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# Title: Generation of Human Induced Pluripotent Stem Cell-derived Planar Hair-bearing Skin Organoids Using an Air—Liquid Interface Culture System

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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? **Yes**

**SCOPE: 2.3.2, 2.4.1, 2.4.2, 2.5.1, 2.5.2**

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. A few meters in the same building**

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: 06
Number of Shots: 13

# Introduction

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

1.1. **Charlotte de Henau:** I am developing a UVB-induced damage model to investigate the pathogenicity of the rare disease, Xeroderma pigmentosum.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.3*

What are the current experimental challenges?

1.2. **Charlotte de Henau:** One challenge with these *in vitro* models is that they take time to develop, and since this is a young field, they need better characterization.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*

What advantage does your protocol offer compared to other techniques?

1.3. **Charlotte de Henau:** This hiPSC-derived 3D model, cultured at an air-liquid interface, recapitulates native human skin architecture with appendages such as hair follicles and sebaceous glands.

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.***

# Protocol

## 2. Preparation of Cystic Skin Organoids

**Demonstrator:** Charlotte M.S. de Henau / Myrthe Flesseman

2.1. To begin, prepare a 1 molar solution by adding sodium hydroxide pellets to an empty 15-milliliter tube **[1].** Add distilled water to the pellets **[2]** and vortex the tube to dissolve the pellets **[3]**. Filter-sterilize 0.1 molar sodium hydroxide solution using a 0.2-micrometer pore-size membrane filter **[4]**. NOTE: The VO is edited for the additional shots

2.1.1. WIDE: Talent weighing sodium hydroxide pellets and adding them to an empty 15ml tube

Added Shot : Add distilled water to the 15ml tube containing the hydroxide pellets (Slated as 2.1.3)

Added Shot: Dissolve the hydroxide pellet solution using a vortex (Slated as 2.1.4)

2.1.2. Talent filtering the sodium hydroxide solution through a 0.2 micrometer membrane filter into a sterile container.

2.2. On ice, dilute collagen type one solution with 10 times PBS, 5 millimolar sodium hydroxide, and distilled water to adjust the final volume **[1-TXT]**. Distribute 150 microliters of this collagen solution into each insert placed in a standard 12-well plate **[2]**. Incubate the plate at 37 degrees Celsius for 30 minutes to allow the collagen gel to polymerize **[3]**.

2.2.1. Talent placing the collagen type I solution on ice and diluting it with phosphate-buffered saline, sodium hydroxide, and distilled water. **TXT: Final concentration: 2 mg/mL** NOTE: close-up shot is filmed

2.2.2. Talent pipetting 150 microliters of the diluted collagen solution into each insert in the 12-well plate.

2.2.3. Talent placing the 12-well plate into a 37 degrees Celsius incubator.

2.3. Next, cut P1000 tips with a heated scalpel to create wide-bore tips **[1].** Then, use the wide-orifice tips to place a selected cystic structure with a small amount of medium on the lid of a 10-centimeter Petri dish **[2]**. Carefully excise any byproducts of the structure

using a sterile scalpel while stabilizing it with sterile forceps on the opposite side **[3]**. NOTE: The VO is edited for the additional shot

Added shot: Talent cutting P1000 regular tips with a heated scalpel to create wide-orifice P1000 tips.

2.3.1. Talent pipetting the cystic structure with medium onto the Petri dish lid using a wide-orifice pipette. NOTE: +close-up shot is also filmed

2.3.2. SCOPE: Flattening-SCOPE-2.3.2.mp4: 00:18-00:25, 00:32-00:40

2.4. Excise approximately 1 millimeter from both ends of a cystic skin organoid adjacent to the first incision using a scalpel **[1]**. Using sterile forceps, gently unfold the upper layer of skin **[2]**.

2.4.1. SCOPE: Flattening-SCOPE-2.4.1.mp4: 00:10-00:27

2.4.2. SCOPE: Flattening-SCOPE-2.3.2.mp4: 00:12-00:19.

2.5. Cut the tissue into 2 to 4 pieces depending on size **[1]**. Then, using sterile forceps, move each piece onto a collagen-coated insert with the epidermis facing up **[2]**.

2.5.1. SCOPE: Flattening-SCOPE-2.5.1.mp4: 00:06-00:15

2.5.2. SCOPE: Flattening-SCOPE-2.5.2.mp4: 00:20-00:27, 01:26-01:31

2.6. Add 600 microliters of OMM *(O-M-M)* to each well of a standard 12-well plate **[1].** Transfer the insert back to the 12-well plate containing OMM **[1-TXT]**. Then place the plate into a 37 degrees Celsius incubator with 5 percent carbon dioxide **[2]**. NOTE: The VO is edited for the additional shot

2.6.1. Talent adding 600 µL of OMM to one well of a 12-well plate.Talent placing the insert back into a 12-well plate filled with 600 microliters of OMM. **TXT: OMM: Organoid Maturation Medium**

2.6.2. Talent returning the 12-well plate to the incubator set at 37 degrees Celsius with 5 percent carbon dioxide. NOTE: +close-up shot is also filmed

# Results

## 3. Results

3.1. A single, dense cellular aggregate incorporating the majority of the cells was formed by Day 0 through careful handling and centrifugation **[1]**. Proper surface ectoderm induction led to a thin, clear epithelium forming on the aggregate's outer layer by Day 3 **[2]**.

3.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the round, compact aggregate at Day 0 in the left column.*

3.1.2. LAB MEDIA: Figure 2A. *Video editor: Highlight the Day 3 panel in the left column*

3.2. By Day 12, a thin layer of mesenchymal cells had accumulated at one pole of the cyst **[1]**. Hair placodes and pegs became visible between Days 50 **[2]** and 80 following epithelial and mesenchymal co-induction **[3]**.

3.2.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the Day 12 panel*

3.2.2. LAB MEDIA: Figure 2A. *Video editor: Highlight the Day 50 panel*

3.2.3. LAB MEDIA: Figure 2B. *Video editor: Highlight the Day 80 panels*

3.3. Organoids lacking visible placodes displayed extremely thin epithelium and were excluded from planar transition **[1]**. Only cysts at least 5 millimeters in diameter with polarized byproducts were selected for planar skin organoid generation **[2]**.

3.3.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the middle Day 50 panel*

3.3.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the third and fourth Day 80 panels*

3.4. Hair follicles elongated progressively throughout the 27 days of air-liquid interface culture **[1]**. Sebaceous glands became visible around Day 14 in the planar configuration **[2]**. Pigmentation gradually increased in the organoids over time **[3]**.

3.4.1. LAB MEDIA: Figure 1C. *Video editor: Sequentially highlight the growth progression from Day 1 to Day 27 panels*.

3.4.2. LAB MEDIA: Figure 1C. *Video editor: Highlight the Day 14 panel and the arrowheads pointing to gland-like structures.*

3.4.3. LAB MEDIA: Figure 1B. *Video editor: Emphasize Day 14, Day 21, and Day 27 panels.*

3.5. Hematoxylin and eosin staining revealed a stratified epithelium and a basement membrane separating it from the dermis **[1]**. Immunofluorescence staining confirmed human skin-like architecture in planar skin organoids **[2]**.

3.5.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the Day 27 panel*

3.5.2. Figure 4B. *Video editor: Highlight the Day 27 panels*

1. **molar**
   IPA: /ˈmoʊlər/
   Phonetic: MOH-lur
2. **sodium hydroxide**
   “sodium” IPA: /ˈsoʊdiəm/ — sod-ee-um
   “hydroxide” IPA: /haɪˈdrɒksaɪd/ — hy-DROX-ide
3. **vortex**
   IPA: /ˈvɔrˌtɛks/ or /ˈvɔrˌtɛks/
   Phonetic: VOR-teks
4. **filter-sterilize**
   “filter” IPA: /ˈfɪltər/ — FIL-ter
   “sterilize” IPA: /ˈstɛrəˌlaɪz/ — STER-uh-lyze
5. **membrane**
   IPA: /ˈmɛmˌbreɪn/
   Phonetic: MEM-brane
6. **collagen**
   IPA: /ˈkɑləˌdʒən/
   Phonetic: KAH-luh-jun
7. **polymerize**
   IPA: /ˈpɑlɪməˌraɪz/
   Phonetic: PAH-li-muh-ryze
8. **organoid**
   IPA: /ɔrgəˈnɔɪd/
   Phonetic: or-guh-NOYD
9. **epithelium**
   IPA: /ˌɛpəˈθiːliəm/
   Phonetic: ehp-uh-THEE-lee-um
10. **mesenchymal**
    IPA: /ˌmɛsənˈkaɪməl/
    Phonetic: MES-en-KYE-muhl
11. **polarized**
    IPA: /ˈpoʊləˌraɪzd/
    Phonetic: POH-luh-ryzd
12. **hematoxylin**
    IPA: /ˌhiːməˈtɒksɪlən/
    Phonetic: HEE-muh-TOCK-siluhn