Peer Review File

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Comment 1:

Line 94: The authors conducted a new rotator cuff model try to mimic the natural history of rotator cuff tear in human by performing partial sharp transection of the tendon bone enthesis. The author stated that this model will be more close to the rotator cuff model in human, however it was not clear which model was the author implying to >> acute or chronic model. Either of these two, what is the scientific background to support this idea? Previous study? Pilot study?

Reply 1:

The goal of the sharp partial transection model was to replicate the chronic degeneration cascade commonly observed within the human rotator cuff tendons. It was hypothesized that surgically induced damage at the tendon-bone enthesis (i.e., sharp surgical transection through 50% of the enthesis; akin to a partial tear), while still leaving the entire width of the tendon attached, would initiate a degree of degeneration which parallels that observed in degenerated human tendons clinically, as evidenced by comprehensive biomechanical, histopathological, histomorphological, and gene expression analyses. The rational for this idea came in part from previous work(1) by our group which transected one half of the width of the tendon. This work resulted in pathologic changes in the non-transected side that were similar to has been documented in humans with chronically degenerated rotator cuff tendons clinically (this work is mentioned on P5 L83-85).

> *To overcome this shortcoming, a partial transection model in which half of the infraspinatus' width was transected from the enthesis, followed by a delayed repair was created.*

The results of this work led us to hypothesize that by transecting half of the thickness of the tendon, that similar changes would be introduced throughout the entire tendon. L88-92 described the inability of current large-animal translational models to accurately replicate the chronic, degenerated human condition. For completeness, the following text was added to the manuscript (P2 L30-31):

> *Therefore, the objective of this study was to develop a large-animal translational model of enthesis damage to the rotator cuff tendons to mimic the chronic degenerative changes that occur in patients that demonstrate clinical manifestations of tendinopathy and/or tendinosis.*

And (P5 L92-93):

> *Therefore, the purpose of this study was to investigate a novel ovine model of chronic rotator cuff degeneration.*

Comment 2:

Specimen of the supraspinatus tendon was taken from the control group with 60s years of mean age >> representing the "true rotator cuff tendinopathy" however I am skeptical on how the study will represent the true nature of the disease.

Reply 2:

The authors acknowledge that by collecting tissue from a limited subset of patients, it is difficult to fully capture all aspects of the disease. However, previous researchers have used similar tissues from these age ranges to represent a population as a positive control of chronically degenerated rotator cuff tendon tissue (1, 2). To address this concern the following text is included in the manuscript (Page 6, Lines 113 -116).

> *PRSA was performed on arthritic patients with well documented chronic rotator cuff injuries. Succinctly, the rotator cuff injuries in these patients are often severe and the tendons exhibit symptoms of chronic degeneration that would prohibit normal shoulder motion if a normal arthroplasty were performed (33, 34).*

Comment 3:

Line 131 >> why data is not shown for model 2?

Reply 3:

The data for the second model was intentionally omitted. We believe that as the surgical insults are created at different regions of the tendon, each model represents a different injury cascade/disease state. As such, discussing both models would confound, and ultimately dilute, the findings. All analyses performed within this manuscript have also been performed on the second model. The authors are in the process of finalizing a manuscript detailing these findings. It is our goal to submit this manuscript to Annals of Translational Medicine in Q2 of 2021, such that both models are available to the readership. For completeness, the following text was added to the manuscript (P7 L133-134):

> *The surgical models (model 1: sharp partial transection of the entheses (n=20 treated shoulders); model 2: combed fenestration of the enthesis and tendon mid-substance (n=20 treated shoulders – data intentionally omitted from this manuscript so that it can be compiled into a standalone publication)) were alternated between shoulders to eliminate the potential of a left/right side bias.*

Comment 4:

How to prevent spontaneous healing of the rotator cuff tendon since the tendon was only separated partially from the humerus?

Reply 4:

The reviewer raises a justifiable concern. The authors did not try to prevent spontaneous healing of the tendon in this model, instead allowing the natural injury response cascade to progress naturally at the damage site. Our rational was based on previous work that has shown that injuries in one area of the tendon can cause cellular and microstructural changes throughout both the tendon thickness and length(1, 3-7). These models in which a partial width injury was imposed on a tendon have produced inconsistent degenerative characteristics between the two halves of the tendon (i.e., released vs. non-released halves). Specifically, on the released side, severe degenerative changes have been noted that exceed what has been documented clinically in humans, with large degrees of tendon retraction precluding and/or significantly limiting the ability to test surgical strategies to repair the damaged tendon as the tissue has retracted to an extent that makes it intractable to reattach. On the intact side, more mild degenerative changes have been noted that tend to better correlate with overuse tendinopathy, not necessarily chronic degeneration. Our model differentiates itself from these previous models in that the entire width of the tendon was still attached, yet the top half of the thickness of the fibers were released (analogous to a partial tear). Through shear loading and the addition of fibrous tissue overlaying the insult, our hypothesis was that the entire tendon would still see mechanical loading, while simultaneously undergoing cellular/microstructural changes due to the injury. For these reasons, we did not wish to prevent "spontaneous healing" of the injury. This strategy is outlined on P8 L141-144:

> *This was done to simulate damage at the tendon insertion that does not result in a complete separation of the tendon from the humerus, similar to what is hypothesized to occur in humans that have suffered a partial thickness tear at the enthesis.*

Comment 5:

Line 147: Why choose 6, 12, 18 weeks for time frame?

Reply 5:

The goal of choosing multiple sacrifice time points was to capture the time-mediated injury response in the ovine model. Previous research in ovine models have frequently used 6 and 12-week timepoints(8), with some studies including longer timepoints to indicate long-term effects(9). We chose to adopt the 6, 12, and 18-week timepoints to capture the standard 6 and 12-week ovine model timepoints with the addition of an equally spaced longer term timepoint at 18-weeks. For completeness, the following text has been added (P8 L148-149):

> *Animals were humanely euthanized at 6, 12, and 18-weeks to capture the time-mediated tissue response to injury and degeneration.*

Comment 6:

Line 156: how to measure the cross-sectional area of an intact tendon?

Reply 6:

The cross-sectional area measurement protocol is detailed in the biomechanical testing methods section of the manuscript. This procedure has been outlined and used in previous publications(10-13). For clarity, the following text has been added (P8 L158-160):

> *Three cross-sectional area (CSA) measurements of all tendons were taken proximal to the insertion using previously validated techniques in which an*

*area micrometer that applied 0.12 MPa of pressure parallel to the cross section of the tendon(23, 37-39).*

Comment 7:

It will be better if figures show the biomechanical testing.

Reply 7:

Figure 1 has been modified to include a picture of the biomechanical testing. The figure caption has been modified (P26 L595-598):

*Intra-operative image illustrating location of surgical insult at the midpoint of the tendon insertion (left) and digital illustration of surgical insult and relevant tissue (center). Rectangular box outlines the tendon insertion, arrow highlights the surgical cut. Image of biomechanical testing (right).*

The biomechanical testing methods section has been modified to add in a reference to the image (P9 L165):

*The humeri were subsequently mounted in polyvinyl chloride (PVC) sleeves using a strong two-part hard casting resin (SmoothCast 321, Smooth-On, Macungie, PA) and mounted in a custom fixture attached to a servo-hydraulic load frame (Model 805, MTS Corp., Eden Prairie, MN) which allowed anatomically accurate loading of the tendon (Figure 1) (23, 25).*

Comment 8:

Line 179 >> does it mean not all specimen underwent both staining?

Reply 8:

The reviewer comment is justified, and the text has been updated to clarify the methods used. Two slides were prepared from each specimen. One slide was stained with hematoxylin and eosin (H&E), and one slide was stained with Picro-Sirius red. The manuscript text has been modified to clarify this point (P9 L180-183):

*These humerus-infraspinatus tendon constructs were then fixed in 10% neutral buffered formalin (≥7 days), demineralized in 8% trifluoroacetic acid, embedded in paraffin, sectioned at the mid-tendon-body, and mounted* *on a glass slide. Two 5 µm thick slides were cut from each sample; one slide was stained with hematoxylin and eosin (H&E) and the other slide was stained with Picro-Sirius red.*

Comment 9:

In general, supraspinatus tendon tear is more commonly found compare to infraspinatus tendon tear. What was the reason to have an infraspinatus tear model?

Reply 9:

This is an interesting topic of debate when choosing the animal model. Considerations typically include the surgical access, functionality of the tendon, motion of the tendon, and the size of the tendon. Previous work has shown that within a sheep, the infraspinatus tendon is most appropriate due to similar sizing and morphology to human tendons, allowing testing of devices intended for human use without the need for device "scaling"(14). The ovine infraspinatus is accepted as a translational model for human rotator cuff tendon tears(1, 10, 13-18).

Comment 10:

Line 194: this algorithm from the machine learning >> was it validated before? It seems like the machine learnt it from the resident.

Reply 10:

The selected software (Image-Pro Plus) is a commercially available platform that has been widely accepted for histomorphometry analyses(19-21). Data collection approach for this study using this software was consistent with previous studies published by our group(19-21). To ensure consistence and accuracy a veterinary pathology resident verified the program was able to accurately segment the areas of organized and disorganized collagen. The following text within the manuscript has been updated to reflect that a commercially available software was used for this analysis (P10 L197-200):

*Commercially available software (Image-Pro Plus, RRID:SCR_007369) was used to calculate percent area of the organized / disorganized collagen seen within the injury region of the tendon.*

Comment 11:

Line 223: why 20 shoulders? Not 40?

Reply 11:

This study included a total of 20 animals, yielding a total of 40 possible shoulder samples. Only 20 shoulders were included in this study as only 20 of the shoulders were operated on with the sharp partial transection model (see Response 3 above). The other 20 shoulders were operated on with a different surgical insult; the data for these shoulders will be included in a separate manuscript (see Response 3 above). For completeness, P7 L132 states that there were 20 treated shoulders in the sharp partial transection model.

Comment 12:

Figure 4 >> collagen organization>> does it means 12-week group is similar to degenerated human? and 18-week group resemble control group?

Reply 12:

Confusion on this topic was inadvertent. As indicated by Figure 4, the collagen organization in the 18-week group was statistically greater than the degenerated human group ($P$=0.002). The collagen organization in the 12-week group was most similar to the degenerated human group. To better clarify this within the manuscript, the following text was added (P20 L 422-423):

*Specifically, the 12-week timepoint appears to most accurately embody the characteristics of chronic tendon degeneration seen clinically in humans.*

Comment 13:

How did you define several subjective statements such as “thick”, “always most dense” at the histologic analysis?

Reply 13:

The term “thick” was used on P13 L258 in the histopathology results section of the paper:

> *Arising from the superficial, adjacent humeral head and overlying this area of the tendon was a variably **thick** band of dense granulation tissue with prominent angiogenesis that extended into the tendon body.*

Here, the word was used subjectively to describe the changes in thickness of the granulation tissue that was overlaying the tendon within and across specimens. The intention was to describe that the granulation tissue was not of uniform thickness.

Similarly, the statement “always most dense” was used to subjectively describe the observation that capillary clusters were most prominent in the injury area and overlying granulation tissue. This statement was included as a subjective description of the locations of angiogenesis. Both terms were not further detailed with unit measurements as there was not a clinical correlate for comparison and thus was outside the scope of the manuscript. The inclusion of these subjective terms is meant to serve as a descriptive aid for the readers.

Comment 14:

Of all time frame, which time frame indicate the point of no return following rotator cuff tear model?

Reply 14:

This is an interesting comment, and holistically we do not have a specific answer to this question yet. While this study was designed to investigate the temporal changes in tendon biomechanics, collagen organization, pathological characteristics, and gene expression following an enthesis injury at 6, 12, and 18-weeks post-surgery, it was impossible to determine a “point of no return” as in this study there was no attempt at repair or apply a therapy at any point in this study. As such, the authors have not collected adequate data to make scientific conclusions regarding “the point of no return” in this model.

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8. . !!! INVALID CITATION !!! (1, 8, 9).
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