Webinar Q&A Report:
Studying Smooth and Striated Muscle Function Using Advanced Tissue Bath Systems

1. What is, or how do you get– the most effective normalisation approach? How many steps are too many steps?

Aaron Stupica: This depends whether we're talking about rings or muscle strips. With rings, it's hard to have too many steps in the normalization approach, you just do not want to significantly over-stretch your tissue. With striated tissue or muscle strips, it can get over-stimulated if you're using a stimulator for your normalization. Part of that step is to find the minimum current or voltage that would evoke a response, so you have a good chance to lessen that likelihood.

Dr. Keshari Thakali: For our contractility experiments where we're using strips of tissue, we usually do two steps once we've figured out what our optimal length is for stretching, and that seems to work well.

2. How would you test the response of an aorta to pathogenic stress such as high blood pressure?

Dr. Keshari Thakali: The way I would set this up experiment is with two animals: one with high blood pressure (through whatever means that you choose induce it) and one that doesn't. Since the 750TOBS has four chambers, you can set it up to have one chamber with the tissue from an animal with high blood pressure and then one chamber with the tissue from an animal with normal blood pressure. Then if you were looking at contractility or endothelial function you could have a control for each pathogenic animal there.

3. How do you separate intact coronary arteries from the mouse heart?

Dr. Keshari Thakali: A Silastic dissection disc would be helpful to dissect off the mouse coronary arteries, but your best bet is to have good quality tools, like Dumont number five forceps, a fine pair of iris scissors, and very steady hands.

4. What is the role of KCl as an agent of precontraction? Is it essential? How should it be administered?

Dr. Keshari Thakali: KCl can be used to 'wake up' tissue and to assess viability, but if we want to look at endothelial function, we typically precontract our tissues. If there is not basal tone on the tissue, it's hard to see any relaxation. But it's not essential to use KCl – you can use any other contractile agent, either for assessing tissue viability or for precontraction and assessing endothelial function.

This also depends on what sort of receptors are present in your tissue. For example, I used to study venous physiology. Veins have a different complement of adrenergic receptors than arteries. Veins don't contract very well to phenylephrine, an α1-adrenergic agonist, but they do contract very well to norepinephrine. So it does depend on the tissue you're studying and some of the questions you're trying to answer.

Aaron Stupica: KCl is not essential. We recommend a high potassium PSS buffer for precontraction, but there are many others you can use. As far as how it should be administered – you can add it directly into the bath as long as you have the gas bubbling to mix up the bath and evenly distribute that agonist.

5. What is the greatest challenge in the use of coronary and cerebral artery using this system?

Aaron Stupica: Size is going to be the greatest challenge here. Each pin that would have to go through an artery is 200 microns, so you would need a vessel that's at least 400 microns, so these vessels might be too small for this system. The 620M Multi Myograph System might be more appropriate.

Dr. Keshari Thakali: Coronary and cerebral arteries in mice are very small in size, so careful dissection of these tissues without handling them too much is a challenge. In my experience with these tissues, the myograph or an isolated perfusion system are more useful in studying these vessels.

6. What is the maximum force we can measure with this system and can we use isotonic force transducers?

Aaron Stupica: The force transducers are isometric or isotonic and have a range of 0 – 1600 millinewtons (about 0 – 160 g). However, each type of transducer is programmable so you can narrow down the range that you're looking for, providing you with more sensitive data.

7. 10- and 20-mL volumes can be too high. Can we fill the chambers to half volume or less? Do you have alternative chambers for other volumes?

Aaron Stupica: We can make custom sized chambers, but with the 10, 20, and 50 mL chambers, that is the maximum you can put in, but using the software you can simply input any volume up to the max, as long as you've calibrated the system with the pressure source that you're using, hit enter and it will fill right up.

Dr. Keshari Thakali: Sometimes you have drugs that are expensive and you don't want to use 20 mL, for example. In this case you just want to make sure that your tissue is still completely immersed in the solution.

8. Can the system accommodate custom mounting clips for unique applications?

Aaron Stupica: DMT already has approximately 6 different mounting options for this system, but we can easily customize other mounting clips/supports for unique applications.

9. Can you use a non-DMT stimulator with the 750TOBS?

Aaron Stupica: Absolutely. There are multiple types of electrodes, but they can easily be connected to any stimulator with a connecting cable that will communicate with any standard stimulator.

10. Basal tissue stretch is required to achieve a good contractile force signal. Is there a way to minimize the direct stretch applied to tissues?

Aaron Stupica: This is a matter of finding the "correct" amount of basal tissue stretch or passive tension. You do not want to overstretch the tissue of course, but there is a plateau where you can get the maximum responses from tissues that are stretched to their proper tension. If you are anywhere in the range of this plateau, your tissue is perfectly healthy.

11. If the natural environment is approximately 21% oxygen, does the 95% O2 influence the results in a non-physiological manner?

Dr. Keshari Thakali: This is an excellent question since patients receiving oxygen can experience hyperoxic vasoconstriction. However, a group from the Netherlands observed that the mouse femoral artery and gracilis arteriole responded similarly to endothelium-dependent vasodilation (acetylcholine, arachidonic acid), endothelium-independent vasodilation (sodium nitroprusside), and vasoconstriction (norepinephrine, PGF2alpha) under normoxia and three levels of hyperoxia (https://doi.org/10.1371/journal.pone.0182637).

12. What advantages does this procedure have in comparison with aortic ring experiments?

Aaron Stupica: This system can perform either muscle strip or aortic ring studies. The advantage of this system is that the mounts for either type of tissue are very easy to swap out in a matter of minutes.

13. When conducting a cumulative response, you are changing the volume with each addition. Is this accounted for in each of your subsequent standard additions within a chamber?

Dr. Keshari Thakali: Based on the solubility of the chemical, we normally make drugs 1000x more concentrated than the final concentration we want in them chamber. So for a 20mL chamber, if we need to reach a final concentration of 10-5 M norepinephrine, we would add 20 uL of a 10-2 M norepinephrine to the chamber, minimizing the volume changes with each addition of drugs.

14. Can we normalise arteries on organ baths similar to myographs?

Aaron Stupica: Yes, this organ bath is a different, larger version of the myographs. You still need to normalize any artery or tissue strip that you are studying. There is a micropositioner for each channel where you can add your stretch and normalize.

15. How do you exclude the mechanical effect of PVAT in the experiment? Because tissue with PVAT is thick.

Dr. Keshari Thakali: There are a number of studies that suggest that PVAT does not actually mechanically inhibit vasoconstriction. For example, while Wistar rats fed a high fat diet had more peri-aortic fat mass than rats fed a chow diet, contraction of aorta with PVAT attached was greater in high fat diet-fed rats compared to aorta with PVAT attached from chow-fed rats. (https://www.nature.com/articles/hr201011)

16. Would this organ bath be good for undergrads, grad students, and post-docs?

Aaron Stupica: The majority of our systems sold are primarily used by post-docs and professors. However, this system is very durable and parts are very easily replaced if anything is blocked or broken. The system would not need to be sent in for repair if something were to break. We have some labs full of these and single-channel versions of this system in Australia dedicated to undergraduate studies due to their durability for researchers unfamiliar with this type of research.

If you have additional questions for DMT regarding content from this webinar or wish to learn more about their hardware or software for *ex vivo* studies in striated and non-striated muscles, click here to send an inquiry or contact them by phone:

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