Silicon Nitride Cantilevers for Muscle Myofibril Force Measurements

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Primary CNF Tools Used: GCA 5X Stepper, SÜSS MA6-BA6 Contact Aligner, Photolith spinners, Oxford 81 ion etcher

Abstract:

To measure muscle forces in the nano-Newton range, silicon nitride cantilever pairs were manufactured and used. Measuring sarcomere length (SL) variation across a myofibril with 20-30 sarcomeres in-series in skeletal muscle myofibrils provides useful information about variation in individual SLs and indirectly, the isoform mass, extensibility, and quantity of the structural protein titin [2]. Myofibril imaging has traditionally been done using phase-contrast (PC) microscopy, but this technique has limited resolution. Promising and reliable fluorescent epitope labelling can be done on sarcomeric structural proteins α-actinin (Z-line) and myomesin (M-line).

The purpose of this study was to 1: Test if antibody labelling techniques affect SL non-uniformities and SL repeatability following a stretch-shortening protocol, and 2: Measure passive stress at matched SL to determine if labelling affects passive force development at long SL. Myofibrils from rabbit psoas muscle were used and labelled with anti-α-actinin, anti-myomesin primary antibodies, and polyclonal IgG (H+L) AlexaFluor488 secondary antibodies and observed using both PC and Fluorescein isothiocyanate (FITC) microscopy.

Myofibrils (Labelled, n=7 and Control, n=7) were attached to force-measuring cantilevers and stretched passively from a mean SL of 2.6 μm (short position; SP) to 3.2 μm (long position; LP). Fluorescently labelled myofibrils showed no change in the range of SL non-uniformities after stretch compared to non-labelled myofibrils, but labelling contributed to a decrease in individual SL repeatability (as seen in the greater variation around the "perfect" identity line for each sarcomere). Passive force was not affected by the presence of the labels. In conclusion, antibody labeling made SL measurements easier by clearly defining the Z- and M-zones, and while they do not affect the passive force, they do alter the time-history of titin extension during stretch, resulting in poor repeatability of individual SL after stretch-shortening.

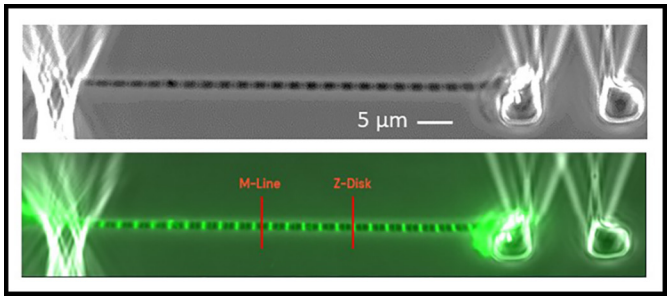

Figure 1: Myofibril attached to a glass needle for stretch-shortening and nano-cantilevers for force measurement. Top panel is a phase-contrast image and lower panel is the same myofibril, when fluorescently labelled.

Summary of Research:

Video images were collected using an Olympus IX83 microscope and analyzed to determine SL in the CellSens® Dimensions software. The maximum number of sarcomeres accurately measurable in each myofibril were chosen, and sarcomeres were pooled together from the seven control and seven antibody-labelled myofibrils, resulting in n=141 control sarcomeres and n=150 antibody-labelled sarcomeres. Individual SL was measured from adjacent M-line centroids throughout the myofibril. SL non-uniformity and individual SL

repeatability were measured after passive stretches, in both control and antibody-labelled myofibrils. An identity line was used for displaying whether each individual sarcomere was the same length, before (x-axis) and after a stretch-shortening test (y-axis). All sarcomeres with perfect repeatability would reside on the identity line. Figure 2 shows the decrease in repeatability in the labelled samples because fewer sarcomeres were close or on, the identity line (x=y).

Force was determined using a microfabricated pair of cantilevers [1], where one end of the myofibril was attached using a mixture of silicon-based adhesive to one of the cantilevers, while the other end of the myofibril was pierced using a glass needle. As passive stretches occurred, the relative movement of the cantilever relative to the stationary cantilever was measured and force calculated based on the known stiffness of the cantilevers. The cross-sectional area of the myofibril was determined and used to determine the stress in the myofibril (nN/μm^2). Figure 3 box plot shows no difference in passive stress at SL of 3.2 μm.

Overall, the introduction of antibodies did not affect the passive force produced by the myofibril at long SL. It did, however, cause a decrease in the repeatability of the length for each sarcomere following the stretch-shortening cycle. This is possibly due to some "internal drag" caused by the label antibodies or by the fluorophores, on the extension/unfolding of titin during stretch. These findings help support further use of protein labeling in myofibril research.

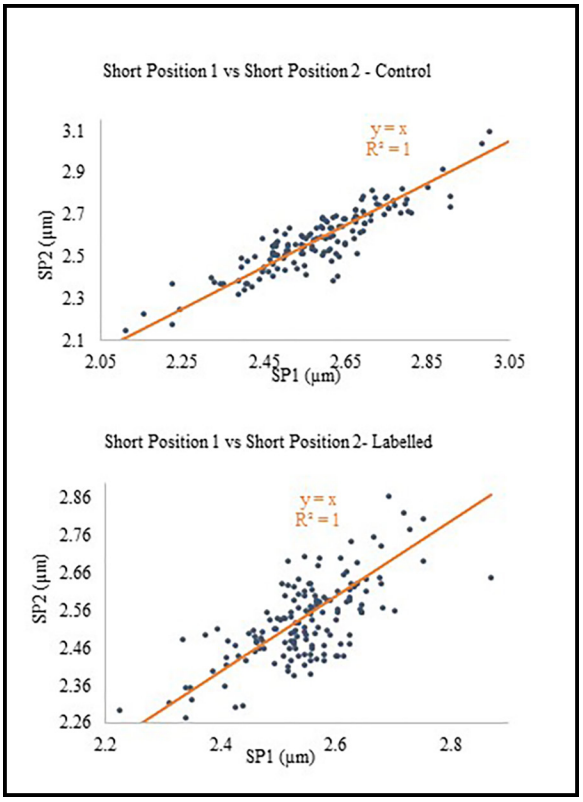

Figure 2: Sarcomere length for control (top) and labelled (bottom) myofibrils. Each sarcomere was measured prior to stretch (SL on x-axis) and then plotted after stretch-shortening (SL on y-axis). If a sarcomere had the identical length following stretch and return to initial length, then each sarcomere length was perfectly repeatable, and the data point would reside on the "perfect identity line".

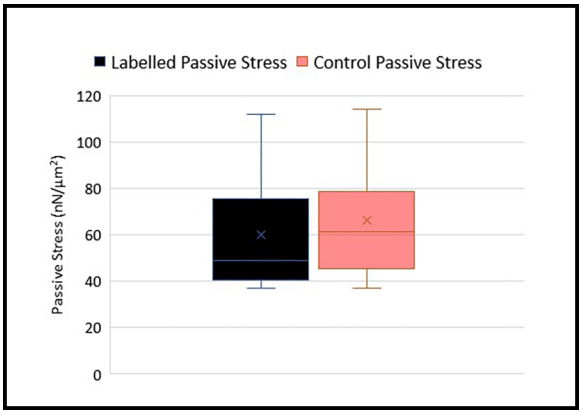

Figure 3: Boxplots comparing passive stress for control and antibody-labelled myofibrils at long sarcomere length (SL 3.2 µm).

References:

[1] M. E. Fauver, et al. IEEE Trans Biomed Eng 45(7):891-898, 1998.
[2] Granzier and Labeit. Muscle Nerve. 36:740-755, 2007.