12-23-2017

# Potential Graft Materials for ACL Reconstruction Surgery

Matthew Jenkins
*Washington University in St. Louis*

Spencer Lake
*Washington University in St. Louis*

Matthew Jenkins
Muscuoskeletal Soft Tissue Laboratory
Spencer Lake
12/20/2017

# A Semester in Review

I began working for the Muscuoskeletal Soft Tissue Laboratory (MSTL) under Spencer Lake early in July of this past summer. Working closely with Ryan Castile, I initially joined the anterior cruciate ligament (ACL) project. However, as Ryan has many projects he works on, I ended up assisting the ulnar collateral ligament (UCL) project as well as Ronak Patel's anterolateral ligament work. Though my primary focus was on the ACL research, most of my time during the summer was working on the other two. My general role in the lab was assisting Ryan until the beginning of the fall semester, where I spearheaded the testing of various graft tendons for ACL and UCL reconstruction surgeries. This testing absorbed almost all the time I invested into research this semester.

The objective of the research I assisted is to gather data on potential graft tendons for reconstruction surgeries. The method employed by MSTL is composed of a tensile test that stretches the sample while a camera captures real time collagen alignment data. This dynamic analysis is a novel technique that MSTL has applied to many soft tissue, both tendons and ligaments. As alluded to above, both the ACL and UCL have been tested with this protocol, and thus the potential graft materials are to be put through the same protocol. As the testing was identical for the actual ligaments and potential grafts, comparisons can be drawn between the tissues. The end goal of the research is to improve the quality and performance of reconstruction surgeries by creating a more specific and useful body of data describing the tissues involved.

This semester I tested three tendons: the gracilis tendon (GR), the semitendinosus tendon (ST), and the patellar tendon (PT). The GR is used in UCL reconstruction surgery whereas the ST and PT are used in ACL reconstruction surgery. These tissues were harvested from 14 human knees (10f, 4m; age range 35-50) post mortem by Ronak Patel. I aided in the dissection, doing whatever Ronak needed so that the dissections could be done accurately and efficiency. These jobs included taking photos, acquiring new blades and tools, marking the proximal end of samples, and organizing the storage of the harvested samples in PBS in a freezer. Ronak harvested 9 total tissues from the knee: LCL, ALL, ALC, PT, ST, GR, quadriceps tendon (QUAD), ACL, and meniscus. The LCL, ALL, and ALC were used in a study done by Ronak to evaluate the ALL as an important ligament to reconstruct during ACL reconstruction surgery. The meniscus was also used by Ronak for a separate study. The PT, ST, and GR were the tendons I tested and evaluated during this past semester.

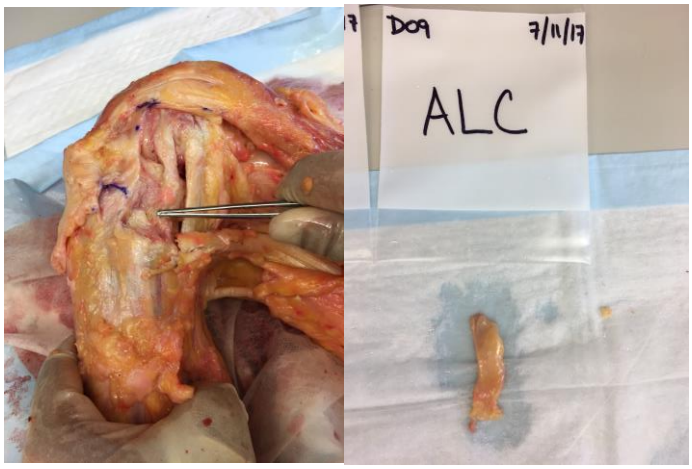


**Figure 1** *On the left is a picture Ronak asked for to show the lateral collateral ligament (LCL) and ALL. On the right is an image of an anterior lateral capsule harvested by Ronak and a bag labeled by me.*

The MSTL protocol for evaluating tendons requires a fair amount of sample prep to create the proper size and shape for testing. First, I would cut the ST and GR into 3 sections: proximal, middle, and distal. Each section was 35mm long. The first cut was always the proximal end such that the tissue taken was just under the suture marking the proximal end of the

overall tissue. This suture was placed at the point of transition of the tendon from connective tissue to the muscle itself and the body of the tendon. As seen in Fig 2, there is some extra tendon left on the proximal side due to this practice. Next, the tendon is shaved down using a freezing stage microtome to the requisite thickness. The thickness was determined by a 600 micron gauge. Once the top layer of OCT was pulled of the gauge, the tissue would be anywhere from roughly 700-1000 microns. The purpose of this thinning is to allow light to pass through the tissue while testing. The final product of the microtome is then imaged for thickness and cross sectional area, and placed between sandpaper such that the gauge length is 25mm between the sandpaper. The sandpaper is to increase the friction between the clamps of the tensile tester and the sample itself. Finally, four 1/32" beads are glued on the top surface, acting as a strain gauge and marker during the analysis. The PT used a different cutting method. Instead of cutting horizontally, I cut longitudinally along the direction of the collagen fibers, separating the PT into lateral, middle, and medial sections. Next, I cut each sample down to 45mm in length. Though Fig 3 depicts shaving the tissue from bone, this was already done and not done by me in this study.

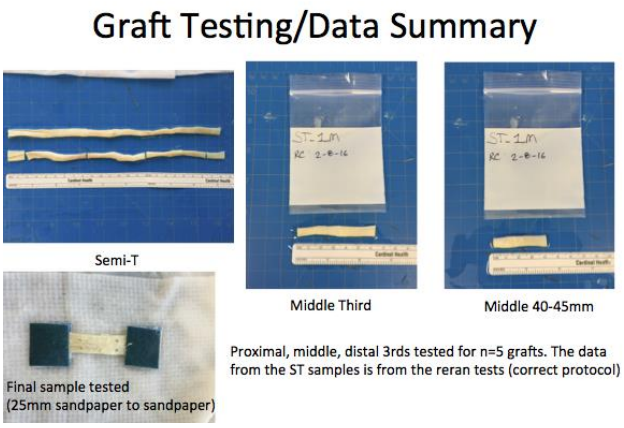


**Figure 2** *A description of the cutting process used to make the proper sized samples for the ST and GR.*

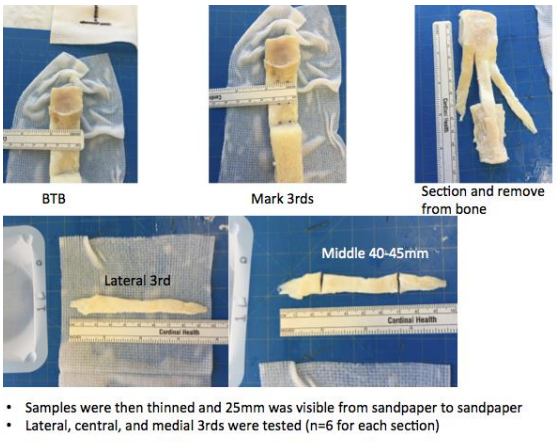


**Figure 3** *A description of the cutting process used to make the proper sized samples for the PT.*

The rest of the sample prep is done in the exact way as described for the ST and GR, the only difference being extra tissue under the sandpaper on each end.

The actual testing of the samples was the same for each specimen, regardless of the type of tendon. This testing is done on a tensile tester as seen in Fig 4. The tendon is placed in the clamps attached to the actuators. These calmps are submerged in a bath of PBS to keep the tissue hydrated. Below the sample is a source emitting polarized light to be captured by the polarization camera placed directly above the camera. This camera evaluates the collagen alignment of the tissue while at various strains. The testing protocol describing these "various strains" is 10 cycles at low strain, 1 min rest, a 5-min stress-relaxation hold at 5% strain, 1 min rest, and a ramp to failure at a rate of 1% strain per second. The 10 cycles are a standard "pre-conditioning" that many of other studies use to prepare the tissues. The stress-relaxation is a measure of the viscoelastic properties of the sample. The ramp to failure is to establish a bi-linear curve fit to the stress-strain curve generated. This curve fit establishes the toe and linear modulus of the tissue tested.

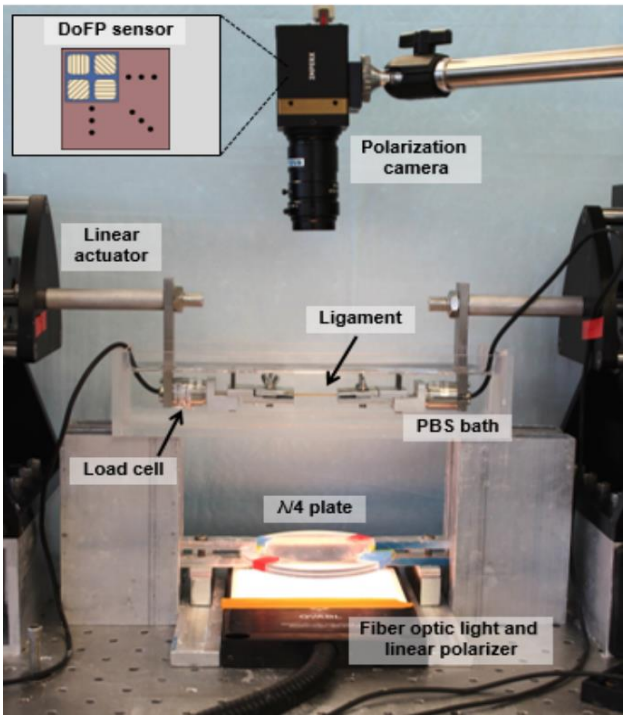


**Figure 4** *A depiction of the testing apparatus used to evaluate soft tissue samples at MSTL.*

The polarization camera evaluates two parameters: degree of linear polarization (DoLP), and angle of polarization (AoP). DoLP is a measure of the strength of alignment of the collagen, and AoP is a measure of the angle of the collagen. The specific numbers evaluated are the

average of DoLP (AVG DoLP) and standard deviation of AoP (STD AoP). The reason that we don't look at AVG AoP is due to the nature of the AoP measurement. Depending on the setup

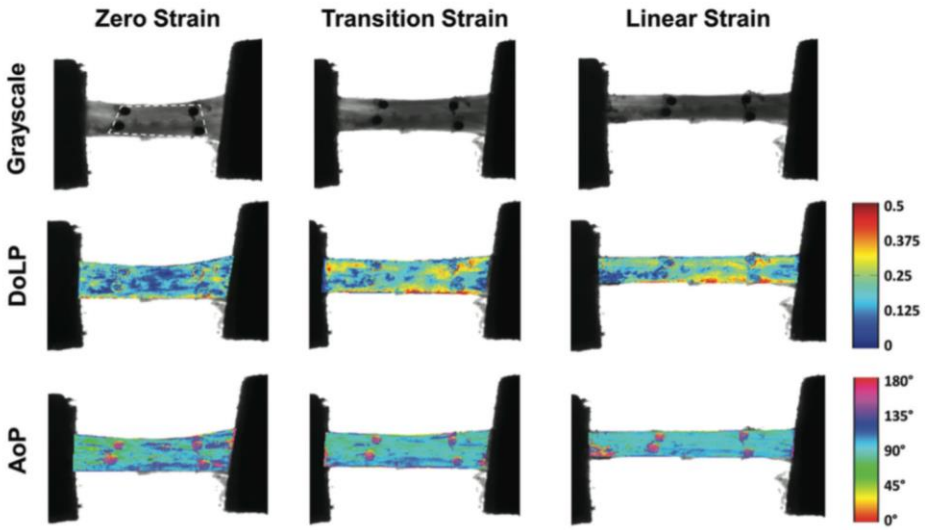


**Figure 5** *An example of the imaging from the polarization camera at various strains.*

and angle of the camera, the direction of the collagen can vary from sample to sample. To correct for this variation, we analyze the STD AoP. The smaller the STD AoP, the more highly aligned the collagen of the sample. Fig 5 contains 3 columns of information: zero strain, transition strain, and linear strain. These columns represent the place on the stress strain curve the image comes from. The zero column is when the tissue is under no strain, and the linear column denotes when the tissue has begun majorly deforming. The transition column represents the change from the initial aspect of the bilinear stress strain relationship of tendons to the final, linear aspect of the bilinear stress strain relationship of tendons.

Throughout the testing process, I encountered several problems that needed to be addressed. The first of these came when cutting the ST and GR samples during the sample preparation. The proximal end of the tissue sometimes was seemingly a different tissue than the rest of the tendon. My hypothesis is that this was either a layer of fat or instead the connective tissue interfacing to the muscle itself. Either way, the resulting couple of smaples where I did not

account for this weaker tissue turned out as seen in Fig 6. The darker area on the left of Fig 6 is the expected visual to be taken by the camera, and the seeming non-existent right portion is the thinner and weaker material described above. The possible consequences of this situation are all the strain and deformation of the material desired will be focused on a structurally useless section and the inaccuracies of the measurements depicted collagen strength and alignment in the proximal sections. Once noticing this issue, I could notice when the standard method of cutting the ST and GR would create such problems and adjust is an optimal way to prevent them.

**Figure 6** *The proximal end of a GR made of different materials*

Another issue that cropped up more often than the cutting of samples was the dirtying of the PBS bath each sample bathed in while be tested. As more and more samples were introduced to the PBC bath, more and more small particles were released into the liquid and, as seen in Fig 7, could cause a disruption in the camera readings. The white flecks encroaching on the dark gray of the sample are these particulates. These disturbances could affect the collagen alignment readings from the polarization data. In addition, the debris effects the ability of the camera to differentiate the sample from the background, which compromises the data analysis code's accuracy. The way I approached this problem was regularly refilling the PBS bath and cleaning the tub thoroughly after each use.

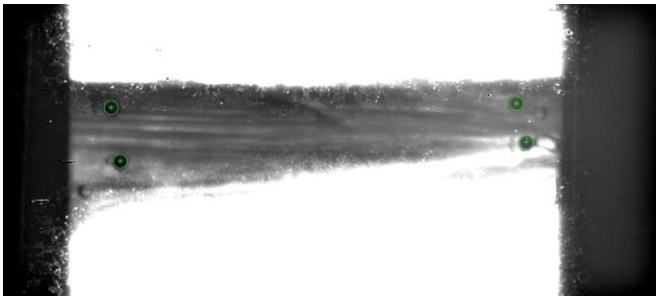

**Figure 7** *A sample suspended in a particularly debris ridden PBS bath*

The first day at MSTL it took Ryan and I 1 full day of testing to test 6 samples from start to finish. By the end of this past semester, we were easily testing 9 samples in 3 hours. Though accuracy is the most important while testing, increasing the rate at which samples could be tested is still important, as there is always something to do in lab and testing can take large chunks of a month even working full time, let alone 10 hours a week over a semester. There were two main causes of this increase in productivity. The first was the discovery that the glue did not need upwards of an hour to dry before testing. In fact, even if the sample was glued less than 5 min before the testing, there was no slippage from the clamps even with forces ranging up to 30N. The second, and more impactful, improvement came from the microtoming of multiple samples at once. Each microtoming session on average took about 30 min per sample, the majority of that time coming from just the freezing of the tissue and only 5 or so minutes of actually cutting. In addition to these changes, Ryan and I developed a very good system of distributing the workload in the most efficient manner. Though less important to the optimization of the time efficiency, it allowed each of us to specialize in certain areas and make each of those processes as accurate as possible.

The work completed testing these potential graft tendons is a step towards learning more about optimal reconstruction surgery techniques. Next semester, the second of the two tendos used in UCL reconstruction surgery, the palmaris tendon, will be tested to obtain a complete set of information concerning UCL reconstruction surgery. Additionally, the ACL surgery graft tendons tested could be examined at the microstructural level. As MSTL is currently working on the histology of the ACL itself, a similar analysis for the potential graft materials would be beneficial for further comparison.