived in Pittsburgh my whole life, and at Pitt I'm only about 3 miles from home. I have two sisters, Colleen and Karin, and one brother, Michael. After graduating from Taylor Allderdice High School in 2001, I came to Pitt to major in Bioengineering.

While I'm not in class or studying, one of my favorite activities is badminton. My friends and I love badminton and when we found out that there was no badminton team at Pitt we decided to start one ourselves. After a year of planning, we finally completed all of the paperwork and were accepted as an official Pitt organization this past winter. I became president of the Badminton Club after we drew names out of a hat. (If anyone is interested in joining let me know!!!)

This summer I have learned firsthand that there is more to engineering besides memorizing formulas. As a result of working at the MSRC, my interest in research has expanded and I now plan on attending graduate school after college. I would like to thank my mentor, Shouchen Dun, for his patience, support, and guidance over the summer. I would also like to thank Dr. Li for his guidance over the summer. I would finally like to thank Drs. Woo and Debski for allowing the summer program to be possible, and the entire MSRC for making this an enjoyable summer.

INTRA-FINGER JOINT COORDINATION OF THE INDEX FINGER DURING REACHING AND PRECISION GRIPPING

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INTRODUCTION

The act of precision gripping (gripping an object with the tips of the index finger and the thumb) may seem simple; however, the motion is carried out through a complex system of muscles, tendons, and joints working together. Precision gripping can be divided into two components: transport and grasp [3, 5, 6]. The transport component occurs when the hand approaches the object to be gripped, while the grasp component involves the shaping of the digits in preparation to grip the object. It has been found that the size of an object influences the grasp component, with maximum grip aperture (the maximum distance between the tips of the index finger and the thumb when preparing to grip the object) increasing with object size [3, 6].

The purpose of this study was to investigate the intra-finger joint coordination of the index finger during reaching and precision gripping. While there has been research on the amount of joint motion during flexion and extension tasks [1, 2], this research has not included precision gripping tasks and the effect of object size on intra-finger coordination. Therefore, in this study we attempted to find a relationship between the angular motion at each joint and the object size during reaching and precision gripping.

METHODS

Ten right-handed participants, seven male and three female, with no previous history of upper extremity disorders participated in the study. The average age, height, weight, and right index finger length were 24.8 ± 6.7 years, 1.75 ± 0.05 m, 72.4 ± 14.7 kg, and 9.6 ± 0.4 cm, respectively. Each participant signed a consent form approved by the Institutional Review Board prior to the experiment.

Two G35 single axis goniometers were used to measure the angular joint motion of the proximal interphalangeal (PIP) and the metacarpophalangeal (MCP) joints of right index finger. The PIP angular motion was measured with a goniometer placed on the dorsal side of the proximal and middle phalanges using double-sided tape. Likewise, the motion of the MCP was measured using a goniometer taped to the dorsal side of the proximal phalanx and of the second metacarpal. The goniometers were secured in place with sports tape.

Each subject underwent ten trials for each of three Styrofoam cubes with lengths of 2 cm, 4 cm, and 6 cm. Styrofoam was used since its light weight minimizes the effect of weight on joint movement. Prior to the initiation of movement, the subject was seated at a table with the frontal plane of the body parallel to the edge of the table as seen in Figure 1. Two lines were drawn on the table with an angle of 45˚ between them. The subject's right elbow was placed on the vertex of the two lines. The upper arm was positioned at 0˚ in the frontal plane and at 45˚ in the sagittal plane.

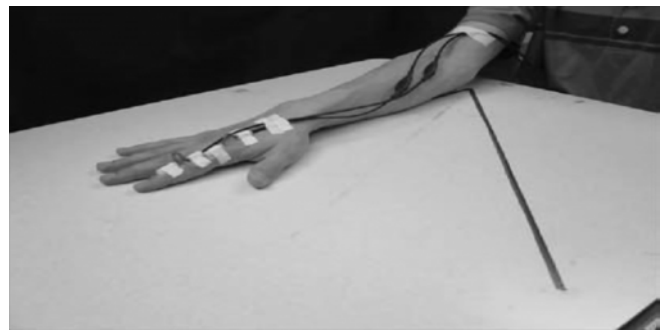

Figure 1. Illustration of experimental set up.

After the subject was positioned at the table and the goniometers were secure, the subject was asked to lift the cube with precision grip and to set the cube at a comfortable position on the line pointing to the left of the subject's body. Prior to the collection of data, the LabVIEW program ran a three second drum roll which alerted the subject to fully extend all of the digits of the hand. After the drum roll, the sound of a gunshot was played. The subject, moving at a comfortable speed, pinched the tips of the index finger and the thumb together. The digits were then slightly extended to allow the subject to use precision grip to grasp the cube, while the middle, ring, and little fingers were naturally flexed. The subject moved the cube by rotating the elbow 45˚ externally until the forearm reached the second line. The cube was then placed on the table and the subject fully extended all five digits, thus completing the experiment. The data was collected using LabVIEW with a sampling frequency of 1000 Hz and a sampling time of eight seconds.

Data Analysis

Correlation coefficients between the MCP and PIP joints were calculated in order to analyze the effect of cube size on the correlation of these two joints. The significance of the data was tested with two-way MANOVA followed by post-hoc one-way ANOVA and t-tests. P-values less than 0.05 were considered to be significant.

RESULTS

It was found that the correlation between the angular motion of the PIP and the MCP joints increases with decreasing cube size ($p < 0.05$), as seen in Figure 2. The average correlation coefficient was 0.810 ± 0.100 for the

large cube, 0.911 ± 0.044 for the medium cube, and 0.954 ± 0.028 for the small cube.

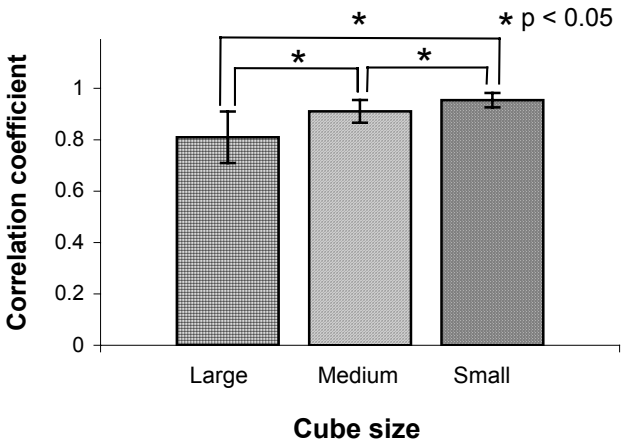

Figure 2. Average correlation coefficients between the MCP and PIP joints.

Three data points, shown in Figure 3, were analyzed, including the point when pinch (a slight contact between the tips of the index finger and thumb) occurred, the point when the index finger and thumb opened in preparation to grip the cube (digits open), and the point when precision grip occurred. A significant effect on angular motion for both the size of the cube (Lambda(4, 160) = 7.35, $p < 0.05$) and the position of the digits (Lambda(4, 160) = 46.86, $p < 0.05$) was found with MANOVA. Follow-up univariate MANOVA showed that the angular motion of the MCP was significantly affected by the size of the cube ($F(2, 81) = 15.98$, $p < 0.05$), while that of the PIP was not affected significantly by cube size ($F(2, 81) = 0.46$, $p > 0.05$). However, the posture of the hand significantly affected both the motion of the MCP ($F(2, 81) = 72.80$, $p < 0.05$) and the PIP ($F(2, 81) = 35.44$, $p < 0.05$).

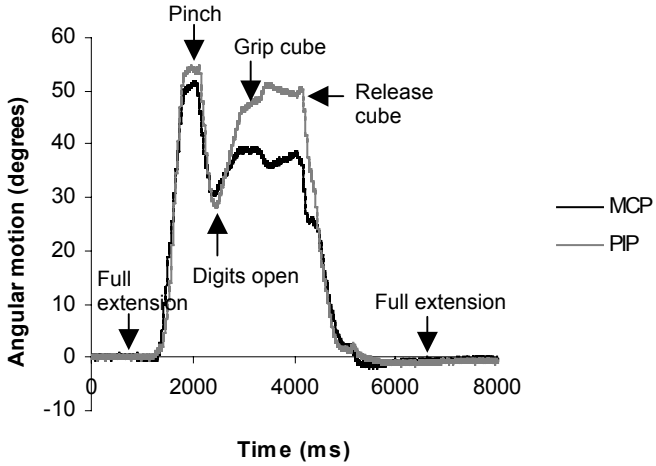

Figure 3. Representative time-series data of the angular displacements of the MCP and PIP joints.

After analysis with MANOVA, follow-up one-way ANOVA indicated that there was a significant difference ($p < 0.05$) between the results of the MCP angular data when the digits opened in preparation for precision grip, with values ranging between 14.7 ± 6.3 degrees for the large cube and 30.7 ± 10.4 degrees for the small cube, and when precision grip occurred, with values ranging between 24.1 ± 6.3 degrees for the large cube and 43.7 ± 7.0 degrees for the small cube. Post-hoc t-tests indicated that the differences were significant ($p < 0.05$) across each combination of cubes.

DISCUSSION

Since the task of pinching does not depend on the size of the cube, the effect of cube size was not significant for the MCP or for the PIP during pinch. However, when the digits opened in preparation to grip the cube and during precision grip, the digits extended more as cube size increased. As a result, the degree of flexion of the MCP decreased significantly with increasing cube size, while that of the PIP did not change significantly, which implies that the difference between the angular motion of these two joints was less for small cubes than for large cubes. Therefore, it can be concluded that the extension of the index finger during precision gripping was controlled mainly by rotation of the MCP, which is more efficient in increasing grip aperture. This could serve as a possible explanation of the finding that as cube size decreased the correlation between the angular motion of the MCP and PIP joints increased.

CLINICAL RELEVANCE

Carpal tunnel syndrome (CTS), which is caused by a compression of the median nerve through the carpal space, can cause recurring pain and result in damage to the sensory and motor function of the hand, causing CTS patients to suffer from clumsiness and weakness while handling objects [4]. Since the median nerve innervates the second lumbrical, which acts to flex at the MCP joint of the index finger, it is predicted that the range of motion at the MCP joint is smaller and the initiation of MCP joint motion is delayed for CTS patients compared to normal subjects. The results of the current study could possibly serve as a baseline for future research in neuromusculoskeletal disorders that may affect intra-finger coordination.

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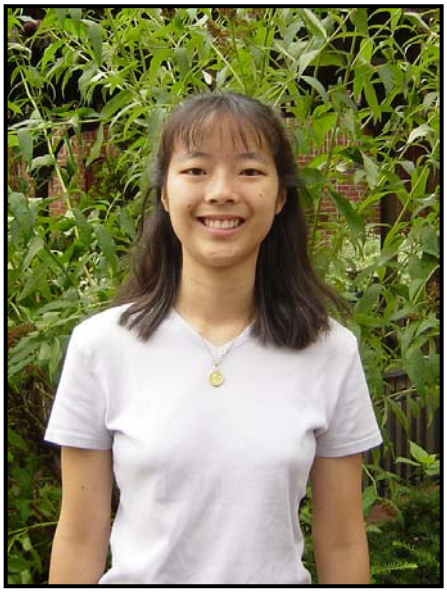

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MCL Group

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I was born in Teaneck, New Jersey on April 19, 1983 and grew up as an only child in Cary, North Carolina, which is near Raleigh. I graduated from a residential high school, The North Carolina School of Science and Mathematics, in 2001, where I had a unique and amazing two year experience. I am going to be a junior at North Carolina State University in the fall of 2003. After I graduate, I hope to pursue a career in medicine and medical research, perhaps by entering an M.D./Ph.D. program.

I study hard, but I also find free time for extracurricular activities I enjoy, including reading, volunteering, and traveling. I am also interested in Chinese studies and have pursued this through activities like being vice president of Chinese Club, attending Asian studies conferences, teaching a Chinese calligraphy seminar, and visiting China and Taiwan.

Many thanks to Sarah Brown for her guidance, support, and patience. Thanks also to Dr. Woo, Dr. Debski, Dr. Niyibizi, and the MSRC—this summer has proved to be a fun and educational research experience, and I have enjoyed working here.

COLLAGEN ANALYSIS OF RABBIT MEDIAL COLLATERAL LIGAMENTS (MCLs) TREATED WITH SMALL INTESTINAL SUBMUCOSA (SIS)

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INTRODUCTION

Isolated medial collateral ligament (MCL) injuries occur frequently during sports- and work-related activities [2, 4]. Although the MCL heals spontaneously after injury, the healing tissue is mechanically and materially inferior to normal tissue, even two years after injury [1, 5, 6, 9]. The use of naturally occurring scaffolds has been applied in an effort to improve ligament healing [3, 5, 8]. Specifically, porcine small intestinal submucosa (SIS), a primarily type I collagen matrix, has been shown to elicit a favorable tissue remodeling response in musculoskeletal applications [3, 5, 7].

In an attempt to restore pre-injury function of the MCL, a recent study in our research laboratory [5] applied a single layer of SIS in a rabbit MCL gap injury to determine how SIS would affect the mechanical and structural properties of healing MCL. The results showed that the stiffness of the femur-MCL-tibia complex (FMTC), the ultimate load at failure, the tangent modulus, and the stress at failure increased with SIS treatment but still remained lower than sham values [5].

To better understand the mechanism behind this improved mechanical response, the aim of this study was to investigate the biochemical aspects of SIS-treated ligaments. Previous studies have shown that type I collagen is the major component of normal MCL, and types III and V collagen are minor components [1, 6]. In healing MCLs, it has been observed that types III and V levels are elevated with respect to type I levels [1, 6]. Therefore, the objective of this study was to compare the ratios of collagen types III to I and types V to I of the SIS-treated MCLs to those of the non-treated controls using SDS-PAGE. It was hypothesized that the collagen ratios of the SIS-treated group would be decreased compared to the ratios of the non-treated group, but increased compared to the sham ratios.

MATERIALS AND METHODS

A 6 mm wide gap was surgically created in the right MCL of female New Zealand white rabbits. In some rabbits, a strip of SIS was sutured onto the two ends of the MCL, while the remaining rabbits were left untreated after MCL rupture, serving as a control group. The left MCLs of all rabbits were surgically exposed but left unruptured to serve as sham controls. The rabbits were allowed free cage activity after surgery. After twelve weeks of healing, they were sacrificed and the legs were harvested at the hip and frozen for storage.

For the present study, the MCLs were harvested and sutures were removed as needed. The ligaments were washed in 1 ml of phosphate buffered saline (PBS) containing protease inhibitors, rinsed in 1 ml of distilled water, minced to homogenize tissue, dried, and stored at –80°C until further analysis.

For tissue digestion, 2 mg of dried ligament was measured and placed in 1 ml of 0.5 M acetic acid and soaked for 24 hours at 4°C. Pepsin was added to each sample at a concentration of 1:30 by weight (enzyme:tissue) and placed in 4°C for 24 hours to stir. Pepsin concentration was raised to 1:10 (enzyme:tissue) and tissue digestion continued for 24 hours at 4°C.

Samples were clarified by centrifugation at max speed. The supernatants were removed, and 1 ml of 0.5 M acetic acid was added to the pepsin residues. The supernatants and the undigested collagen solutions were placed in 4°C for 24 hours. The second supernatant was obtained and combined with the first, and the pH was adjusted to 7.0 using ammonium bicarbonate (NH_4HCO_3). Aliquots of ¼ of the total volume of the supernatants were measured, dried for approximately 5 hours, and stored at –80°C until further analysis.

The samples and the types I, III, and V collagen standards were resolved using SDS-PAGE. Trial and error experiments to optimize the electrophoresis protocol varied the separating gel concentrations (6%, 7.5%, and 10%), the collagen standard sources (Sigma types I, III, and V collagen and Dr. Niyibizi's types I and V collagen), and collagen standard concentrations (20, 30, 40, 50, 60, and 80 µg of 2 µg/µl collagen in 3X buffer). To dissolve the collagen for loading, sample buffer was added. The samples were spun down in a small centrifuge for 2 minutes and heated for 10 minutes at 70°-90°C. Between 60-80 µL of each sample and 40 µL of each collagen standard were loaded in the wells and run on ice at a constant current of 32 mA. After 2 hours, 20 µL of DTT was added to the type III collagen standards and to each of the samples, and the current was set to 10 mA to run overnight.

The gels were removed from the chamber and placed in Coomassie brilliant blue staining overnight. After destaining with a methanol/ethanol solution, the gels were dried and analyzed using an imaging densitometer and Quantity One software.

RESULTS

The results of the optimum standards obtained are shown in Figure 1.

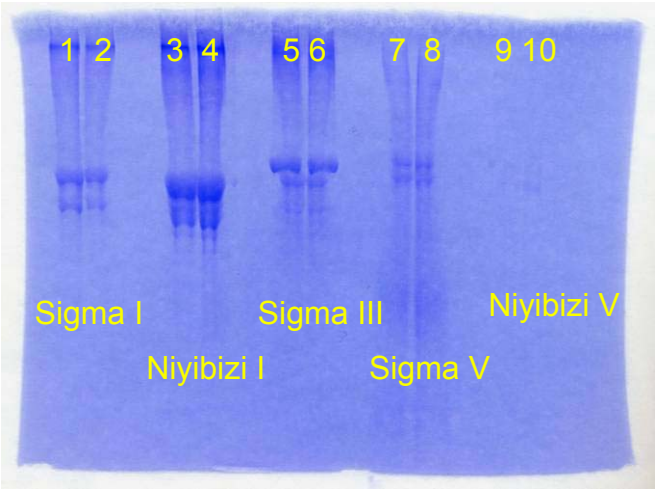

Figure 1. Standards, 7/9/03, 7.5% separating gel

The SDS-PAGE analysis of the SIS-treated group, the non-treated group, and their sham controls are shown in Figure 2. Preliminary densitometric scan results are shown in Chart 1.

Figure 2. Healing MCL, 7/14/03, 7.5% separating gel

Lane	III:I	V:I
4	11.7%	8.3%
5	12.8%	11.1%
6	19.7%	8.1%
7	27.0%	28.5%
8	20.9%	25.4%
9	10.1%	14.1%
10	16.9%	22.7%
11	29.7%	23.4%

Chart 1. Collagen Type Ratios for Figure 2

The success of the trial and error experiments was determined qualitatively. The clearest and most separated experimental and standards bands were produced using a 7.5% separating gel, Sigma types I, III, and V collagen, and 40 µl of 60 µg at 2 µg/µl of collagen in 3X buffer.

DISCUSSION

Trial and error experiments helped to optimize the results produced by the electrophoresis protocol. In some gels, bands were faint, blurry, or unclear. In addition, band separation between the three different types of collagen posed significant problems. Several possible sources leading to these inconsistencies were suggested, including separating gel concentration, the source and concentration of the standards, the amount of protein loaded, and the timing of the DTT addition.

Preliminary densitometric scan data resulted in inconsistent and widely varying ratios of collagen types III:I and types V:I; thus, conclusions regarding the effect of SIS treatment on collagen ratios III:I and V:I in healing rabbit MCL cannot be made at this time. Possible sources leading to these inconsistencies include poor band separation, insufficient collagen extraction, impurities in the collagen, and the subjectivity involved in obtaining the densitometric scans.

Future work for this study calls for further trial and error experimentation with the SDS-PAGE protocol in order to improve band separation, obtain clean and clear bands, and result in consistent and repeatable ratios for collagen types III:I and V:I.

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It all started in Ashland, PA on September 29, 1981….and my parents lives have never been the same…. After a year living in Ashland, my family and I moved to the village of Weishample in Barry Township. To say that it is the middle of nowhere would be an understatement, however, it is a nice small place that is quiet, and serves as a nice break from city life.

I started playing sports at an early age, about six or seven I believe because my father was the coach of the baseball team. In about fifth grade I started playing football, and played both sports up until I graduated from high school. My senior year of high school, my high school baseball team won the district championship, and my football team set several school records. It was the first time in school history to make it to the state semifinals. We, unfortunately, lost by literally a yard. I did however manage to break three school records and be named the scholar athlete for my school.

Next came Pittsburgh, quite a culture shock from home, but definitely for the better. I enrolled in the University of Pittsburgh's School of Engineering, and later chose to follow the bioengineering path, more specifically the biomechanical path. And that's how I ended up at the MSRC. Thus far it has been great. People have been fun to work with, and it has been a great opportunity. I would just like to thank Drs. Debski and Woo for giving me the opportunity to partake in such a wonderful program. I would also like to thank my mentor, Susan Moore, for all of her help and support.

THE DEVELOPMENT OF A METHODOLOGY TO DETERMINE THE 3D STRAIN DISTRIBUTION OF THE AXILLARY POUCH

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INTRODUCTION: The glenohumeral joint is the most commonly dislocated diarthroidal joint in the body. Therefore, glenohumeral stability is a major concern within the orthopaedic community, as indicated by the numerous surgical and non-surgical treatments. In order to improve current surgical and rehabilitation techniques, our laboratory is developing subject-specific finite element models of the glenohumeral capsule. The one-dimensional strain in the axillary pouch of the glenohumeral capsule has already been investigated using strain gages; however, the axillary pouch has been shown to be a structure capable of supporting a multi-dimensional strain field. [2, 3] The 3D strain of the axillary pouch during a simulated joint motion is needed for validation of our FEMs. Moreover, for the past decade our laboratory has utilized robotic techniques to simulate loading conditions and/or joint kinematics of the glenohumeral joint. Therefore, the objective of this study was to develop a methodology to determine the 3D strain distribution of the axillary pouch while the glenohumeral joint is rigidly mounted within the robotic testing environment.

METHODS: An optical tracking system (Vicon)was selected for this project which allows for 3D data collection of strain during dynamic motions by passively tracking reflective markers. Developing a methodology using the Vicon system involved several steps: (I) fabrication of a fixation device (II) identify optimal camera configuration (III) fabrication of calibration tools for the Vicon camera system placement (IV) camera calibration and (V) determination of accuracy and repeatability of the camera configuration. Strain will be determined by comparing the X, Y, and Z positions of the capsule markers.

I) Development of a fixation device: An aluminum fixation device (Figure 1 - Left) was developed to simulate the robotic environment (Figure 1 - Right) and to allow for a rigid fixation of both the humerus and scapula while allowing for 5 DOF.

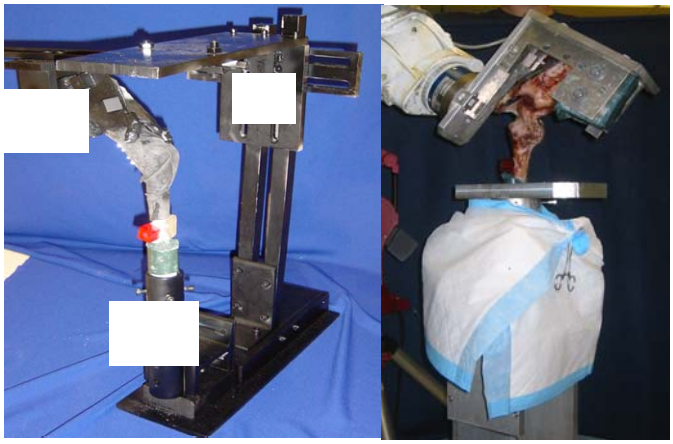

Figure 1. Left: Fixation device with A) X-Y plates B) Scapular bolt C) Hollow humeral cylinder Right: Robotic Environment

X-Y plates (Figure 1A) provided anterior-posterior as well as proximal-distal translations, and a bolt (Figure 1B) passed through the scapula perpendicular to the scapular plane, which allowed glenohumeral abduction. A thin walled hollow aluminum cylinder (Figure 1C) allowed the humeral shaft to be freely rotated (internal-external rotations) along its long axis until rigidly fixed using four set screws. Preliminary testing of the fixation device indicated that it was highly reflective, and therefore introduced noise to the Vicon infrared light camera system. Therefore it was necessary to paint the fixture matte black.

II) Camera Placement: Once the fixation device and shoulder model were placed within the Vicon working volume, the ideal camera placement was determined. The Vicon system will only detect a reflective marker if it is visible to at least three cameras at that particular point in time. Two camera configurations were tested: 1) 3 camera Vicon system and 2) 5 camera Vicon system.

For the 3 camera system (Figure 3), the external cameras (1and 3) were focused on the anterior and posterior portion of the capsule, respectively. The center camera (2) was focused on the inferior portion of the capsule. (Figure 2)

For the 5 camera system (Figure 4), cameras one and five (external) were placed focusing at the most anterior and posterior points of the axillary pouch. Cameras two, three, and four (internal) were focused on the center of the capsule, with camera three set at an inferior view of the capsule.

Figure 2. The shoulder capsule with A) anterior view B) inferior view and C) posterior view

For both camera configurations the cameras were re-positioned until all markers were visible to each camera with the joint in neutral rotation. Once all markers were visible for the shoulder model, the exact locations (angles and distances) of the cameras were recorded with respect to the most anterior point of the greater tuberosity.

III) Calibration Tools: Once all markers were visible, the shoulder model was removed. It was then necessary to calibrate the camera configuration. A calibration frame and calibration

wand were designed and developed for static and dynamic calibration of the Vicon cameras, respectively. During camera calibration, the Vicon system uses the marker distances of the calibration tools, measured and input by the user, as a baseline to compare distances it detects with the cameras. Therefore, the calibration frame was designed to have four 3mm hemisphere reflective markers positioned on a flat surface in an "L" pattern with the distance between the markers measured by calipers. The distance between the markers was determined by making a 3.5:1 ratio of the original Vicon calibration frame, thus producing a working volume corresponding to the size of the axillary pouch. The calibration wand was designed such that two markers were placed on a long slender rod at a known distance apart (20.8 mm), which approximated the distance of marker movement during preliminary 3D strain testing.

IV) Calibration and Testing: Static calibration began by placing the fabricated calibration frame in the humeral clamp. A matrix of marker distances of the calibration frame was input into the Vicon system to serve as a baseline for the cameras to compare their measurements. After static calibration was performed, the calibration frame was removed and the calibration wand was utilized to dynamically calibrate by moving the wand in a random fashion throughout the working volume (defined by the static calibration frame). After both calibration protocols were completed, the shoulder model was remounted within the fixation device at 60° of glenohumeral abduction and neutral flexion-extension. The model was then repositioned at approximately neutral rotation, 30° external rotation, and 30° internal rotation while the marker locations were tracked using the Vicon camera configurations. It was shown that the 3-camera configuration was capable of capturing locations of only 5 of 16 (31%) markers at all times, while the 5-camera configuration was capable of locating 13 of 16 (81%) markers at all times.

V) Accuracy and Repeatability Determination: The accuracy and repeatability of the two configurations were determined using four additional wands. These wands were the same design as the calibration wand, with differing distances between the reflective markers. The lengths of the wands used for the 5 camera setup were 11.28 mm, 20.81 mm, 28.21 mm, and 41.25 mm, for wands 1, 2, 3 and 4, respectively. The wands using the 3 camera setup had lengths of 7.48 mm, 15.85 mm, 23.1, and 41.25 mm for wands 1, 2, 3, and 4, respectively. In both setups, each wand was moved throughout the work volume in a random fashion. Five separate trials of each wand were performed for a total of 20 trials for each camera setup. The 3-camera system was shown to have an error of 1.3 ± 1.1% and was repeatable to within 0.7 mm during accuracy and repeatability studies. The 5-camera system was shown to have an average percent error of 1.4 ± 0.5 % and to be repeatable to within .1 mm during accuracy and repeatability studies.

DISCUSSION: This study determined a methodology to collect 3D strain distributions of the axillary pouch while the glenohumeral joint is rigidly mounted within the robotic testing environment. The testing apparatus accurately represented the robotic testing environment in that it allowed for 5-DOF and had a metallic finish, mimicking the noise that will be observed during testing in the robotic environment. The calibration tools allowed for accurate calibration of the Vicon systems and were proved to accurately represent the shoulder capsule working volume. It was shown that with an infrared Vicon system metallic/reflective environments need to be coated/covered before the cameras can accurately track marker movement. The robotic environment is highly metallic, and therefore will need to be coated or covered to prevent excessive noise during data acquisition. Due to the amount of markers lost during internal-external rotations using the 3 camera setup, the 5 camera setup is the recommended setup to collect 3D strain data on the axillary pouch. The 5-camera setup is also recommended due to its greater repeatability. To accurately assess the strain distribution in the axillary pouch, an accuracy of 0.5% is needed. Although the 5-camera system had an error of 1.4 ± 0.5%, it may have been attributed to high standard deviations of caliper measurement between trials. A coordinate measuring machine will be used to obtain a more accurate marker distance of the calibration wand and therefore will serve as a more accurate golden standard. To date, few studies have examined multi-dimensional strain fields in the glenohumeral capsule during stationary positions and small ranges of movement. [2] Future experiments will utilize the optimal camera positioning to collect strain fields in the glenohumeral capsule during a simulated clinical exam using a 6-DOF robotic/universal force-moment sensor (UFS) testing system. The multi-dimensional strain data collected during the simulated clinical exam will then be used as an input for our specimen-specific FEM and be used to validate that FEM.

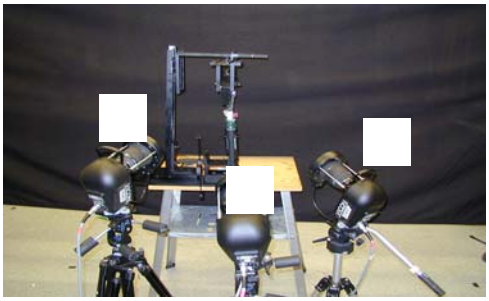

Figure 3. 3-Camera Vicon configuration focused on the shoulder model with cameras 1, 2, and 3 labeled.

Figure 4. 5-Camera Vicon configuration focused on the shoulder model with cameras 1, 2, 3, 4, and 5 labeled.

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It was a hot, summer day back in August of ’78 when I decided to make my way into the world. I started out in the city of Torrance, CA, which is in L.A. County, where I lived until the age of 13. Deciding we needed a change, my family and I moved north to San Ramon, CA, where I attended California High School. Throughout high school, I enjoyed playing sports, especially basketball and soccer.

Aspiring to one day work at NASA, I entered the Mechanical Engineering program at California State University, Chico. I was involved in activities such as the Human Powered Vehicle, ASME, SWE, and other various clubs. In my spare time, I enjoyed spending time outdoors with my friends, playing soccer, running, hiking, snowboarding, and camping.

In May 2001, I graduated from Chico State with a B.S. in Mechanical Engineering. Alas, my childhood dream of becoming an astronaut had faded, and I started working as a Quality Control Engineer at N.U.M.M.I., an automobile manufacturing plant. However, after a year and a half, my career interests had changed, and I have decided to pursue further education in the area of biomechanics/bioengineering.

Working at the MSRC this summer has been a great experience. As a result, my interests in the area of bioengineering research have grown. I’d like to thank my mentor Jesse “Cartesior” Fisk, and the rest of the ACL group, for the help and guidance I received, and for the knowledge I have acquired. I would also like to thank Dr. Woo for giving me the opportunity to work at the MSRC and explore my interests. And to the rest of the MSRC, thank you for making this experience so enjoyable…good times.

SIMULATION OF SKIN MOTION AND EFFECT ON KINEMATICS

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INTRODUCTION

Anterior Cruciate Ligament (ACL) injuries are one of the most common injuries in sports today. A partial tear or complete rupture of the ACL can lead to joint instability. Due to the frequency of this injury, finding an efficient treatment for this condition is crucial. It is necessary to understand the role of the ACL in the intact knee in order to assess the efficacy of ACL reconstructions. Determining kinematics of knees with an intact ACL will provide a standard for comparing kinematics of ACL reconstructed patients. The kinematics of knees with an intact ACL can subsequently be utilized to evaluate the success of various techniques of surgical reconstruction of the ACL and help to improve ACL reconstruction techniques.

An essential aspect of kinematic analysis is to determine the position and orientation of each body segment in space. In the past, markers have been attached directly to bones, using intracortical pins, to obtain accurate kinematic data. Due to the invasiveness of this method, most studies place markers on the skin. These methods involve using cameras to track reflective markers, or sensors attached to the skin, over bony landmarks. The main limitation of this method is skin motion artifact (SMA) [1]; skin movement relative to underlying bone.

The most common marker set was developed by the Helen Hayes Clinic. This method is used to determine knee rotations in the clinical setting; this method does not account for translations. While several studies have observed SMA up to 30 mm for markers on the thigh and shank [2,3,4], to our knowledge, no studies have been done to show how knee kinematics obtained with the Helen Hayes marker set are affected by skin motion artifact.

OBJECTIVE

The objective of this study was to determine the effect of random skin motion artifact on knee kinematics measured with the Helen Hayes method during a simulated step up activity.

METHODS

The knee kinematics of a step up activity used for this simulation study were generated using MATLAB (The MathWorks, Inc., Natick, MA). A VICON 4 camera motion system (VICON Motion Systems, Inc., Lake Forest, CA) was used to record the position of the Helen Hayes markers on a normal human subject. (The marker set is pictured in Figure 1.) Markers were attached to: the heel, lateral malleolus, metatarsal head, lateral epicondyle, right and left anterior superior iliac spine (ASIS), sacrum, and to wands attached to the shank and thigh. These marker locations were incorporated in a MATLAB program. SMA was simulated by adding random motion of 5, 10, 15, and 20 mm.

Using a uniform probability function, SMA was randomly chosen for each marker at each time frame, in each direction.

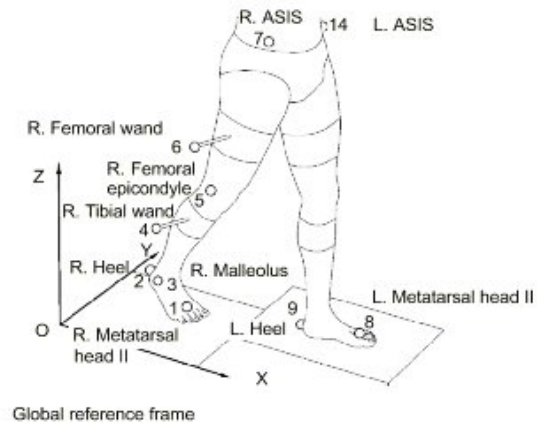

Figure 1. Helen Hayes marker locations [5].

The simulated step-up activity consisted of 55 frames of data over which knee flexion increased from 1 to 55 degrees, adduction from 0-1 degree, and internal rotation from -4 to +2 degrees [6]. Euler angles were used to calculate the orientation of the tibia with respect to the femur in anatomical directions. The marker locations with respect to global were then generated via transformation matrices.

A second program was written to calculate kinematics from marker locations. Using the 3-D position of these markers, as well as anthropometric measurements (ASIS breadth, knee diameter, malleolus width, malleolus height, and foot length), the 3-D positions of joint centers were calculated for the hip, knee and ankle [6]. From this data, anatomical coordinate systems were calculated, and the tibia with respect to the femur was determined. Knee rotations were generated using Euler angles about anatomical axes. These rotations were calculated without and with varying amounts of SMA.

Error was calculated as the magnitude of the difference in knee rotation obtained from the marker data with and without added SMA. The error between these trials illustrates the accuracy of the Helen Hayes marker set in representing actual joint movement.

RESULTS

As the amount of SMA increased, the mean error in knee rotations increased up to 4 degrees (Figure 2). For 5

mm of added artifact, the error for flexion/extension, abduction/adduction, and internal/external rotation were all approximately 1 degree. For 20 mm artifact, the mean error for flexion/extension and internal/external rotations was 3 degrees and the error for abduction/adduction rotation was 4 degrees. Standard deviation of error also increased as the magnitude of artifact increased. For 5 mm of artifact, the standard deviation for each rotation was approximately 0.6. For 20 mm of artifact standard deviation was 2.5 - 3 degrees for each rotation.

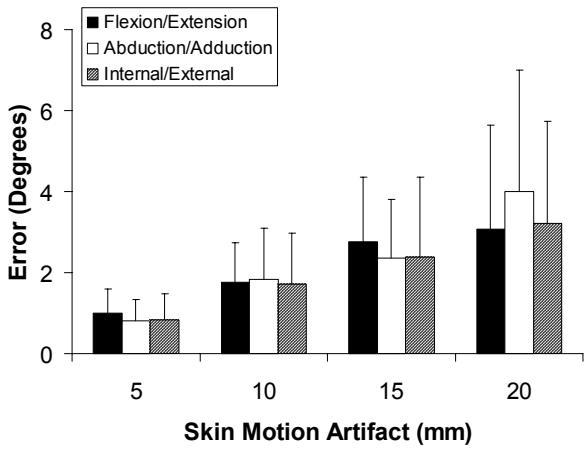

Figure 2. Effect of simulated skin motion artifact on knee rotation, calculated with the Helen Hayes method.

DISCUSSION

The objective of this study was to determine the effect of random skin motion artifact on knee kinematics measured with the Helen Hayes method during a simulated step up activity. With video based systems becoming a common method of determining human kinematics, it is important to understand the accuracy of these methods. As this study shows, mean error of knee rotations calculated with the Helen Hayes marker set can be up to 4 degrees for 20 mm of simulated artifact.

The ultimate goal of this research is to determine how knee kinematics change with ACL deficiency and reconstruction. In a study using radiostereometric techniques [6], tibial rotations of ACL deficient and normal knees were compared. At 55 degrees of knee flexion, ACL deficient knees were reported as 2 degrees more externally rotated than normal knees. At full extension, deficient knees were also approximately 2 degrees more externally rotated. Abduction/adduction rotations were also examined at 0 and 55 degrees of flexion. At 55 degrees of flexion, the deficient knees were 3 to 4 degrees more adducted than normal knees. At zero degrees of flexion, these positions changed by 1-2 degrees.

Error due to SMA should be an order of magnitude less than the differences between knee rotations of normal compared to ACL deficient knees to detect these differences. The error in calculated rotations due to SMA was similar to the difference between ACL deficient and normal knees. While Helen Hayes has been proven to be greatly effective for a variety of clinical applications, the error is too large for the purposes of this study. Therefore, a more accurate method than the Helen Hayes method is needed to meet our objective.

In order to reduce error in rotation kinematics, the SMA must be reduced or corrected for. The Helen Hayes marker set places markers near joints where skin motion has been reported to be the largest [2,3,4]. Data has also shown that skin motion follows a trend through rotational range of motion [2]. However, due to the lack of information on skin motion artifact of markers on the pelvis, as well as attached to wands, it was necessary to model skin motion artifact as random error in this study.

Several methods have been proposed to reduce error from skin motion artifact. In the future, the interval deformation technique with the point cluster marker set, will be evaluated. The point cluster marker set has been reported to reduce error in SMA, while interval deformation technique further corrects for SMA mathematically [7]. In addition, these methods and techniques provide knee translations in addition to rotations. Future works include repeating this study with systematic SMA using the point cluster marker set and interval deformation technique to determine if sufficient accuracy is attainable. Once an acceptable technique is determined, these methods can be used to evaluate treatment, reconstruction, and prevention techniques of ACL injuries.

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I was born in Butler, Pennsylvania on June 6, 1981. I graduated from the University of Pittsburgh in April 2003 with a BS in neuroscience. During college and high school, I spent a lot of time competing in taekwondo. Skiing (i.e., not snowboarding) is also one of my favorite things to do. I will be attending the University of Chicago Pritzker School of Medicine beginning this August.

This was my second summer in the MSRC and I have learned more than I could have ever expected over the past year and a half. By now, I finally feel that I have learned enough to consider myself a "bioengineer" despite my neuroscience background. Also, by working with the surgeons in the lab, I have confirmed my goal of becoming an orthopaedic surgeon.

I would like to thank everyone who has taught me everything from suturing to how the robot functions. Maribeth Thomas has been a great mentor; she has become one of my best friends and few people know and understand "Hector" the robot as well as she does. I would also like to thank Dr. Debski and Dr. Woo for their guidance and direction. Finally, I have made a lot of friends during my time at the MSRC – I want you all to come visit me in Chicago!

ASSESSING HOW THE ERROR IN REPRODUCING KINEMATICS AFFECTS THE EXTERNAL LOADS ON THE KNEE AND FORCES IN THE ANTERIOR CRUCIATE LIGAMENT

Mara L. Schenker, Maribeth Thomas, B.S., Susan M. Moore, B.S., Robert Kilger, M.D., Richard E. Debski, Ph.D., Savio L-Y. Woo, Ph.D., D.Sc.

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INTRODUCTION

Six degree-of-freedom (DOF) clinical examinations are frequently used to diagnose joint instability and soft tissue damage [9,10]. However, the forces and moments applied by the clinician to the joint as well as the *in situ* forces in soft tissues during these exams have not yet been quantified. A method has recently been developed to collect kinematics during a clinical examination and reproduce the exact kinematics on the same cadaveric specimen on a robotic/universal force-moment sensor (UFS) testing system. With this method, the external forces and moments applied by the clinician as well as the *in situ* forces in soft tissues can be determined. The average position and orientation errors associated with this method have been shown to be 0.7 mm and 0.6°, respectively [1].

The effect of this error in the method of reproducing kinematics on the external loads and *in situ* forces of biological tissues such as those in the knee, however, is still unknown. Therefore, the objective of this study was to determine the effect of the novel method of reproducing kinematics on the external loads applied to the knee and the *in situ* force in the ACL. By understanding the effect of this error, the method of reproducing kinematics may be further validated for its use with biological tissues.

METHODS

One fresh-frozen porcine knee was prepared and rigidly attached to a robotic/UFS testing system as described in previous literature [2]. Registration blocks were fixed to the femur and tibia of the specimen.

To examine the effect of the error in the reproducing kinematics method on the external loads on the knee and the *in situ* force in the ACL, the porcine knee was tested with a robotic/UFS testing system. The robotic manipulator (Puma Model 762, Unimate, Inc.) is capable of achieving position control in 6 DOF with a repeatability of 0.2 mm for translations and 0.2° for rotations. The UFS (Model 4015, JR3, Inc., Woodland, California) can measure three orthogonal forces and moments with a repeatability 0.2 N and 0.01 Nm for forces and moments, respectively. With the force feedback from the UFS, the robotic/UFS testing system can also operate in a force control mode. In both human and porcine knees, this system has been successfully used to apply external loads to the joint at preselected flexion angles, while the kinematics of the resulting 5 DOF (medial-lateral, proximal-distal, and anterior-posterior (A-P) translations, internal-external, and varus-valgus rotations) joint motions are measured [6,7,8]. By repeating these positions with a high level of accuracy, the principle of superposition can be applied, thus allowing the *in situ* forces in the ACL to be determined [3,4,5].

The robotic/UFS testing system operating in force control was used to collect the kinematics for the reproducing kinematics method. The 5 DOF kinematics were determined in response to a 100 N anterior tibial load and a 5 Nm valgus tibial torque at 30°, 60°, and 90° of knee flexion.

After collecting the kinematics, the tibia was detached from the end of the robotic manipulator. The robotic arm was removed from the working volume of the robot and it was manually returned to the specimen. These steps simulated the beginning of a new test as the tibia was not in the same initial position as it had been in the force control.

Prior to biomechanical testing, the precise location of the registration blocks attached to the femur and tibia were determined with respect to a frame coordinate system mounted on the robot according to the method described by Moore et al. [1]. An external digitizing device (Microscribe) was used to determine these relationships.

After the tibia was re-attached to the end of the robotic manipulator, the intact kinematics were reproduced with the method described by Moore et al. [1]. Ten positions were reproduced in this study. Positions corresponding to 0% applied load, 33% of maximal applied load, 50% of maximal applied load, 66% of maximal applied load, and 100% of maximal applied load were reproduced at 30° of flexion for valgus and anterior loads. With the force-feedback provided by the UFS, the external loads on the knee were recorded. These loads could be directly compared to the external loads applied to the knee during the force control. To assess the position repeatability of this test, the registration blocks were digitized at each position.

RESULTS

In response to a 100 N anterior tibial load at 30° of knee flexion, the external loads on the knee during the force control at 0%, 33%, 50%, 66%, and 100% of maximal load were 0 N, 17.9 N, 49.6 N, 71.5 N, and 96.1 N, respectively. When the kinematics obtained from this force control were reproduced using the method described by Moore, et al. [1], the external loads measured were 4.9 N, 59.9 N, 101.3 N, 132.6 N, and 155.8 N. The difference between external loads applied by the robot and the new method for the 100 N anterior load at 30° of knee flexion were 4.9 N, 42.0 N, 51.7 N, 61.1 N, and 59.7 N. Figure 1 illustrates the external anterior loads on the knee applied by the robot and those measured by the new method at each of positions reproduced by the new method.

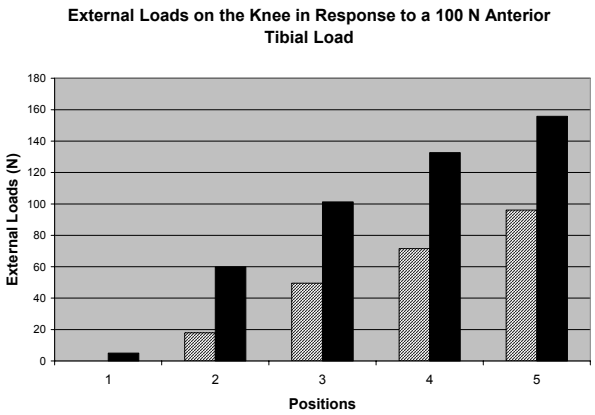

Figure 1: External Loads on the Knee in Response to a 100 N Anterior Tibial Load at 30° of Knee Flexion: The striped bars correspond to the external loads applied to the tibia in increments of 0%, 33%, 50%, 66%, and 100% of maximal applied load. The solid bars correspond to the external loads measured by the reproducing kinematics method described by Moore et al. [1].

In response to a 5 Nm valgus tibial torque at 30° of knee flexion, the external loads on the knee during the force control at 0%, 33%, 50%, 66%, and 100% of maximal load were 0 N, 4.1 N, 3.6 N, 5.1 N, and 4.1 N. When the kinematics obtained from this force control were reproduced using the method described by Moore et al. [1], the external loads measured were 4.9 N, 44.1 N, 61.9 N, 89.4 N, and 86.4 N. The difference between external loads applied by the robot and those measured by the new method for the 5 Nm valgus torque at 30° of knee flexion were 4.9 N, 39.9 N, 58.3 N, 84.3 N, and 82.4 N. Figure 2 illustrates the external valgus loads on the knee applied by the robot and those measured by the new method at each of positions reproduced by the new method.

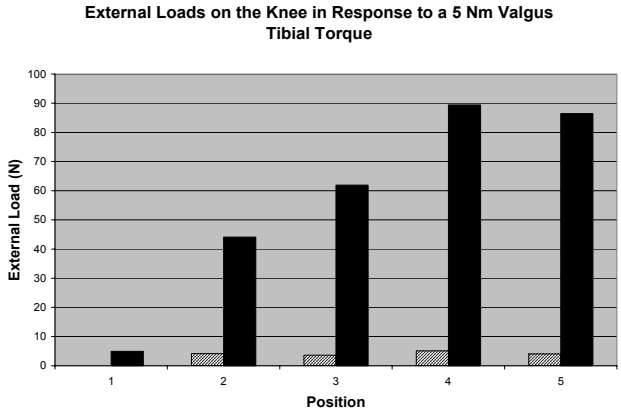

Figure 2: External Loads on the Knee in Response to a 5 Nm Valgus Tibial Torque at 30° of Knee Flexion: The striped bars correspond to the external loads applied to the tibia in increments of 0%, 33%, 50%, 66%, and 100% of maximal applied load. The solid bars correspond to the external loads measured by the reproducing kinematics method described by Moore et al. [1].

In order to validate the positional repeatability of the reproducing kinematics method, the registration blocks were digitized at each position during the force control when the kinematics were being collected. In addition, the registration blocks were digitized at each position during the reproducing kinematics method. The positional error measured in this study was 2.77 mm.

DISCUSSION

A novel method has been developed to collect kinematics during a clinical examination and reproduce the exact kinematics on the same specimen on a robotic/UFS testing system [1]. This method allows the quantification of external forces and moments applied by a clinician to a joint in addition to the *in situ* forces in soft tissue structures. By understanding the applied loads and forces in soft tissues associated with clinical examinations, clinicians may hopefully improve injury diagnoses and treatments.

The average error associated with the new method of reproducing kinematics has been shown to be 0.7 mm for position and 0.6° for orientation [1]. The purpose of this study was to evaluate the effect of this error on anisotropic biological tissues, specifically, on the external loads applied to the knee and the force in the ACL.

Currently, the results of this study indicate that this method is not sufficient for reproducing kinematics to obtain the external loads on the knee and the force in the ACL. The positional error for this specimen was 2.77 mm. This was much greater than 0.7 mm error explained by Moore, et al. [1] for this protocol. Unfortunately, during this particular porcine knee test, the protocol had to be aborted before the test was completed. At mid-experiment, prior to reproducing kinematics on the intact knee at 60° and 90° of flexion, the reproducing kinematics program was no longer able to recognize the tool coordinate system. Therefore, it was unable to recognize any of the desired positions at 60° and 90° of flexion. As a result, the external loads on the knee could not be measured with the reproducing kinematics method at these flexion angles.

Since the positional error was very large in this study at 30° of flexion, the resultant external loads that were measured in the new positions were inaccurate when compared to the applied external loads. In the future, the effect of the random error in the reproducing kinematics method on the external loads and the force in the ACL will be assessed when the positional error is reduced.

ACKNOWLEDGEMENTS

I would like to thank my mentor, Maribeth Thomas, for teaching me a lot about the robot and for being a great friend. I would also like to thank Susan Moore for her absolute persistence and perseverance in these tests even late at night after LONG days! Also, thanks to Robert Kilger for helping me with the dissections and for teaching me a lot about arthroscopy and orthopaedic surgery in general. I will definitely be a step ahead in medical school because of him! Finally, I would like to thank Drs. Debski and Woo for providing me with the opportunity to work in this great lab for the summer.

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Upper Extremity Group
Mentor: Danny Harkness, B.S.
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I knew from day one that my time in the lab this summer would expose me to all sorts of i*nteresting* things. But it would have been difficult to prepare me for the robots, appendages, technology, tools, and resources that were readily available in the lab. *Every* task was a learning experience.

So on to a little personal history. I was the second and final child for my parents, Scott and Susan, who decided that two boys were enough. My older brother, Ryan, my parents, and I packed up and moved from Ohio to south Texas when I was nine years old. We returned to Ohio while I was in high school, and it was nice to be back home, although I have just recently become accustomed to the rain and the snow and the gray days that abound in the northeast.

During high school, my participation in sports and interest in the sciences developed, and I began to orient my goals towards the field of sports medicine. Pitt was a natural choice for me; I loved the urban environment, the proximity to home, the surrounding city, and the relationship Pitt shares with UPMC.

Upon my arrival to Pitt, my love of contact sports landed me on the rugby pitch as the newest member of Pitt's Rugby Football Club. It is always a fun time, and serves as an excellent stress outlet as well. My other pleasant distraction from schoolwork comes in the form of my campus job; a Pitt Pathfinder, one of the official undergraduate recruiters for the university.

My studies, involvement in research, experiences within the field of orthopaedics and my interest in sports medicine motivated me to apply to the MSRC internship program. I was very honored to be asked to join the MSRC team for this summer. I will leave the lab with new knowledge and original ideas; however, I believe the most important thing that I take with me is the knowledge that research is an intelligent and constant struggle that requires determination and patience to get to where you want to be.

"Genius is one percent inspiration and ninety-nine percent perspiration."
-*Thomas Alva Edison*

DETERMINATION OF JOINT MOMENT AND JOINT CENTER

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INTRODUCTION

Motor function within the musculoskeletal system allows for the performance of many daily activities. Force production required to perform tasks is based upon the available moment about articulating joints of the body. Prediction of these moments, however, is not well understood due to the difficultly in determining the moment arms of tendons crossing the joints and the relative joint center. Limited techniques, however, have been investigated to provide a means of determining hand joint moments and joint centers *in vivo*.

A joint moment is determined by summing the products of all forces acting on the system and their respective moment arms. Moment arms of musculoskeletal systems are defined as the perpendicular distance from a tendon line of action to the joint center of rotation. A study conducted by Li et. al [1] involved using anthropometric moment arm data [2] and isometric force measurements to estimate the contribution of the intrinsic and extrinsic muscles *in vivo* joint moments about various joints of the hand. Methods to calculate these tendon moment arms, however, have typically relied on calculating the slope of the joint angle vs. tendon excursion data [2, 3, 4]. These data are limited in that the reported variability of their values is large. Finally, Hollister et al. [5] developed a means of estimating the center of rotation of the metacarpophalangeal joint of the thumb based on an axis finder. The axis finder, however, is limited due to skin movement and initial placement of the device over the joint.

Therefore, our objective was to develop a novel method to calculate a joint moment and center of rotation using a mechanical model.

METHODS

An apparatus was developed consisting of a customized aluminum bar, three known weights, and a 6 DOF force/torque transducer capable of being positioned within a 3D environment via an aluminum slide rail system (80/20 Inc., Columbia City, IN, USA) (Figure 1). The aluminum bar was machined with eight notches (approximately 1mm x 2 mm) approximately 1 cm apart. A screw was inserted into the bar to hold the three weights of 4.5 N, 9.0 N, and 13.5 N during testing. A metal pin was inserted into the end of the bar perpendicular to its longitudinal axis to limit movement of the bar to one-degree of freedom. A groove was cut around the rod 11.2 mm from the center of rotation to be used as a reference marker location for subsequent calculations. A metal ring, positioned perpendicular to the longitudinal axis of the bar, was attached to the transducer (ATI Industrial Automation, Apex, NC, USA) with a customized adapter plate. Force measurements were collected at 100 Hz and instantly displayed using a LabVIEW program (National Instruments, Austin, TX, USA).

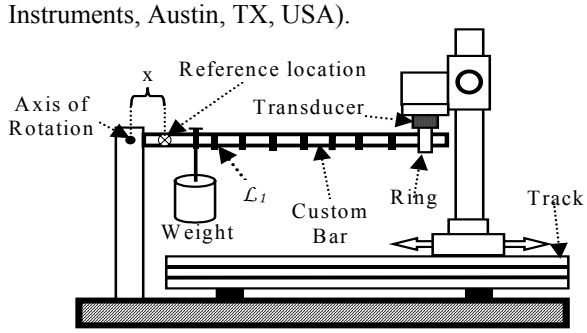

Figure 1. Entire system consisting of apparatus, suspended load, and 6 DOF force/torque transducer.

Making use of static equilibrium, known moments about the center of rotation were calculated using the weight of the bar and the applied load. The 8 notches designate known arbitrary positions at which the ring/transducer system was positioned to acquire force output data. The distance from the reference point to the center of a respective notch was designated L_i, and the distance from the reference point to the center of rotation was designated x. Three output force measurements were made for each loading condition (4.5 N, 9.0 N, and 13.5 N) at locations L_i along the bar. Thus, the moment arm of the output force was the sum of the known distance, L_i and the unknown distance, x.

Unknown variables M (joint moment) and x were determined by minimizing the following objective function:

$$f(M,x) = \Sigma\, [M - F_i * (L_i + x)]^2$$

The output of this objective function yielded the theoretical joint moment and theoretical joint center location.

RESULTS

The known and predicted joint moments increased with increasing load as expected (see Table 1). The known joint moments, calculated from actual weight and joint center location, were 366.6, 700.7, and 1032.7 Nm, respectively, under the three increasing loading conditions. The corresponding predicted joint moments were 373.5, 712.1, and 1050.7 Nm, respectively. The percentage differences between the known and predicted joint moments were 1.9%, 1.6%, and 1.8%, respectively.

	Mass #1	Mass #2	Mass #3
Known Joint Moment	366.6	700.7	1032.7
Predicted Joint Moment	373.5	712.1	1050.7

Table 1. Known and predicted joint moment (Nm) under three separate loading conditions.

The known and predicted distances from the reference point to the center of rotation (x) are shown in Table 2. The known distance measured was 11.2mm. The predicted value was 12.5, 12.0 and 12.6mm for subsequent increasing loading conditions. The theoretical distances were larger than the experimental distances by 1.3, 0.8, and 1.3 mm, respectively with an average position error of 1.1mm. Thus, the percentage differences were 11.2%, 7.2%, 11.9%, respectively (see Table 2).

	Mass #1	Mass #2	Mass #3
Known Joint Center Location	11.2	11.2	11.2
Predicted Joint Center Location	12.5	12.0	12.6

Table 2. Known and predicted distance (mm) from reference point to center of rotation (x).

SUMMARY

We have developed a means of determining a joint moment and joint center of rotation based on force measurements at arbitrary distances from an unknown center of rotation. The moment arm data [2] used in previous calculations of joint moment [1] contains a large variance. However, the joint moment data presented herein is not reliant on previous moment arm data. Our novel method provided error slightly higher for location of joint center of rotation when compared to previous works [5]. Limitations of the current system include the tolerances inherent to our measurement devices, human measurement error, and potential movement of our system with applied load.

This method, however, has potential utility as a means of predicting the joint moment and joint center of rotation *in vivo*. Our methodology therefore contributes to the current research into the determination of joint moments, moment arms, and location of the center of rotation. Determination of the joint moment and joint center could also serve to provide accurate attachment sites for tendon transfers. During tendon transfer procedures, moments created by individual tendons of the digits can potentially aid decisions as to location of tendon insertions to most closely resist normal physiological loads. Additionally, our future plans are to incorporate this method in both *in vitro* and *in vivo* experiments to determine joint moments of the extrinsic and intrinsic muscles of the hand.

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I was born in Pittsburgh on October 19, 1983, and I've lived my entire life in Penn Hills, PA with my parents and younger brother Alex. I graduated from Penn Hills Senior High School as a National Merit Scholar and valedictorian of the class of 2001. I also spent ten years competing in gymnastics. It was my experience in gymnastics that inspired me to pursue a career in medicine and my aptitude for math and science that led me to engineering; when I discovered biomedical engineering, I knew it was the perfect combination of the two.

In the fall of 2003 I will be a junior at Carnegie Mellon University. My two majors keep me busy, but in my free time I enjoy taking ballet classes and playing the viola in the All University Orchestra. I am also a member of the National Society of Collegiate Scholars. After graduation, I plan on attending graduate school for biomedical engineering, perhaps focusing on biomaterials.

I would like to thank my mentor, Steve Abramowitch, for his support this summer. I would also like to thank Dr. Woo and Dr. Debski for giving me the opportunity to spend my summer at the MSRC learning about biomechanics and the research process. Finally, thanks to the MCL group and the entire MSRC for making this summer a great educational experience and also a lot of fun.

EFFECT OF STRAIN RATE ON THE VISCOELASTIC PROPERTIES OF THE RABBIT MCL

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INTRODUCTION

Knee injuries are a common occurrence in the general population, with 3 million people visiting orthopaedic surgeons yearly for injury related knee problems [1]. The MCL is one of the most frequently injured ligaments in the knee, with the majority MCL injuries occurring during sports activities [3]. MCL injuries usually occur when an excessive valgus load is applied to the knee. This type of loading is common in beginning skiers who are taught to ski in the "snowplow" position with legs apart and feet turned in, as well as in athletes who play contact sports such as rugby or football.

Ligaments behave as viscoelastic structures. The viscoelasticity of a ligament dictates its response to various loading conditions; its response in turn is an indicator of its potential for injury. It has been shown by Woo et al (1981) that the viscoelastic properties of the rabbit MCL do not change at strain rates between 0.001 and 1 Hz [4]. However, viscoelastic properties of the MCL at frequencies higher than 1 Hz are only starting to be investigated [2] and strain rate sensitivity of the healing MCL needs to be explored.

The goal of this research was to develop a protocol for strain rate testing of the normal rabbit femur-MCL-tibia complex (FMTC) under sinusoidal tensile loading, with the eventual application of this protocol to testing of the healing MCL.

METHODS

Specimen Preparation

Fresh frozen rabbit knees were used in this study. Throughout the specimen preparation, the MCL was kept moist using saline solution. Each knee was allowed to thaw and then dissected. All soft tissues were removed except for the MCL and its insertions. The femur and tibia were potted in polymethyl methacrylate (PMMA). After solidification of the PMMA, the FMTC was secured in custom counterweighted clamps. A 50 lb. submersible load cell (Sensotec Model 31) was attached to the bottom clamp. The entire load cell-clamp-FMTC assembly was loaded into a materials testing machine (EnduraTEC Model ?). The alignment of the FMTC in the machine was adjusted using an XY table so that the MCL lay in a straight line between the top and bottom attachments.

Strain Rate Testing

Once loaded into the testing machine, a 2N preload was applied to the specimen. The gauge length was then set to 0. The specimen was then subjected to cyclic elongation between 0 and 1 mm at a frequency of 0.1 Hz. The specimen was allowed to come to equilibrium at this frequency before beginning data collection. The corresponding displacement and load were recorded at 0.1 second intervals during each test. The frequency of the waveform was then increased to 1 Hz and the procedure was repeated with no rest period between frequencies. This was repeated at 5, 10, and 15 Hz. The specimen was kept moist using saline solution throughout testing.

Data Analysis

A program was written to analyze the data using the *Mathematica* software package (Wolfram Research, Inc). The data recorded by the EnduraTEC system is read into *Mathematica* as tables of load versus elapsed time and displacement versus elapsed time. The program finds the maximum and minimum values in a series of 30 consecutive load or displacement readings using *Mathematica*'s Max[ ] and Min[ ] functions. A point is classified as a peak if its value matches this maximum, is greater than or equal to the value of the point immediately before it, and is strictly greater than the value of the point immediately following it. In this way, if several consecutive data points share the same maximum load or displacement value, only the last point is selected as a peak. A similar process is used to find the valleys. This entire procedure is repeated until all data points have been analyzed.

Because the data being analyzed are not ideal, the program also checks for and eliminates values that were incorrectly identified as peaks or valleys by the above algorithm. A peak is eliminated if its value is less than 80% of the maximum overall value; a valley is eliminated if its value is greater than 120% of the minimum overall value; and a peak or valley is eliminated if the time elapsed between it and the next peak or valley is less than half of the test wavelength.

The program then graphs the hysteresis loop for the specimen and calculates the phase angle (δ) for each cycle via the equation $\delta = 2\pi f(\Delta t)$, where f is the test frequency and Δt is the time lag between the load and displacement curves.

RESULTS

One specimen was tested using the current protocol. Hysteresis loops generated by *Mathematica* for the specimen at 0.1, 1, 5, 10 and 15 Hz are shown below.

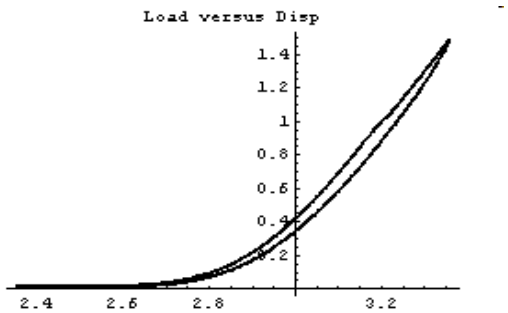

Figure 1. Hysteresis loop at 0.1 Hz, 2 cycles

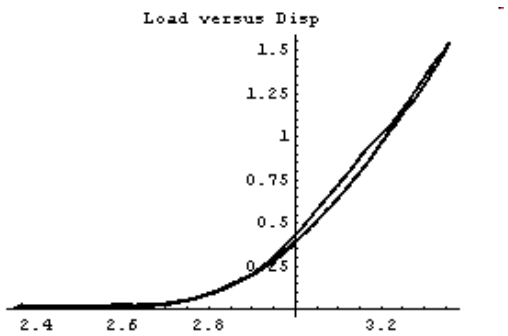

Figure 2. Hysteresis loop at 10 Hz, 50 cycles

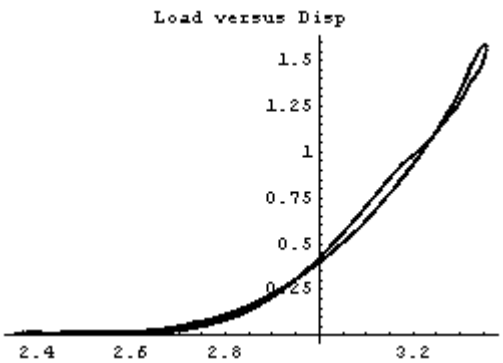

Figure 3. Hysteresis loop at 15 Hz, 65 cycles

Hysteresis loops for 0.1, 1, and 5 Hz were very similar in shape (Figure 1), which confirms the findings of previous studies [4] showing that viscoelastic properties of the MCL do not change between these frequencies. The area enclosed in the hysteresis loop appears to decrease in figures 3, 4, and 5, suggesting that the MCL behaves more elastically at higher frequencies.

Phase angle calculations were carried out by the *Mathematica* program for the specimen at 0.1 and 10 Hz. The phase angle at 0.1 Hz was 0.15 radians while the phase angle at 10 Hz was 0.06 radians. The *Mathematica* program was unable to calculate a phase angle at 1 and 5 Hz due to problems locating the peaks and valleys in the data, and the calculated phase angle at 15 Hz was very inconsistent despite the peaks being located correctly.

DISCUSSION

The goal of this study was to develop a protocol that can be used for frequency testing of the healing rabbit MCL. A protocol has been developed and a *Mathematica* program was written to perform data analysis. This study tested one rabbit MCL with the current protocol. It was found that the area of hysteresis was very similar from 0.1 to 5 Hz, but became smaller at higher frequencies. The phase angle also decreased from 0.1 to 10 radians. This data suggests that the MCL behaves more elastically at higher frequencies.

The results obtained in this study at 0.1 and 1 Hz concur with the study by Woo et al [4] which demonstrated that the MCL's properties do not change in this range of frequencies. However, the high-frequency tests at 10 and 15 Hz would appear to contradict the results of previous studies. A study by Weiss et al [2] found that the phase angle increased sharply in this frequency range, suggesting a more viscous material; conversely, this study found the MCL to be more elastic in this range. The previous study tested dog-bone cutouts of the MCL, whereas this study used a femur-MCL-tibia complex. Thus, there may be differences in behavior resulting from the ligament's insertions. It is also unclear whether clamp vibration, which could change the calculated phase angle and hysteresis, affected the data in one or both of these studies

Although progress has been made toward a protocol for frequency testing the healing MCL, further work must be done before the protocol can be applied. The *Mathematica* program now identifies peaks and valleys in data consistently, but improvements are necessary to make the program more efficient and accurate in its calculations. Also, the experimental setup must be examined to determine if a different method of attachment to the EnduraTec would reduce clamp vibration and sensitivity to alignment.

Once these issues have been addressed, specimens will be tested in a saline-filled tank to simulate the MCL's environment within the body. Motion Analysis will be incorporated in order to calculate strain on the MCL. When this protocol has been established for the normal MCL, it can then be applied to the healing MCL.

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I was born on June 6, 1981 in Baltimore, Maryland. I lived there until the fourth grade when my family moved to Fox Chapel in Pittsburgh. I went to O'Hara Elementary, Dorseyville Middle School, and Fox Chapel Area High School. I've played club soccer since I was in fourth grade, and in high school I lettered in swimming and volleyball. I also rowed on the Fox Chapel crew team. I started taking piano lessons when I was 5 and continued for 10 years. I spent many years in the Boy Scouts of America and am now an Eagle Scout. In 1998, the summer before my senior year, I went to the Pennsylvania Governor's School for Health Care to learn more about careers in medicine and the field in general.

I recently graduated from the University of Pittsburgh at Johnstown where I spent all 4 years of my boss undergraduate experience. Intramurals were the center of life at UPJ and my volleyball team "Juggernaut" won championships 5 times. I worked as a lifeguard at our swimming pool for 4 years and became a certified lifeguard and water safety instructor. I will be attending Nova Southeastern University School of Dentistry in the fall located in beautiful Ft. Lauderdale, Florida.

I would like to thank Dr. Woo for this unique opportunity to work at the MSRC. I would also like to thank Dr. Jia and Dr. Takakura for their guidance and patience in mentoring me throughout the summer. It has been very interesting and educational.

DEVELOPMENT OF A METHODOLOGY TO ANALYZE COLLAGEN FIBRIL DIAMETERS OF HEALING LIGAMENTS

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INTRODUCTION

Medial collateral ligament (MCL) injuries occur with high frequency during sports [1]. The MCL of the knee has the potential to heal from injuries without surgical intervention, however the healing ligament displays inferior histomorphological appearance, biochemical composition, and biomechanical properties in comparison to the normal ligament. In terms of histomorphological appearance, the collagen fibril diameters in the healing ligament are homogenously smaller compared to the bimodal distribution of small and large collagen fibril diameters in the normal ligament. Larger fibrils are not typically present in the healing ligaments until up to 2 years after injury. The small diameter fibrils are believed to be responsible for the inferior tensile strength properties of healing ligament [2, 3, 4]. The assessment of collagen fibril diameters is important because it correlates the ultrastructure of the tissue to its tensile strength. Previous ligament healing studies have demonstrated the necessity of transmission electron microscope (TEM) images of ligament cross-sections to make tissue comparisons. Collagen fibril diameter measurements can provide important information regarding the ligament healing process. Previous studies have typically analyzed at least 1000 fibrils per specimen. Some problems associated with TEM studies of collagen fibrils have included artifacts of fixation, oblique cutting, varied fibril diameters along tissue length, and fibril measurement techniques [3]. As for measurement techniques, issues concerning image processing and repeatability tend to be associated more with human error from using free analysis programs such as Scion Image or NIH Image. Potential possibilities to address these problems might require a certain degree of automation in the software used to obtain measurements as well as performing image processing of a single image more than once by several different observers to reduce possible error from user subjectivity.

OBJECTIVE

Because image analysis software currently on the market is not designed specifically for measuring collagen fibril diameters in TEM images, it was our objective to design a protocol for the measurement of collagen fibrils from TEM images of ligament. It was also our goal to utilize Scion Image analysis software and evaluate the error associated with human subjectivity.

METHODS

Two skeletally mature female New Zealand White rabbits were used in this study. A 6 mm gap injury was made in the right MCL of each rabbit, while the left side served as a sham control. The rabbits were allowed free cage activity for 12 weeks. Animals were sacrificed and samples were excised from the center of ligament at the joint level and placed in Karnovsky's fixative for preparation of transmission electron microscopy.

NIH image capture software was used in conjunction with a TEM to take images with background subtraction of the various MCL cross sections at a magnification of x70,000 (Model H-7100 electron microscope, Hitachi). We followed the practice of avoiding artifacts, regions subject to oblique cutting, and pericellular regions [3]. Scion Image analysis computer software was used for the analysis of the collagen fibrils in each image (Scion Corporation, 2000). Before analysis was performed on the images, a 21,574 lines/cm calibration grid image at a magnification of x70,000 was utilized in conjunction with the programs calibration function to convert all measurements from pixels to nanometers. Regions of interest were selected and cropped from each image to measure a sample of fibrils for each specimen. Images were subject to an adjustable threshold mode to differentiate the fibrils from their background and each other. Program separation functions were implemented to differentiate any fibrils that were touching or appeared to overlap. Only minimal amounts of fibril silhouettes were erased between touching fibrils at the thinnest point(s) of attachment. Stray pixels were erased so that they would not be measured as individual fibrils. A particle count and measurement function was used to count and obtain the smallest diameter measurements of each fibril in the selected region. At the same time, we also measured the length of time required to carry out the process of measuring collagen fibrils using the image analysis software. The obtained measurements were exported to a Microsoft *Excel* spreadsheet and organized into a histogram. This protocol of image processing was repeated by a second person. The two observers compared the same fibrils in the same order for each image. A paired t-test was used to compare the differences in measurements between the observers. Statistical significance was set at $p<0.05$.

RESULTS

Examples of typical regions of interest from Sham and Injury TEM images are shown in figure 1.

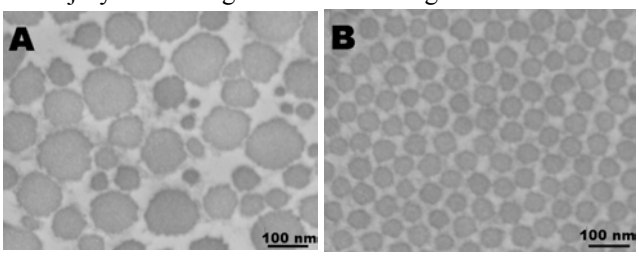

Figure 1. A. Sham operated ligament B. Healing ligament

Histograms from the study showed healing subject collagen fibrils in the 45-60 nm diameter range were most frequent. Two observers performed image analysis of identical images. Two fibril diameter peaks occurred in Sham subjects in the 30-45 nm and 75-90 nm ranges (figure 2).

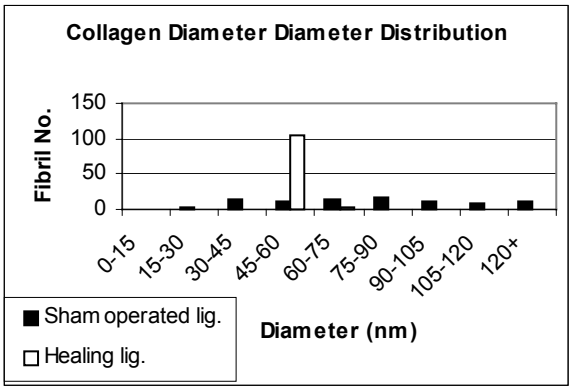

Figure 2. Observer #1 histogram of sham operated and healing ligament measurements

Observer 1 performed measurements at an average rate of about 9 minutes per 10 fibrils (n=378 fibrils). Observer 2 performed fibril measurements at an average rate of about 4 minutes per 10 fibrils (n=378 fibrils). The average rate between the two observers for measurements is 7 minutes/10 fibrils. Our mean minimum fibril diameter for sham operated ligament was 78.4±33.3 nm. Our mean minimum fibril diameter for healing ligament was 55.7±4.36 nm.

A paired t-test revealed that measurements obtained by the two observers were significantly different from each other ($p<0.05$). The diagram of user subjectivity reveals that observer 1 (mean minimum fibril diameter value of 65.3±25.7) typically obtained diameter measurements that were smaller than those of observer 2 (mean minimum fibril diameter value of 66.6±25.9 nm). Their measurements of identical fibrils could differ by up to 12 nanometers.

DISCUSSION

Our mean minimum fibril diameter of healing ligament (55.7±4.36 nm) was fairly consistent with previous results although our mean minimum fibril diameter of sham operated ligament (78.4±33.3 nm) was much smaller than that of C. Frank (131±0.7 nm) [3, 4]. This is possibly due to the fact that this study was conducted on a much smaller sample of fibrils (n=400 vs. n>11,911). Another factor that may account for the difference in mean minimum fibril diameter was the set of ranges in which fibril diameters were organized. Our study organized fibrils into 9 ranges of 15 nanometer increments. One of C. Frank's previous studies organized fibrils into 60 ranges of 8 nanometer increments [3].

After working with the various manual software programs such as Scion Image and NIH Image, several problems became evident with the free software. The measurement/analysis tool of the Scion software could not accurately measure overlapping or touching fibrils. This meant manual intervention using the eraser tool was required to separate them. Determining how and where to separate fibrils by using manual eraser tools posed significant problems. Such a "free hand" method of eraser correction changed the area of manipulated fibril silhouettes measured and could not be performed consistently to each adjusted fibril due to limitations in the precision of the human eye. Different measurements of the same fibrils obtained by separate observers indicated that this method of manual image manipulation and measurement was subject to a great degree of human error. Thus it was inadequate for the purposes of accuracy and consistency. It was also time consuming due to the great degree of trial and error involved. The image must be checked for proper separation by repeatedly manipulating and performing automated analysis labeling. An estimated time calculation based upon counting and analysis attempts on smaller field images reveal that a great deal of time would be required to use this method in order to measure the required number of fibrils: ex. 7 min/10 fibrils x 100 fibril/image x 10 images = 700 min/1000 fibrils per subject.

In order to address these problems of inconsistency, inaccuracy, and excessive time consumption, appropriate computer image analysis software and a consistent method of measurement are necessary. Analysis functions are essential capabilities the software must possess to insure that the fibrils are measured in an accurate and consistent manner. Marketed software, such as Simple PCI (Compix Inc., Imaging Systems), has the capabilities ideal for addressing such problems. A particle separation function makes counting and analysis of adjacent, touching bodies possible by automatically drawing a line between them. This process is carried out by a program and so may be applied in a similar manner for each circumstance of particle overlap/touching to insure consistency. In collaboration with this separating capability, Simple PCI is also able to automatically count and measure the collagen fibrils and then display the results within seconds. An image that may have taken 70 minutes to measure with manual intervention, using free software such as Scion or NIH Image, potentially requires only 10 minutes or less to measure the same number of fibrils using automated analysis software programs such as Simple PCI.

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ACL Group
Lab Mentor: Xinguo Ning, Ph.D.
Faculty Advisor: Savio L-Y. Woo, Ph.D., D.Sc.

I grew up in Pleasant Hills, PA, which is a suburb about half an hour south of Pittsburgh. I have three younger sisters and a golden retriever named Tootsie.

One of my favorite activities is running. I ran on the Cross Country and Track teams throughout high school and currently compete in local 5K, 10K, and half-marathon road races. In addition to running, I also enjoy many other outdoor activities including rock climbing and roller-blading. When the Pittsburgh weather prevents me from enjoying the outdoors, I can often be found in the kitchen. Cooking is one of my favorite activities. I have been studying the culinary art for the past decade under some of the greatest chefs in the area (my mom, aunt, and grandmas)!

I am currently majoring in Mechanical Engineering. My career goals are continuously changing, but as of right now I would like to be involved in the design of athletic footwear. In fact, I became interested in the MSRC because I wanted to see how my Mechanical Engineering education could be applied to the biomechanical area. In the future, I would also like to do some traveling. My traveling adventures will start next spring when I will be heading to Australia to study abroad.

I would like to thank Xinguo Ning for all of his help and support with my project. I have learned a lot about 3D finite element modeling and I think that this will be very beneficial in my future. I have also learned so many interesting things about China and its culture. Overall, I would like to thank Dr. Woo and the rest of the MSRC for giving me the opportunity to work in such an interesting lab.

RECONSTRUCTION OF A GEOMETRY MODEL OF THE KNEE JOINT AND ACL USING MIMICS

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INTRODUCTION

The rising popularity of sports has led to an increase in the incidence of anterior cruciate ligament (ACL) injuries. The primary function of the ACL is to prevent anterior translation of the tibia in relation to the femur [1]. In order to better understand the functions of the ACL, reconstruct the ACL, and aid in rehabilitation of the ACL it is beneficial to create a finite element model of the knee joint and ligaments. The reconstruction of the geometrical model of the bones and ligaments should be as efficient and accurate as possible.

In studies by Li [2,3], a three dimensional computational model of the knee joint was constructed from magnetic resonance (MR) images that were digitized and then imported into MSC/Patran© for meshing. Song et al. [4] reconstructed the model of the knee joints and ACL by using MIMICS© (Materialise, Ann Arbor, MI). MIMICS© is a tool for processing images from a CT or MRI scan. First, a CT scan was taken on the intact knee, and the CT images were used to reconstruct the bones. After a robotic test, another CT scan was taken on the knee joint with the bony contact cut away. The ACL geometry was then reconstructed from this image. The two geometry models were then converted into the same frame and assembled together. In this study the challenge was to make sure the insertion sites in bones and the ACL matched accurately.

Once a model of the knee joint and ligaments is created it can be utilized to analyze the shape and stress distribution of the ACL as well as analyze different types of ACL reconstructions [5, 6].

OBJECTIVE

The purpose of this project was to develop an efficient procedure to reconstruct a model of the knee joint and the ACL using MIMICS© software based on MRI images. Possible solutions to the problems encountered in the reconstruction will be discussed.

METHODS AND MATERIALS

The MIMICS© software may be used to create either a 3D or a surface model. A surface model takes up less memory and allows for faster computation. In the finite element analysis the femur and tibia can be considered rigid when compared to the ACL, thus it was only necessary to reconstruct them using a surface model. The ACL was reconstructed as a 3D model because future studies will look at the deformation in the ligament. The following is a brief description of the whole reconstruction procedure.

First, the images from the MRI or CT slices in the format of DICOM were imported into the MIMICS software. In order to create multiple models from the same scans, different masks were created by setting the threshold value. Next, the image was further broken down to the specific bones or ligaments by region growing, which selects separate objects in the mask. After region growing, a surface model or a 3D model may be generated.

To reconstruct a surface model the first step is to create polylines. In this case polylines were created around each slice of the bone. These polylines were then touched up to eliminate parts of the image that appeared in the thresholding range but were not really desired in the mask. Once the polylines were touched up the "Grow polylines" command was executed so that the polylines in all of the slices were grouped together. Finally, after the polylines were grown a surface was fitted to them.

To reconstruct a 3D model, the "edit" command is used so that images only contain the desired object and no unwanted artifact or soft tissue. It was necessary to go through each slice and erase everything but the ACL. After editing, the 3D representation was calculated.

In order to reconstruct a geometrical model of the bones and ACL, a test was done. First, one fresh-frozen human cadaveric left knee was thawed overnight (Age = 64, Female). The designed water-based registration blocks were rigidly fixed to the femur and tibia. The knee was placed in an acrylic fixture and MRI (Sigma, Horizon; GE, Milwaukee, WI) scans were taken on the knee using two five-inch surface coils placed on each side of the knee joint. The thickness of the MRI slices was 0.7 mm and the resolution was 512 x512 pixels. After the MRI scan the knee was tested using the robotic/UFS system to determine the knee kinematics. Next, CT scanning was conducted to verify the geometry of the ACL. Before processing the above image data, a whole preliminary analysis was performed on existing CT scan data of a human knee using MIMICS software. This reconstructed model is presented.

RESULTS

After the reconstruction process an entire geometry model of the femur, tibia, and ACL was created. Figure 1 shows the reconstructed surface and 3D models of the femur. The overall geometry of the surface model is comparable to the 3D model.

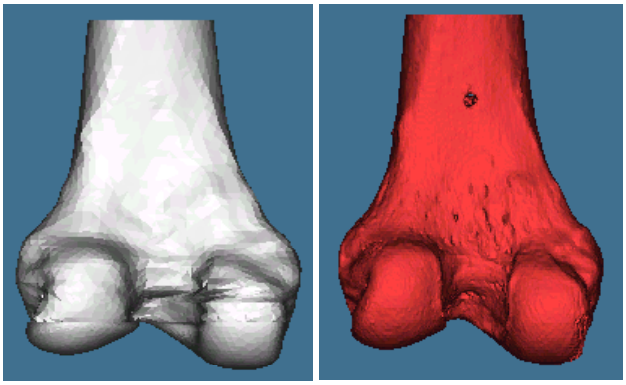

Figure 1. The picture on the left is a surface model, the one on the right is a 3D model

The reconstructed 3D model of the ACL is shown in Fig. 2. The two separate bundles of the ACL can be seen, however there was a problem separating the ACL from the rest of the soft tissue. This problem was encountered during the thresholding procedure. In the image obtained from the CT the intensity of the pixels making up the bones and soft tissue and the intensity of the pixels making up the ACL were not differentiable. In order to create a model of the ACL and get accurate insertion sites it would be beneficial to work closely with a clinician to be able to separate the ACL from the bone and soft tissue in the image.

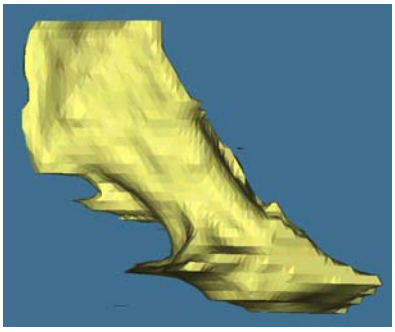

Figure 2. 3D model of the ACL

DISCUSSION

There are several possible sources of error in the reconstruction of surface and 3D models. One possible source of error was the resolution of the slices and the pixel size of the CT scan. In this CT scan, each slice had 512x512 pixels and each pixel size was 0.3 mm x 0.3 mm x 1 mm. Another possible source of error was human judgment in determining the threshold values and utilizing the edit command.

In addition, the surface models are not continuous. This is due to the fact that there are slices with more than one contour. In slices where there are more than one contour present two separate masks had to be made. These separate masks are necessary because MIMICS can only fit curves to one contour per slice. As can be seen in Figure 1 this problem occurs right near the insertion sites, in the direction running from the medial to the lateral side of the bone. The discontinuities on the surface model can be glued using other engineering software. However, a better solution is to scan the knee in the sagittal plane instead of the transverse plane. This would create more accurate geometry around the insertion sites.

The models of the femur, tibia and ACL will be imported into finite element analysis software for assembling and meshing. A study will then be done to analyze the effects of the insertion sites on the stress distribution in the ACL.

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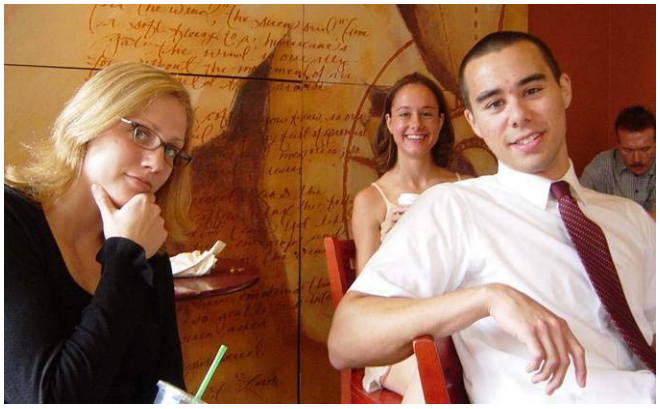
SUMMER 2003 FUN PICTURES!

Fun with the panther

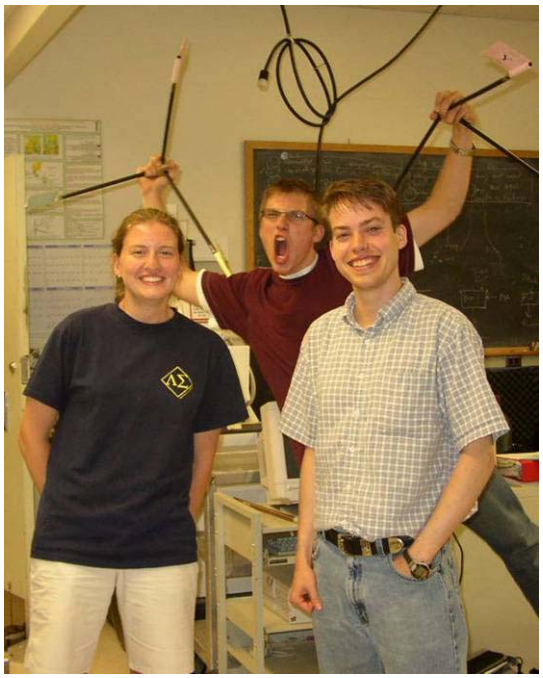


Melissa, Mary, and Kevin

The lovely ladies of the MSRC

Melissa, Lily, and Steph

'Messe Fiskins'



CARTESIOR!

Picnic time!

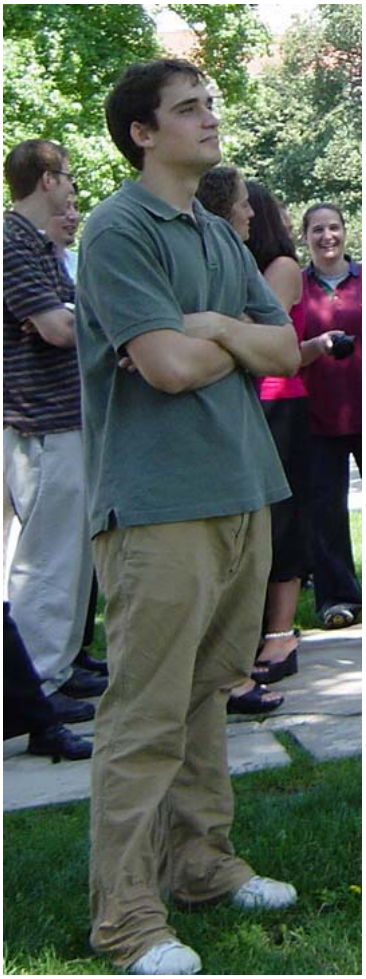
Mmmm… Starbucks.

Melissa, Mara, Steph, and Eric

Ron

The productivity never ends in the computer lab!

Josh in a rare moment – outside the MSRC!



Zack "Attack"

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