Submission ID #: 67854
Scriptwriter Name: Debopriya Sadhukhan
Project Page Link: https://review.jove.com/account/file-uploader?src=20695518

Title: Generating Primary Cultures for Keratinocyte Live Cell Imaging

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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? **Yes**

   How far apart are the locations? **4 miles**

Current Protocol Length
Number of Steps: 22
Number of Shots: 52

Introduction

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

REQUIRED:

1.1. **Ruby Ghadially:** We study hyper and hypoproliferative diseases including psoriasis and aging, focusing on the effects of disease on stem cell and progenitor proliferation. Pinpointing where the defect in proliferation is will allow more precisely targeted therapies. NOTE: This statement was changed during the shoot.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.13.4.*

What are the most recent developments in your field of research?

1.2. **Ruby Ghadially:** Recent advances in live cell imaging have provided deeper insights into the behavior of stem cells and committed progenitors in keratinocytes. This has improved the understanding of the biological changes underlying both hyperproliferation and hypoproliferation, particularly in the context of aging.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.14.2.*

What significant findings have you established in your field?

1.3. **Ruby Ghadially:** We have used live cell imaging to demonstrate that, although stem cells divide less frequently than committed progenitors, their divisions occur more rapidly. In response to tissue damage, stem cells are capable of rapid turnover.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 2.*

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

Ethics Title Card

This research has been approved by the UCSF Committee on Human Research

Protocol

2. Separation of Epidermis from the Dermis

Demonstrator: Brook Abegaze

2.1. To begin, spray the collection container containing the keratinocyte isolated tissue sample with 70 percent alcohol **[1]** and place it in the fume hood **[2]**. Position a cooling pad beneath the tissue to maintain its integrity and prevent warming to room temperature **[3-TXT]**.

2.1.1. WIDE: Talent spraying the collection container with alcohol.

2.1.2. Collection container being placed inside the fume hood.

2.1.3. Cooling pad positioned beneath the tissue. **TXT: If the tissue is large, cut a portion for processing and keep the rest moist**

2.2. Place two 100-millimeter by 15-millimeter Petri dishes inside the cabinet **[1]**. Add 10 milliliters of 10 percent chlorhexidine gluconate to one of the Petri dishes **[2]**. Then, add 10 milliliters of 5x *(five-ex)* penicillin, streptomycin, amphotericin, and gentamycin or PSA *(P-S-A)* in Hank's Balanced Salt Solution or HBSS *(H-B-S-S)* to the second Petri dish **[3]**.

2.2.1. Talent opening two Petri dishes and positioning them inside the biosafety cabinet.

2.2.2. Talent pipetting 10 milliliters of chlorhexidine gluconate into a Petri dish.

2.2.3. Talent pipetting the antibiotic mixture into the second Petri dish.

2.3. For the Dispase step, take a six-well plate and add 3 milliliters of Dispase per well for neonatal tissue **[1-TXT]**.

2.3.1. Pipetting 3 milliliters of Dispase into wells for neonatal tissue. **TXT: For aged tissue samples, use 4 mL Dispase per well**

2.4. Using a scalpel, cut the tissue into smaller pieces to enhance Dispase penetration **[1]**. Cut long strips 0.5 to 1 centimeter wide, as Dispase penetrates only up to 5 millimeters from the edges **[2-TXT]**.

2.4.1. Talent using a scalpel to cut tissue into small pieces. NOTE: 2.4.1 and 2.4.2 are combined.

2.4.2. Talent trimming the tissue into long strips 0.5 to 1 centimeter wide. **TXT: Cut thicker tissues like foreskins into 3 – 4 pieces (~5 mm × 7 mm); For thinner abdominal flaps, cut into longer strips (~5 mm × 15 mm)**

2.5. If the tissue contains excessive subcutaneous fat, trim it off to allow complete Dispase penetration **[1]**. Place a maximum of four strips per well of Dispase **[2]**.

2.5.1. Trimming subcutaneous fat from the tissue.

2.5.2. CU: A shot of four strips placed in a well.

2.6. Next, place the tissue epidermal side down in the first Petri dish containing 10 percent chlorhexidine gluconate for 10 to 20 seconds **[1]**. Then, transfer the tissue epidermal side down to the second Petri dish containing 5x PSA in HBSS, and agitate for 10 to 20 seconds **[2]**. Transfer the tissue again to the third Petri dish, containing the same solution, and agitate for another 10 seconds **[3-TXT]**.

2.6.1. Talent submerging the tissue epidermal side down in the Petri dish containing chlorhexidine gluconate. Videographer's NOTE: 2.6.1 to 2.6.3 are shot together as 2.6.1.

2.6.2. Talent transferring the tissue to the Petri dish containing PSA in HBSS and agitating the tissue.

2.6.3. Talent transferring the tissue to the third Petri dish containing PSA in HBSS and agitating the tissue. **TXT: Processing multiple samples with the same solution is acceptable**

2.7. Transfer the tissue similarly into the six-well Dispase plate prepared earlier **[1]**.

2.7.1. Talent transferring the tissue into the six-well plate.

2.8. Label the six-well plate containing the Dispase and tissue samples **[1]** and transfer the plate to a refrigerator **[2-TXT]**. NOTE: The VO has been edited.

2.8.1. Talent labeling the six-well plate.

2.8.2. Talent placing the plate inside a refrigerator. **TXT: Keep at 4 °C; Neonatal**

samples: 16 - 18 h; Aged samples: 24 h NOTE: This shot was slightly modified during the shoot.

3. Isolation of Keratinocytes

3.1. Place 0.05% Trypsin-EDTA, Trypsin neutralizing solution, and keratinocyte media in a 37-degree Celsius water bath **[1]**.

3.1.1. Talent placing Trypsin-EDTA, Trypsin neutralizing solution, and keratinocyte media in a water bath.

3.2. After removing the six-well plate from the refrigerator, spray the plate with 70 percent ethanol **[1]** and place it inside the biosafety cabinet **[2]**. NOTE: The VO has been slightly edited.

3.2.1. Talent spraying the six-well plate with ethanol. NOTE: 3.2.1 and 3.2.2 are combined.

3.2.2. Six-well plate being placed inside the biosafety cabinet.

3.3. Place sterile toothed and untoothed forceps in a 50-milliliter conical tube containing 20 milliliters of 70 percent ethanol **[1]**.

3.3.1. Talent submerging sterile toothed and untoothed forceps in a 50-milliliter conical tube containing ethanol.

3.4. Using these chemically sterilized forceps, grasp a piece of tissue and place it on a sterile surface, epidermal side up **[1]**. Hold the dermal portion of the tissue with toothed forceps using the non-dominant hand while simultaneously firmly grasping the epidermis with the nontoothed forceps and peeling off the epidermis from edge to edge **[2]**. The epidermis should come off smoothly as one piece **[3]**.

3.4.1. Talent using sterilized forceps to grasp the tissue and place it on a sterile surface (a Petri dish or the inside of the top half of the 6-well plate).

3.4.2. Talent holding the dermal portion of the tissue with toothed forceps and grasping the epidermis with the nontoothed forceps and peeling off the epidermis.

3.4.3. A shot of the epidermis removed in one piece.

3.5. Place the epidermis on the inside of the lip of a 15-milliliter conical tube, ensuring each conical tube has an epidermis of two foreskins maximum **[1]**.

3.5.1. Talent placing the epidermis on the lip of a 15-milliliter conical tube.

3.6. Add 4 milliliters of 0.05% Trypsin-EDTA into the conical tube **[1]**. Close the tube and invert it to ensure the epidermis is fully suspended in the Trypsin and not stuck to the lid or sides **[2]**. Place the tube in a 37-degree Celsius water bath for 10 minutes **[3]** and agitate it every 2 minutes by hand **[4]**.

3.6.1. Talent pipetting 4 milliliters of Trypsin-EDTA into the conical tube. NOTE: 3.6.1 and 3.6.2 are combined.

3.6.2. Talent closing the tube and inverting it to suspend the epidermis.

3.6.3. Conical tube being placed in a 37-degree Celsius water bath. NOTE: This shot is labeled as 3.7.1.

3.6.4. Talent agitating the tube every 2 minutes by hand.

3.7. Next, place a 50-milliliter conical tube in the biosafety cabinet, unscrew the lid **[1]**, and position a 100-micrometer cell strainer over the opening **[2]**.

3.7.1. Talent placing a 50-milliliter conical tube in the biosafety cabinet and unscrewing the lid. NOTE: This shot is labeled as 3.7.1.A. NOTE: 3.7.1A and 3.7.2 are combined.

3.7.2. Talent positioning a cell strainer on top.

3.8. Remove the 15-milliliter conical tube containing the digested sample from the water bath **[1],** and after drying the tube thoroughly, spray it with 70 percent ethanol before placing it back in the biosafety cabinet **[2]**. Tap the tube three times against the surface of the fume hood **[3]**, then invert it six times. Repeat the tapping-inversion cycle three times **[4]**.

3.8.1. Talent removing the conical tube from the water bath.

3.8.2. Talent spraying the dried tube with ethanol. NOTE: 3.8.2 to 3.8.4 are combined.

3.8.3. Talent tapping the tube three times against the fume hood.

3.8.4. Talent inverting the tube six times.

3.9. Add 6 milliliters of Trypsin neutralizing solution into the conical tube **[1]**. Pour the

contents through the cell strainer into the 50-milliliter conical tube prepared earlier **[2]**.

3.9.1. Talent pipetting 6 milliliters of Trypsin neutralizing solution into the conical tube.

3.9.2. Talent pouring the solution through the cell strainer into the 50-milliliter conical tube.

3.10. Rinse the 15-milliliter conical tube containing the sample with 5 milliliters of Trypsin neutralizing solution **[1]** and pour the rinse through the cell strainer into the 50-milliliter conical tube **[2]**. Centrifuge at 300 *g* for 5 minutes **[3]**.

3.10.1. Talent adding 5 milliliters of Trypsin neutralizing solution into the conical tube.

3.10.2. Talent pouring the rinse through the cell strainer into the 50-milliliter tube.

3.10.3. Talent placing the tube in a centrifuge.

3.11. After 5 minutes, remove the tube from the centrifuge **[1]**. Using a pipette, carefully remove the supernatant to prevent cell loss **[2]**.

3.11.1. Talent removing the tube from the centrifuge.

3.11.2. Talent pipetting the supernatant from the tube.

3.12. Resuspend the keratinocyte pellet in 2 milliliters of keratinocyte media **[1]** and count the cells **[2]**.

3.12.1. Talent pipetting keratinocyte media to resuspend the pellet.

3.12.2. Talent counting the cells.

3.13. To seed the keratinocyte cells, resuspend the cells in the total amount of medium to be plated **[1]**. Gently agitate the cells by inversion **[2]**, and vortex lightly for 1 to 2 seconds **[3]** before plating **[4]**. Move the microplate in a cross-like pattern three times inside the incubator **[5]**.

3.13.1. Talent resuspending keratinocytes in medium. NOTE: 3.13.1 and 3.13.3 are combined.

3.13.2. Talent agitating the cells by inversion.

3.13.3. Talent vortexing the sample for 1 to 2 seconds.

3.13.4. Talent plating the cell solution.

3.13.5. Talent moving the plate in a cross-like pattern inside the incubator.

3.14. After 24 hours, place the plate inside the incubator of the live cell imaging system **[1]**. Perform live cell imaging at 10x *(ten-ex)* magnification, capturing images every 20 minutes **[2]**. NOTE: The VO has been edited.

3.14.1. Talent placing the plate inside the live cell imaging system.

3.14.2. SCREEN: Live cell imaging system set to 10x magnification with image capture every 20 minutes. **TXT: Place the samples at 37 °C and 5% CO_2 in the microscope-attached incubator** Videographer's NOTE: This shot was filmed at 3 different angles and exposures.

~~3.14.3. Talent placing the samples in the incubator attached to the microscope.~~ NOTE: This shot was not filmed. The VO has been added concisely as an onscreen text at 3.14.2.

Results

4. Results

4.1. Seeding density directly affects the ability to track cells, as excessive density hinders tracking accuracy, while insufficient density leads to poor growth **[1]**. The difference in cell adherence and colony formation at 2 days and 23 hours with and without collagen is shown here **[2]**.

4.1.1. LAB MEDIA: Figure 2.

4.1.2. LAB MEDIA: Figure 2.

4.2. The results indicate that collagen coating significantly enhanced initial keratinocyte adherence and colony formation **[1]**. In contrast, the absence of collagen resulted in poor adherence, lower cell density, and uneven colony formation **[2]**.

4.2.1. LAB MEDIA: Figure 2. *Video Editor: Highlight the left image*.

4.2.2. LAB MEDIA: Figure 2. *Video Editor: Highlight the right image.*

Pronunciation Guides:

1. Keratinocyte
Pronunciation link: https://www.merriam-webster.com/dictionary/keratinocyte
IPA: /ˌkɛr.əˈtɪn.əˌsaɪt/
Phonetic Spelling: keh-ruh-TIN-uh-syte
2. Chlorhexidine
Pronunciation link: https://www.merriam-webster.com/dictionary/chlorhexidine
IPA: /klɔːrˈhɛk.səˌdiːn/
Phonetic Spelling: klor-HEK-suh-deen
3. Gluconate
Pronunciation link: https://www.merriam-webster.com/dictionary/gluconate
IPA: /ˈgluː.kəˌneɪt/
Phonetic Spelling: GLOO-kuh-nayt
4. Penicillin
Pronunciation link: https://www.merriam-webster.com/dictionary/penicillin
IPA: /ˌpɛn.əˈsɪl.ɪn/
Phonetic Spelling: pen-uh-SILL-in
5. Streptomycin

Pronunciation link: https://www.merriam-webster.com/dictionary/streptomycin
IPA: /ˌstrɛp.təˈmaɪ.sɪn/
Phonetic Spelling: strep-tuh-MY-sin
6. Amphotericin
Pronunciation link: https://www.merriam-webster.com/medical/amphotericin
IPA: /ˌæm.fəˈtɛr.ə.sɪn/
Phonetic Spelling: am-foh-TER-uh-sin
7. Gentamicin
Pronunciation link: https://www.merriam-webster.com/dictionary/gentamicin
IPA: /ˌʤɛn.təˈmaɪ.sɪn/
Phonetic Spelling: jen-tuh-MY-sin
8. Epidermis
Pronunciation link: https://www.merriam-webster.com/dictionary/epidermis
IPA: /ˌɛp.ɪˈdɜːr.mɪs/
Phonetic Spelling: ep-ih-DUR-mis
9. Dermal
Pronunciation link: https://www.merriam-webster.com/dictionary/dermal
IPA: /ˈdɜːr.məl/
Phonetic Spelling: DUR-muhl
10. Trypsin
Pronunciation link: https://www.merriam-webster.com/dictionary/trypsin
IPA: /ˈtrɪp.sɪn/
Phonetic Spelling: TRIP-sin
11. EDTA
Pronunciation link: https://www.howtopronounce.com/edta
IPA: /ˌiː.diː.tiːˈeɪ/
Phonetic Spelling: EE-dee-tee-AY
12. Conical
Pronunciation link: https://www.merriam-webster.com/dictionary/conical
IPA: /ˈkɒn.ɪ.kəl/
Phonetic Spelling: KON-ih-kuhl
13. Centrifuge
Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
IPA: /ˈsɛn.trəˌfjuːʤ/
Phonetic Spelling: SEN-truh-fyooj
14. Supernatant
Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant
IPA: /ˌsuː.pərˈneɪ.tənt/
Phonetic Spelling: SOO-per-NAY-tuhnt
15. Collagen
Pronunciation link: https://www.merriam-webster.com/dictionary/collagen
IPA: /ˈkɒl.ə.ʤən/
Phonetic Spelling: KOL-uh-jen