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Title: Preparation of Adipose Progenitor Cells from Mouse Epididymal Adipose Tissues

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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. Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one.**

☒ Interviewees wear masks until videographer steps away (≥ 6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

- 4. Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 13

Number of Shots: 40

Introduction

1. Introductory Interview Statements

REQUIRED:

- 1.1. **Dong Seong Cho**: This protocol makes it possible to isolate high quality adipose progenitor cells from mouse epididymal adipose tissue for sensitive downstream analyses.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.
- 1.2. **Dong Seong Cho**: This technique yields highly viable adipose progenitor cells and has been validated in a recent single cell RNA sequencing study.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

- 1.3. Procedures involving animal subjects have been approved by the Mayo Clinic Institutional Animal Care and Use Committee.

Protocol

2. Dissection of Epididymal Fat Pad and Tissue Dissociation

- 2.1. After euthanizing the male mouse, locate the epididymal fat pad [1] and pull on it gently with blunt forceps to liberate it [2]. Use scissors to remove the testes [3] and incubate the epididymal fat pad in 5 milliliters of HBSS supplemented with 3% BSA in a 50-milliliter conical tube for 15 minutes [4]. *Videographer: This step is difficult and important!*
 - 2.1.1. WIDE: Establishing shot of talent preparing to dissect the mouse.
 - 2.1.2. Talent liberating the epididymal fat pad. Vid NOTE: Talent only liked the second half of take 2.
 - 2.1.3. Talent removing the testes.
 - 2.1.4. Talent placing the epididymal fat pad in the tube with HBSS, with the HBSS container in the shot.
- 2.2. After the incubation, centrifuge the tube at 150 x g for 7 minutes at 4 degrees Celsius [1]. Remove the floating epididymal fat pad from the conical tube [2] and finely mince it with clean scissors [3].
 - 2.2.1. Talent putting the tube in the centrifuge and closing the lid. Vid NOTE: Use Second half of Take 1
 - 2.2.2. Talent taking the tissue out of the tube.
 - 2.2.3. Talent mincing the tissue.
- 2.3. Add 200 microliters of 2% collagenase solution to 3.8 milliliters of HBSS in a 13-milliliter culture tube [1], then add the minced tissue [2] and incubate it in a rotating incubator at 5 rpm for 1 hour at 37 degrees Celsius [3]. *Videographer: This step is important!*
 - 2.3.1. Talent adding collagenase solution to HBSS, with the collagenase container in the shot.
 - 2.3.2. Talent adding the minced tissue to the tube.
 - 2.3.3. Talent putting the tissue in the incubator and starting the rotation.
- 2.4. Transfer the entire contents into a 50-milliliter conical tube and add 10 milliliters of neutralization medium [1]. Mix gently by inverting the tube 2 to 3 times [2]. Prepare another 50-milliliter conical tube fitted with a 70-micrometer cell strainer [3] and filter the digested tissue into the new tube [4].
 - 2.4.1. Talent transferring the tissue into the conical tube and adding neutralization buffer.

- 2.4.2. Talent inverting the tube.
- 2.4.3. Talent fitting the strainer on the tube.
- 2.4.4. Talent filtering the tissue.
- 2.5. Transfer the flow-through to a 15-milliliter tube [1] and centrifuge it at 350 x g for 10 minutes at 4 degrees Celsius [2]. Carefully remove the supernatant [3] and resuspend the cell pellet with 5 milliliters of DPBS [4], then centrifuge the tube for another 10 minutes [5].
 - 2.5.1. Talent transferring the flow-through to a 15mL tube.
 - 2.5.2. Talent putting the tube in the centrifuge and closing the lid. *Videographer: Obtain multiple usable takes, this will be reused in 2.5.5.*
 - 2.5.3. Talent removing the supernatant.
 - 2.5.4. Talent resuspending the cells in DPBS, with the DPBS container in the shot. **Vid**
NOTE: Use the beginning of Take 1 and then Take 2, if possible
 - 2.5.5. *Use 2.5.2.*
- 2.6. Carefully remove the supernatant [1] and add 50 microliters of flow cytometry buffer. Mix the cells by pipetting gently [2] and keep them on ice. The total volume of the cells in the flow cytometry buffer will be approximately 100 microliters [3].
 - 2.6.1. Talent removing the supernatant.
 - 2.6.2. Talent adding flow cytometry buffer and gently pipetting.
 - 2.6.3. Talent putting the cells on ice.

3. Antibody Labeling and Fluorescence Activated Cell Sorting (FACS)

- 3.1. After mixing the cells, transfer 54 microliters of the cell suspension into a 1.7-milliliter microcentrifuge tube [1]. Add 6 microliters of FcR blocking reagent and pipet gently to mix [2], then incubate the tube at 4 degrees Celsius for 10 minutes [3]. Keep any leftover cells on ice to use as unstained control [4]. *Videographer: This step is important!*
 - 3.1.1. Talent transferring cells to a microcentrifuge tube.
 - 3.1.2. Talent adding FcR to the tube and pipetting.
 - 3.1.3. Talent putting the tube in the refrigerator and closing the door.
 - 3.1.4. Leftover cells on ice.
- 3.2. During the incubation, prepare an antibody cocktail by combining 11 microliters each of anti-Sca1-APC (*pronounce 'anti-Skah-one-A-P-C'*), anti-Ter119-FITC (*pronounce 'anti-ter-one-nineteen-fit-C'*), anti-CD31-FITC, and anti-CD45-FITC antibodies in a

microcentrifuge tube [1-TXT]. Gently pipet the mixture and keep it on ice protected from light [2]. *Videographer: This step is important!*

3.2.1. Talent preparing the antibody cocktail. **TEXT: Antibody concentrations may need to be adjusted**

3.2.2. Talent putting the antibody cocktail on ice.

3.3. After the incubation with FcR blocking reagent, add 40 microliters of the antibody cocktail and mix well with gentle pipetting [1]. Incubate the cells at 4 degrees Celsius for another 10 minutes [2]. *Videographer: This step is important!*

3.3.1. Talent adding antibody cocktail to the cells and pipetting them.

3.3.2. Talent putting the cells in the refrigerator and closing the door.

3.4. Add 500 microliters of flow cytometry buffer supplemented with 1 microgram per milliliter DAPI into the stained cells [1]. Mix well and filter the cells using a 5-milliliter test tube with a cell strainer snap cap [2]. Then, keep the cells on ice [3].

3.4.1. Talent adding flow cytometry buffer with DAPI to the cells.

3.4.2. Talent filtering the cells.

3.4.3. Talent putting the cells on ice.

3.5. Add 500 microliters of flow cytometry buffer to the unstained cells and mix by pipetting gently [1]. Use a 5-milliliter test tube with a cell strainer snap cap to filter the cells [2] and keep them on ice [3]. Transport the stained cells and unstained control to the FACS (*pronounce 'faks'*) analysis or sorting instrument [4].

3.5.1. Talent adding flow cytometry buffer to the unstained cells.

3.5.2. Talent filtering the cells.

3.5.3. Talent putting the cells on ice.

3.5.4. Talent bringing the cells to the FACS instrument.

3.6. Identify and isolate the APC-positive, FITC-negative, DAPI-negative population using the gating strategies described in the text manuscript [1]. Use the unstained controls to aid in setting gating parameters [2]. *Videographer: This step is important!*

3.6.1. Talent at the computer setting the gating parameters.

3.6.2. LAB MEDIA: Second row of Figure 1.

3.7. [1].

3.7.1. ~~Cells collecting into the microcentrifuge tube.~~

Results

4. Results: Isolation of Viable Sca1⁺ Adipose Progenitor Single Cells

- 4.1. After exclusion of debris and doublets using FSC-SSC plots, viable cells were gated, followed by the selection of the APC-positive-FITC-negative population [1]. DAPI, APC, and FITC gates were drawn based on the unstained control [2]. The following results show the isolation of adipose progenitor cells from 4-month-old male FVB mice [3].

4.1.1. LAB MEDIA: Figure 1.

4.1.2. LAB MEDIA: Figure 1. *Video Editor: Emphasize the Unstained control gates, bottom row.*

4.1.3. LAB MEDIA: Figure 1.

- 4.2. After 1 hour of sorting, the quality of isolation was quantitatively evaluated by flow cytometry analysis [1], which demonstrated that the cells maintained high viability and purity [2].

4.2.1. LAB MEDIA: Figures 2 and 3.

4.2.2. LAB MEDIA: Figure 3.

Conclusion

5. Conclusion Interview Statements

5.1. **Dong Seong Cho**: Sensitive downstream analyses can be performed after this protocol, such as single cell RNA sequencing, quantitative real time PCR, and flow cytometry. This will enable further characterization of the adipose progenitor cells.

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

5.2. **Dong Seong Cho**: Adipose progenitor cells are heterogeneous populations that require further single cell studies. This method makes it possible to isolate the cells suitable for single cell studies.

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

