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Title: Isolation and Differentiation of Primary White and Brown Preadipocytes from Newborn Mice

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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 similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **N**

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 the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

   ☒ Interviewees self-record interview statements outside of the filming date. JoVE can provide support for this option.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **42**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Andrea Galmozzi**: This protocol provides a simple strategy for generating cell culture models to study white and brown adipose tissues that faithfully represent the physiology of adipose tissue in animals **[1]**.

1.1.1. LAB MEDIA 1.1 1.2 7.2.MOV, last part.

REQUIRED:

1.2. **Andrea Galmozzi**: This protocol enables the simultaneous isolation of both white and brown preadipocytes from newborn mice. Cells obtained using this protocol possess a high proliferative capacity and differentiation potential **[1]**.

1.2.1. LAB MEDIA: IMG_8868.MOV, first part.

OPTIONAL:

1.3. **Enrique Saez**: Cells isolated using this approach reflect the complexity of adipose tissue better than other culture models and can be used to more accurately study adipocyte dysfunction in obesity and diabetes **[1]**.

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

OPTIONAL:

1.4. **Enrique Saez**: We focus primarily on understanding adipose tissue dysfunction in obesity. However, cells prepared with this method can also be used to study basic questions about cell and developmental biology **[1]**.

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

OPTIONAL:

1.5. **Bernard Kok**: The key to a successful experiment is determining how to identify the white adipose depots, as these depots, which are small but very rich in proliferating preadipocytes, can be difficult to distinguish in pups **[1]**.

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

OPTIONAL:

1.6. **Bernard Kok**: Visual demonstration illustrates how simple this protocol is and allows the provision of tips for locating and dissecting the white and brown adipose depots. Seeing the depot isolation is very helpful **[1]**.

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

Ethics Title Card

1.7. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Scripps Research and the University of Wisconsin-Madison.

Protocol

2. Adipose Depot Collection and Digestion

2.1. To collect subcutaneous white adipose tissue, cut the skin around the abdomen of a euthanized newborn pup **[1-TXT]** and gently pull the skin below the legs **[2]**.

2.1.1. WIDE: Talent cutting skin *Videographer: More Talent than pup in shot* **TEXT: Euthanasia: hypothermia + decapitation**

2.1.2. Skin being pulled *Videographer: Important step*

2.2. Without collecting any skin tissue, carefully harvest the fat depot, which will appear as a clear or white, thin, elongated tissue attached to the inside of the skin or on top of the quadriceps **[1-TXT]**.

2.2.1. Shot of depot, then depot being harvested *Videographer: Important/difficult step* **TEXT: ≤P1: clear fat depot; ≥P2: white fat depot**

2.3. Rinse the harvested depot in PBS **[1]** and place the adipose tissue a 1.5-milliliter tube containing 250 microliters of PBS and 200 microliters of 2x isolation buffer on ice **[1-TXT]**.

2.3.1. Talent rinsing tissue, with PBS container visible in frame

2.3.2. Talent placing tissue into tube on ice, with PBS and buffer containers visible in frame **TEXT: See text for all buffer and medium preparation details**

2.4. To collect interscapular brown adipose tissue, pull the skin from the shoulder blades over the head **[1]** and lift the deep red brown adipose tissue located between the shoulder blades **[2]**.

2.4.1. Skin being pulled from shoulder blades *Videographer: Important step*

2.4.2. Shot of tissue, then tissue being lifted *Videographer: Important step*

2.5. Carefully make incisions all around the adipose to detach it from the body **[1]** and check the tissue for consistency and color **[2-TXT]**.

2.5.1. Incision(s) being made *Videographer: Important/difficult step*

2.5.2. Tissue being checked *Videographer: Important/difficult step* **TEXT: Caution: Do not remove WAT with BAT in ≥P2 mice**

2.6. After rinsing, place the tissue in a second 1.5-milliliter tube containing 250 microliters of PBS and 200 microliters of 2x isolation buffer on ice **[1]**.

2.6.1. Talent placing tissue into tube, with rinsing, PBS, and buffer containers are visible in frame

2.7. When all of the depots have been collected, use scissors to gently mince each depot 4-6 times directly inside the tubes **[1]** and add 50 microliters of 10x collagenase in 2x isolation buffer to each tube **[2]**.

2.7.1. Depot being minced *Videographer: Important step*

2.7.2. Talent adding collagenase to tube, with collagenase and buffer containers visible in frame *Videographer: Important step*

2.8. Then invert the tubes quickly to mix **[1]** and place the tissues in a temperature-controlled mixer for 30 minutes at 37 degrees Celsius and 1400 revolutions per minute **[2]**.

2.8.1. Talent inverting tube(s)

2.8.2. Talent placing tube(s) into mixer

3. Plating Preadipocytes

3.1. At the end of the digestion, place the tubes back on ice **[1]** and filter the digested tissues through 100-micron cell strainers into new 50-milliliter tubes **[2-TXT]**.

3.1.1. WIDE: Talent placing tube(s) on ice

3.1.2. Talent filtering tissue through strainer *Videographer: Important step* **TEXT: See text for known and unknown genotype preadipocyte plating details**

3.2. To maximize the cell yield, rinse each tube with 1 milliliter of isolation medium **[1]** and filter this wash through the strainer **[2]**.

3.2.1. Talent rinsing tube

3.2.2. Talent filtering wash through strainer

3.3. Dilute the brown and white adipose tissue solutions to 2 milliliters of tissue suspension per well per depot in fresh isolation medium **[1]** and plate 2 milliliters of white adipose tissue suspension to each of 2-3 wells and 2 milliliters of brown adipose tissue suspension to each of 4-6 wells of a 6-well plate through one 100-micron filter per well **[2]**.

3.3.1. Talent adding medium to tube(s), with medium container visible in frame

3.3.2. Talent adding cells to well(s)

3.4. When all of the cells have been plated, place the plate in the cell culture incubator for 60-90 minutes **[1]**.

3.4.1. Talent placing plate into incubator

3.5. At the end of the incubation, wash each well with 2 milliliters of serum-free medium **[1]** and gently agitate the plate to detach any blood cells from the bottom of the wells **[2]**.

3.5.1. Talent washing well(s)

3.5.2. Plate being agitated

3.6. After three washes, add 2 milliliters of fresh isolation medium to the wells **[1]** and return the plate to the cell culture incubator **[2-TXT]**.

3.6.1. Talent adding medium to well(s), with medium container visible in frame
NOTE: Use 3.4.1

3.6.2. Talent placing plate into incubator **TEXT: Wash cells and refresh culture medium every 2 d**

4. Preadipocyte Culture Expansion

4.1. When the cells reach 80-90% confluency, coat new destination plates with sterile 0.1% gelatin in distilled water at 37 degrees Celsius for 10 minutes **[1]**.

4.1.1. WIDE: Talent adding gelatin to plate(s), with gelatin container visible in frame

4.2. While the plates are incubating, wash the cells with PBS **[1]** before treating with trypsin for 3 minutes in the cell culture incubator **[2]**.

4.2.1. Talent washing well(s), with PBS container visible in frame

4.2.2. Talent adding trypsin to well(s), with trypsin container visible in frame

4.3. When the cells have detached, pipette the cell suspension several times to maximize the cell recovery **[1]** and transfer the cells to a new tube **[2]**.

4.3.1. Talent pipetting cells

4.3.2. Talent adding cells to tube

4.4. When all of the cells have been collected, wash each well with 1 milliliter of isolation medium **[1]** and pool the washes with the cell suspension **[2]**.

4.4.1. Talent washing well(s)

4.4.2. Talent adding wash to tube

4.5. Collect the cells by centrifugation **[1-TXT]** and resuspend the pellet in 3-5 milliliters of isolation medium for counting **[2]**.

4.5.1. Talent adding tube(s) to centrifuge **TEXT: 5 min, 800-1200 x g, RT**

4.5.2. Shot of pellet if visible, then medium being added to cells, with medium container visible in frame

4.6. Dilute the cells to the appropriate concentration for plating in fresh isolation medium **[1]** and wash the gelatin-coated plates with PBS to remove any excess gelatin **[2]**.

4.6.1. Talent adding medium to tube(s), with medium container visible in frame

4.6.2. Talent washing plate, with PBS container visible in frame NOTE: Only 1 take, can use 4.2.1.

4.7. Then seed the cells onto the gelatin-coated plates **[1]** and return the cells to the cell culture incubator **[2]**.

4.7.1. Talent adding cells to plate(s)

4.7.2. Talent placing plate into incubator

5. White and Brown Preadipocyte Differentiation

5.1. When the cells reach confluency, replace the isolation medium with differentiation medium **[1]**. If differentiating brown adipose tissue preadipocytes, also add 1-nanomolar triiodothyronine **[2]**.

5.1.1. WIDE: Talent adding medium to plate(s), with medium container visible in frame

5.1.2. Talent adding T3 to plate(s), with T3 container visible in frame

5.2. After 48 hours, the accumulation of small lipid droplets within the cells should become visible under brightfield microscopy **[1]**. When lipid is visible, refresh the medium with the appropriate volume of maintenance medium per culture **[2]**.

5.2.1. LAB MEDIA: Figure 2B Brightfield images *Video Editor: please emphasize droplet(s) in both images*

5.2.2. Talent adding medium to plate(s)

Results

6. Results: Representative Adipocyte Progenitor Collection and Differentiation

6.1. Even after filtering **[1]**, some cellular debris, blood cells, and mature adipocytes will be present within the cell suspension **[2]**.

6.1.1. LAB MEDIA: Figure 1C
6.1.2. LAB MEDIA: Figure 1C *Video Editor: please emphasize After filtration images*

6.2. Gentle washes 1 hour after plating will remove non-relevant cells **[1]** as the preadipocytes attach rapidly to the bottom of the well **[2]**.

6.2.1. LAB MEDIA: Figure 1C *Video Editor: please emphasize After wash images*
6.2.2. LAB MEDIA: Figure 1C *Video Editor: please emphasize After 24 h images*

6.3. Although both white and brown preadipocytes isolated from newborn pups have a high proliferative capacity **[1]**, the yield of white preadipocytes is generally twice that **[2]** of brown preadipocytes on a per-depot basis **[3]**.

6.3.1. LAB MEDIA: Figure 2A
6.3.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize WAT data bar*
6.3.3. LAB MEDIA: Figures 2A *Video Editor: please emphasize BAT data bar*

6.4. Therefore, if synchronized cultures are desired, the starting density of the white preadipocytes must be calculated accordingly **[1]**.

6.4.1. LAB MEDIA: Figure 2A

6.5. At the end of differentiation **[1]**, the cells will appear to be loaded with lipid droplets **[2]** and will express classical markers of white **[3]** and brown adipocytes, respectively **[4]**.

6.5.1. LAB MEDIA: Figures 2B and 2C
6.5.2. LAB MEDIA: Figures 2B and 2C *Video Editor: please emphasize red signal in right images of Figure 2B*
6.5.3. LAB MEDIA: Figures 2B and 2C *Video Editor: please emphasize white data bars in Figure 2C*
6.5.4. LAB MEDIA: Figures 2B and 2C *Video Editor: please emphasize black data bars in Figure 2C*

6.6. In this comparative analysis of oxygen consumption in a mitochondrial stress test of primary white and brown adipocytes under basal conditions **[1]** as well as in response to known stimulators of mitochondrial function **[2]** the specific bioenergetic capabilities of each cell type can be observed **[3]**.

6.6.1. LAB MEDIA: Figure 2D *Video Editor: please emphasize left graph*

6.6.2. LAB MEDIA: Figure 2D *Video Editor: please emphasize right graph*

6.6.3. LAB MEDIA: Figure 2D *Video Editor: please sequentially emphasize black data lines then blue data lines*

Conclusion

7. Conclusion Interview Statements

7.1. **Bernard Kok**: Remember that we are isolating live cells and that we want to culture them for several days, so be sure to maintain sterile conditions during the isolation to avoid contamination, which can compromise the subsequent culture **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *suggested b-roll: 3.1.1, 3.1.2*

7.2. **Andrea Galmozzi**: This method generates fully mature adipocytes that can be used for diverse applications, including functional assays, genetic or chemical screens, and omics profiling of cell-autonomous mechanisms that impact adipocyte function. **[1]**.

7.2.1. LAB MEDIA: IMG_8868.MOV, last part.