Webinar Q&A Report:

How to Plan and Execute Contractile Measurements in Permeabilized Muscle Fibers

Q: What is the expected shelf life of relaxing/activating solution at 4C and -20C?

[T. West] We keep 1 ml aliquots of relaxing/pre-activating/activating solutions at -20 C for several months. For our experiments, we add Creatine Phosphokinase (300 – 400 U/ml final activity, from a stock solution that requires addition of only 5 ul CPK to the 1 ml of thawed solution). Usually we use the 1 ml solutions entirely on the same day they were thawed from -20C. But on rare occasions we have used the thawed solutions over a period of 4 days; the solutions are kept ice-cold the whole time.

Q: Would you know if the methodology you use with the wild animal fibers (freezing them in high sucrose solution for later analysis) would work for mouse isolated fibers?

[T. West] We deep freeze our fibres in trehalose, as routine. Sucrose is just as good…maybe better, as it is cheaper. We have treated and frozen mouse EDL and soleus and thawed them from -80C after several months. They seem OK. But we do seem to have to 'search' for good (ie straight, unblemished) fibre fragments when working with skinned mouse fibres. I'm not sure why that is. We have a much, much, easier time finding long and unblemished fibre fragments with other mammalian fibres that we use. But really, our group does not have extensive experience with mouse or human skinned skeletal muscle fibres. It may be that our solution-set is not optimal for mouse fibres.

Q: Is it possible to flash freeze a tissue and run kinetics experiment after thawing the tissue? maybe the water crystal formed could interfere these measurements by damaging the preparation.

[T. West] I have not worked with flash-frozen muscle. We skin fibres soon after retrieving a biopsy and we either store in a glycerol-relaxing solution at -20 C for use over a period of several weeks, or we treat with trehalose (or sucrose) and then deep freeze the bundle for transport or storage. Other groups do seem to have some success with skinning fibres from flash-frozen muscle.

Q: Do you know of any efficient way to transfer a muscle fiber onto the plate where you attach the arms of the force transducer and the motor. My colleague and I would try to transfer the muscle fiber to the arms and would frequently lose the fiber in the transferring process.

[T. West] We always use a small glass rod to transfer fibres onto to the motor and force-transducer hooks. This works very well if you have T-shaped clips attached to the fibre end. Often the T-clip can be placed from the glass rod directly onto one of the hooks. If the fibre drops away from the glass rod into the bath solution it is relatively easy to use very fine forceps to pick it from the bottom of the chamber and place it over the hook.

[M. Borkowski] I have seen two common methods for transporting the fiber from a mounting/prep dish to the experimental bath. The first is to take a small, circular glass coverslip and heat it up over a Bunsen burner or open flame such that the edges curl up and form a small dish (sometimes called a 'boat'). The 'boat' can then be filled with solution and used to transfer the fiber. The mounting technique then involves floating the boat near the hooks for the transducer/motor and tipping the ends onto the hooks.

The other method is to use a cut pipette tip to form a small trough. The fiber is then deposited into the bath and can be teased onto the hooks by holding the pipette tip nearby.

Q: Is it possible to isolate Baths 7 & 8 rather than 1 & 2 on the 802D plate?

[M. Borkowski] Yes, it is possible, but would require a custom-built plate and some other minor modifications.

Q: Did you try the staircase approach in order to obtain multiple force-control events within a single activation?

[T. West] We tried the staircase approach early on in our assays, using 3 consecutive force-controlled step-downs before switching back to length-control and re-lengthening the fibre back to its starting length. It works OK, but we found that the second and third force-control steps generated different force-velocity curve(s) to the one made by just fitting data from the first step-down is a series of activations. Our feeling was that the fibre was shortening too far, into the ascending side of a force-length curve. This is likely because at 25C (our assay temperature) the fibre shortening speed is quite fast (sometime > 10 muscle-lengths per s). At lower temperature (12 – 15 C) a staircasing approach is likely to suffer less from the issues we found at higher temperatures.

Q: How was skinning/freezing/transport co-ordinated?

[T. West] Of course, skinning biopsies, or small muscle bundles, in the lab is relatively straightforward. We skin by either ice-cold treatment of the fibre bundle in relaxing solution with 2% triton added (30 min on ice), or by taking the fibre bundles through a series of increasing glycerol concentrations (up to 50% made up in relaxing solution) over several hours. For our field studies, we used the triton-based approach to skin fibre bundles to save time. The researchers had premade solution sets with them in the field and they had a source of liquid nitrogen for freezing the samples; they could skin, cryoprotect, and freeze in the field. For transport back to the UK, up to thirty 0.5 ml cryotubes would fit into the storage compartment of a liquid-nitrogen dry shipper. Samples were taken from the dry shipper and placed directly into a -80C freezer in the UK.

Q: How can I speed up the measurements to be able to measure more fibres per day?

[T. West] Maximizing the number of fibres tested per day is one reason that we adopted the multiple force-control approach described in the webinar. If all went well, then we would need only one activation to determine fibre stiffness for the Aurora software. Then we would require 3 activations, each with 4 separate force-control events, to collect 12 data points for curve fitting (power-force or force-velocity) for each individual fibre. We would need perhaps 20 min per fibre. Reality was, however, that we generally worked toward a rate of 7 to 8 fibres per day. This included the hour or so needed in the morning to prepare fibres for mounting onto the Aurora apparatus. The temperature jump approach helps because the activation is quicker than if using calcium diffusion activate and de-activate fibres.

Q: What microscope do you use/would you recommend?

[T. West] We have very old Nikon Diaphot that has been stripped of its fluorescent optics. It has a large manual x-y stage with 10cm cut-away, which is essential accommodate the 802D bath changer. The Diaphot has a hinged condenser so we can move it out of the way and then position a boom-mounted stereoscope for mounting the fibres between the force transducer and motor hooks.

[M. Borkowski] The inverted microscope doesn't need to be anything fancy, but there are 4 key elements to look for:

1. Flat, 3 plate stage with a minimum 100mm circular cut out
2. Side mount camera port with a c-mount adaptor
3. Long working distance objectives (at least 3mm)
4. A tilting/moveable condenser arm is a plus

For more reading see the attached blog. https://aurorascientific.com/how-to-select-a-microscope-for-an-asi-muscle-physiology-system/

Q: Do you have any best practices to share on the subject of bath cleaning procedure?

[T. West] We use only de-ionised water to clean the baths between fibres or between solution changes. We have a mini suction pump set up to draw away solution. With practise, it is possible to feed water into one side of a bath and withdraw it from the opposite side; this way the chamber gets flooded with several volumes of water before loading up a new solution. A bead of vacuum grease, used to hold the bottom coverslip onto the 802D plate, is often unavoidable *inside* the 802D baths. It smears quite easily and once this can be no longer 'tolerated' it is time to replace the bottom coverslip; while doing this we take the opportunity clean the bath walls using dilute ethanol on a cotton swab. The prisms are cleaned with lens-paper soaked in ethanol and/or water.

Q: Is your success-rate improved with the x4 approach compared to the 1-event-per-activation approach?

[T. West] The success rate with the multiple force-control per activation procedure, as described in the webinar, does not match the success rate (98%) we achieved by making 6 – 8 activations and measuring only one force-step per activation. We 10 – 15 % rejection rates with the 4-steps per activation approach. However, the gains include (i) minimizing overall activation time per fibre, (ii) expanding the number of data points (to 12) for curve-fitting per fibre, and (iii) maximizing the overall number of fibres tested per day.

If you have additional questions for Aurora Scientific or Dr. Tim West regarding content from their webinar or wish to receive additional information about muscle experimentation please contact them by email.

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