Alveolar Organoid Generation Using Corning® Matrigel® Matrix and Transwell® Permeable Supports

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Protocol

The lung is comprised of at least 40 to 60 resident cell types, each with specific physiologic functions[1-2]. Within the alveolus, alveolar epithelium comprises two cell types: alveolar epithelial type II cells that secrete surfactant proteins and are considered as tissue stem cells, and type I alveolar epithelial cells that form a thin wall for gas exchange[3]. Alveolar epithelial type II cells are stem cells of the alveoli and play crucial roles in maintaining lung homeostasis and the pathogenesis of lung diseases. One of the challenges in researches of distal respiratory system is that alveolar epithelial type II cells lose functional markers within a few days of cultivation in 2D culture. It has been reported that alveolar epithelial cells can better maintain the functional markers when cultured in 3D cell culture.[4]

Here, a method for generating alveolar organoids derived from human alveolar epithelial cells is described. The Transwell permeable support inserts from Corning provide a platform for *in vitro* generation of alveolar organoids in combination with Corning Matrigel matrix.

Reagents and Materials

- Human pulmonary alveolar epithelial cells (HPAEpiC; ScienCell Cat. No. 3200)
- Corning Matrigel matrix for organoid culture (Corning Cat. No. 356255)
- 6.5 mm Transwell insert with 0.4 µm pore Polyester membrane (Corning Cat. No. 3470) or 6.5 mm Falcon permeable supports
- Small airway epithelial cell growth medium (SAGM; Lonza Cat. No. CC-3118)
- Jagged-1 peptide (AnaSpec Cat. No. AS-61298)
- FGF7 (Novoprotein Cat. No. CH73)
- Noggin (Novoprotein Cat. No. CB89)
- SB431542 (MCE Cat. No. HY10431)
- CHIR99021 (MCE Cat. No. HY10182)
- Y27632 (MCE Cat. No. HY10071)
- Cell Recovery Solution (Corning Cat. No. 354253)
- Accutase® (Corning Cat. No. 25-058-CI)
- Anti-HT2-280 antibody (mouse IgM; Terrace Biotech Cat. No. TB-27AHT2-280)
- Anti-mouse IgM microbeads (Miltenyi Biotec Cat. No. 130-047-302)
- LS columns (Miltenyi Biotec Cat. No. 130-122-729)

Protocol

Generation of Alveolar Organoid

1. Prior to use, thaw Corning Matrigel matrix overnight by submerging the vial in ice in a 4°C refrigerator before use. Once Matrigel matrix is thawed, swirl vial to ensure the material is dispersed.
2. Prepare alveolar organoid culture medium by adding FGF7 (100 ng/mL), Noggin (100 ng/mL), SB431542 (10 µM), and CHIR99021 (3 µM) to small airway epithelial cell growth medium.

   NOTE: Small airway epithelial cell growth medium (SAGM) was prepared by adding all additives except epinephrine to SABM (a basal medium).
3. Place the frozen vial of HPAEpiC in a 37°C water bath. Gently hold and rotate the vial until the contents completely thaw.
4. Gently resuspend the contents of the vial, transfer the suspension to a sterile centrifuge tube, adjust cell density to 1 x 10^5/mL with SAGM.

   NOTE: Centrifugation after cell thawing is not recommended since it is more harmful to the cells than the effect of residual DMSO in the culture. Refreshing culture medium the next day to remove residual DMSO is optional.
5. Mix cell suspension at a 1:1 ratio with Matrigel matrix containing Jagged-1 peptide T 1 µM of final concentration.
6. Gently place aliquots of the mixture (90 µL/well) to the membrane of 24-well Transwell inserts.
7. Place the vessel to 37°C for 20 minutes to allow Matrigel matrix to polymerize.
8. After polymerization, add 500 µL of alveolar organoid culture medium to the lower chambers.
9. Return the culture vessel to the 37°C, 5% CO_2 incubator.
10. Continuously culture for 14 days, changing the medium every three days. For the first three days of culture, add Y-27632 at 10 µM to the alveolar organoid culture medium.

Sorting and Passaging of Alveolar Organoid

1. After 2 weeks of culturing, alveolar organoids form on the membrane of the Transwell insert.
2. Remove the medium in lower chambers, add 200 µL of cell recovery solution to the insert and place the vessel at 4°C for 20 min. to dissociate alveolar organoid from Matrigel matrix.

3. Transfer the depolymerized matrix mixture to a sterile pre-chilled centrifuge tube, wash alveolar organoids twice with pre-chilled PBS.
4. Pellet the organoids by centrifugation at 300 xg for 5 min. at 4°C.
5. Resuspend the pellet with Accutase®, followed by incubating at 37°C for 10 min. to prepare single cell suspension. Terminate digestion with PBS, centrifugate at 300 xg for 5 min.
6. To sort alveolar type II cells, apply according to manufacturers' instructions MACS® Column Technology which is developed for the gentle isolation of microbeads-labeled cells.
7. Resuspend cells with anti-HT2-280 antibody (diluted at 1:100 with antibody diluent) at 4°C for 20 min.
8. Wash cells with recommended buffer and centrifuge at 300 xg for 10 min. Aspirate supernatant completely.
9. Resuspend cell pellet in 80 µL of recommended buffer, add 20 µL anti-mouse IgM microbeads to the suspension. Mix and incubate for 20 min at 4°C.
10. Wash cells with recommended buffer and centrifuge at 300 xg for 10 min. Aspirate supernatant completely.
11. Resuspend cell pellet in 500 µL of recommended buffer.
12. Separate and harvest HT2-280$^+$ alveolar type II cells with MACS LS column and MACS separator.
13. After sorting, resuspend HT2-280$^+$ alveolar type II cells with SAGM to 1 x 10^5/mL of concentration and mix with Matrigel matrix, followed by placing the mixture to the membrane of Transwell inserts according to the Generation of Alveolar Organoid Protocol on the previous page.

References

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