jove

One Tissue, Many Models: Comprehensive Toolkit For Skeletal Muscle Studies

Matthew J. Borok[1], **Despoina Mademtzoglou**[1]

[1] Univ Paris Est Creteil, INSERM, IMRB, F-94010 Creteil

Corresponding Author

Despoina Mademtzoglou
despoina.mademtzoglou@pasteur.fr

Citation

Borok, M.J.,Mademtzoglou, D. One Tissue, Many Models: Comprehensive Toolkit For Skeletal Muscle Studies. *J. Vis. Exp.* (197), e64965, doi:10.3791/64965 (2023).

Date Published

July 14, 2023

DOI

10.3791/64965

URL

jove.com/video/64965

Editorial

Covering ~40% of the human body, skeletal muscle is a major organ, supporting critical functions from movement to respiration and metabolism. Importantly, it has a unique capacity to self-repair after injury and with this regenerative capacity ensures complete functional and structural recovery from serious damage in less than a month. Several cell types have been implicated in this remarkable repair capacity, with the muscle stem cells having a central role.

Muscle researchers around the world have developed several assays to analyze skeletal muscle on the functional, molecular, and cellular levels. Each technique has its own strengths, with *in vivo* testing on animal models representing the option with the most relevance to humans and the complexity of living organisms. The *in vitro* and *ex vivo* counterparts are gaining ground as research groups worldwide are striving to minimize animal research in view of the internationally recognized Replacement-Reduction-Refinement (3R) principle. This Methods Collection aimed to provide *in vivo* and *ex vivo* models for molecular, cellular, and functional muscle studies across species.

Montandon et al.[1] describe techniques to rapidly characterize muscle regeneration phenotypes in zebrafish of control and mutant genotypes. First, embryos are genotyped using material that is shaken off of anesthetized animals. This is an exciting prospect as most protocols involving the genotyping of genetically modified embryos require a simultaneous sacrifice of the embryo. Next, regeneration is induced by stabbing a specific region of the zebrafish larva musculature. Thanks to the optical properties of the sarcomere, the integrity of the injured muscle can be assessed using imaging post-injury. This protocol is of great interest to scientists studying muscle regeneration as it is a rapid, potentially high-throughput method to investigate different mutants for regenerative defects.

Hüttner et al.[2] and Pegoli et al.[3] present a method that allows cellular and molecular studies of muscle stem cells, the cell population at the core of muscle regeneration. The method is highly interesting to researchers focusing on early activation studies and cell-niche interactions. The method of single myofiber cultures allows culturing and studying muscle stem cells up to 96 h while they are still associated with the muscle fibers, receiving signals from this proximate, endogenous, niche. The technique is particularly challenging to establish

due to the many delicate steps that Hüttner et al.[2] carefully explain (e.g., gentle muscle dissection and dissociation). Going one step further, the authors detail siRNA targeting for functional analyses of factors that can influence stem cell behavior, and they underline the importance of comparing siRNA-transfected to control-transfected cells from the same mouse and muscle. In parallel, Pegoli et al.[3] describe how this method can be adjusted to study muscle stem cells in the context of Emery-Dreifuss muscular dystrophy and early postnatal life, focusing on the challenges linked to muscle fragility in this context. To increase protocol efficiency, they guide readers with images of fibers with good and bad survival and they highlight the need to "empirically validate" digestion time.

Zaidan et al.[4] describe an *ex vivo* culture system of the skeletal muscle that favors the production of quiescent-like reserve cells, while also maintaining a number of other cell types, both presenting challenges under alternative culture systems. Thus, this method can be useful for researchers oriented in quiescence studies or niche-stem cell interactions, including under conditions that modify molecular pathways using drugs, siRNAs, or vectors. The protocol entails rapid dissection of the hindlimb muscles, followed by enzymatic digestion and filtration. Afterward, cells are placed in culture, with the appearance of reserve cells within one week. The protocol allows large-scale amplification of these reserve cells, with the possibility of live cell imaging using genetically tagged mice, such as *Pax7*^{nGFP}.

Park et al.[5] demonstrate how nitrate and nitrite can be measured in skeletal muscle, one of the major nitrate storage organs. The described method is of particular interest to researchers studying exercise physiology and nitric-oxide-cycle-related neuromuscular or metabolic disorders. Even though there is significant technological development to facilitate nitrate and nitrite measurements, there are several parameters that can affect the final result and are carefully described and compared by the authors (*e.g.*, homogenization method, biopsy size, muscle type).

Hessel et al.[6] describe in great detail a procedure for taking biopsies from the *Tibialis anterior* muscle of human patients in order to obtain fibers of sufficient length for mechanical experiments. The authors emphasize that patients with recent damage to the muscle should be excluded. Once informed consent of eligible patients is taken, ultrasound is used to assess the best site in the muscle for biopsy collection. After discussing the ideal injection route, the team performs the biopsy using a specialized needle. Finally, post-operative patient care and tissue processing methods are detailed. This method is of particular interest to physician-scientists, who want to standardize tissue collection from patients for mechanical studies of muscle fibers.

In this Methods Collection, mechanical studies are presented by Brightwell et al.[7] and Corona et al.[8]. These articles discuss methods to measure strength in small and large animal models. Neuromuscular disease or injury negatively affects muscle strength; thus, exercise, nutrition, and therapeutic interventions aim to restore it. Brightwell et al.[7] detail a method to evaluate quadriceps muscle function in mice in an analogous way to methods used in human patients. First, they provide steps to prepare the equipment and software required for the experiment. Next, the mouse is anesthetized and put in position for the extensor function to be evaluated. Electrodes are placed in the distal part of the quadriceps for induction of contraction, an optimal current is assessed, and measurements of peak isometric tetanic torque are recorded and analyzed. Using a larger animal model, Corona et al.[8]

assess isometric torque in pig hindlimb dorsiflexor muscle, showing the step-by-step equipment settings and strength measurement protocol. Of note, they show high- and low-quality wavefronts to ensure optimal result acquisition. They recognize the strengths of this non-invasive technique that allows longitudinal analyses of the same animal, but they also discuss in length the critical steps (*e.g.*, nerve stimulation, mechanical alignment) and method limitations.

There are still many outstanding questions in the field of skeletal muscle research: which genes are crucial for muscle regeneration, how satellite cells maintain regenerative potential over the lifetime of so many different organisms, and how is muscle function affected in different disease conditions. This collection showcases a number of modern techniques to investigate muscle function and repair on molecular and mechanical levels, using a wide range of model systems. By providing detailed protocols, the authors of these papers have already helped to increase consistency and transparency for basic and applied skeletal muscle research.

Disclosures

The authors have nothing to disclose.

Acknowledgments

No external funding supported this work.

References

1. Montandon, M., Currie, P. D., Ruparelia, A. A. Examining muscle regeneration in zebrafish models of muscle disease. *Journal of Visualized Experiments.* (167), e62071 (2021).
2. Hüttner, S. S. et al. Single myofiber culture assay for the assessment of adult muscle stem cell functionality ex vivo. *Journal of Visualized Experiments.* (168), e62257 (2021).
3. Pegoli, G., Lucini, F., Mozzetta, C., Lanzuolo, C. Single myofiber isolation and culture from a murine model of Emery-Dreifuss muscular dystrophy in early post-natal development. *Journal of Visualized Experiments.* (161), e61516 (2020).
4. Zaidan, L. et al. Unfractionated bulk culture of mouse skeletal muscle to recapitulate niche and stem cell quiescence. *Journal of Visualized Experiments.* (196), e65433 (2023).
5. Park, J. W., et al. Preparation of rat skeletal muscle homogenates for nitrate and nitrite measurements. *Journal of Visualized Experiments.* (173), e62427 (2021).
6. Hessel, A. L., Hahn, D., de Marées, M. Collection of skeletal muscle biopsies from the superior compartment of human musculus tibialis anterior for mechanical evaluation. *Journal of Visualized Experiments.* (163), e61598 (2020).
7. Brightwell, C. R. et al. In vivo measurement of knee extensor muscle function in mice. *Journal of Visualized Experiments.* (169), e62211 (2021).
8. Corona, B. T., Call, J. A., Borkowski, M., Greising, S. M. In vivo measurement of hindlimb dorsiflexor isometric torque from pig. *Journal of Visualized Experiments.* (175), e62905 (2021).