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Development of a Custom Tendon Explant Frame Compatible with Reflectance Quantitative Polarized Light Imaging

Matthew Riak
Washington University in St. Louis

Lila Dickstein
Washington University in St. Louis

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Abstract

Tendons and ligaments are collagenous tissues in the body which attenuate load. Reflectance quantitative polarized light imaging (rQPLI) is being developed to combat some of the issues present in many of the current imaging modalities used to study tendons and ligaments. To establish its utility in continuous monitoring of tendon healing, rQPLI will be used to monitor a tendon explant defect over a four week period in both normal and super healer mice strains. In this study, we discuss fabrication of a custom frame built to exert a constant force on tendon explants for these imaging studies. Additionally, the frame needed to be compatible with rQPLI: it must be non-reflective and not interfere with the optical path during sample monitoring. Many design iterations were created using CAD software before the design was finalized. To ensure the frame was sturdy, and would not corrode, high corrosion resistant stainless steel was used for the frame and connector screws. A constant force spring attached to a fishing line was used to ensure the tendon was kept at 2.5 N, and alligator clips were used to hold the tendon in place.

Introduction

Tendons and ligaments are musculoskeletal soft tissues which attenuate load. Each year in the United States there are over 33 million injuries to musculoskeletal soft tissues, 50% of which are to tendons and ligaments. [1] Injuries to tendons and ligaments are extremely prevalent, and better understanding the changes in the underlying structure during injury can help inform therapeutics and prevention strategies. One of the many ways to probe microstructural changes during injury is by imaging collagen, one of the main building blocks of tendons and ligaments. Although there are many different types of imaging modalities including magnetic resonance imaging (MRI), computed tomography (CT), optical coherence tomography (OCT), many have numerous limitations including spatial resolution issues, tissue processing requirements, and a limited field of view [2].

An imaging technique called reflected quantitative polarized light imaging (rQPLI) is being developed for microstructural analysis of musculoskeletal soft tissues. Collagenous tissues exhibit form birefringence, and thus alter polarization state of light in proportion to collagen structural anisotropy. Based on this principle, QPLI can be used to measure and quantify collagen fiber alignment in real time of different musculoskeletal soft tissues and tissue phantoms. In rQPLI, a custom division of focal plane sensor is used to determine the polarization state of light (Figure 1**)**. The sensor is made of superpixels that contain four oriented linear polarizers, each oriented 45 degrees apart from each other, which only absorb light oscillating in a specific direction.

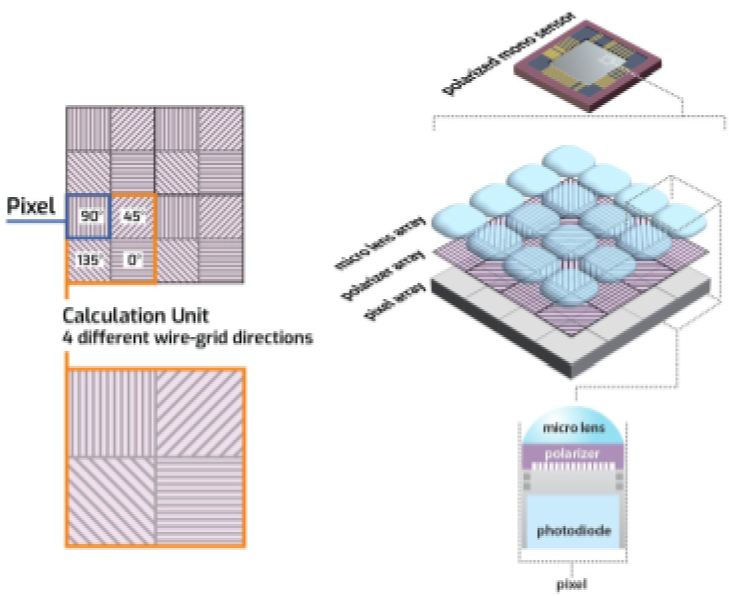

Figure 1. Schematic of superpixels used in custom polarized light sensor. [10]

The science behind tendon healing is still widely unknown and misunderstood. The Murphy Roths Large (MRL/MpJ) mouse strain, also known as a "super healer," is unique in the fact that these mice can heal wounds without production of a fibrotic scar. [3] The increased rate of healing was first discovered by observing ear hole closings used to identify mice. Since this study, research has been done on mouse patellar tendons, which also demonstrate an increased healing rate in superhealer mice. In particular, superhealer tendons exhibited improved matrix and cell alignment factors after healing compared to normal healer controls. [4]

rQPLI's utility to monitor microstructural changes in musculoskeletal soft tissues in real time will be leveraged to assess collagen dynamics which are associated with the healing process. MRL patellar tendon explants will be compared to a C57Bl/6 (control) mouse patellar tendon explants to analyze differences in the quality of scar formation as well as the time-dependent differences in the healing time course between them. Ideally, this can establish rQPLI as a technique with sufficient spatiotemporal resolution to monitor nuanced changes in healing tendon tissue quantity for use in real time regenerative medicine therapies. Tendon healing is still widely misunderstood, and hopefully rQPLI will be able to help us better understand healing mechanisms.

One hurdle to overcome before these experiments can be performed is answering the question of tendon explant culture conditions. Tenocytes are cells which reside in the extracellular matrix in tendons. [5] It has previously been shown that tenocytes which were stress deprived were much more likely to apoptose than those which were cyclically loaded. [6] Therefore, it is important to maintain some level of physiological tension on tendon explants during culture.

The specific purpose of this independent study was to create a custom frame which could hold these tendon explants at a specific static tensile force of 2.5 N while allowing for simultaneous imaging with rQPLI. There were many design constraints associated with creating a frame which is compatible with rQPLI and can house tendon explants. A few design constraints were present when designing the frame. It needed to be compatible with rQPLI, meaning it must be non-reflective and allow light to pass through at a 30º angle from the upright direction, it must non corrosive, it must be able to be placed in an autoclave, and finally, it must hold the tendon at a constant force. In this paper, we will describe the design process used to create the frame, and will explain the materials and why they were chosen.

Methods

Literature Search. Prior to initiating the design process, a thorough literature search was conducted to evaluate designs from other similar studies. As it has been previously established that tenocytes in explants apoptose when stress deprived during organ culture, explant culture systems typically involve housing tendons in culture media under a constant force. We searched the Washington University Open Scholarship database, and other biomedical journals va PubMed and Google Scholar to find such studies. We were able to identify three different types of designs present in the literature that accomplished the same goal of housing tendon explants under a constant force.

The first type of frame observed (Figure 2) was made up of a clear acrylic case that had two metal bars inserted horizontally across it. The tendon explant is tied to one of the bars, and the other bar serves as a pulley. A string is tied to the tendon, then the string loops under the pulley and out of the acrylic case, where a weight is hung from it. It accomplished the same goal of exerting a constant force on the bone-tendon-muscle assembly

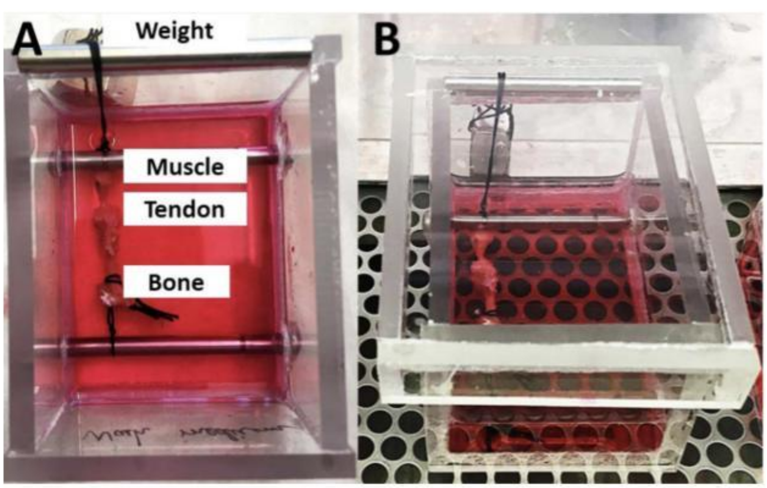

Figure 2: Fang et al. tendon explant frame using weight-pulley system [7]

within culture media. However, the tendons cultured in Fang et al. were Achilles tendons and the tendons that were planned to be used in our frame are patellar tendons. There is a non-negligible difference in size between the two tendon types. This affects our design by requiring a method of securing the tendon on either end without a large piece of bone that can be easily tied with suture. Additionally, this design is not cost efficient due to the large amount of culture media it requires for one tendon. We were inspired by the use of clear acrylic casing for the frame, which is required for the rQPLI. Additionally, we liked the idea of using weights as a way to exert a constant tensile force on the tendon.

Another existing tendon explant apparatus involved a similar setup with the use of weights to exert the constant force, tied to the tissue with suture (Figure 3). This design is more efficient in terms of culture media consumption, due to the small individual tubes that each tendon is placed in. This design also could not be fully adopted for our purposes, however, due to the issue of tying suture around the small tendons. Loading the tendons into this design seems to be logistically difficult because of its vertical setup. Additionally, purchasing weights for each tendon in the system is not cost efficient. We liked the idea of

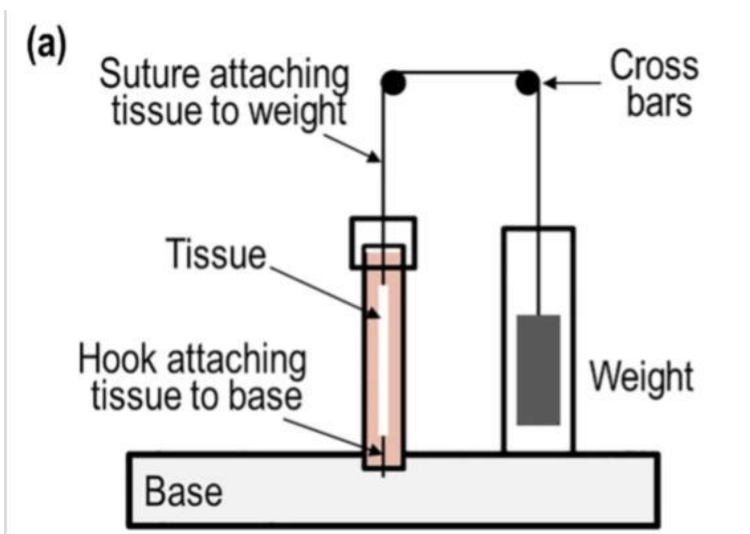

Figure 3: Soreide et al. vertical pulley-weight tendon explant system [8]

having a small tube for each tendon. This is efficient in regards to the amount of culture media that is required for each tendon.

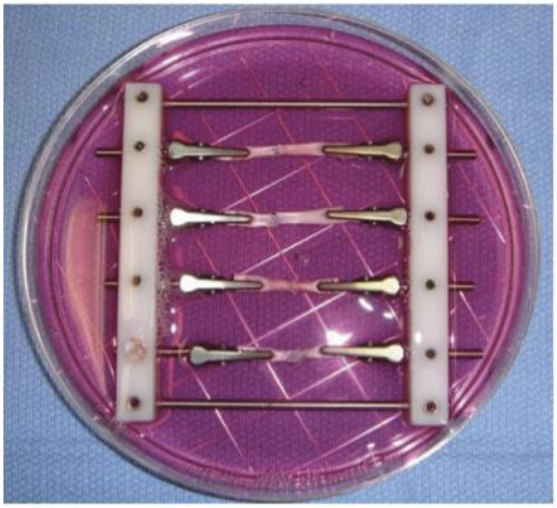

Figure 4: Ikeda et al. horizontal tendon explant frame using alligator clips [9]

The third type of frame that was considered during this literature search held several tendons with an alligator clip on either side (Figure 4). The frame was then placed in a petri dish with culture media. This is an effective way to house several tendons in culture media, and would work with the size constraint of the patellar tendons. However, this design does not provide a way to measure and standardize the force exerted upon the tendons in the frame. Additionally, patellar tendons are significantly shorter than the tendons used with the design. We hypothesized that shorter tendons would be hard to load into a frame like this because of the set distance between the two alligator clips. We liked the use of two alligator clips to secure both ends of the tendon. These seemed like an effective method to hold something as small as a patellar tendon.

The final design explained in this paper uses the best parts from each of these previous studies, working around the specific design constraints of media use and tendon size. Additionally, we had to consider constraints associated with rQPLI imaging. There will be a light source above the frame, offset at a 30º angle. No part of the frame can obstruct this light source because that would form a shadow. Additionally, the bottom of the design must be transparent and non-birefringent so that the tendons can be imaged from below.

Prototyping and Testing. There were several different iterations of the design (Figure 5), with the initial idea looking drastically different from the final product. Fusion 360 was used to model each iteration. Computer modeling was useful to visualize new design ideas, and so that designs could be 3D printed. This helped with testing each iteration and determining how feasible it was in practice, especially in terms of loading the tendons into it.

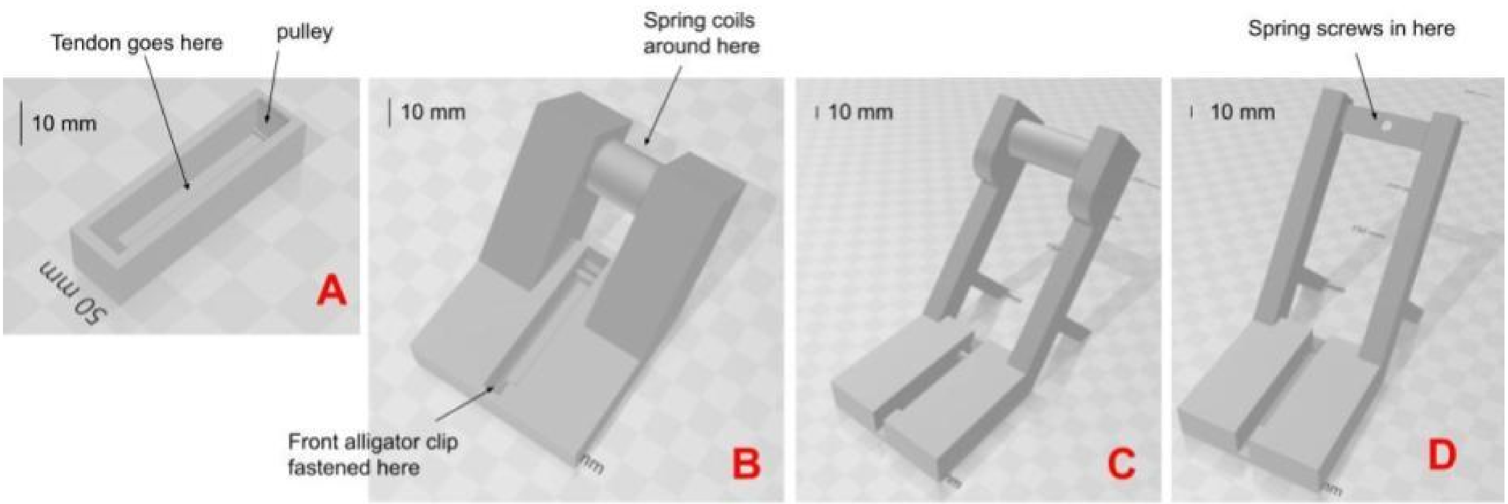

Figure 5: Computer-Modeled Iterations of Frame Design

The initial design (Figure 5A) was intended to use an alligator clip to secure the tendon on one side (the left), and then a weight-suture system attached to the other side to hold the tendon in place while exerting a constant force. The weight would hang off the side of the apparatus. The weights proved to be impractical due to the large amount of space they would take up and how imbalanced they would make the frame, since they would all hang off of one side of the frame. In terms of cost efficiency, it did not make sense to buy a set of weights for each tendon that would be tested simultaneously. Additionally, the suture-weight system proved to be impractical because of the difficulty involved with securing it to what is approximated to be a 5 mm long tendon. The aspects of this design that we decided to keep were the following: the two alligator clip system with the left clip secured to the frame, and the use of the pulley to exert a tensile force on the tendon.

The next design (Figure 5B) moved away from the use of weights to the use of a constant force spring. A constant force spring is a coiled strip of metal with typically a clip or hole on the end. These springs exert a constant, predetermined force regardless of how far they have been extended, unlike a regular spring, which exerts more force the more it is displaced. We decided to try these instead of ways as a way to exert a constant tensile force on the tendon explants, because they are more space and cost efficient. In this iteration, the tendon would be secured on either side by an alligator clip. The clip on the left would be secured to the frame, while the clip on the left would be attached to a string that looped under the bar/pulley, and was tied to the spring looped around the bar on the top. This design conceptually seemed to work, but after 3D printing it and putting the spring on it, we determined that we would need more space between the spring and the base of the frame, where the tendon would be.

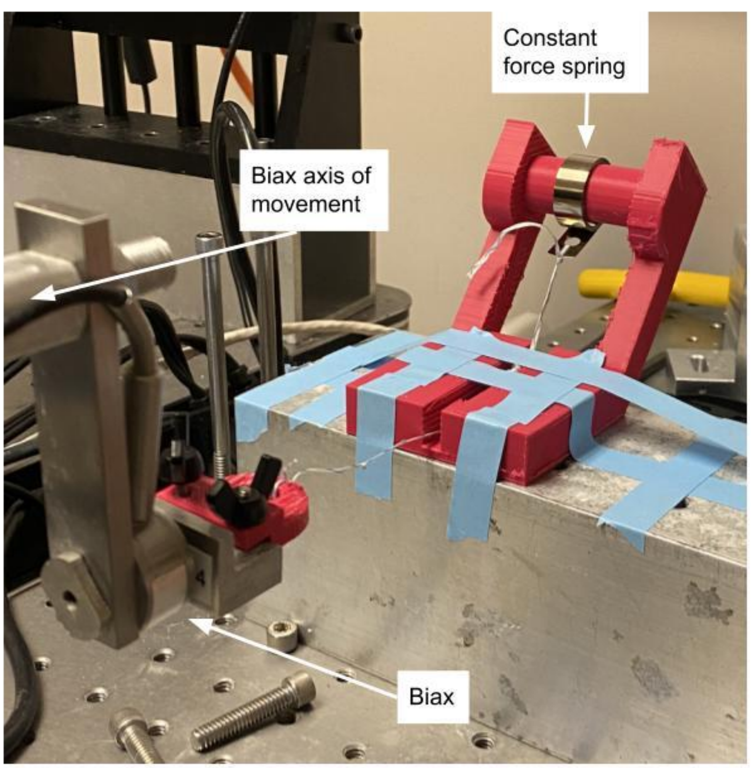

Figure 6: Biaxial Tensile Tester Setup with Prototype

The next design (Figure 5C) underwent several rounds of testing to evaluate the force exerted by the spring at various amounts of extension. A biaxial tensile tester was used to conduct these tests. The frame was 3D printed without the front wall (Figure 5C), allowing the left alligator clip to be tied to the biaxial tester (Figure 6), instead of being fastened to the frame as it would be during normal operation. The purpose of this testing was to determine the amount of force that would be exerted on the tendons (a piece of string was used to model this), and how far the spring needed to be extended to reach that constant force (so that changing its extension depending on tendon length would not impact force). We defined success as 2.5-4.0 N of force on the tendon phantom. However, it was necessary to ensure consistency across the four tendons in the apparatus and consistency across repeated tests (because the apparatus will likely be used several times to test a higher volume of tendons). The initial design tested on the biax (Figure 5C) produced inconsistent test results. This is believed to be due to inefficient incorporation of the spring into the design. There was likely high amounts of friction between the spring and the frame, and it often got caught trying to unravel.

This testing process led to two changes in the following iteration (Figure 5D). First, the distance between the spring and the cell that would house the tendon was extended. Constant force springs must be extended a certain amount before that predetermined force is reached. The modified design allowed for more space to reach that desired force. The next modification changed the way that the spring was incorporated into the design. Previously, the coiled part of the spring wrapped around the top bar, and the string was tied to the hole at the end. In this modification, it was flipped so that the end part of the spring with the hole was screwed down to the frame, and the string looped through the coiled part. This was an effective way to reduce friction between the apparatus and the spring. We know this to be effective, because the results from the tensile tests of this iteration showed consistent force readings at reasonable levels of spring extension that were within the bounds of the frame.

The final design (Figure 6) uses a constant force spring with a clip at the end, instead of a hole. These will clip onto the bar at the top, and fishing line will be used to connect the spring to one of the alligator clips that holds the tendon. Each frame is designed to hold four tendons at once. It was broken up into smaller pieces for ease of machining. A separate metal dowel will be incorporated to serve as the pulley system that the string can loop under.

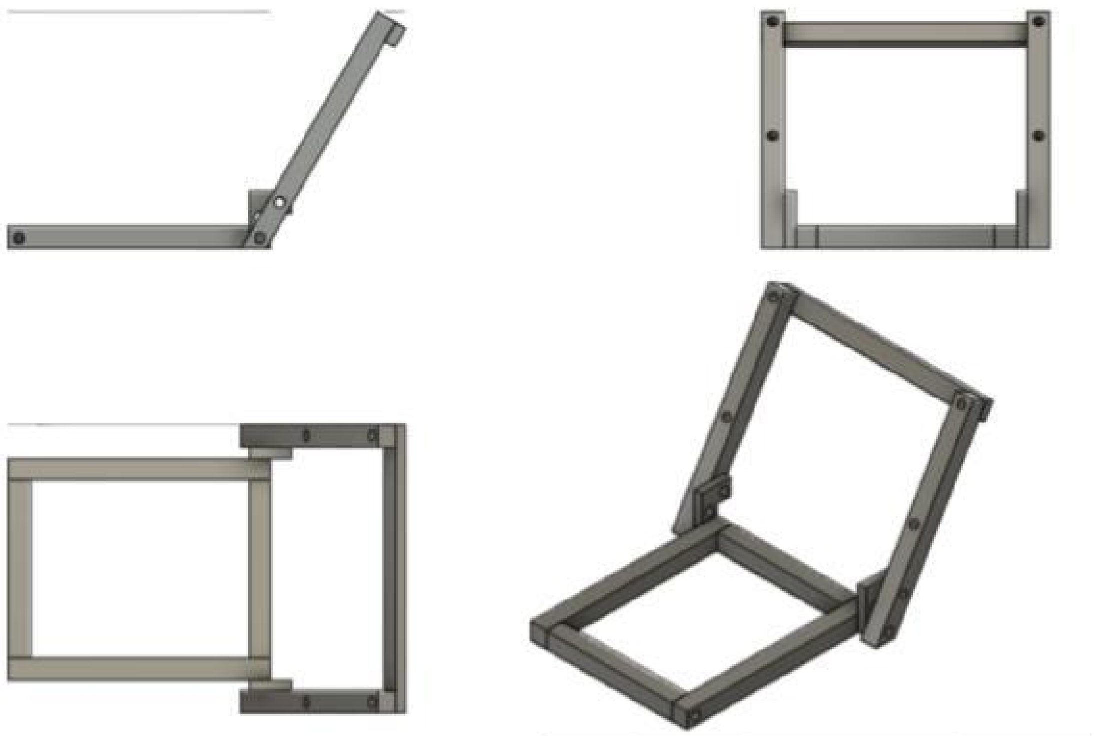

Figure 7: CAD Drawing of Final Design

Hardware. Table 1 shows the materials used while creating the custom frame.

Table 1: Materials List

Material	**Quantity**	**Description**
Clip-on constant force springs	4	0.8 lbs

Fishing line	1 roll	For connecting spring to alligator clip
Alligator clips	8	To secure both ends of the tendon
Stainless steel bar	0.25” x 0.25” x 2’	316 stainless steel, for frame
Stainless steel sheet	0.125” x 0.5” x 0.5’	304 stainless steel, for frame
Stainless steel rod	0.125” diameter, 0.5’ long	316 stainless steel, for pulley
Screws	4 1” long, 6 0.5” long	4-40 screws
Acrylic sheet	0.25” thick	Clear, for sides of casing
Glass	0.125” thick	For top and bottom of casing
Silicone sealant	1 tube	For connecting and sealing casing together

Results/Discussion

The objective of this study was to create a custom frame which could hold a tendon explant at a constant force. Tendons from different mouse strains will be compared using the custom frame, which is necessary because tenocytes apoptose when they are stress deprived. There were some additional design constraints beyond just maintaining constant tension. The frame needed to be compatible with rQPLI and be noncorrosive.

The design for the frame was created using CAD software (Fusion 360), and the parts were 3D printed in PLA. While designing the frame, many iterations were created using hand drawings, which were then transferred to the CAD software before the final design. During this process, one of the necessary steps involved testing the model to ensure it would meet the design constraints. A biaxial loading machine was used to test each of the designs to ensure they were able to hold a constant force of 2.5 Newtons. Many trials were conducted, but during this process it was clear that friction was a major issue in the design, as the force values were smaller than anticipated. To solve this, the design was altered to minimize friction by using a material with a lower coefficient of friction.

Deciding which material to use was a major decision when creating the frame. The frame needed to be sturdy and durable so the obvious material choice was a type of metal. Both aluminum and stainless steel were considered for their relatively low prices and high durability and strength. Because of the necessary corrosion resistance, we decided to use a high corrosion resistant stainless steel. Although stainless steel is more difficult to machine, aluminum most likely will not be able to last in the humid and corrosive environment, so stainless steel was chosen for the frame. The frame consists of nine different pieces which are screwed together with 4-40 stainless steel screws. Springs with strings and alligator clips are attached at the top to hold the tendon at a constant force. Alligator clips are attached at the bottom to hold the other side of the tendon. The entire frame is encased in an acrylic and glass housing. The glass pieces were ordered to size, and the acrylic was laser cut to size.

Although the design is finalized, the pieces have not yet been machined and assembled, but the design has been sent to the machine shop and the materials have been ordered. Once the pieces are machined, they will be assembled into the first final design for the custom frame.

Tendons will be loaded into the frame to ensure it will work correctly for the experiment. After verifying the validity of the first frame, more materials will be ordered and three more separate frames will be machined. Finally, the study will be conducted and tendons will be loaded into the frames, held at a constant force of 2.5 Newtons, and imaged using rQPLI. The frame will give insight to the Murphy Roths Large mouse strain and further research about rQPLI.

Conclusion

rQPLI is being used as an imaging technique to study collagen, which is the main building block of tendons and ligaments. It will be used to monitor a mouse patellar tendon over a four week period in both normal and super healer mice strains. A custom frame was necessary to hold the tendon at a constant force over the 4 week period because tenocytes apoptose when stress deprived. The frame will be created out of high corrosion resistant stainless steel. It will be surrounded in a glass and silicone housing which will prevent contaminants from entering. This frame will help establish rQPLI's utility in continuous monitoring of tendon healing.

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