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Title: Isolation and Culture of Single Myofibers from *Macaca fascicularis* Extensor Digitorum Longus Muscle

Authors and Affiliations:

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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

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

Scope shots: 4.1.1, 4.2.1, 4.3.1, 4.4.1

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. **No**

Current Protocol Length

Number of Steps: 17

Number of Shots: 34

Interviews

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. **Yuguo Liu:** Our research focuses on isolating and culturing a single myofiber from the monkey EDL 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. **Yuguo Liu:** Most existing protocols were performed in rodents. This is the first protocol to isolate a single myofiber from non-human primates.
 - 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. **Yuguo Liu:** This protocol provides a unique in vitro model for studying non-human primate MuSC behaviors within the niche.
 - 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. **Yuguo Liu:** The most important consideration is careful myofiber dissociation to avoid damaging the live myofibers.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Yuguo Liu:** Additionally, this system provides a prime platform for mechanistic studies, siRNA transfection, and drug screening.
 - 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.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Conghua Huazhen animal center

Protocol

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

2. Preparation of HS-Coated Cultureware and Glass Pipettes

Demonstrator: Yuguo Liu

- 2.1. To begin, obtain the required medium and the isolated hindlimb extensor digitorum longus, or EDL (*E-D-L*), muscle from the euthanized *Macaca fascicularis* [1-TXT].
 - 2.1.1. Talent arranging the isolated extensor digitorum longus muscle and the reagents on the laboratory bench. **TEXT: Euthanasia: IV Pentobarbital Sodium (100 mg/kg BW)**
- 2.2. In a biosafety cabinet, using a 10-milliliter pipette, add 5 milliliters of horse serum, or HS (*H-S*), into a 150-milliliter dish [1]. Swirl the dish to coat the bottom evenly, then let it stand for 5 minutes [2].
 - 2.2.1. Talent adding 5 milliliters of horse serum into a 150-milliliter dish with a 10-milliliter pipette. **Videographers NOTE: 2.2.1 and 2.2.2 were combined in one shot.**
 - 2.2.2. Talent swirling the dish to evenly coat the bottom and inner wall with horse serum and leaving it undisturbed.
- 2.3. Using a 10-milliliter pipette, transfer all the HS into another 150-milliliter dish [1] and repeat 2 more times to prepare 4 coated dishes [2].
 - 2.3.1. Talent transferring all the horse serum from one 150-milliliter dish into another 150-milliliter dish with a 10-milliliter pipette.
 - 2.3.2. Show 4 prepared coated dishes.
- 2.4. Collect the remaining HS into a 50-milliliter tube [1]. Cap the tube tightly and seal it with parafilm for later use [2].
 - 2.4.1. Talent transferring the remaining horse serum into a 50-milliliter tube. **Videographers NOTE: 2.4.1 and 2.4.2 were combined in one shot.**
 - 2.4.2. Talent capping the tube tightly and sealing it with parafilm.
- 2.5. Coat a 100-millimeter dish with 3 milliliters of HS [1] and each well of a 6-well plate with 1 milliliter of HS as previously demonstrated [2]. Collect the remaining HS into a new 50 milliliter tube [3]. Cap the tube tightly and seal it with parafilm for later use [4].
 - 2.5.1. Talent adding 3 milliliters of horse serum into a 100-millimeter dish and swirling the dish to evenly coat the bottom and inner wall with horse serum, and leaving

- it undisturbed. Videographers NOTE: 2.5.1 to 2.5.4 were combined in one shot.
- 2.5.2. Talent coating each well of a 6-well plate with 1 milliliter of horse serum by swirling the plate to evenly coat the bottom and inner wall of each well with horse serum and leaving it undisturbed.
 - 2.5.3. Talent transferring the remaining horse serum into a new 50 milliliter tube.
 - 2.5.4. Talent capping the tube tightly and sealing it with parafilm.
 - 2.6. Dry all the coated dishes for 5 minutes without lids [1]. Cover the dishes and the 6-well plate with parafilm [2] and store at 4 degrees Celsius [3].
 - 2.6.1. Show all the coated dishes and the 6-well plate without lids.
 - 2.6.2. Talent covering the dishes and the 6-well plate with parafilm.
 - 2.6.3. Talent placing the wrapped dishes and the 6-well plate in the refrigerator.
 - 2.7. Prepare a large-bore glass pipette by cutting a 10-milliliter glass pipette to approximately 4 millimeters in bore diameter [1]. Prepare a small-bore glass pipette by cutting a 10-milliliter glass pipette to an approximately 1-millimeter bore diameter [2]. Sterilize and round the edges of both glass pipettes with a flame, then allow them to cool to room temperature [3].
 - 2.7.1. Talent cutting a 10-milliliter glass pipette to prepare a large-bored pipette with an approximately 4 millimeter bore diameter. Videographers NOTE: 2.7.1 and 2.7.2 were combined in one shot.
 - 2.7.2. Talent cutting another 10-milliliter glass pipette to prepare a small-bored pipette with an approximately 1 millimeter bore diameter.
 - 2.7.3. In a biosafety cabinet, talent rounding the cut ends of both glass pipettes, through a flame sterile the pipette body parts through a flame and keeping them aside.
 - 2.8. Gently pipette the previously collected HS through each glass pipette 3 times to coat it [1]. Allow the coated glass pipettes to dry for at least 5 minutes [2].
 - 2.8.1. Talent pipetting the horse serum through each glass pipette 3 times to coat the inner surface. Videographers NOTE: 2.8.1 and 2.8.2 were combined in one shot.
 - 2.8.2. Talent placing the coated glass pipettes on the work surface to dry for at least 5 minutes.

3. Enzymatic Digestion of the EDL Muscle

- 3.1. Hold the distal tendon [1]. Transfer the EDL muscle into a 50-milliliter centrifuge tube containing 40 milliliters of 37 degrees Celsius pre-warmed muscle digestion medium [2]. NOTE: VO is also edited for inverted shots.
 - 3.1.2. Talent holding the distal tendon. Videographers NOTE: 3.1.2 and 3.1.1 were combined in one shot.

- 3.1.1. Talent transferring the EDL muscle into a 50-milliliter centrifuge tube containing muscle digestion medium. **Author's NOTE: 3.1.2 was placed before 3.1.1**
- 3.2. Cap the tube tightly and seal it with parafilm [1]. Fix the tube to a bracket [2] and immerse it horizontally in a water bath at 37 degrees Celsius [3].
 - 3.2.1. Talent capping the 50-milliliter centrifuge tube tightly and sealing it with parafilm.
 - 3.2.2. Talent securing the tube in a bracket.
 - 3.2.3. Talent placing the tube horizontally in a water bath.
- 3.3. Incubate for 6 hours, gently inverting the tube 3 times every 2 hours [1]. ~~Stop the digestion once the muscle begins to loosen and a small number of free single myofibers start to emerge [2].~~ **NOTE: For the removed shot, VO is added in the concise format in the next step.**
 - 3.3.1. Talent taking the tube out of the water bath and inverting it.
 - ~~3.3.2. Shot of the digested muscle beginning to loosen with a small number of free single myofibers emerging.~~ **Author's NOTE: Remove it.**
- 3.4. After stopping the digestion, using the HS-coated large-bored glass pipette, transfer the digested muscle into the first washing dish containing 20 milliliters of pre-warmed fiber washing medium, or FWM (*F-W-M*) [1].
 - 3.4.1. Talent transferring the digested muscle with the horse serum-coated large-bored glass pipette into the first washing dish containing 20 milliliters of pre-warmed fiber washing medium.

4. Myofiber Dissociation and Suspension Culture

- 4.1. Under a stereomicroscope, with the large-bored glass pipette, slowly flush the muscle in the first dish with warm FWM to release the desired number of single muscle fibers [1].
 - 4.1.1. **SCOPE:** View of the muscle being slowly flushed in the dish with warm fiber washing medium using the large-bored glass pipette.
- 4.2. Using the small-bored glass pipette, transfer the intact single myofibers one by one into the second washing dish, avoiding the transfer of debris and damage to the myofibers [1].
 - 4.2.1. **SCOPE:** Shot of transferring an intact single myofiber into the second washing dish with the small-bored glass pipette while debris remains behind.
- 4.3. Repeat the wash 2 more times and retain the intact living myofibers in the fourth washing dish [1].
 - 4.3.1. **SCOPE:** View of intact living myofibers in the fourth washing dish.

- 4.4. Using the small-bored glass pipette, transfer the washed myofibers one by one into a 100-millimeter HS-coated dish containing 10 milliliters of pre-warmed fiber culture medium [1]. Transfer the dish into a humidified carbon dioxide incubator and culture the myofibers in suspension for 72 hours at 37 degrees Celsius with 5 percent carbon dioxide [2].
 - 4.4.1. **SCOPE**: Talent transferring washed myofibers one by one into the 100-millimeter horse serum-coated dish containing pre-warmed fiber culture medium.
 - 4.4.2. Talent placing the culture dish into a humidified carbon dioxide incubator for suspension culture.
- 4.5. After completing the staining procedure, mount the myofibers onto an adhesion microscope slide, and image the sample [1].
 - 4.5.1. Talent placing the mounted slide on the microscope stage and adjusting it for imaging.

Results

5. Results

- 5.1. Live myofibers were long and straight, with a uniform diameter across the whole fiber [1], while dead or dying fibers appeared short, contracted, and swollen in certain regions [2].
 - 5.1.1. LAB MEDIA: Figure 3A. *Video editor: Highlight all the black arrows and the white lines it indicates.*
 - 5.1.2. LAB MEDIA: Figure 3A. *Video editor: Highlight all the blue arrows and the white lines it indicates.*
- 5.2. Clean living myofibers were obtained, with most debris removed [1].
 - 5.2.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the long, thin myofiber indicated by the black arrowheads.*
- 5.3. Sarcomere repeats appeared as striations on the surface of isolated myofibers during bright-field live myofiber imaging at higher magnification [1].
 - 5.3.1. LAB MEDIA: Figure 3C.
- 5.4. Only 2 activated muscle stem cells, or MuSCs (*M-U-S-C-S*), were observed on the 72-hour cultured myofibers, with an average of 2 Pax7-positive (*pax-seven-positive*) MuSCs detected per myofiber [1].
 - 5.4.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the two touching oval-shaped cells inside the box. One cell appears red and green, while the other appears mainly red.*
- 5.5. After 72 hours of in vitro culturing, only 1 MuSC entered the cell cycle, forming a PAX7- and MYOD-positive cell doublet on the myofibers [1].
 - 5.5.1. LAB MEDIA: Figure 4B.

Pronunciation guides:

1. Macaca fascicularis
 Pronunciation link: <https://www.merriam-webster.com/dictionary/Macaca>
 IPA: /məˈkɑːkə fəˌsɪkjəˈleɪs/
 Phonetic Spelling: muh·kaa·kuh fuh·sik·yuh·lair·iss
2. Euthanasia
 Pronunciation link: <https://www.merriam-webster.com/dictionary/euthanasia>

IPA: /ˌjuː.θəˈneɪ.ʒə/, /ˌjuː.θəˈneɪ.zi.ə/

Phonetic Spelling: yoo-thuh-nay-zuh; yoo-thuh-nay-zee-uh

3. Pentobarbital

Pronunciation link: <https://www.merriam-webster.com/medical/pentobarbital>

IPA: /ˌpen.təʊˈbɑːr.bɪ.tɑːl/

Phonetic Spelling: pen-toh-bar-bih-taal

4. Extensor

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

IPA: /ɪkˈsten.sə/

Phonetic Spelling: ik-sten-ser

5. Digitorum

Pronunciation link: <https://www.howtopronounce.com/digitorum>

IPA: /ˌdɪdʒɪˈtɔːrəm/

Phonetic Spelling: dij-ih-tor-uhm

6. Biosafety

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

IPA: /ˌbaɪ.əʊˈseɪf.ti/

Phonetic Spelling: bye-oh-sayf-tee

7. Pipette

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

IPA: /paɪˈpet/

Phonetic Spelling: pie-pet

8. Reagents

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

IPA: /riˈeɪ.dʒənt/

Phonetic Spelling: ree-ay-juhnt

9. Parafilm

Pronunciation link: <https://www.howtopronounce.com/parafilm>

IPA: /ˈpærəfɪlm/

Phonetic Spelling: pair-uh-film

10. Sterilize

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

IPA: /ˈster.ə.laɪz/

Phonetic Spelling: stair-uh-lyze

11. Enzymatic

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

IPA: /ˌen.zaɪˈmæt.ɪk/

Phonetic Spelling: en-zye-mat-ik

12. Digestion

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

IPA: /daɪˈdʒes.tʃən/

Phonetic Spelling: dye·jes·chuhn

13. Distal

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

IPA: /ˈdɪs.təl/

Phonetic Spelling: dis·tuhl

14. Tendon

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

IPA: /ˈten.dən/

Phonetic Spelling: ten·duhn

15. Centrifuge

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

IPA: /ˈsen.trə.fjuːdʒ/

Phonetic Spelling: sen·truh·fyooj

16. Myofibers

Pronunciation link: <https://www.merriam-webster.com/medical/myofiber>

IPA: /ˈmaɪ.oʊˌfaɪ.bər/

Phonetic Spelling: my·oh·fye·ber

17. Stereomicroscope

Pronunciation link: <https://www.howtopronounce.com/stereomicroscope>

IPA: /ˌster.i.oʊˈmaɪ.krəˌskoʊp/

Phonetic Spelling: stair·ee·oh·my·kruh·skohp

18. Dissociation

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

IPA: /dɪˌsoʊ.siˈeɪ.ʃən/

Phonetic Spelling: dih·soh·see·ay·shuhn

19. Humidified

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

IPA: /hjuːˈmɪd.əˌfaɪd/

Phonetic Spelling: hyoo·mih·duh·fied

20. Incubator

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

IPA: /ˈɪŋ.kjəˌbeɪ.tər/

Phonetic Spelling: ing·kyuh·bay·ter

21. Suspension

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

IPA: /səˈspen.ʃən/

Phonetic Spelling: suh·spen·shuhn

22. Adhesion

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

IPA: /əd'hi:.ʒən/

Phonetic Spelling: ad·hee·zhuhn

23. Microscope

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

IPA: /'maɪ.krəˌskoʊp/

Phonetic Spelling: my·kruh·skohp

24. Sarcomere

Pronunciation link: <https://www.merriam-webster.com/medical/sarcomere>

IPA: /'sɑ:r.kəˌmɪr/

Phonetic Spelling: sar·kuh·meer

25. Striations

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

IPA: /straɪ'eɪ.ʃənz/

Phonetic Spelling: stry·ay·shunz

26. Magnification

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

IPA: /ˌmæɡ.nə.fə'keɪ.ʃən/

Phonetic Spelling: mag·nuh·fuh·kay·shuhn

27. Vitro

Pronunciation link: <https://www.merriam-webster.com/dictionary/in%20vitro>

IPA: /ɪn 'vi:.troʊ/

Phonetic Spelling: in·vee·troh