Submission ID #: 67600
Scriptwriter Name: Debopriya Sadhukhan
Project Page Link: https://review.jove.com/account/file-uploader?src=20618048

Title: Purification, Expansion, and Flow Cytometry-Based Phenotyping of Mouse-Derived Bone Marrow Mesenchymal Stem Cells

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? **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.**

Current Protocol Length
Number of Steps: 17
Number of Shots: 21

Introduction

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

1.1. **Yanping Yang:** Our research provides a low-cost method to purify and expand homogeneous mouse bone marrow mesenchymal stem cells. This helps researchers get sufficient high-quality cells with strong growth and differentiation abilities **[1]**.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4.1., 3.4.2.*

What are the current experimental challenges?

1.2. **Yanping Yang:** A key challenge in bone marrow stem cell research is developing a straightforward and effective protocol for isolating bone marrow mesenchymal stem cells **[1]**.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.7.1, 3.7.2.*

What advantage does your protocol offer compared to other techniques?

1.3. **Yanping Yang:** This method lets bone marrow mesenchymal stem cells grow naturally by avoiding disturbance. It uses slow passaging, avoids added growth factors, and keeps hematopoietic cells around to support the stem cells' health and function **[1]**.

1.3.1. 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 in accordance with the Laboratory Animal Ethics guidelines of Shanghai University of Traditional Chinese Medicine

Protocol

2. Harvesting Femurs and Tibias from Mouse Hind Limbs

Demonstrator: Chenglong Wang

2.1. To begin, soak the euthanized mouse in 75 percent ethanol for 5 minutes **[1]**.

2.1.1. WIDE: Talent placing the mouse in a beaker containing 75% ethanol and soaking it.

2.2. Using sterile dissection scissors, make an approximately 1-centimeter incision at the hind limb near the Achilles tendon **[1]**.

2.2.1. Talent making 1-centimeter incision at the hind limb near the Achilles tendon using scissors.

2.3. Then, perform a circular incision around the hind limb **[1]** to expose the distal tibia **[2]**.

2.3.1. Talent performing a circular incision around the hind limb near the Achilles tendon.

2.3.2. A shot of the exposed distal tibia.

2.4. Peel the skin from the incision site upward toward the hip to remove the skin from both hind limbs **[1]**.

2.4.1. A shot of the mouse after peeling the skin from both hind limbs. @Authors: If possible, please record this shot after the skin has been peeled from the limbs. It is okay to omit the skin-peeling procedure itself, as some viewers may find it disturbing.

2.5. Cut along the iliac bone to detach the femoral head from the hip bone **[1]**.

2.5.1. Talent cutting along the iliac bone to detach the femoral head from the hip bone.

~~2.6. Place the isolated femurs and tibias in a 60-millimeter dish containing sterile, pre-cooled PBS with 2 percent penicillin and streptomycin **[1]**.~~ NOTE: The authors deleted step 2.6 during filming as they felt it was not needed.

~~2.6.1. Talent transferring bones into a petri dish filled with PBS and antibiotics.~~

2.7. Using a sterile scalpel, remove as much tendon and muscle as possible from the tibia and fibula to ease later flushing **[1]**.

2.7.1. Talent trimming tissue from the bones using a scalpel. Videographer's NOTE: 2.7.1 and 2.8.1 are combined in one CLIP C2275

2.8. Return the cleaned bones to the dish containing PBS with 2 percent penicillin and streptomycin before moving them into a sterile hood **[1]**.

2.8.1. Talent placing the cleaned bones back in the PBS dish.

3. Purification and Culture of Mouse Bone Marrow Mesenchymal Stem Cells (BMSCs)

3.1. Take a 50-milliliter syringe and attach the needle that comes with the syringe.**[1]**.

3.1.1. Talent attaching the needle to the syringe on a sterile workspace.

3.2. Fill the syringe with 20 milliliters of αMEM *(Alpha-M-E-M)* medium **[1]** and replace the needle with the one that comes with the 1-milliliter syringe **[2].**

3.2.1. Talent drawing alphaMEM medium into the syringe.

3.2.2. Talent attaching a 1 mL syringe needle to the 50 mL syringe.

3.3. In the sterile hood, use scissors to cut off both ends of the separated femur or tibia **[1]**.

3.3.1. Talent holding bone pieces and cutting off both ends cleanly using scissors.

3.4. Now, insert the needle into the bone at the cut edge and flush the bone marrow into a 100-millimeter cell culture dish containing complete αMEM Medium. Continue flushing **[1]** until the bone becomes visibly pale, indicating successful marrow extraction **[2]**.

3.4.1. Talent inserting the needle into the bone at the cut edge and flushing marrow into the dish.

3.4.2. A shot of the pale bone after repeated flushing.

3.5. Pipette up and down 40 times using a 1-milliliter pipette tip to disperse any cell aggregates **[1]**.

3.5.1. Talent pipetting cell suspension up and down repeatedly to break up clumps.

3.6. Now, filter the cell suspension through a 40-micrometer cell strainer into a 50-milliliter centrifuge tube to remove debris, tissue fragments, and undispersed clumps **[1]**.

3.6.1. Talent using a pasteur pipette to draw cell mix into the strainer attached to the centrifuge tube.

3.7. Adjust the total volume to 15 milliliters with complete αMEM medium **[1]** and transfer the filtered cell suspension into a 100-millimeter culture dish **[2]**.

3.7.1. Talent adding αMEM medium to the centrifuge tube to adjust the total volume to 15 milliliters.

3.7.2. Talent using a Pasteur pipette to draw the filtered cell suspension into the culture dish.

3.8. Hold the dish with both hands and gently swirl it 15 times in a figure-eight motion to distribute the cells evenly across the dish **[1]**.

3.8.1. Talent swirling the dish slowly in a figure-eight motion with both hands.

3.9. Finally, place the culture dish in a 37 degrees Celsius, 5 percent carbon dioxide incubator and culture for 5 days **[1]**.

3.9.1. Talent placing the dish into the incubator and closing the door.

Results

4. Results

4.1. This figure illustrates the morphological progression of bone marrow stromal cells from initial extraction to the third passage in culture **[1]**.

4.1.1. LAB MEDIA: Figure 3.

4.2. Freshly extracted bone marrow cells appeared predominantly mononuclear with scattered fat droplets visible throughout the field **[1]**.

4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight A.*

4.3. By the 5th day of culture, spindle-shaped cells began to emerge and reached approximately 60% to 80% confluency **[1]**, continuing to increase in density by day 7 **[2]**.

4.3.1. LAB MEDIA: Figure 3. *Video editor: Highlight B.*

4.3.2. LAB MEDIA: Figure 3. *Video editor: Highlight C*.

4.4. From passage 1 to passage 3, bone marrow stromal cells maintained a spindle or fusiform morphology, formed a clear monolayer with interconnected colonies, and showed no signs of apoptosis, such as cytoplasmic granularity or nuclear fragmentation **[1]**.

4.4.1. LAB MEDIA: Figure 3D, 3E, 3F. *Video editor: Highlight 3D when the VO says "passage 1", and 3F when the VO says "passage 3".*

4.5. Flow cytometry confirmed that CD29 *(C-D-twenty-nine)*, CD44 *(C-D-forty-four)*, and Sca-1 *(S-C-A-one)* were highly expressed in bone marrow stromal cells **[1]**.

4.5.1. LAB MEDIA: Figure 4A, 4B, 4C. *Video Editor: Highlight A when the VO says CD29, highlight B when the VO says CD44, and highlight C when the VO says Sca-1.*

4.6. The expression of CD31 *(C-D-thirty-one)* and CD45 *(C-D-forty-five)* markers of endothelial and hematopoietic lineages was negligible **[1]**.

4.6.1. LAB MEDIA: Figure 4. *Video editor: Highlight D and E.*

4.7. Marker quantification further confirmed high expression levels of CD29, CD44, and Sca-1 compared to CD31 and CD45 **[1]**.

4.7.1. LAB MEDIA: Figure 4F. *Video editor: Highlight the three tall bars.*