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James Abraham Spring Independent Study Report

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Abstract:**Background:**

Tendons are one of the most important parts of a human's musculoskeletal system. Even with their great importance, there is relatively little known about how the non-collagenous components of a tendon influence its mechanical properties. Dr. Spencer Lake's research lab has done extensive research on tendons, and more specifically, the mechanics of a small protein in tendons called elastin. Elastin exists predominantly in the interfascicular matrix (IFM), which is located between collagen fascicles and may link them together. It has been proposed that elastin has great mechanical importance in a tendon, for it gives the tendon its elastic properties and may allow it to recoil after being exposed to different stresses. However, there is little experimental evidence on how elastin affects the tensile strength of the tendon. Previous research in the Musculoskeletal Soft Tissue Lab has suggested that elastin does have some effect on its strength, but not as much as anticipated. However, the tests were done on mouse models rather than larger species. Because of the smaller tendon size, the distribution of the elastin within the tendon and IFM may be quite different compared to larger animals (2). Testing these large animals without elastin could lead to data suggesting that elastin does have a significant mechanical effect on the tensile strength of tendons. So, to test these larger tendons a new machine must be used for testing and a new method to degrade the elastin in the tendon must be formulated, as well as using a new clamp design, for the previous clamps used are not able to provide the loads needed for larger, and therefore stronger, tendons.

So far, the testing completed on the new machine has been on six samples of bovine deep digital flexor tendon, or DDFT. These tendons are located behind the hoof of the cow, and are loaded in tension, shear, and compression (1). These tendons were used specifically for preliminary testing, and so the sample sizing is very small, which can be seen in the results section. In future experiments, the tendon will be removed from the Instron and put in a solution of elastase in order to degrade the elastin. Then the tendon will be tested again using the same protocol. The degradation of elastin using elastase has not been completed yet due to two major design problems with this process. First, new clamps will need to be designed to hold the larger tendons, as the tendons used for this testing are larger than mouse tendon, meaning new sized clamps are needed. The next problem, is that while the tendon is removed from the Instron and undergoes elastase treatment, the gauge length must be kept constant to allow for comparisons between the first and second test. A gauge length is where the displacement for the testing is zero. This is necessary in order to have a consistent gauge length and preload between pre and post elastase treatment, and doing this will ensure the only change between tests is the degradation of elastin. By doing this preliminary testing and prototype clamp design, and with these future intentions in mind, I was able to confirm that using new clamps and different machine is viable for these larger species of tendon.

Methods, Procedures, and Design:

In order to study the effects of elastin, we will use a protein in solution called elastase to render the elastin in the tendon non-functional. This will allow us to study the properties of a tendon with and without elastin and determine if there is a noticeable effect on a tendon's tensile strength. However, in order to use the elastase for larger tendons, it is first necessary to transfer the loading regimen from the mouse tendon experiments previously conducted on a Biax

Machine in the Musculoskeletal Soft Tissue Lab (2), to the larger Instron Machine which can be used to study larger tendons. Making this transition will involve first testing a tendon in tension before any elastase treatment. To do this, we will use a 5542 Instron Testing Machine as shown in fig. 1, that puts the tendon in tension to a specified percent elongation or strain.

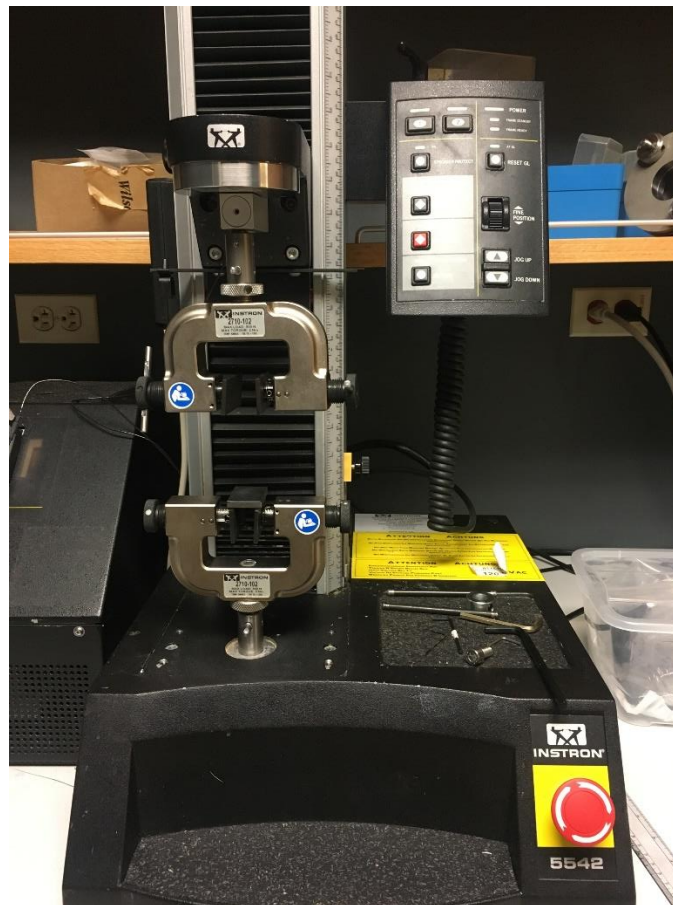


Figure 1: Instron tensile testing device

A single test involves three separate processes. First, the tendon will be put into cyclic tension, in which the sample will be cycled between 0 - 3% strain for 10 cycles. This is to establish a consistent strain history in the sample, which can affect further testing in a viscoelastic material such as tendon. Next, the tendon will be held in tension at 5% elongation for five minutes. This is to measure the viscous properties of the tendon as the stress relaxes

during the test. Finally, the tendon will undergo three increasing ramps of tension. The first goes to 2% elongation, followed by 4% elongation, and finally 8% elongation. A sample of the loading regimen, which graphs displacement of the clamps versus time can be seen in fig. 2 below.

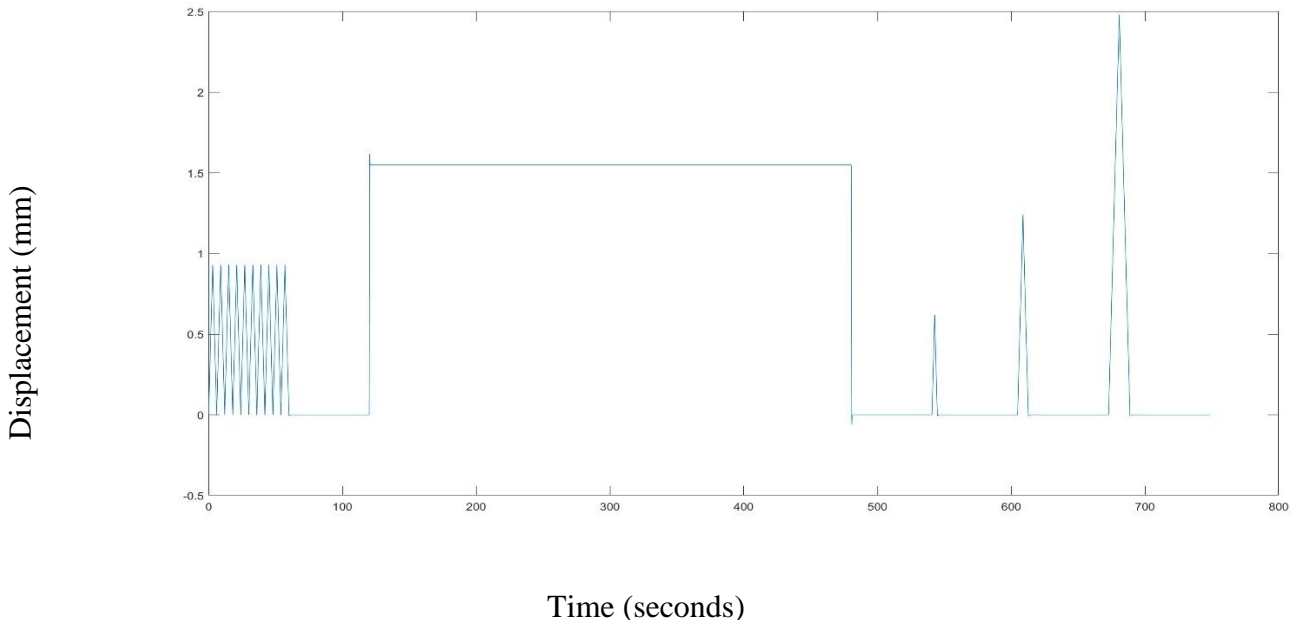


Figure 2: Displacement versus time graph of testing protocol used for elastin testing

During the testing, load, displacement, and time data points will be recorded at a frequency of 10 Hz. In the future this data acquisition rate will be increased to 100 Hz. To test the tendon, the samples were first dissected from six cow hooves, and then they were freshly frozen. Roughly a week later, the tendons were slowly thawed and tested on two separate days. To test the tendons, we used the preexisting clamps as shown in fig. 1, and used the protocol described above. From this testing, we will be able to extract data about the elastic modulus, hysteresis, and many other mechanical properties of the tendon, and those results done on bovine

testing can be seen in the results section below. The next portion of my research involved designing the necessary clamps for larger tendon testing.

Currently, the design of these clamps is still in the prototype phase. I used a computer aided design, or CAD, software called SolidWorks to make the design, and 3D printed the designs to do preliminary testing. A picture of the clamps can be seen below in fig. 3.



Figure 3: 3D printed prototype clamps

The design in fig. 3 is only half of the current design, as a tendon will be laid on top of the two flat pieces, and this exact design will “sandwich” the tendon between the two layers of clamping. The two main components of this design include the two long bars on each side and the two square pieces between the two bars. The two square pieces are where the tendon will be

placed. This design for the square pieces works well as it is enough space to properly clamp the tendon without slipping. If some slipping does occur, sandpaper can be placed between the clamp and tendon to hold it in place. The two bars on the side will be screwed to the square pieces after testing, and they are the component that ensures the gauge length stays at zero. In the future months, the design will be improved so that the clamp surface area is smaller, screws can be properly placed in the sides of the clamp, and finally machined into metal to be used for testing.

The specified testing protocol defined above will be completed twice per tendon, before and after elastase treatment, and then will be compared to see if elastin has a mechanical effect on large tendons. This analysis will be done using a custom MATLAB code.

Results:

Much of the Spring semester was focused on replicating the protocol of testing from the Biax machine to the Instron machine and designing clamps. That being said, preliminary data on the four cow DDFT was collected, and it is likely that the deviations in the samples were due to the small sample size. Below in fig. 4, a load versus time graph shows how much force was placed on the tendon using the protocol described above. This graph only represents one sample's load versus time graph and but representative of the other samples.

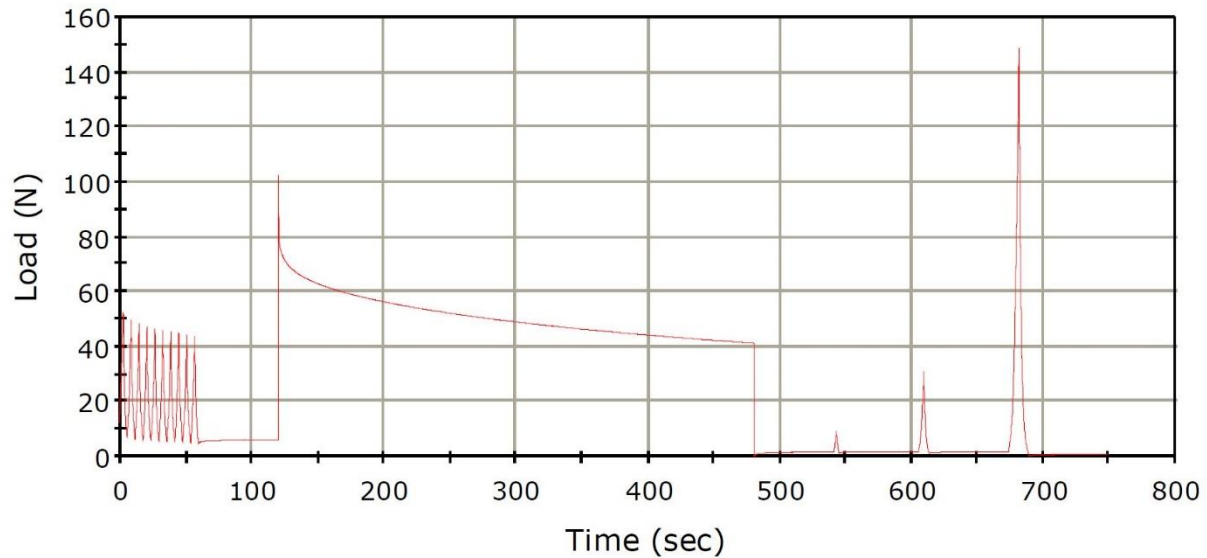


Figure 4: A load versus time graph of a bovine DDFT testing in tension

The graph displayed in fig. 4 directly corresponds to each of the three portions of that were described in the methods section, with the first portion of the graph being the pre-cycling, the central curve being the 5 minute tensile test, and then 3 varying peaks that show the 2, 4, and 8 percent ramping. With this, a displacement versus time graph, shown in the methods section, was analyzed using a custom MATLAB code in order to find the peak load for the 5 minute holding period and each of the ramps. A stress relaxation value as also found using the 5 minute holding period based on peak and minimal loading values, and finally hysteresis loops and values were analyzed for each of the ramps to a percent elongation. Below, in fig. 5, a chart can be seen showing the peak load for the 5 minute hold period, an equilibrium load, which is the minimal peak value for the 5 minute hold period, and peak loads for each of the 2, 4, and 8 percent ramps.

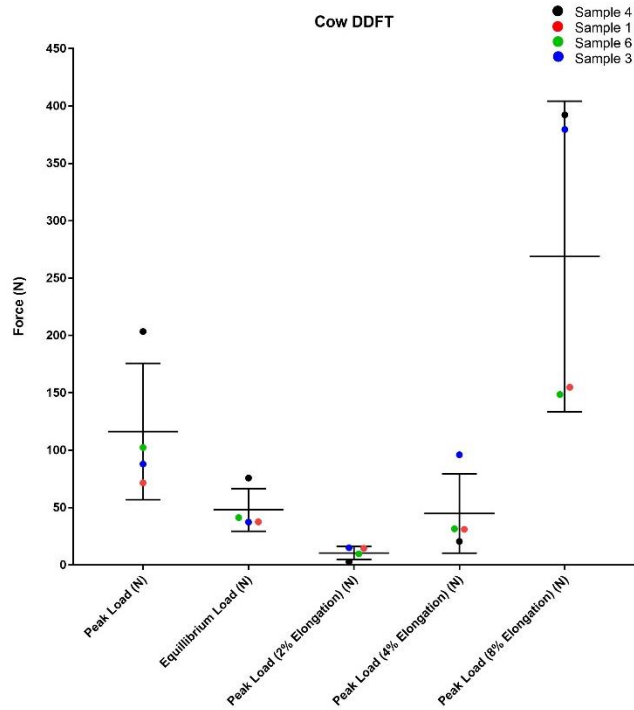


Figure 5: Loading values for 4 bovine DDFT samples

Next, using the same force values, but normalized over the cross sectional area of the tendon, the stress values were computed and are shown in fig. 6 below.

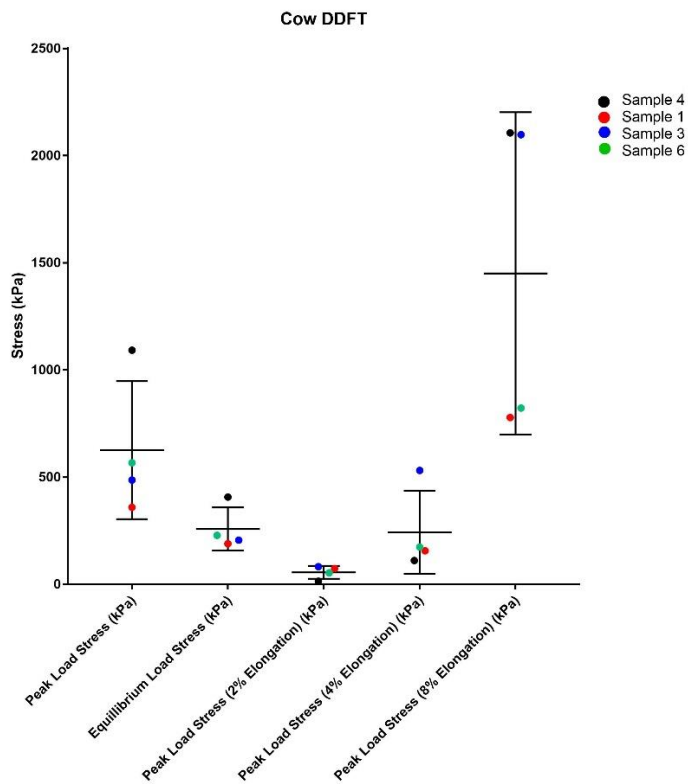


Figure 6: Stress values for 4 bovine DDFT samples

Next, we used the same custom MATLAB code to analyze hysteresis loops from each of the percent elongation ramps as well as a stress relaxation value for each of the four samples. A sample of a hysteresis loop for one sample can be seen below in fig 7.

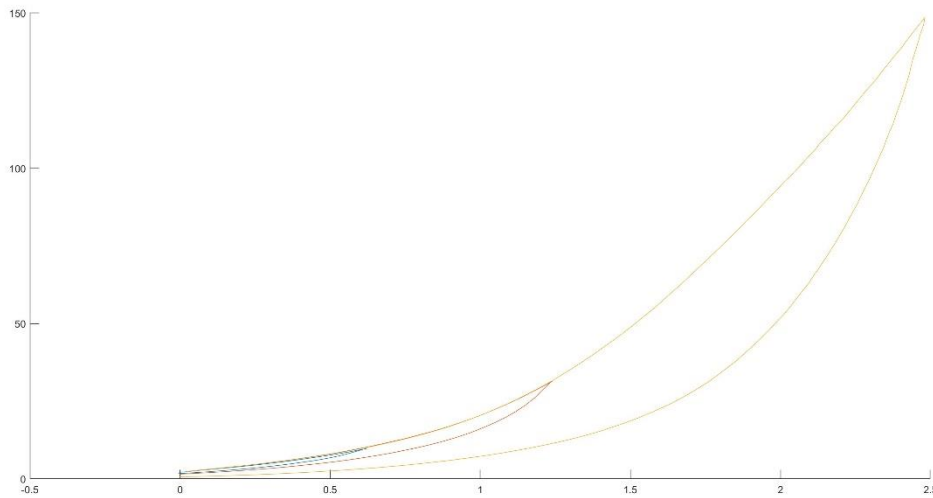


Figure 7: Hysteresis loops found from 2, 4, and 8 percent elongation values

On the chart above, the smallest green loop shows the 2% ramp hysteresis loop, the middle red loop shows the 4% hysteresis loop, and the large yellow loop shows the 8% hysteresis loop. Finally, the percent hysteresis was calculated from these loops. This was done by subtracting the area under the curve of the bottom bound of each loop from the area under the curve of the top bound of the loop. This subtraction was repeated for each sample and each percent elongation ramp. Finally, the stress relaxation was found for the 5 minute hold portion using the equation shown below.

$$\text{Stress Relaxation} = \frac{\text{Peak Load} - \text{Equilibrium Load}}{\text{Peak Load}}$$

The results from this analysis can be seen in figure 8 below.

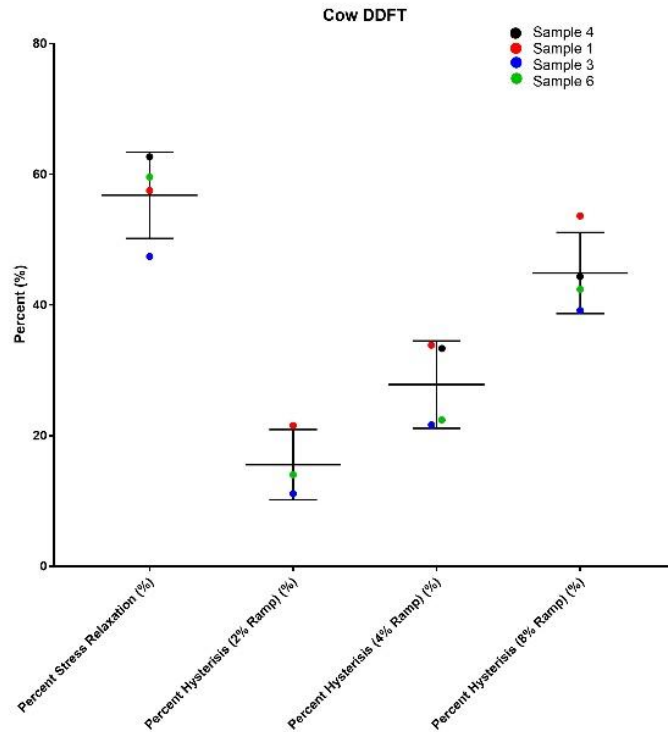


Figure 8: Percent Hysteresis and Stress Relaxation Values

These results conclude the data gathered from the bovine DDFT samples tested. Once this was completed, I went on to design the clamps shown in the methods, procedure, and design section above.

Discussion:

As stated previously, these results were from preliminary testing of four samples. These tests were done specifically to confirm that the transition from Biax machine to the Instron machine was viable. The criteria for determining whether the switch was viable include whether or not there were any major inconsistencies in the data and whether the data found was similar to the data gathered from the mouse tendon testing on the Biax machine. Overall, there were not significant enough inconsistencies to decide the Instron Machine was not viable for this

experiment. Furthermore, because this was the first time the Instron Machine was used to test tendons of this size using this procedure, there were likely inconsistencies in the placement and execution of the testing, which would have contributed to inconsistencies in the results. With more practice of loading these tendons in the machine, standardization of the testing procedure, and a larger sample size, we believe that the Instron Machine is a comparable way to test these tendons in tension using the specified protocol.

In terms of the clamping mechanism designed thus far, we believe with small design changes and well crafted machining the clamps will work well for various tendon sizes. The major problems with the clamps as of now is finding a strong enough design to keep the gauge length constant as well while being small enough to minimize the amount of the enzyme solution, elastase, that is needed in order to minimize cost. That being said, we believe using two square plates with two connecting bars is the best way to keep the gauge length constant, which is its most important function.

Conclusion:

Overall, this semester has been a success, as we have started to transition from using a protocol defined for mouse tendon, into an experiment that will eventually be applicable to testing various tendon sizes of various species. This is all done in the hopes of determining the mechanical properties of elastin in tendons, and finding the elastic fiber has any significant effects on the tendon's strength. In the future, I will continue to develop and perfect the design of the clamps. I will do this by making them slightly smaller as well as making sure they are able to keep a constant gauge length. Furthermore, once they are machined from metal, I will begin to use elastase treatment to further confirm the Instron Machine is a viable way to test the tendons

for the effects of elastin. Furthermore, I will continue to increase my sampling size, and begin testing other tendons to see if different species also gives as promising results as the bovine DDFT.

References:

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