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Title: Isolation of Skeletal Muscle Satellite Cells for In Vitro Myogenesis Studies

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**

Videographer: Please carry a scope kit to capture some shots as backup, if authors request it

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: 20

Number of Shots: 41

Introduction

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

INTRODUCTION:

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

- 1.1. **Carlos Acosta Gallo**: We developed an optimized protocol to isolate viable satellite cells from neonatal and adult mouse skeletal 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. **Carlos Acosta Gallo** : Existing methods yield variable purity, low neonatal efficiency, and stress-induced cell loss. Our method preserves viability, high purity, as well as reproducibility.
 - 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. **Lilya Lehka**: This method enables direct observation of satellite cell behavior, fusion parameters, marker expression, and responses to environmental or chemical stimuli.
 - 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. **Lilya Lehka**: The main challenge is controlling digestion, plating density, and fibroblast removal to ensure healthy, high-purity myogenic cells.

- 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. **Carlos Acosta Gallo:** Isolated cells can support research on muscle regeneration, disease modeling, drug testing, and mechanisms controlling satellite cell differentiation and self-renewal.

- 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 article address?

1.3. **Lilya Lehka, Senior researcher**: (authors will present their testimonial statements live)

1.3.1. 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?

1.4. **Carlos Acosta-Gallo, PhD Candidate**: (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the Director of the Nencki Institute of Experimental Biology, in compliance with the European Communities Council directives. Since all studies used isolated tissues from non-suffering animals, formal ethics committee approval was not required

Protocol

2. Isolation of Mononuclear Cells from Mouse Skeletal Muscle Tissue

Demonstrator: Lilya Lehka

- 2.1. To begin, obtain skeletal muscle tissue from mice [1]. With a pair of autoclaved forceps, transfer the washed muscles into two 1.5-milliliter tubes [2]. Using autoclaved scissors, mince the tissue finely into fragments measuring approximately 1–2 millimeters [3].
 - 2.1.1. WIDE: Talent holding a dish with mouse skeletal tissue.
AUTHORS: Please note that JoVE requires the opening shot to be a wide, non-screen capture. This shot was added specifically to meet that requirement
 - 2.1.2. Talent transferring washed hind limb muscles into labeled 1.5 milliliter tubes using autoclaved forceps.
 - 2.1.3. Shot of muscle tissue being minced with autoclaved scissors into small uniform fragments.
- 2.2. Transfer the minced muscles into tissue dissociation tubes containing 0.2 percent collagenase in DMEM (*D-M-E-M*) [1-TXT]. Incubate the tubes at 37 degrees Celsius for 30 minutes to initiate enzymatic digestion [2].
 - 2.2.1. Talent transferring minced muscle into tissue dissociation tubes containing collagenase solution. **TXT: Maintain a ratio of 500 mg/3 mL collagenase**
 - 2.2.2. Talent placing the dissociation tubes into a 37 degrees Celsius incubator.
- 2.3. Using a mechanical tissue dissociator, run the predefined program to facilitate enzymatic digestion while preserving cell viability [1]. Apply the program twice to enhance mechanical disruption of the tissue [2].
 - 2.3.1. Talent placing dissociation tubes into the mechanical tissue dissociator and selecting the m underscore muscle underscore 01 program.
 - 2.3.2. Shot of the dissociator screen showing the program running a second time.
- 2.4. Then place the samples back into the incubator at 37 degrees Celsius for an additional 30 minutes to complete the digestion process [1].
 - 2.4.1. Talent placing the tubes back into the 37 degrees Celsius incubator.
- 2.5. Repeat mechanical dissociation once more to ensure thorough dissociation [1]. Confirm that the digestion medium becomes cloudy, with few or no visible tissue fragments remaining [2].
 - 2.5.1. Talent placing the tubes back in the mechanical dissociator.

- 2.5.2. Shot of the dissociated muscle suspension showing a cloudy appearance with minimal fragments.
- 2.6. Next, place a 100-micrometer cell strainer on top of a 50-milliliter conical tube [1]. Using a pipette, prewet the strainer with 1 milliliter of prewarmed neutralizing medium [2-TXT].
 - 2.6.1. Talent positioning a 100-micrometer cell strainer onto a 50-milliliter conical tube.
 - 2.6.2. Talent pipetting prewarmed neutralizing medium onto the strainer. **TXT: Neutralizing Medium: High glucose DMEM, 10% FBS, 1% P/S**
- 2.7. Pour the digested muscle suspension over the prewetted 100-micrometer strainer [1]. Then rinse the strainer with an additional 1 milliliter of neutralizing medium to remove residual debris [2-TXT].
 - 2.7.1. Talent pouring the digested suspension onto the 100-micrometer strainer.
 - 2.7.2. Talent rinsing the strainer with neutralizing medium. **TXT: Similarly, strain over 70 µm and 40 µm strainers**
- 2.8. After straining the suspension over 70 and 40-micrometer cell strainers, place a 30-micrometer cell strainer on a 15-milliliter conical tube [1]. Prewet it with 1 milliliter of neutralizing medium [1]. Then pass the filtrate through the strainer [3] before washing with 1 milliliter of neutralizing medium to maximize cell recovery [4].
 - 2.8.1. Talent setting up a 30-micrometer strainer on a 15-milliliter tube.
 - 2.8.2. Shot of 1 mL NM being pipetted over the strainer. **Videographer's NOTE: Use Shot number 2.8.3 instead of 2.8.2 (Probably he is meaning it's mislabeled. So please check once.)**
 - 2.8.3. Talent pouring the cell suspension over the strainer. **Videographer's NOTE: please use shot no. 2.8.2 instead of 2.8.3**
 - 2.8.4. Talent pipetting 1 mL NM over the strainer.
- 2.9. Centrifuge the collected cell suspension at 300 g for 15 minutes at room temperature [1]. Under a sterile biosafety cabinet, carefully remove and discard the supernatant [2]. Confirm the presence of a white cell pellet at the bottom of the tube [3].
 - 2.9.1. Talent placing the tube into a centrifuge.
 - 2.9.2. Talent aspirating the supernatant inside a biosafety cabinet.
 - 2.9.3. Shot of the white pellet at the tube bottom.

3. Magnetic-Activated Cell Sorting of Satellite Cells from Skeletal Muscle Cell Suspension

Demonstrator: Carlos Acosta

- 3.1. To perform magnetic-activated cell sorting, first suspend the cell pellet in freshly prepared MACS (*M-A-C-S*) buffer [1-TXT].
 - 3.1.1. Talent resuspending the pellet in magnetic-activated cell sorting buffer. **TXT: MACS Buffer: 1x PBS (pH 7.2), 0.5% FBS, 2 mM EDTA; Use 80 µL per g tissue**
- 3.2. Add 20 microliters of magnetic labeling reagent [1]. Then, gently mix and incubate for 15 minutes at 2 to 8 degrees Celsius on ice [2].
 - 3.2.1. Talent adding magnetic labeling reagent to the suspension. **NOTE: Please add shot no. 3.2.1.A between: 3.2.1 and 3.2.2, this might be a close-up**
 - 3.2.2. Shot of tube being placed on ice.
- 3.3. Adjust the total volume to 500 microliters with sorting buffer for samples up to 5 grams of tissue [1]. For larger tissue amounts, divide the suspension equally into multiple tubes [2].
 - 3.3.1. Talent adding sorting buffer to reach 500 microliter total volume.
 - 3.3.2. Shot of suspension being divided into multiple tubes.
- 3.4. Place a separation column in the magnetic field of a magnetic separator [1]. Wash the column with 3 milliliters of sorting buffer to equilibrate it [2].
 - 3.4.1. Talent positioning the column in the magnetic separator.
 - 3.4.2. Talent pouring 3 mL sorting buffer into the column.
- 3.5. Apply the labeled cell suspension onto the column [1]. Collect the eluent containing the satellite cell-enriched population [2]. Then wash the column twice with 1 milliliter portions of sorting buffer [3]. Combine the collected fractions [4].
 - 3.5.1. Talent pouring the cell suspension to the column.
 - 3.5.2. Shot of the flow-through being collected.
 - 3.5.3. Talent pipetting sorting buffer into the column.
 - 3.5.4. Shot of the collected fractions being combined in a new tube.
- 3.6. Centrifuge the combined suspension at 300 *g* for 10 minutes at room temperature to pellet the isolated satellite cells [1].
 - 3.6.1. Talent placing the tube into the centrifuge.

4. Collagen Coating and Culture of Mouse Myoblasts

Demonstrator: Lilya Lehka

- 4.1. Dilute type I (*one*) rat collagen in 20 millimolar acetic acid to a final concentration of 50 microgram per milliliter [1]. Add the solution to the culture plates and incubate [2-TXT].
 - 4.1.1. Talent adding type 1 rat collagen into 20 mM acetic acid.

- 4.1.2. Talent adding diluted collagen solution to culture plates. **TEXT: Incubation: RT, 1 h**
- 4.2. Carefully aspirate the collagen solution from the plates [1]. Then rinse the plates three times with sterile PBS using equal volumes to remove residual acid [2].
 - 4.2.1. Talent aspirating solution from coated plates.
 - 4.2.2. Talent pipetting sterile PBS into the plates.
- 4.3. Resuspend the cell pellet in 10 milliliters of myoblast proliferating medium prewarmed to 37 degrees Celsius [1].
 - 4.3.1. Talent resuspending the pellet in 10 mL prewarmed medium.
- 4.4. Next, prepare myoblast proliferating medium for adult mouse myoblasts or neonatal mouse myoblasts [1].
 - 4.4.1. Talent preparing MPM in labeled bottles.
AND
TEXT ON PLAIN BACKGROUND:
MPM For Mouse Myoblasts:
High glucose DMEM (adult) or F10 (Neonatal)
20% FBS
10% horse serum (HS)
0.5% of chicken embryo extract (CEE)
20 ng/mL basic fibroblast growth factor (bFGF) dissolved in sterile distilled H₂O
1% of P/S
Video Editor: Please play both shots side by side in a split screen
- 4.5. Seed adult myoblasts onto the collagen-coated plates [1]. Then incubate the plates at 37 degrees Celsius in a carbon dioxide incubator for 24 hours [2].
 - 4.5.1. Talent plating cells onto collagen-coated culture plates.
 - 4.5.2. Talent placing plates into a 37 degrees Celsius carbon dioxide incubator.

Results

5. Results

5.1. After the optimized preplating step, most fibroblasts and other non-myogenic cells remained adherent to the initial collagen-coated surface [1], whereas myoblasts stayed in suspension and subsequently attached to the Matrigel-coated plates [2].

5.1.1. LAB MEDIA: Figure 3.

5.1.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the images in panel B labeled “Pre-plating”.*

5.2. Within 72 hours of culture in myoblast proliferation medium, small, round to spindle-shaped myoblasts with a high nuclear-to-cytoplasmic ratio predominated [1]. A minor population of large, flattened fibroblast-like cells remained in culture, typically representing less than 3% of the total cells [2].

5.2.1. LAB MEDIA: Figure 2C. *Video editor: Sequentially highlight each image panel*

5.2.2. LAB MEDIA: Figure 2C.

5.3. More than 97% of the cells isolated from adult and neonatal mice were myogenic progenitors as indicated by Pax7 (*Pax-Seven*) positive expression [1].

5.3.1. LAB MEDIA: Figure 4A,B. *Video editor: Highlight the row with green Pax7-positive cells in A and the corresponding Pax7+ dots in B*

5.4. Neonatal myoblasts achieved high purity with or without magnetic-activated cell sorting [1].

5.4.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the images for “Neonatal” and “Neonatal + MACS”*

5.5. After switching to differentiation medium at 80–90% confluency, myoblasts fused to form elongated, multinucleated myotubes within 48–72 hours [1].

5.5.1. LAB MEDIA: Figure 2D. *Video editor: Sequentially highlight each image panel*

5.6. Differentiated myotubes were confirmed by immunostaining for myosin heavy chain

[1]. The presence of organized sarcomeric structures in differentiated myotubes indicated structural maturation under these culture conditions [2].

5.6.1. LAB MEDIA: Figure 5A *Video editor: Highlight the green-stained elongated structures*

5.6.2. LAB MEDIA: Figure 5B. *Video editor: Show the zoomed in images*

1. Skeletal
Pronunciation link: <https://www.merriam-webster.com/dictionary/skeletal>
IPA: /'skɛlɪtəl/, /'skɛlɪtəl/
Phonetic Spelling: skel·uh·tl
2. Autoclaved
Pronunciation link: <https://www.merriam-webster.com/dictionary/autoclave>
IPA: /'ɔːtoʊˌklɛv/
Phonetic Spelling: aw·tow·klayvd
3. Collagenase
Pronunciation link: <https://www.merriam-webster.com/dictionary/collagenase>
IPA: /kəˈlædʒəˌneɪs/
Phonetic Spelling: kuh·laj·uh·nays
4. Enzymatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/enzymatic>
IPA: /ˌɛnzəˈmætɪk/
Phonetic Spelling: en·zai·mat·ik
5. Dissociation
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissociation>
IPA: /dɪˌsoʊsiˈeɪʃən/, /dɪˌsoʊʃiˈeɪʃən/
Phonetic Spelling: dih·soh·see·ay·shuhn
6. Dissociator
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissociate>
IPA: /dɪˌsoʊʃiət/, /dɪˌsoʊsiət/
Phonetic Spelling: dih·soh·see·ay·tor
7. Viability
Pronunciation link: <https://www.merriam-webster.com/dictionary/viability>
IPA: /ˌvaɪəˈbɪləti/
Phonetic Spelling: vai·uh·bil·uh·tee
8. Micrometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
IPA: /maɪˈkræˌmɛtər/
Phonetic Spelling: my·krah·mee·ter

9. Neutralizing
Pronunciation link: <https://www.merriam-webster.com/dictionary/neutralize>
IPA: /'nu:trəˌlaɪz/, /'nju:trəˌlaɪz/
Phonetic Spelling: noo·truh·lyze·ing
10. Conical
Pronunciation link: <https://www.merriam-webster.com/dictionary/conical>
IPA: /'kɑ:nɪkəl/
Phonetic Spelling: kah·nuh·kl
11. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sentrəˌfju:dʒ/
Phonetic Spelling: sen·truh·fyooj
12. Biosafety
Pronunciation link: <https://www.merriam-webster.com/dictionary/biosafety>
IPA: /ˌbaɪoʊ'seɪfti/
Phonetic Spelling: bye·oh·sayf·tee
13. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /'su:pərˌneɪtənt/
Phonetic Spelling: soo·per·nay·tnt
14. Pellet
Pronunciation link: <https://www.merriam-webster.com/dictionary/pellet>
IPA: /'pelɪt/
Phonetic Spelling: pel·it
15. Magnetic
Pronunciation link: <https://www.merriam-webster.com/dictionary/magnetic>
IPA: /mæg'netɪk/
Phonetic Spelling: mag·net·ik
16. Reagent
Pronunciation link: <https://www.merriam-webster.com/dictionary/reagent>
IPA: /ri'eɪdʒənt/
Phonetic Spelling: ree·ay·jnt
17. Separator
Pronunciation link: <https://www.merriam-webster.com/dictionary/separator>
IPA: /'sepəˌreɪtər/
Phonetic Spelling: sep·uh·ray·ter
18. Eluent
Pronunciation link: <https://www.merriam-webster.com/dictionary/eluent>
IPA: /'ɛluənt/
Phonetic Spelling: eh·loo·uhnt
19. Satellite
Pronunciation link: <https://www.merriam-webster.com/dictionary/satellite>
IPA: /'sætəˌlaɪt/
Phonetic Spelling: sat·uh·lyte

20. Myoblasts
Pronunciation link: <https://www.merriam-webster.com/dictionary/myoblast>
IPA: /'maɪoʊˌblæst/
Phonetic Spelling: my·oh·blast
21. Proliferating
Pronunciation link: <https://www.merriam-webster.com/dictionary/proliferate>
IPA: /prə'lifəˌreɪt/
Phonetic Spelling: ɹpɹuh·lif·uh·ray·tɪŋ
22. Acetic
Pronunciation link: <https://www.merriam-webster.com/dictionary/acetic>
IPA: /ə'siːtɪk/
Phonetic Spelling: uh·see·tɪk
23. Confluency
Pronunciation link: <https://www.merriam-webster.com/dictionary/confluence>
IPA: /'kɑːnfluəns/
Phonetic Spelling: kahn·floo·en·see
24. Multinucleated
Pronunciation link: <https://www.merriam-webster.com/dictionary/multinucleated>
IPA: /ˌmʌlti'nuːkliˌeɪtɪd/
Phonetic Spelling: mul·tee·noo·klee·ay·tuhd
25. Myotubes
Pronunciation link: <https://www.merriam-webster.com/dictionary/myotube>
IPA: /'maɪoʊˌtuːb/
Phonetic Spelling: my·oh·toob
26. Immunostaining
Pronunciation link: <https://www.merriam-webster.com/dictionary/immunostaining>
IPA: /ɪˌmjuːnoʊ'steɪnɪŋ/
Phonetic Spelling: ih·myoo·noh·stay·nɪŋ
27. Sarcomeric
Pronunciation link: <https://www.merriam-webster.com/dictionary/sarcomere>
IPA: /'sɑːrkoʊˌmɪr/
Phonetic Spelling: saar·koh·meer
28. Progenitors
Pronunciation link: <https://www.merriam-webster.com/dictionary/progenitor>
IPA: /proʊ'dʒɛnɪtər/
Phonetic Spelling: proh·jen·uh·ter
29. Pax7
Pronunciation link: No confirmed link found
IPA: /pæks 'sevən/
Phonetic Spelling: paks·sev·uhn