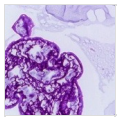

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How to Culture Human Bronchial Epithelial Cells as Airway Organoids

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ABSTRACT

Human bronchial epithelial cells (HBECs) are typically cultured at the air-liquid interface (ALI) to functionally recapitulate the human airway. ALI cultures are formed by plating primary HBECs onto porous cell culture inserts and allowing the cells to achieve confluence prior to removing the culture medium from the apical surface of the cells. However, the requirement of porous culture inserts can limit the application of ALI culture to smaller scale experiments, thus largely precluding high-throughput drug screening of differentiated epithelial cultures. Alternatively, HBECs may be cultured and differentiated as spherical aggregates, providing a means for high-throughput study of the differentiated human airway. This protocol describes a method for the culture of differentiated HBECs as spherical structures known as broncospheres or airway organoids.

EXTERNAL LINK

https://www.stemcell.com/sphere-culture-method-mucociliary-differentiation-primary-human-bronchial-epithelial-cells-lp.html?utm_source=protocolsio&utm_medium=referral

MATERIALS

NAME	CATALOG #	VENDOR
Trypan Blue	07050	Stemcell Technologies
PneumaCult™-ALI Medium	05001	Stemcell Technologies
Hydrocortisone Stock Solution	07925	Stemcell Technologies
Heparin Solution	07980	Stemcell Technologies
Trypsin-EDTA (0.05%)	07910	Stemcell Technologies
Corning® Matrigel®	354277	Corning
Soybean Trypsin Inhibitor	T6522	Millipore Sigma

BEFORE STARTING

The instructions below describe an optimized procedure for use with 24-well tissue culture plates. If using alternative cultureware, adjust volumes accordingly. Human bronchial epithelial cells (HBECs) can be cultured in a serum-and BPE-free expansion medium (e.g. PneumaCult™-Ex) in T-25 flasks according to the instructions on the Product Information Sheet (PIS). The following procedure should be initiated with HBECs (P1-P4) that are approximately 70-90% confluent in PneumaCult™-Ex.

Preparation of Reagents and Materials

1 Prepare PneumaCult™-ALI Complete Base Medium by adding 50 mL PneumaCult™-ALI 10X Supplement to 450 mL PneumaCult™-ALI Basal Medium. This Complete Base Medium can be aliquoted and stored at -20°C for up to 6 months. Avoid additional freeze/thaw cycles.

Note: the PneumaCult™-ALI Medium kit contains:
1. PneumaCult™-ALI Basal Medium
2. PneumaCult™-ALI 10X Supplement
3. PneumaCult™-ALI Maintenance Supplement

2 Prepare PneumaCult™-ALI Maintenance Medium by adding the following components per 1 mL PneumaCult™-ALI Complete Base Medium:
- 10 µL PneumaCult™-ALI Maintenance Supplement
- 2 µL Heparin Solution
- 5 µL Hydrocortisone Stock Solution

Note: Prepare only enough PneumaCult™-ALI Maintenance Medium needed for immediate use.

3 Prepare a sufficient volume of a 40% Matrigel® solution by promptly mixing the following components (will require 500 µL/ well of a 24-well plate):
- 300 µL cold PneumaCult™-ALI Maintenance Medium
- 200 µL cold Matrigel®

4 Aliquot 500 µL 40% Matrigel® solution per well of a 24-well plate.

5 Incubate the plate at **37 °C** in a humidified incubator at 5% CO_2 for **00:30:00** to allow for the Matrigel® layer to solidify. Steps 6 through 12 can be completed during this 30 minute incubation. 30m

Preparation of a Single Cell Suspension

6 Prepare a sufficient volume of a 5% Matrigel® solution by mixing the following components (require 500 µL/well of a 24-well plate):
- 475 µL cold PneumaCult™-ALI Maintenance Medium
- 25 µL cold Matrigel®

7 Warm 5% Matrigel® solution, Ca^{2+}/Mg^{2+}-free PBS, 0.025% Trypsin-EDTA [1/2 dilution of 0.05% Trypsin-EDTA] and 1 mg/mL Soybean Trypsin Inhibitor to **37 °C**.

8 Wash each T-25 flask of HBECs twice with 2 mL warm Ca^{2+}/Mg^{2+}-free PBS. Aspirate the Ca^{2+}/Mg^{2+}-free PBS and add 2 mL warm 0.025% Trypsin-EDTA to each flask.

9 Incubate flask at **37 °C** for 3 - 5 minutes, until the cells can be dislodged with gentle tapping of the flask. Neutralize the Trypsin-EDTA by adding an equivalent volume of 1 mg/mL warm Soybean Trypsin Inhibitor to each T-25 flask. 5m

10 Collect the cell suspension into a 15 mL conical tube and centrifuge at **350 x g for 5 minutes** 5m

11 Remove the supernatant and resuspend the cell pellet in 1 - 2 mL warm 5% Matrigel® solution.

12 Perform a viable cell count using Trypan Blue and a hemocytometer.

Airway Organoid Culture

13 Prepare cells at a concentration of 180,000 cells/mL in 5% Matrigel® solution. Plate 500 µL cell solution per well of a 24-well plate (90,000 cells/well or approximately 50,000 cells/cm^2).

Note: This seeding density has been optimized for use with P4 cells. If using lower passage number cells, the optimal seeding density may be lower.

14 Incubate at 37 °C, 5% CO_2 in a humidified incubator. Medium changes should be performed three times per week (e.g. Monday/Wednesday/Friday) by gently aspirating the medium above the semi-solid Matrigel® layer and replacing with 500 µL fresh warm 5% Matrigel® solution.

HBECs will grow into spheres within approximately one week, continually increasing in size throughout the entire four week culture period. Between week one and two, a lumen will become visible within some of the bronchospheres (Figure 1).

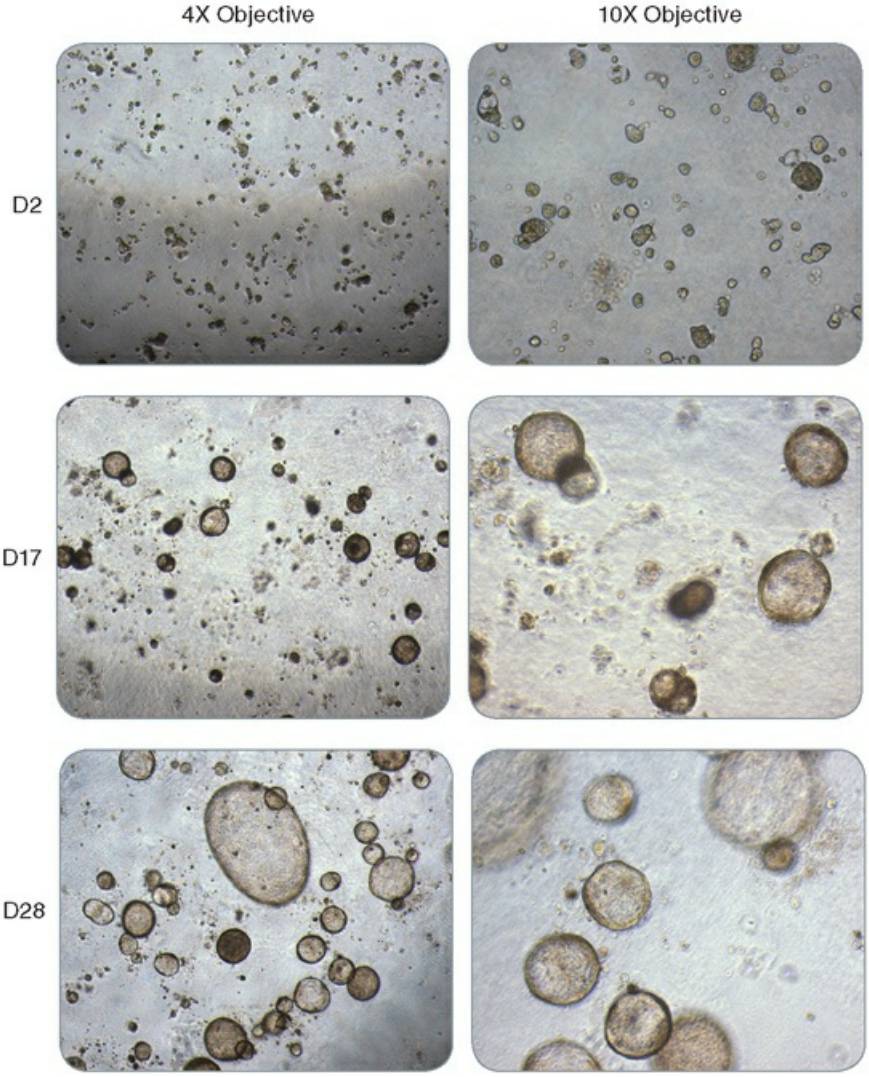

Figure 1. Morphology of bronchospheres generated in PneumaCult™-ALI Medium.

At Day 2, bronchospheres were small in size. By Day 17 the bronchospheres were larger in size with approximately 70% containing a visible lumen. By Day 28 almost all bronchospheres contained a lumen.

By two weeks, the cells within the wall of each bronchosphere will start to differentiate and self-organize to create a pseudostratified epithelium with the apical surface of goblet and ciliated cells pointing into the lumen (Figure 2).

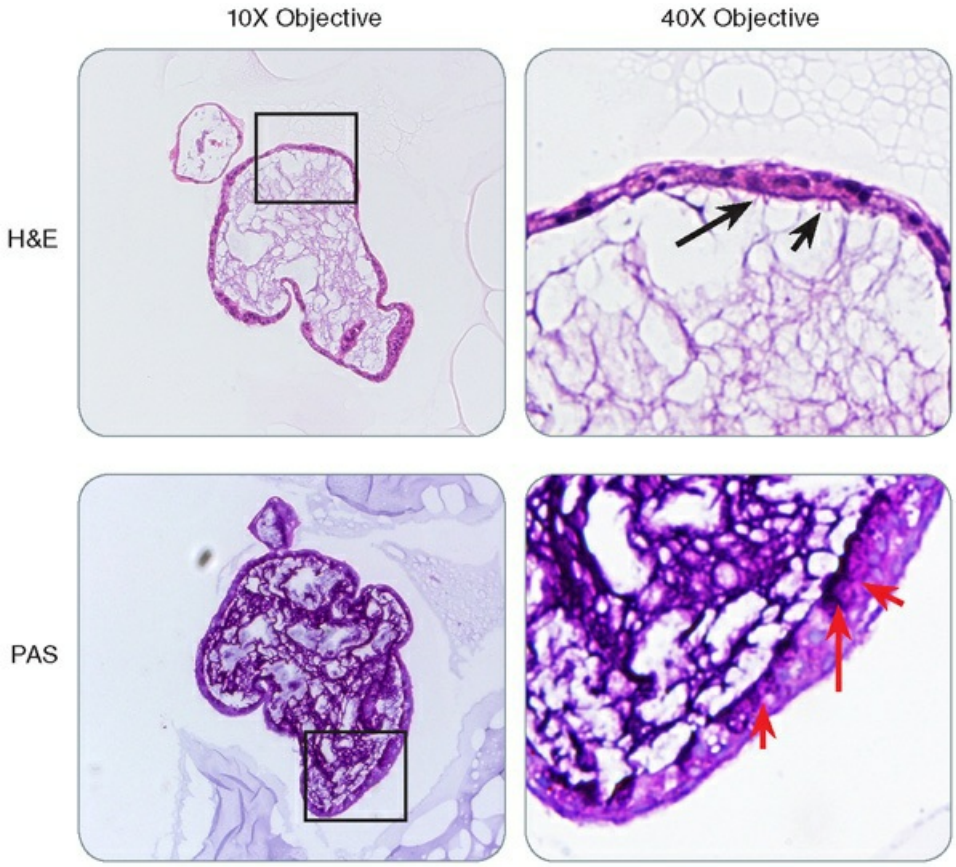

Figure 2. Histological staining of bronchospheres reveal the presence of goblet cells and ciliated cells.

Top Panels: H&E staining of representative bronchospheres. Black arrows indicate beating cilia pointing towards the lumen. Bottom Panels: PAS staining of representative bronchospheres. Red arrows indicate goblet cells.

By three weeks, visualization under bright field microscopy will reveal beating cilia:

Video 1. Visualization of Bronchospheres.

Light microscope visualization showing a bronchosphere cultured in PneumaCult™-ALI. This video shows mucus in the lumen being swirled around by the beating of synchronized cilia lining the lumen of the bronchosphere.

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