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Title: Efficient Isolation of Adipose-derived Stem Cells and Adipocytes from Porcine Adipose Tissue

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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? **Yes**
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: 14

Number of Shots: 50

Interviews

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. **Lu Lu:** The purpose of this research is to build a standard method to isolate cells from adipose tissues rapidly and efficiently.
 - 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. **Lu Lu:** Traditional digestion is time-cost and may over-digest; our enzyme and mechanical-wave method offers gentle dispersion and reduces isolation time.
 - 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. **Yating Xiao:** This protocol allows researchers to compare the isolated cell characters across adipose depot.
 - 1.3.1. 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?~~

- 1.4. **Yating Xiao:** The isolated SVFs from this protocol can be used for organoid construction and adipose stem cell purification.
 - 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. **Yating Xiao**: Future studies could further optimize isolation methods, considering the adipose tissue variations in extracellular matrix across depot.

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 Animal Ethical and Welfare Committee of Sichuan Agricultural University

Protocol

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

2. Porcine Adipose Tissue Harvesting and Preparation

Demonstrator: Yating Xiao

- 2.1. To begin, put a piece of back subcutaneous adipose tissue of a euthanized pig in a culture dish ~~To begin, obtain a piece of back subcutaneous adipose tissue from a euthanized pig~~ [1]. Dissect along the natural plane between the adipose tissue and dermis to obtain a complete 2 to 3 millimeters thick ULB (U-L-B) sheet [2-TXT]. Rinse the ULB once in ice-cold sterile saline [3]. Place the tissue in a sterile culture dish [4].

NOTE: Author made slight adjustment in [1]. VO and shot has been edited accordingly.

- 2.1.1. WIDE: Talent putting a piece of back subcutaneous adipose tissue from a euthanized pig in a culture dish. ~~Talent obtaining a piece of back subcutaneous adipose tissue from a euthanized pig.~~
- 2.1.2. Talent dissecting along the natural plane between the adipose tissue and dermis to isolate the ULB sheet. **TXT: ULB: Upper layer of back subcutaneous adipose tissue**
- 2.1.3. Talent taking the ULB sheet and rinsing it in sterile ice-cold saline.
- 2.1.4. Talent placing the tissue in a sterile culture dish.
- 2.2. After taring the dish, weigh the tissue in the sterile culture dish on a pre-sterilized balance [1]. For every 1.8 grams of tissue, add 10 milliliters of ice-cold D-Hanks (D-Hanks) buffer to the dish [2]. Keep the tissue fully submerged [3].
- 2.2.1. Talent weighing the tissue on a pre-sterilized balance
- 2.2.2. Talent adding ice-cold D-Hanks buffer to the dish containing the tissue.
- 2.2.3. Shot of the tissue fully submerged in D-Hanks buffer.
- 2.3. Use sterile surgical scissors to identify and excise all visible blood vessels from the tissue [1]. Remove any connective tissue and fibrotic components [2]. Discard the excised materials into designated biohazard containers [3]. Rinse the tissue while keeping it fully submerged in D-Hanks buffer [4].
- 2.3.1. Talent excising visible blood vessels from the tissue with sterile surgical scissors.
- 2.3.2. Talent removing connective tissue and fibrotic components from the tissue.
- 2.3.3. Talent discarding the excised materials into a designated biohazard container.

- 2.3.4. Talent rinsing the tissue in D-Hanks buffer while ensuring that it is fully submerged.

3. Dissociation of Adipose Tissue for Cell Isolation

- 3.1. Weigh the tissue in the sterile culture dish on a pre-sterilized balance after taring the dish [1]. Transfer 1.8 grams of the rinsed tissue fragments to a sterile 1.5-milliliter microcentrifuge tube [2]. Add 800 microliters of A Type Tissue Dissociation Buffer to the tube [3]. Use sterile ophthalmic scissors to mince the tissue in the tube into pieces approximately 1 to 2 cubic millimeters in size [4]. Transfer the minced tissue along with the surrounding buffer to a new microcentrifuge tube [5].
 - 3.1.1. Talent weighing the tissue using a pre-sterilized weighing balance.
 - 3.1.2. Talent transferring 1.8 grams of rinsed tissue fragments into a sterile 1.5 milliliter microcentrifuge tube.
 - 3.1.3. Talent adding 800 microliters of A Type Tissue Dissociation Buffer to the tube.
 - 3.1.4. Talent mincing the tissue in the microcentrifuge tube using sterile ophthalmic scissors.
 - 3.1.5. Talent transferring the contents of the tube into a fresh microcentrifuge tube.
- 3.2. Add 200 microliters of A Type Tissue Dissociation Agent to the tube [1]. Gently rotate and shake the tube by hand thrice to mix the contents [2]. Ensure that the adipose fragments are fully immersed in the dissociation solution [3]. Check if the tube cap is tightly closed [4]. Place the tube in a 37 degrees Celsius metal bath and incubate for 5 to 10 minutes [5].
 - 3.2.1. Talent adding 200 μ L of A Type Tissue Dissociation Agent to the minced adipose tissue in a new microcentrifuge tube.
 - 3.2.2. Talent gently rotating and shaking the microcentrifuge tube by hand.
 - 3.2.3. Shot of the adipose fragments fully immersed in the dissociation solution.
 - 3.2.4. Talent securing the microcentrifuge tube cap tightly.
 - 3.2.5. Talent placing the microcentrifuge tube in a 37 °C metal bath.
- 3.3. Transfer the partially digested adipose tissue in buffer into a sterile 1.5-milliliter microcentrifuge tube designated for the mechanical dissociation system [1]. Run the preset program for **Adipose Tissue** in the mechanical dissociation system to perform mechanical dissociation using a 200 hertz sinusoidal wave [2].
 - 3.3.1. Talent transferring the partially digested adipose tissue fragments and surrounding buffer into a sterile 1.5-milliliter microcentrifuge tube.
 - 3.3.2. Talent placing the tube in the mechanical dissociation system and starting the preset adipose tissue program.

4. Isolation of Adipocyte and Stromal Vascular Fractions (SVF)

- 4.1. Collect the cell suspension and pass it through a 200-micrometer cell strainer into a new 50-milliliter centrifuge tube [1]. Add 5 milliliters of precooled D-Hanks solution at 4 degrees Celsius to the strainer [2]. Rinse the strainer twice with the D-Hanks solution [3].
 - 4.1.1. Talent filtering the cell suspension through a 200- μ m cell strainer into a 50-milliliter centrifuge tube.
 - 4.1.2. Talent adding precooled D-Hanks solution to the cell strainer.
 - 4.1.3. Shot of the talent rinsing the cell strainer with D-Hanks solution.
- 4.2. Centrifuge the filtered cell suspension at 500 *g* for 5 minutes at 4 degrees Celsius [1]. Use a sterile Pasteur pipette to aspirate and discard the supernatant lipid layer while leaving 1 milliliter of buffer to protect the adipocyte layer [2]. Collect the second layer having the adipocytes, without disturbing the third and fourth layers [3].
 - 4.2.1. Talent placing the 50-mL centrifuge tube into the centrifuge.
 - 4.2.2. Talent aspirating and discarding the supernatant lipid layer with a sterile Pasteur pipette.
 - 4.2.3. Talent collecting the second layer having adipocytes without disturbing the lower layers.
- 4.3. Aspirate and discard the third layer [1]. Add 1 milliliter of D-Hanks buffer to the bottom pellet containing the stromal vascular fraction or SVF (*S-V-F*) [2]. Resuspend the cell pellet gently in the buffer to achieve a cell concentration of 2 to 5 times ten to the power of six cells per milliliter [3].
 - 4.3.1. Talent aspirating and discarding the third layer
 - 4.3.2. Talent adding 1 milliliter of D-Hanks buffer or phosphate-buffered saline to the bottom pellet.
 - 4.3.3. Talent gently resuspending the SVF cell suspension in the buffer.

5. Analysis of SVF Cell Suspension and Adipocyte Samples

- 5.1. Prepare the SVF cell suspension in a 1.5-milliliter microcentrifuge tube [1]. Keep the suspension on ice until use [2].
 - 5.1.1. Talent preparing the SVF cell suspension in a 1.5 milliliter microcentrifuge tube.
 - 5.1.2. Talent keeping the SVF cell suspension on ice.
- 5.2. Add 10 microliters of the SVF cell suspension and 10 microliters of 0.4 percent Trypan Blue solution in a sterile 0.5-milliliter microcentrifuge tube [1]. Gently pipette up and

down 3 times [2]. Transfer 10 microliters of the stained cell mixture to a counting slide appropriate for the Automated Cell Counter [3]. Verify that the sample fills the chamber completely without overflowing [4].

- 5.2.1. Talent adding 10 μ L of SVF cell suspension and 10 μ L of 0.4% Trypan Blue solution into a sterile 0.5 mL microcentrifuge tube.
- 5.2.2. Shot of the talent gently pipetting the mixture up and down.
- 5.2.3. Talent transferring the stained cell mixture onto the counting slide.
- 5.2.4. Shot of the counting chamber completely filled without any overflow.

- 5.3. Insert the counting slide into the Automated Cell Counter [1]. Select the **Trypan Blue** assay type [2] and adjust the cell concentration range if necessary [3]. Press **Count** to initiate automated cell counting [4]. Monitor the counting chamber images and observe the automatic distinction of live and dead cells [5].

- 5.3.1. Talent inserting the counting slide into the Automated cell counter.
- 5.3.2. SCREEN: 70464_screenshot_1.mp4 00:00-00:12
- 5.3.3. SCREEN: 70464_screenshot_1.mp4 00:12-00:20
- 5.3.4. SCREEN: 70464_screenshot_1.mp4 00:20-00:31
- 5.3.5. SCREEN: 70464_screenshot_1.mp4 00:31-00:41

- 5.4. Record the cell viability percentage and the total number of viable cells displayed on the instrument screen [1]. Perform 3 independent measurements for each sample [2]. Calculate the mean viability and the cell yield per gram of adipose tissue [3].

- 5.4.1. SCREEN: 70464_screenshot_2.mp4 00:00-00:12
- 5.4.2. Shot of the talent repeating the cell counting measurement for the sample.
- 5.4.3. Talent calculating the mean viability and cell yield per gram of adipose tissue.

- 5.5. Prepare a staining solution with 5 micrograms per milliliter Hoechst (*HOE-kst*) and 1 micromolar BODIPY (*BOH-dih-pee*) in D-Hanks [1]. Incubate the isolated adipocytes in the staining solution for 15 minutes at 37 degrees Celsius [2]. Use a self-made adipocyte-friendly slide-cover glass system for slide preparation suitable for fluorescence microscopy [3]. ~~Observe the cells under a fluorescence microscope [4].~~

Author's NOTE: Delete this shot

NOTE: VO has been merged.

- 5.5.1. Talent preparing the staining solution containing 5 mg/mL Hoechst and 1 μ M BODIPY in D-Hanks or phosphate-buffered saline.
- 5.5.2. Talent placing the adipocyte sample into the staining solution.
- 5.5.3. Talent preparing the stained adipocyte sample with a self-made adipocyte-friendly slide-cover glass system.

~~5.5.4. SCOPE: The cells under a fluorescence microscope.~~

Results

6. Results

- 6.1. The homemade adipocyte-friendly mounting system enabled uniform distribution of intact adipocytes for clear imaging [1], unlike traditional methods that produced crowded, multi-layered arrangements [2].
 - 6.1.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the evenly spaced round structures that are clearly separated from each other*
 - 6.1.2. LAB MEDIA: Figure 3D. *Video editor: Highlight the area where many round structures are packed closely together and overlapping*
- 6.2. The extracted adipocytes showed intact structure and normal morphology [1].
 - 6.2.1. LAB MEDIA: Figure 3C. *Video editor: Zoom in on a single circular cell having a smooth, unbroken blue outer border.*
- 6.3. The survival rate of adipocytes obtained by the mechanical dissociation method was significantly higher [1] compared to the traditional enzymatic hydrolysis method [2].
 - 6.3.1. LAB MEDIA: Figure 3F. *Video editor: Highlight the taller bar*
 - 6.3.2. LAB MEDIA: Figure 3F. *Video editor: Highlight the shorter bar*
- 6.4. The purified and cultured adipose-derived stem cells exhibited a uniform fibroblast-like morphology with elongated spindle-shaped cells arranged in an orderly manner [1].
 - 6.4.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the long, thin cells aligned in the same direction and evenly spaced across the image*
- 6.5. Most cells remained unstained, indicating viability [1], whereas only a small proportion stained blue, representing dead cells [2].
 - 6.5.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the many clear, unstained round structures spread across the image*
 - 6.5.2. LAB MEDIA: Figure 4B. *Video editor: Highlight one or two small blue dots at the top of the image, slightly smaller than the clear structures*

Pronunciation Guides:

- 1. Porcine

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

IPA: /'pɔːr,saɪn/

- Phonetic Spelling: por·syne
2. Adipose
Pronunciation link: <https://www.merriam-webster.com/dictionary/adipose>
IPA: /'ædəˌpoʊs/
Phonetic Spelling: a·duh·pohs
 3. Subcutaneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>
IPA: /ˌsʌbkjuˈteɪniəs/
Phonetic Spelling: sub·kyoo·tay·nee·uhs
 4. Euthanized
Pronunciation link: <https://www.merriam-webster.com/dictionary/euthanize>
IPA: /'juːθəˌnaɪz/
Phonetic Spelling: yoo·thuh·nyze
 5. Dermis
Pronunciation link: <https://www.merriam-webster.com/dictionary/dermis>
IPA: /'dɜrməs/
Phonetic Spelling: dur·muhs
 6. Sterile
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterile>
IPA: /'sterəl/
Phonetic Spelling: steh·ruhl
 7. Fibrotic
Pronunciation link: <https://www.merriam-webster.com/dictionary/fibrotic>
IPA: /faɪˈbrɑːtɪk/
Phonetic Spelling: fy·braa·tik
 8. Biohazard
Pronunciation link: <https://www.merriam-webster.com/dictionary/biohazard>
IPA: /ˈbaɪoʊˌhæzərd/
Phonetic Spelling: by·oh·ha·zurd
 9. Dissociation
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissociation>
IPA: /dɪˌsoʊsiˈeɪʃən/
Phonetic Spelling: dih·soh·see·ay·shuhn
 10. Microcentrifuge
Pronunciation link: <https://www.merriam-webster.com/medical/microcentrifuge>
IPA: /ˌmaɪkroʊˈsentɹəˌfjuːdʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj
 11. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /ˌmaɪkroʊˌliːtər/
Phonetic Spelling: my·kroh·lee·tur
 12. Ophthalmic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ophthalmic>

IPA: /ɒf'θælmɪk/

Phonetic Spelling: off·thal·mik

13. Sinusoidal

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

IPA: /ˌsaɪnə'sɔɪdəl/

Phonetic Spelling: sy·nuh·soy·duhl

14. Stromal

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

IPA: /'stroʊməl/

Phonetic Spelling: stroh·muhl

15. Vascular

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

IPA: /'væskjələr/

Phonetic Spelling: vas·kyuh·lur

16. Centrifuge

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

IPA: /'sentrəˌfjuːdʒ/

Phonetic Spelling: sen·truh·fyooj

17. Adipocytes

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

IPA: /'ædəpəˌsaɪt/

Phonetic Spelling: a·duh·puh·syte

18. Resuspend

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

IPA: /ˌriːsə'spend/

Phonetic Spelling: ree·suh·spend

19. Trypan

Pronunciation link: <https://www.merriam-webster.com/medical/trypan%20blue>

IPA: /'traɪˌpæn/

Phonetic Spelling: try·pan

20. Viability

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

IPA: /ˌvaɪə'bɪləti/

Phonetic Spelling: vy·uh·bil·uh·tee

21. Hoechst

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

IPA: /hɛkst/

Phonetic Spelling: hekst

22. BODIPY

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

IPA: /'boʊdɪpi/

Phonetic Spelling: boh·dih·pee

23. Fluorescence

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

IPA: /flɒˈresəns/

Phonetic Spelling: flo·reh·suhns

24. Morphology

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

IPA: /mɔrˈfɑːlədʒi/

Phonetic Spelling: mor·faa·luh·jee

25. Enzymatic

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

IPA: /ˌenzəˈmætɪk/

Phonetic Spelling: en·zuh·ma·tik

26. Hydrolysis

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

IPA: /haɪˈdrɑːləsəs/

Phonetic Spelling: hy·draa·luh·suhs

27. Fibroblast

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

IPA: /ˈfaɪbrəˌblæst/

Phonetic Spelling: fy·bruh·blast

28. Spindle-shaped

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

IPA: /ˈspɪndəl ˌʃeɪpt/

Phonetic Spelling: spin·duhl·shaypt

29. Microscopy

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

IPA: /maɪˈkrɑːskəpi/

Phonetic Spelling: my·kraa·skuh·pee

30. Phosphate-buffered

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

IPA: /ˈfɑːsˌfeɪt/

Phonetic Spelling: faas·fayt