Comparative Stress Production of Native and Quantum Dot Labeled Rabbit Psoas Muscle Myofibrils

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SUMMARY
Studying muscle mechanics is often aided by pairing visualization techniques with mechanical measurements. However, we must first characterize any change in muscle properties with the introduction of these muscle specific labels. By introducing titin and myomesin specific antibodies conjugated with quantum dots into a myofibrillar preparation, we noted very little variation in the stress required to passively stretch these muscle specimens. This work represents first steps into a thorough evaluation of sarcomeric protein label introduction, suggesting passive stress remains unaltered with labeling.

INTRODUCTION
Utilizing fluorescent markers in subcellular muscle preparations is a well documented procedure [1,3]. However, before embarking on mechanical measurements with fluorescent markers, it is necessary to evaluate any potential change in the mechanical properties of the preparation attributed to label introduction. It is our goal to use a new muscle testing apparatus to characterize the effect of antibody and quantum dot conjugation into the lattice spacing of skeletal muscle myofibrils. The visualization of different proteins or regions of a sarcomere will then be possible, alongside real-time force measurement. Additionally, we will compare the stress recorded from unlabeled experiments to a well characterized complimentary system in our possession [2,4]. Using this technique to demarcate different parts of the myofibril, specifically the titin spring within myofibrils, we will be able to track how different parts elongate with passive and active lengthening. We believe this titin spring may exhibit different segmental elongation in the passive stretch condition, when compared to the active stretch. Should this deviation arise between conditions, it may be indicative of a unique titin behavior in myofibrils that could render the spring shorter during active stretch if titin were to transiently bind to actin. This mechanism could explain some of the dramatic increases in force we observe during active stretch, and illustrate a way in which titin can prevent muscle overextension and damage from occurring.

METHODS
Rabbit psoas muscle myofibrils were homogenized fresh prior to experimentation, and mounted directly to the muscle testing apparatus, or labeled with anti-titin antibody T12 (Enzo Life Sciences) and anti-myomesin (Developmental Studies Hybridoma Bank) antibody. These were then conjugated with quantum dots (Qdot525, Invitrogen) for visualization (Figure 1). Solutions used are detailed elsewhere [2].

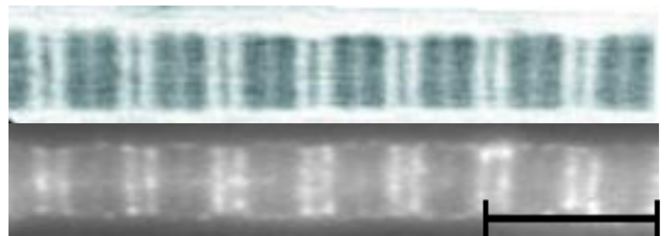

Figure 1: An unlabeled myofibril under phase contrast (Top), contrasted with a labeled myofibril with Qdot525 illuminating titin (Bottom). Scale bar: 4µm

The custom muscle testing apparatus consists of a myofibril attached between a rigid glass needle and a force transducing cantilever (Figure 2, inset). This cantilever is coupled to a laser and quadrant detector system, converting any change in cantilever deflection into a force.

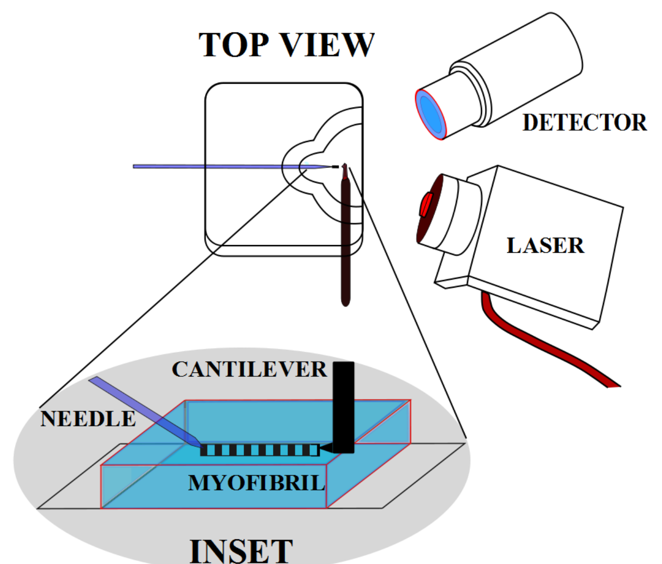

Figure 2: Schematic representation of the myofibril testing apparatus. Inset: the myofibril is attached between a glass needle and cantilever. This sits in a fluid bath, through which a laser is used to measure cantilever deflection.

RESULTS AND DISCUSSION

Preliminary results suggest that labeled myofibrils resist passive lengthening equally well to unlabeled myofibrils (Figure 3). Although there is a marginal increase in passive force observed with labeling, this change is well within the variation in force recorded using this apparatus.

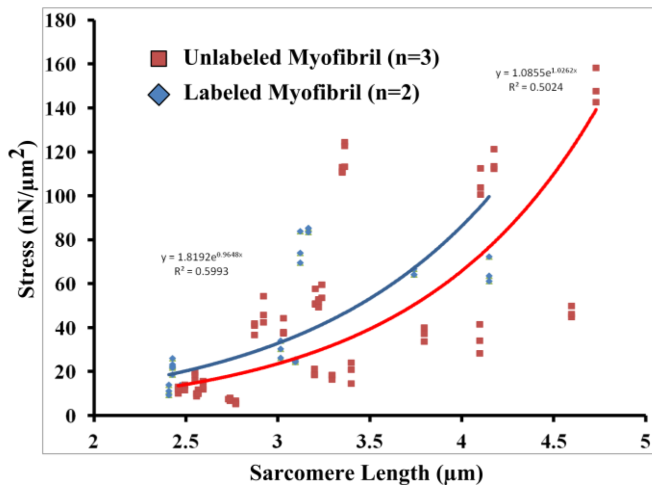

Figure 3: Stress comparison of antibody and Qdot525 labeled myofibrils (Blue Diamonds), with unlabeled native myofibrils (Red Squares).

These labeling experiments represent a "worst-case" scenario, in which double labeling is performed to exaggerate any effect that may arise in future single-labeling studies. The titin T12 antibody localizes to 100nm on either side of a sarcomeric Z-line, which is where titin is thought to interact with actin rendering this spring inextensible [3]. The anti-myomesin antibody resides in the center of the A-band, where myomesin is found. Should an appreciable difference arise, then some cross-linking may be speculated upon in the lattice spacing between parallel filaments (myosin, actin, titin). However, using a similar system and the same antibody and muscle, Telley et al., [1] found no change in the active force kinetics with their immunofluorescence labeling, which suggests the passive properties may also be unaffected. These two antibodies localize to regions where actin and myosin interaction would largely be unaffected and titin remains inextensible, thus it could be the case that no change is seen simply due to antibody pair selection. Further combinations of antibodies will need to be explored.

The variable nature of the stress collected during passive stretch requires more experimentation to draw conclusions. However, we have agreement between the unlabeled passive stress data collected on our new system and that on a similar well-tested system in our possession [4].

CONCLUSIONS

The experiments conducted thus far suggest minimal effect associated with introducing antibodies and fluorescent labels into the lattice spacing of sarcomeres. Whether this result remains correct for other sites in the I-band region, and with other antibodies, is still under experimentation.

ACKNOWLEDGEMENTS

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