

Submission ID #: 70637

Scriptwriter Name: Deepthi Das

Project Page Link: https://review.jove.com/files_upload.php?src=21544333

Title: Single Fiber Isolation Assay for the Assessment of Oxidative Myofiber Behavior

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

If **Yes**, can you record movies/images using your own microscope camera? **No**

SCOPE shots: 3.4.3, 3.5.1, 3.5.2, 3.5.3, 3.6.1, 3.7.2, 4.3.2, 4.3.4, 4.4.1, 4.4.3

Videographer: Please film the abovementioned shots using a scope kit

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 18

Number of Shots: 47

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Teng Hu:** This research focuses on the isolation of intact single muscle fibers from the oxidative soleus muscle.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Pedro:** Existing single-fiber isolation methods inadequately preserve fragile, long soleus fibers; this optimized protocol overcomes damage, enabling recovery of intact fibers.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Pedro:** This protocol enables high-resolution analysis of molecular behavior within single fibers while yielding a rich, viable satellite cell population.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Teng Hu:** Preserving soleus structural integrity during dissection and precisely optimizing type I collagenase digestion are critical to preventing fiber damage loss.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Teng Hu:** Future studies can utilize enriched population of soleus fibers for comprehensive analyses of molecular mechanisms underlying muscle behavior.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

1.6 Teng Hu, Project Assistant Professor and Post doctoral fellow, Tokyo Metropolitan University: (authors will present their testimonial statements live)

1.6.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What value do you expect this video article to have for other researchers working in your field?

1.7 Teng Hu, Project Assistant Professor and Post doctoral fellow, Tokyo Metropolitan University: (authors will present their testimonial statements live)

1.7.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research strictly adhered to the guidelines of the Safety and Ethics Committee of Tokyo Metropolitan University.

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Mouse Hindlimb Dissection and Soleus Isolation

Demonstrator: Teng Hu

- 2.1. To begin, lay out the equipment required for muscle dissection [1]. Use the preprepared triple anesthetic mixture [2] to sedate the C57BL/6 (C-57-black-6) mouse by intraperitoneal injection [3-TXT].
 - 2.1.1. WIDE: Show the equipment setup and the talent arranging dissection tools neatly on the work surface.
 - 2.1.2. TEXT ON A PLAIN BACKGROUND:
Triple Anesthetic Mixture Composition:
1 mg/mL - Medetomidine hydrochloride
5 mg/mL - Midazolam
5 mg/mL - Butorphanol tartrate
Dose: 0.5 mL per g body weight
 - 2.1.3. Talent administering intraperitoneal injection to the mouse.
TXT: Euthanize by cervical dislocation
- 2.2. Make sequential skin incisions on the hindlimb [1]. Peel away the skin to expose underlying tissue [2]. Remove the surface fascia [3].
 - 2.2.1. Talent making incisions on the hindlimb skin.
 - 2.2.2. Talent peeling back the skin.
 - 2.2.3. Talent removing fascia from the exposed muscle.
- 2.3. Separate the plantaris tendon from the gastrocnemius and soleus tendon bundles [1]. Gently remove the plantaris by pulling from its tendon [2].
 - 2.3.1. Talent isolating the plantaris tendon from adjacent tendons.
 - 2.3.2. Talent pulling the plantaris muscle away from its tendon.
- 2.4. Cut the gastrocnemius and soleus tendon bundles to expose the muscles [1]. Insert a pin into the gap between the proximal tendon of the gastrocnemius and soleus [2].

- 2.4.1. Talent cutting the tendon bundles.
- 2.4.2. Talent inserting a needle between the proximal tendon of the gastrocnemius and soleus.
- 2.5. Carefully slide this needle toward the distal end to separate the soleus and gastrocnemius muscles [1]. Now insert a second needle into proximal tendon of the gastrocnemius and soleus [2]. Cut the tendon bundles from the gastrocnemius side [3].
 - 2.5.1. Talent sliding the needle between the muscles to separate the soleus and gastrocnemius muscles.
 - 2.5.2. Talent inserting a second needle into proximal tendon of the gastrocnemius and soleus.
 - 2.5.3. Talent cutting tendon bundles on the gastrocnemius side.
- 2.6. Rest the soleus on the gastrocnemius [1]. Adjust the position of the soleus to expose the proximal tendon [2]. Cut the proximal tendon with micro scissors [3].
 - 2.6.1. Talent placing the soleus muscle onto the gastrocnemius.
 - 2.6.2. Talent repositioning the soleus to expose the tendon.
 - 2.6.3. Talent cutting the proximal tendon with micro scissors.
- 2.7. Position the soleus on the dissection table [1]. Remove the remaining tendon [2].
 - 2.7.1. Talent placing the soleus on the table.
 - 2.7.2. Talent removing residual tendon from the soleus.

3. Isolation and Purification of Single Soleus Muscle Fibers

- 3.1. Transfer the intact soleus into a 25-milliliter centrifuge tube containing 0.5 percent collagenase solution [1]. Incubate at 37 degrees Celsius for 130 minutes [2].
 - 3.1.1. Talent placing the soleus into the centrifuge tube with solution.
 - 3.1.2. Talent placing the tube into the incubator.
- 3.2. Cut two Pasteur pipettes to create one with a wide bore [1] and one with a narrow opening [2]. Sterilize and smooth the openings under flame to prevent muscle damage during trituration [3].
 - 3.2.1. Talent cutting a Pasteur pipette to create one with a wide bore.
 - 3.2.2. Talent cutting a Pasteur pipette to create one with a narrow opening
 - 3.2.3. Talent flaming the openings of both the pipettes.

3.3. Use serum solution containing 5 percent BSA (*B-S-A*) in PBS (*P-B-S*) to coat the Pasteur pipettes [1] and three 50-millimeter Petri dishes to prevent fiber adhesion [2].

3.3.1. Talent coating pipette with serum solution.

3.3.2. Talent coating Petri dish with serum solution.

3.4. After digestion, discard the excess collagenase solution [1]. Transfer the soleus into a dish containing DMEM (*D-M-E-M*) with 1 percent antibiotic-antimycotic [2]. Use the wide-bore Pasteur pipette to gently pipette up and down to dissociate fibers [3].

3.4.1. Talent removing collagenase solution from the tube.

3.4.2. Talent transferring the muscle into a Petri dish with medium.

3.4.3. SCOPE: Talent pipetting the muscle tissue gently with a wide-bore pipette.

3.5. Use the narrow-bore Pasteur pipette to transfer intact single fibers along their length, to a second dish [1]. Remove debris and shrunken fibers during transfer [2]. Now, transfer high-quality intact single fibers to a third dish using the narrow-bore Pasteur pipette [3].

3.5.1. SCOPE: Talent transferring intact fibers with a narrow-bore pipette.

3.5.2. SCOPE: Talent discarding debris and damaged fibers.

3.5.3. SCOPE: Talent transferring selected fibers into a clean dish.

3.6. Collect the fibers into a BSA-coated 25-milliliter centrifuge tube [1]. Gently stir the suspension and leave it at room temperature for 5 minutes to allow fibers to precipitate [2].

3.6.1. SCOPE: Talent transferring fibers into the centrifuge tube.

3.6.2. Talent stirring the suspension gently and placing the tube on the bench for settling.

3.7. Use a narrow-bore Pasteur pipette to discard the supernatant, leaving approximately 500 microliters of the medium [1]. Retain the precipitate containing purified single muscle fibers [2].

3.7.1. Talent removing supernatant with a pipette.

3.7.2. SCOPE: Shot of remaining fiber pellet in the tube.

4. Establishment of highly purified myoblast cultures from soleus fibers

4.1. Dilute Matrigel Matrix to a concentration of 0.1 milligrams per milliliter in DMEM [1]. Use it to coat a 100-millimeter culture dish [2]. Incubate the dish at 37 degrees Celsius

for 30 to 60 minutes or until polymerization is complete [3].

- 4.1.1. Talent diluting the Matrigel Matrix in DMEM.
- 4.1.2. Talent coating the culture dish with Matrigel.
- 4.1.3. Talent placing the dish into the incubator.

4.2. Add around 500 microliters of Accutase to the previously prepared muscle fiber suspension and incubate at room temperature for 10 minutes [1]. Then, add 10 milliliters of growth medium containing glucose-free DMEM with supplements [2].

- 4.2.1. Talent adding Accutase to the tube and leaving it undisturbed.
- 4.2.2. Talent adding growth medium to the suspension.

4.3. Transfer 10 milliliters of the Accutase-treated muscle fiber suspension into the Matrigel-coated dish [1]. Add approximately 4 single fibers per square centimeter to the 100-millimeter culture dish [2]. Transfer the fibers evenly throughout the culture dish [3]. Use a microscope to check the even distribution of the fibers [4].

- 4.3.1. Talent pipetting the muscle fiber suspension into the coated culture dish.
- 4.3.2. SCOPE: Show the talent distributing fibers the dish at defined density.
- 4.3.3. Talent gently moving the dish to evenly spread the fibers.
- 4.3.4. SCOPE: Shot of the fibers evenly spread out in the dish.

4.4. Observe myoblast colonies after 3 days in culture [1]. Replace the growth medium daily until the cells reach confluence [2]. Continue culture until confluence is achieved by day 6 [3].

- 4.4.1. SCOPE: Shot of myoblast colonies forming in the dish.
- 4.4.2. Talent replacing culture medium in the dish.
- 4.4.3. SCOPE: Shot of confluent myoblast culture.

Results

5. Results

- 5.1. After 6 days in proliferation medium, isolated soleus fibers yielded a pure myoblast culture [1].
 - 5.1.1. LAB MEDIA: Figure 3A. *Video editor: Show the full image, then emphasize the central area to highlight the dense, uniform layer of closely packed dark cell shapes forming a continuous sheet.*
- 5.2. Soleus-derived single fibers [1] and extensor digitorum longus-derived single fibers, immediately after isolation, showed satellite cells immunolabelled for paired box 7 [2].
 - 5.2.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the row labelled "SOL myofiber" at "0 day" and emphasize the green signals.*
 - 5.2.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the row labelled "EDL myofiber" at "0 day" and emphasize the green signals.*
- 5.3. Soleus-derived single fibers [1] and extensor digitorum longus-derived single fibers cultured for 3 days in floating culture medium, satellite cells immunolabelled for paired box 7 [2].
 - 5.3.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the row labelled "SOL myofiber" at "3 days" and emphasize the green signals.*
 - 5.3.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the row labelled "EDL myofiber" at "3 days" and emphasize the green signals.*

Pronunciation guides:

1. Medetomidine
 Pronunciation link: <https://www.merriam-webster.com/dictionary/medetomidine>
 IPA: /məˈdɛtəˌmɪdiːn/
 Phonetic Spelling: muh·deh·tuh·mih·deen
2. Midazolam
 Pronunciation link: <https://www.merriam-webster.com/dictionary/midazolam>
 IPA: /mɪˈdæzəˌləm/
 Phonetic Spelling: mih·daz·uh·lam

3. Butorphanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/butorphanol>
IPA: /bjuːˈtɔːrfəˌnɔːl/
Phonetic Spelling: byoo·tor·fuh·nol
4. Intraperitoneal
Pronunciation link: <https://www.merriam-webster.com/dictionary/intraperitoneal>
IPA: /ˌɪntrəˌpɛrɪtəˈniːəl/
Phonetic Spelling: in·truh·peh·ruh·tuh·nee·uhl
5. Fascia
Pronunciation link: <https://www.merriam-webster.com/dictionary/fascia>
IPA: /ˈfæʃiə/
Phonetic Spelling: fash·ee·uh
6. Plantaris
Pronunciation link: <https://www.merriam-webster.com/dictionary/plantaris>
IPA: /plænˈtɛrɪs/
Phonetic Spelling: plan·teh·ruhs
7. Gastrocnemius
Pronunciation link: <https://www.merriam-webster.com/dictionary/gastrocnemius>
IPA: /ˌɡæstrəˈkniːmiəs/
Phonetic Spelling: gas·trok·nee·mee·uhs
8. Soleus
Pronunciation link: <https://www.merriam-webster.com/dictionary/soleus>
IPA: /ˈsoʊliəs/
Phonetic Spelling: soh·lee·uhs
9. Proximal
Pronunciation link: <https://www.merriam-webster.com/dictionary/proximal>
IPA: /ˈprɒːksɪməl/
Phonetic Spelling: prok·suh·muhl
10. Distal
Pronunciation link: <https://www.merriam-webster.com/dictionary/distal>
IPA: /ˈdɪstəl/
Phonetic Spelling: dis·tuhl
11. Collagenase
Pronunciation link: <https://www.merriam-webster.com/dictionary/collagenase>
IPA: /kəˈlædʒəˌneɪs/
Phonetic Spelling: kuh·la·juh·nays
12. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsentrɪˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
13. Trituration
Pronunciation link: <https://www.merriam-webster.com/dictionary/trituration>

IPA: /ˌtrɪtʃəˈreɪʃən/

Phonetic Spelling: tri·chuh·ray·shuhn

14. Serum

Pronunciation link: <https://www.merriam-webster.com/dictionary/serum>

IPA: /ˈsɪrəm/

Phonetic Spelling: seer·uhm

15. Adhesion

Pronunciation link: <https://www.merriam-webster.com/dictionary/adhesion>

IPA: /ədˈhiːʒən/

Phonetic Spelling: uh·hee·zhuhn

16. Antibiotic-antimycotic

Pronunciation link: No confirmed link found

IPA: /ˌæntaɪbaɪˈɑːtɪk ˌæntaɪmaɪˈkɑːtɪk/

Phonetic Spelling: an·tie·bye·aa·tik an·tie·my·kaa·tik

17. Supernatant

Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>

IPA: /ˌsuːpərˈnextənt/

Phonetic Spelling: soo·per·nay·tuhnt

18. Precipitate

Pronunciation link: <https://www.merriam-webster.com/dictionary/precipitate>

IPA: /prɪˈsɪpəˌteɪt/

Phonetic Spelling: pruh·sip·uh·tayt

19. Matrigel

Pronunciation link: No confirmed link found

IPA: /ˈmeɪtrɪˌdʒel/

Phonetic Spelling: may·trih·jel

20. Polymerization

Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerization>

IPA: /pəˌlɪməˈreɪʃən/

Phonetic Spelling: puh·lih·muh·ruh·zay·shuhn

21. Accutase

Pronunciation link: No confirmed link found

IPA: /ˈækjʊˌteɪs/

Phonetic Spelling: ak·yoo·tays

22. Myoblast

Pronunciation link: <https://www.merriam-webster.com/dictionary/myoblast>

IPA: /ˈmaɪəˌblæst/

Phonetic Spelling: my·uh·blast

23. Confluence

Pronunciation link: <https://www.merriam-webster.com/dictionary/confluence>

IPA: /ˈkɒnfluəns/

Phonetic Spelling: kon·floo·uhns

24. Immunolabelled

Pronunciation link: No confirmed link found

IPA: /ɪ,mjuːnoʊˈleɪbəld/

Phonetic Spelling: ih·myoo·noh·lay·buhld

25. Extensor

Pronunciation link: <https://www.merriam-webster.com/dictionary/extensor>

IPA: /ɪkˈstɛnsər/

Phonetic Spelling: ik·sten·ser

26. Digitorum

Pronunciation link: <https://www.merriam-webster.com/dictionary/digitorum>

IPA: /ˌdɪdʒɪˈtɔːrəm/

Phonetic Spelling: dih·jih·tor·uhm

27. Satellite

Pronunciation link: <https://www.merriam-webster.com/dictionary/satellite>

IPA: /ˈsætəˌlaɪt/

Phonetic Spelling: sa·tuh·lyte