Webinar Q&A Report:

How to Study Structural and Functional Properties of Tendon

1. **Are you aware of any difference between males and females in human tendons?**

   [D. Sarver] The epidemiological data presented in the study is of course from human. We know that males have an increased for tendon rupture as age increases and females have an increased burden associated with tendon injury. There are other more specific differences between the two, I'm sure. However, an extensive study, such as the one I presented in the webinar, has not been conducted in human tissue.

2. **Can you customize the software to do some viscoelastic measurements (stress relaxation or creep, hysteresis, or frequency sweeps). If yes, are there resources that show how to do this?**

   [M. Borkowski] Yes, you can write these sorts of protocols with the latest version of DMC. (V5 and higher). We do have resources for writing protocols on our website in our blog section. See http://aurorascientific.com/blog/ to access these articles.

   If you do not see the protocols you wish to write there, please reach out to us at info@aurorascientific.com and we would be glad to write you examples based on your requirements and specifications. If you require customization which is not currently in the protocol, please also reach out to us as we will try to add it into future versions of the software.

3. **Is it possible to use this test machine with synthetic scaffold tendon (before the scaffold is implanted in the animal)?**

   [M. Borkowski] We would probably require some more information about the size and shape of the sample, however, I would say that testing these synthetic scaffolds is extremely likely as there is experimental precedent for testing scaffolds for other tissues with these instruments.

4. **How would you recommend calculating CSA and cell density on synthetic scaffold?**

   [M. Borkowski] Personally, I would believe that using the prism method that Dylan was using for his tendon samples would work very well to calculate CSA in synthetic scaffold. I am not familiar with measuring cell density.

   [D. Sarver] I have been able to calculate cell density after mechanical testing using histological techniques. After stretching the tissue, I would freeze it in OCT for cryo-sectioning and subsequent histological analysis with WGA/DAPI staining. DAPI is a nuclear stain that can be used to calculate cell density with relative ease. Longitudinal and cross-sections both supply a unique view of the cell nuclei spread across the tissue, and either could be used for cell density calculations.

5. **Is it possible to switch load control mid test? For example, could we apply a certain strain, and maintain the stress at that strain for the remainder of the test?**

   [M. Borkowski] Yes definitely. This example would be certainly attainable for the system. You could even attain this if the amount of stress produced after applying the strain were unknown.

6. **Can you use this system for testing "suturing" of ruptured tendons? Any thoughts on that?**

   [D. Sarver] Yes, I am doing something like this for another study in which we tear the tendon then immediately repair the area with a suture. While we are then waiting days after to see how the tendon has healed, it is possible to tear the tendon, suture, and immediately test the strength of the suture knotting technique. Both destructive and non-destructive testing can be useful investigative tools for this.

7. **What is the minimum size/length of tendon to be used? I am working with shoulder tendons from rats, Its like 2-3 mm length.**

   [M. Borkowski] The length noted (2-3mm) seems attainable. More critical than the length of the tendon itself is what material exists at either end of the tendon sample to attach on to.

8. **What is the size of the suture thread used to mount the tendon**?

   [M. Borkowski] This will depend on the size and type of tendon sample, but using Dylan's plantaris tendon as an example I believe the preferred size was 6-0.

   [D. Sarver] Durability and grip are the two things to consider. I used 4-0-gauge suture because this was the smallest suture that was strong enough to survive the testing protocol. A larger suture will not grip as tight, but will be needed for higher testing loads.

9. **What type (size) of controller (in N) is needed for mouse tendon study and for mouse ligament (specifically ACL)?**

   [M. Borkowski] This will naturally depend on the strain percentage applied to the tendon, but if we use Dylan's plantaris as a guide, it produced less than 1N of tensile force when stretched on the order of 10-20%. We could safely assume that a 5N unit would be more than enough to handle these tendon samples.

10. **Are there any issues with slippage in destructive testing? Can you suggest tips or best practices for destructive testing?**

    [M. Borkowski] Slippage in destructive testing is a huge problem. Some "tips" I have encountered are folding over the sample where it is sutured and tying a second knot to create two "parallel" knots for strength, applying glue or adhesive to the suture knot, crimping or clipping on to the sample and then attaching the suture to it, etc.

    [D. Sarver] While suture is fine for non-destructive testing, clamp grips are the best thing to prevent slipping at higher testing loads. With suture, glue (usually veterinary glue, GLUture) has been effective to prevent slipping of the proximal end of the tendon from the stationary hook.

11. **Are there specific limitations (for example size, or sample compliance) when using this system for stress/strain assays?**

    [M. Borkowski] Sample size can be a limiting factor, but it is more from the perspective of how to attach to the sample with suture than it is for the system to perform measurements. Compliance as mentioned, is another important consideration. Samples must have some elastic properties and have some level of compliance when stretches. Samples such as bone are not compliant enough for testing.

12. **How long can you study a single tendon sample? Are there any surgical tips for excising samples and removing muscle?**

    [M. Borkowski] I would say since you don't need to oxygenate the solution you can test a tendon sample pretty well indefinitely as long as it doesn't dry out. Surgical tips are extremely specific to the sample type but Dylan may be able to share his tips for plantaris tendon and some of the other muscles he's worked with here.

    [D. Sarver] I have tested bubbling O2 through the solution, in fact I did this for the sex differences study, but there does not seem to be a difference with and without oxygenation of the solution. The samples preserve mechanical properties for a very long time, on the order of hours to a full day. I have even tested the properties of a tendon stored at 4°C for up to three days, and that was still maintained fine. The biggest surgical tip I would give is, take too much always. It is much easier to take away then it is to add

on. You can always trip samples down later prior to testing. For the plantaris, I remove half of the calcaneus, the entire tendon body, and about one third of the muscle. Prior to testing I will remove the muscle from the tendon and use that excess as the tendon loop.

13. **Mr. Sarver: is there any particular reason why you chose to omit destructive testing from your experiment?**

    [D. Sarver] Destructive testing is great for investigating some of the mechanical properties of tendon, but this has been done many times before and omits important hysteresis non-destructive testing can provide. Normal tendon movement occurs with approximately 5-12% material strain, so it makes sense to test the mechanical properties within this physiologically relevant range.

14. **Is it possible to use this same system to study muscle samples? If yes, how would the system need to be optimized, and what factors should be taken into account when planning your tests?**

    [M. Borkowski] Definitely. First, if studying active force produced by muscle consult the literature to get a sense for how much force production would be expected and compare against the tensile loads of any connective tissue samples being tested. To produce active contraction of muscle an electrical stimulator will also be required. A slightly different chamber or experimental apparatus may be a consideration but in general they are multi-purpose. Otherwise this is probably everything. The equipment is well equipped already to deal with muscle samples.

If you have additional questions for the presenters, please contact them by email:

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