

Submission ID #: 70894

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21622718>

Title: Isolation of Small Extracellular Vesicles from Murine Skeletal Muscle and Bone Marrow by Size-Exclusion Chromatography

Authors and Affiliations:

Reine Khoury^{1,2}, Laura Montermini³, Nadim Tawil³, and Corina Nagy^{1,2,4}

¹Integrated Program in Neuroscience, McGill University

²McGill Group for Suicide Studies, Douglas Mental Health University Institute

³Center for Applied Nanomedicine, Research Institute of the McGill University Health Center

⁴Department of Psychiatry, McGill University

Corresponding Authors:

Corina Nagy corina.nagy@mcgill.ca

Email Addresses for All Authors:

Reine Khoury	reine.khoury@mail.mcgill.ca
Laura Montermini	laura.montermini@muhc.mcgill.ca
Nadim Tawil	nadim.tawil@mail.mcgill.ca
Corina Nagy	corina.nagy@mcgill.ca

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? **No**

- 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: 25

Number of Shots: 47

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. **Reine Khoury**: Our research focuses on isolating small extracellular vesicles from mouse muscle and bone marrow to understand how tissues exchange signals.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Nadim Tawil**: This protocol can be applied to studies pertaining to aging, muscle regeneration, exercise physiology, and bone marrow disease in mouse models.
 - 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. **Nadim Tawil**: This protocol allows researchers to measure the size, concentration, and molecular cargo of extracellular vesicles from muscle and bone marrow.
 - 1.3.1. 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.~~

- 1.4. **Reine Khoury**: Future research can extend this protocol to disease models and use the isolated vesicles to identify biomarkers and therapeutic targets.
 - 1.4.1. 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 McGill University's Animal Care Committee

Protocol

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

2. Murine Bone Marrow Extraction

Demonstrator: Reine Khoury

Authors: During filming, please point out the parts before dissecting them.

- 2.1. To begin, place the murine femur and tibia samples in a biosafety cabinet [1].
 - 2.1.1. Talent placing the sample in a biosafety cabinet.
- 2.2. Pre-weigh and label 1.5-milliliter centrifuge tubes [1].
 - 2.2.1. Talent checking the weight of the tube on a scale and labeling the 1.5-milliliter centrifuge tube.
- 2.3. Next, puncture the bottom of a 0.2-milliliter tube with a sterile 16-gauge needle held perpendicular to the base, producing a single uniform opening [1]. Insert the punctured tube into the pre-weighed 1.5-milliliter centrifuge tube with a snug fit to prepare the poked-tube setup [2].
 - 2.3.1. Talent puncturing the bottom of a 0.2-milliliter tube once with a sterile 16-gauge needle.
 - 2.3.2. Talent inserting the 0.2-milliliter PCR tube into the 1.5-milliliter centrifuge tube.
- 2.4. Place the leg bones in a sterile Petri dish. Separate the femur and tibia by cutting through the joint space [1].
 - 2.4.1. Talent placing the bones in a dish and cutting through the joint space to separate the femur and tibia.
- 2.5. Cut both ends of the femur and tibia to expose the red marrow [1]. Place the bones into the punctured PCR tube, which is positioned inside the 1.5-milliliter tube [2], and seal the assembly with parafilm [3].
 - 2.5.1. Talent cutting both ends of the femur and tibia to expose the red marrow.

2.5.2. Talent placing the bones into the punctured PCR tube positioned inside the 1.5-milliliter tube.

2.5.3. Talent sealing the assembly with parafilm.

2.6. Centrifuge the sample for 10 seconds in a benchtop microcentrifuge at approximately 8,000 *g* at room temperature [1]. Verify successful marrow extraction by the presence of visibly white bones devoid of residual red marrow in the upper tube and the dark red and compact pellet at the bottom of the lower tube [2]. **Note: The narration and numbers are adjusted to accommodate the added shot.**

2.6.1. Talent placing the sealed sample tubes into a benchtop microcentrifuge.

Added shot: 2.6.1.b. Talent showing the result: extracted marrow in the 1.5 mL tube and the bones in the upper 0.2 mL tube.

2.7. Then, weigh the 1.5-milliliter tube again to determine the mass of the extracted marrow to ensure it falls within the expected range of approximately 15 to 30 milligrams per mouse [1].

2.7.1. Talent weighing the tube containing the extracted marrow.

3. Bone Marrow Resuspension and Clarification

3.1. Add 250 microliters of RPMI-1640 to each tube containing the bone marrow [1] and place the samples on ice [2].

3.1.1. Talent adding 250 microliters of RPMI-1640 to each tube.

3.1.2. Talent placing the tubes on ice.

3.2. Resuspend the marrow by gently pipetting up and down 10 to 15 times using a P-1000 pipette [1] until a homogeneous suspension is obtained with no remaining clumps or intact tissue fragments [2].

3.2.1. Talent gently pipetting the marrow suspension up and down multiple times using a P1000 pipette.

3.2.2. Shot of the homogeneous suspension, with no visible clumps or intact tissue fragments remaining.

3.3. Centrifuge the sample at 300 *g* for 10 minutes at 4 degrees Celsius to pellet undigested

debris, intact cells, and remaining tissue fragments [1].

3.3.1. Talent placing the sample tube into a centrifuge.

3.4. Carefully transfer the supernatant to a new sterile 1.5-milliliter microcentrifuge tube without disturbing the pellet [1]. Centrifuge the transferred supernatant at 2,000 *g* for 15 minutes to remove large debris [2]. Collect the supernatant into a new tube [3] and centrifuge again at 10,000 *g* for 30 minutes at 4 degrees Celsius [4]. Then, collect at least 200 microliters of the final supernatant in a fresh tube [5].

3.4.1. Talent carefully transferring the supernatant to a new sterile 1.5-milliliter microcentrifuge tube while avoiding disturbance of the pellet.

3.4.2. Talent placing the tube with supernatant in a centrifuge.

3.4.3. Talent collecting the supernatant and adding in into a fresh tube.

3.4.4. Talent placing the tube in a centrifuge, closing the lid and starting the spin.

3.4.5. Talent transferring the resulting supernatant into a fresh 1.5 ml tube and showing in close-up that the volume collected is around 200 microliters.

4. Size-Exclusion Chromatography and Enrichment of Small Extracellular Vesicles

4.1. Equilibrate the size-exclusion chromatography column by flushing it with 6 milliliters of filtered PBS, maintaining the column and the PBS at room temperature [1].

4.1.1. Talent flushing the size-exclusion chromatography column with 1× PBS.

4.2. Allow the entire volume to pass through the column by gravity [1].

4.2.1. Shot of the buffer passing through the size-exclusion chromatography column and being collected into a tube.

4.3. Then, load 150 microliters of the filtered supernatant onto the column and allow the sample to enter the resin completely [1].

4.3.1. Talent loading 150 microliters of filtered supernatant onto the size-exclusion chromatography column.

4.4. The endpoint is reached when the liquid meniscus reaches the surface of the resin bed, with no visible liquid remaining above the wet resin [1].

- 4.4.1. Shot of the liquid meniscus reaching the surface of the resin bed with no visible liquid above the wet resin.
- 4.5. Immediately add 550 microliters of PBS to initiate elution [1] and collect the void volume of 700 microliters [2].
 - 4.5.1. Talent adding 550 microliters of PBS to the size-exclusion chromatography column.
 - 4.5.2. Talent collecting the 700-microliter void volume from the size-exclusion chromatography column in a tube.
- 4.6. Discard the void volume or retain it as a non-extracellular vesicle fraction [1].
 - 4.6.1. Talent labelling the collected solution as 'non-extracellular vesicle fraction'.
- 4.7. Begin collecting the extracellular vesicle-enriched fraction immediately after the 700-microliter void volume has eluted [1]. Ensure that 850 microliters of extracellular vesicle-enriched eluate is collected [2].
 - 4.7.1. Talent positioning a labelled collection tube and elution being collected in the tube.
 - 4.7.2. Talent picking up and showing the collection tube with 850 microliters of extracellular vesicle-enriched eluate.
- 4.8. To concentrate the extracellular vesicle, load the sample into a pre-rinsed centrifugal filter with a 100-kilodalton molecular weight cutoff [1].
 - 4.8.1. Talent loading extracellular vesicle samples into centrifugal filter.
- 4.9. Centrifuge the sample at 4,000 *g* at 4 degrees Celsius, checking every 2 to 3 minutes until the desired final volume is reached [1] and recover the retentate by gentle pipetting [2].
 - 4.9.1. Talent placing the centrifugal filters containing samples into a centrifuge.
 - 4.9.2. Talent gently pipetting the retentate from the centrifugal filter.
- 4.10. Keep the extracellular vesicle-enriched fractions on ice for immediate assays such as transmission electron microscopy [1].
 - 4.10.1. Talent placing extracellular vesicle-enriched fractions on ice or in 4-degree-

Celsius storage.

Results

5. Results

- 5.1. Western blot analysis showed the presence of small extracellular vesicles or sEV (*S-E-V*) markers in early fractions [1]. Fractions 1 to 5 were pooled for the subsequent characterization of small extracellular vesicles [2].
 - 5.1.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the dark bands in the columns labelled F3, F4, and F5 for all the 5 “sEV marker” rows*
 - 5.1.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the vertical lanes F1 to F5*
- 5.2. The absence of calnexin and binding immunoglobulin protein signals in these fractions ruled out contamination from intracellular membranes and endoplasmic reticulum components [1].
 - 5.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the lanes F1 to F5 in the rows labelled “Calnexin” and “BiP.”*
- 5.3. Transmission electron microscopy of sEV-enriched fractions F1 to F5 revealed intact, round vesicles with clearly defined membrane bilayers and diameters below 200 nanometers [1].
 - 5.3.1. LAB MEDIA: Figure 2C. *Video editor: Zoom in on the round vesicle shown in the center of the image.*
- 5.4. Nanoparticle tracking analysis of the pooled sample demonstrated a unimodal size distribution centered below 200 nanometers, with a median particle diameter of 147.8 nanometers [1].
 - 5.4.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the bar group for F1–F5*
- 5.5. Particle concentration normalized to tissue mass was highest in sEV-enriched fractions F1 to F5 at 3.7×10^8 particles per milliliter per milligram tissue [1] and slightly reduced in later fractions F6 to F8 at 3.6×10^8 particles per milliliter per milligram tissue [2].
 - 5.5.1. LAB MEDIA: Figure 2E. *Video editor: Highlight the bar labelled F1–F5.*
 - 5.5.2. LAB MEDIA: Figure 2E. *Video editor: Highlight the bar labelled F6–F8.*

Pronunciation Guide:

1. Murine
Pronunciation link: <https://www.merriam-webster.com/dictionary/murine>
IPA: /'mjʊr.aɪn/, /'mjʊr.ɪn/
Phonetic Spelling: myoor·ine, myoor·in
2. Femur
Pronunciation link: <https://www.merriam-webster.com/dictionary/femur>
IPA: /'fi:.mər/
Phonetic Spelling: fee·mer
3. Tibia
Pronunciation link: <https://www.merriam-webster.com/dictionary/tibia>
IPA: /'tɪb.i.ə/
Phonetic Spelling: tib·ee·uh
4. Biosafety
Pronunciation link: <https://www.merriam-webster.com/dictionary/biosafety>
IPA: /ˌbaɪ.əʊ'seɪf.ti/
Phonetic Spelling: bye·oh·sayf·tee
5. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sen.trəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
6. Sterile
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterile>
IPA: /'ster.əl/, /'ster.aɪl/
Phonetic Spelling: stair·uhl, stair·ile
7. Gauge
Pronunciation link: <https://www.merriam-webster.com/dictionary/gauge>
IPA: /geɪdʒ/
Phonetic Spelling: gayj
8. Marrow
Pronunciation link: <https://www.merriam-webster.com/dictionary/marrow>
IPA: /'mer.əʊ/
Phonetic Spelling: mair·oh
9. Parafilm
Pronunciation link: <https://www.howtopronounce.com/parafilm>
IPA: /'pær.ə.fɪlm/
Phonetic Spelling: pair·uh·film
10. Microcentrifuge

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

IPA: /ˌmaɪ.kroʊˈsen.trəˌfjuːdʒ/

Phonetic Spelling: my·kroh·sen·truh·fyooj

11. Resuspension

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

IPA: /ˌriː.səˈspen.ʃən/

Phonetic Spelling: ree·suh·spen·shuhn

12. Homogeneous

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

IPA: /ˌhoʊ.məˈdʒiːni.əs/

Phonetic Spelling: hoh·muh·jee·nee·uhs

13. Supernatant

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

IPA: /ˈsuː.pərˌneɪ.tənt/

Phonetic Spelling: soo·per·nay·tuhnt

14. Chromatography

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

IPA: /ˌkroʊ.məˈtɑːɡrə.fi/

Phonetic Spelling: kroh·muh·taa·gruh·fee

15. Equilibrate

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

IPA: /ˌiː.kwəˈlaɪ.breɪt/

Phonetic Spelling: ee·kwuh·lye·brayt

16. Elution

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

IPA: /iˈluː.ʃən/

Phonetic Spelling: ee·loo·shuhn

17. Extracellular

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

IPA: /ˌek.strəˈsel.jə.lər/

Phonetic Spelling: ek·struh·sel·yuh·ler

18. Vesicle

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

IPA: /ˈves.ɪ.kəl/

Phonetic Spelling: ves·ih·kuhl

19. Retentate

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

IPA: /rɪˈten.teɪt/

Phonetic Spelling: rih·ten·tayt

20. Microscopy

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

IPA: /maɪˈkrɑːskəpi/

Phonetic Spelling: my·krah·skuh·pee

21. Kilodalton

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

IPA: /ˈkɪl.ɒdɔːl.tən/

Phonetic Spelling: kil·oh·dawl·tuhn

22. Resin

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

IPA: /ˈrez.ɪn/

Phonetic Spelling: rez·in

23. Pellet

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

IPA: /ˈpel.ət/

Phonetic Spelling: pel·uht

24. Undigested

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

IPA: /ˌʌn.daɪˈdʒes.təd/

Phonetic Spelling: un·dye·jes·tuhd

25. Microliter

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

IPA: /ˈmaɪ.kroʊ.liː.tər/

Phonetic Spelling: my·kroh·lee·ter

26. RPMI-1640

Pronunciation link: No confirmed link found

IPA: /ɑːr.piː.əmˈaɪ wʌn sɪks fɔːr ti/

Phonetic Spelling: ar·pee·em·eye one six four oh

27. PBS

Pronunciation link: No confirmed link found

IPA: /piː.biːˈes/

Phonetic Spelling: pee·bee·ess