Kidney Organoid Formation on Transwell® Permeable Supports from Corning

Protocol

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Introduction

The organoid is a collection of 3D self-organized tissue or organ-like structures which resemble the living organ. As the *in vitro* organoid possesses key structural and functional features of the counterpart *in vivo*, this innovative model has significant advantages over traditional 2D cell culture and holds promise for organ development (organogenesis)[1], disease modeling[2] and toxicity testing[3]. Prediction of nephrotoxicity is one of the key areas of safety evaluation in drug development[4]. Various approaches for drug screening and nephrotoxicity testing have been developed with human-induced pluripotent stem cell (hiPSC)-derived kidney organoids emerging as an attractive *in vitro* system in drug discovery[5].

Materials

- Human-induced pluripotent stem cell line DYR0100 (ATCC® ACS-1011™, derived from foreskin fibroblast)
- Corning® Matrigel® hESC-qualified matrix (Corning 354277)
- Transwell-Clear Polyester (PET) inserts in 6-well plate with 0.4 µm pore membrane (Corning 3450)
- Costar® 6-well Clear TC-treated multiwell plates (Corning 3516)
- DMEM/F12 50:50 mix (Corning 15-090-CV)
- Essential 8™ (E8) medium (Thermo Fisher A1517001)
- Y27632 dihydrochloride (Tocris 1254)
- STEMdiff™ APEL™ 2 medium (STEMCELL Technologies 05270)
- Recombinant Human FGF9 (Peprotech 100-23)
- CHIR99021 (Tocris 4423/10)
- 0.5 mM EDTA (CellApy Bio CA3001500)
- Ca^{2+}/Mg^{2+}-free PBS (Corning 21-040-CV)
- 25 cm^2 TC-treated flask (Corning 430168)
- Heparin (MilliporeSigma H3149-500KU-9)
- 1.5 mL Axygen® microcentrifuge tube (Corning MCT-150-C-S)
- 15 mL centrifuge tube (Corning 430053)
- 200 µL Axygen wide-bore pipet tip (Corning TF-205-WB-R-S)
- 5 mL pipet (Corning 4487)
- 1 mL Axygen pipet tip (Corning TF-1000-R-S)
- Positive displacement pipet (e.g., Corning Step-R™ Repeating pipettor (Corning 4217)

Procedure[6,7,8]

1. **Maintaining hiPSCs on Corning Matrigel Matrix**

   NOTE: This procedure describes passaging hiPSCs from one well of a 6-well plate coated with Corning Matrigel hESC-qualified matrix using enzyme-free, EDTA solution.

   a) Thaw Matrigel hESC-qualified matrix by submerging the vial in ice in a 4°C refrigerator, in the back, overnight. Once the Matrigel matrix is thawed, swirl the vial to ensure the material is evenly dispersed.

   b) Dilute Matrigel matrix using DMEM/F-12 according to the manufacturer's instructions. Add 1 mL into three wells of a pre-chilled 6-well plate. Incubate plate at room temperature (15°C to 25°C) for at least 1 hour before use to polymerize the Matrigel matrix.

   NOTE: Matrigel matrix is a viscous liquid at 4°C but begins to quickly polymerize at higher temperatures (above 10°C). For consistent results, it is essential that the Matrigel matrix is kept cold during use and that any plasticware or media that will come in contact with the Matrigel matrix is pre-chilled/kept on ice. For the most accurate pipetting, we recommend using positive displacement pipets or another instrument designed for viscous liquids, such as the Corning Step-R Repeating pipettor.

   c) Passage cells when the borders and edges of the hiPSC colonies become adjacent and the confluency reaches approximately 70%. Do not allow cell colonies to overgrow.

   d) Aspirate medium in the well and wash once with Ca^{2+}/Mg^{2+}-free PBS.

   e) Add 1 mL of 0.5 mM EDTA to the well and maintain at 37°C in a humidified atmosphere containing 5% CO_2 (37°C/5% CO_2) for 3 to 5 minutes. After 1 to 2 minutes of incubation, observe the cells by microscope. When tight hiPSC colonies become loose, immediately gently aspirate all EDTA without disturbing cells when the cells start to separate from each other.

   NOTE: Do not over-incubate the cells, and discard the EDTA as soon as the cells within the colonies start separating from each other. As the cells are still attached to the plate at this stage, do not rinse.

f) Using a 5 mL pipet, gently add 3 mL pre-warmed E8 medium to the cells in the well. Pipet mix the cell solution up and down 2 to 3 times until the majority of cells have detached to obtain a cell aggregate suspension.

NOTE: This step must be performed very carefully. Do not over-pipet to avoid single cell formation. Optimal cell density per aggregate or cluster is 15 to 20 cells. Do not use a 1 mL pipet tip in this step as this may result in a single cell suspension.

g) Retrieve the Corning® Matrigel® matrix pre-coated 6-well plate, and remove the residual solution from the three wells using a pipet, taking care not to damage the coating. Add 1 mL pre-warmed E8 medium to each well.

h) Transfer 3 mL of the cell aggregate suspension to 3 wells of a 6-well plate (1 mL for each well) using a 5 mL pipet to make a 1:3 split ratio.

NOTE: The split ratio is adjustable. 1:3 to 1:6 is recommended.

i) Ensure cells maintain pluripotency during maintenance culture. Pluripotent markers (e.g., SSEA4 and OCT4 expression should be ≥98% by FACS or immunofluorescence analysis).

2. Inducing the Intermediate Mesoderm

a) Prepare a Matrigel matrix pre-coated 25 cm^2 TC-treated flask following the manufacturer's instructions.

b) Once cell confluency reaches approximately 70% with dense colony growth in the 6-well plate, wash hiPSCs once with Ca^{2+}/Mg^{2+}-free PBS and add 1 mL of 0.5 mM EDTA to each well. Incubate plate at 37°C/5% CO_2 for at least 5 minutes.

c) Gently aspirate all EDTA without disturbing cells when the cells start to separate from each other.

d) Add 1 mL pre-warmed E8 medium to each well and pipet mix the cells up and down using a 1 mL pipet tip at least 10 times to dissociate colonies into single cells.

e) Transfer the single cell suspension into a 15 mL centrifuge tube. Count and seed 5 x 10^5 cells in 5 mL E8 medium supplemented with 10 µM Y27632 onto the Matrigel matrix pre-coated 25 cm^2 flask. Incubate the cells in a 37°C/5% CO_2 incubator overnight.

f) After 16 to 24 hours, aspirate the E8 medium from the 25 cm^2 flask, and add 5 mL of STEMdiff™ APEL™ 2 medium containing 8 µM CHIR99021 **[Day 0]**.

g) Culture the cells in a 37°C/5% CO_2 incubator for 4 days, refreshing APEL medium containing 8 µM CHIR99021 every 2 days **[Days 0-4]**.

h) After 4 days of CHIR99021 treatment, change the medium to 8 mL of APEL medium supplemented with 200 ng/mL FGF9 and 1 µg/mL heparin. Incubate the cells in a 37°C/5% CO_2 incubator and change the medium every 1.5 days until day 7 of differentiation **[Days 4-7]**.

3. Forming the Developing Kidney Organoid

a) Aspirate medium and wash once with Ca^{2+}/Mg^{2+}-free PBS **[Day 7]**.

b) Add 2 mL of 0.5 mM EDTA to the cells and incubate the cells at 37°C/5% CO_2 for at least 5 minutes.

c) Monitor the cells by microscope and gently aspirate all EDTA without disturbing cell layer when the cells start to separate from each other and begin to lift off the surface.

d) Add 5 mL pre-warmed APEL medium and pipet mix the cells up and down using a 1 mL pipet tip at least 10 times to ensure cells are detached from the flask.

NOTE: At day 7 of culture, the cells are highly confluent in the flask. Ensure most cells are dissociated following EDTA treatment and subsequent pipet mixing.

e) Transfer cell suspension into a 15 mL centrifuge tube

f) Count cells and prepare aliquots containing a suspension of 2 x 10^6 cells in a 1.5 mL microcentrifuge tube.

g) Centrifuge the microcentrifuge tube at 400 x g for 2 minutes to make a cell pellet.

h) Add 1.3 mL of APEL medium containing 5 µM CHIR99021 to the lower chamber of a 6-well Transwell® cell culture plate.

i) Discard all supernatant medium without disturbing the cell pellet at the bottom of the microcentrifuge tube.

j) Carefully transfer the cell pellet to the middle of the Transwell insert using a 200 µL wide-bore pipet tip.

k) Place the Transwell plate with insert into a 37°C/5% CO_2 incubator for 1 to 1.5 hours.

l) Replace the medium in the lower chamber with 1.3 mL of APEL medium containing 200 ng/mL FGF9 and 1 µg/mL heparin.

m) Culture the cell pellet for 5 days, refreshing medium with FGF9- and heparin-supplemented APEM medium every 2 days **[Days 7-12]**.

4. Maturing the Kidney Organoid

a) At Day 12, change the medium to heparin-supplemented APEL medium without FGF9 **[Day 12]**.

b) Culture the organoid for another 13 days, refreshing medium with heparin-supplemented APEL every 2 days **[Days 12-25]**.

c) Confirm kidney organoid maturation by immunostaining with appropriate markers of interest[7] and observing with confocal microscope. Stained organoids are not usable for other downstream applications. Culturing several organoids at the same time can be a good solution.

d) Use kidney organoid for downstream studies.

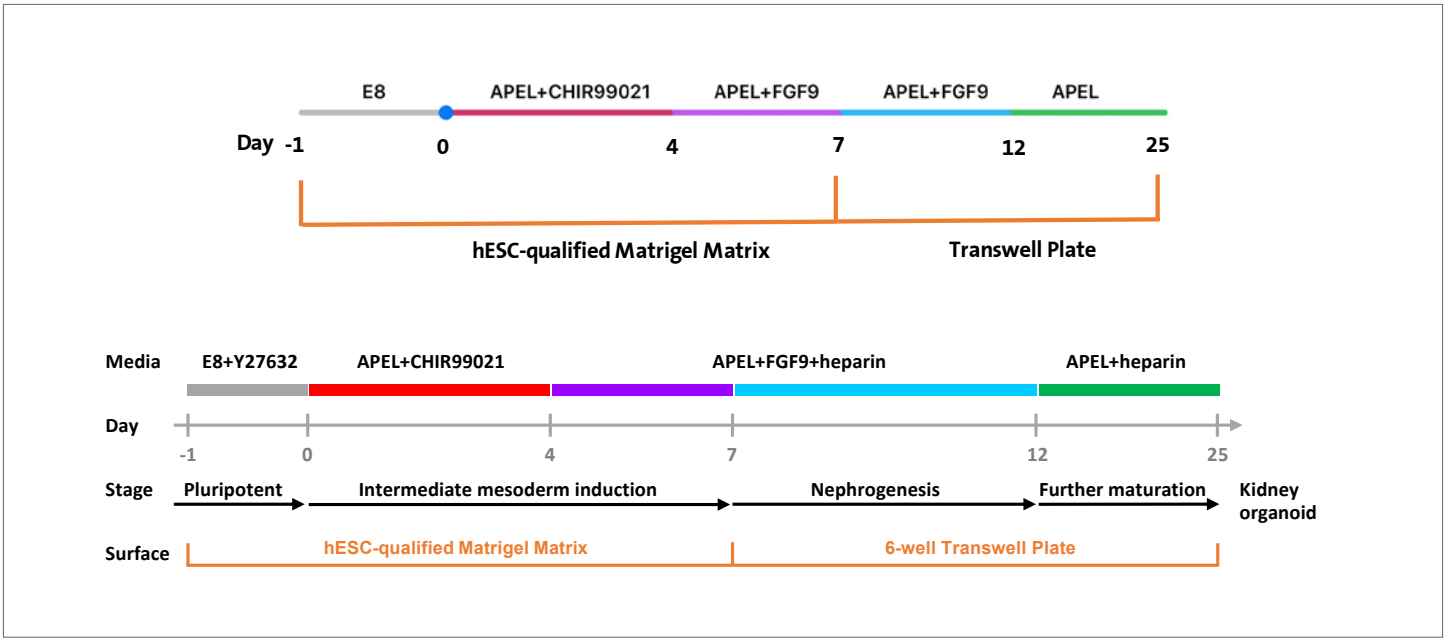

Figure 1. Workflow of hiPSC-derived kidney organoid maturation.

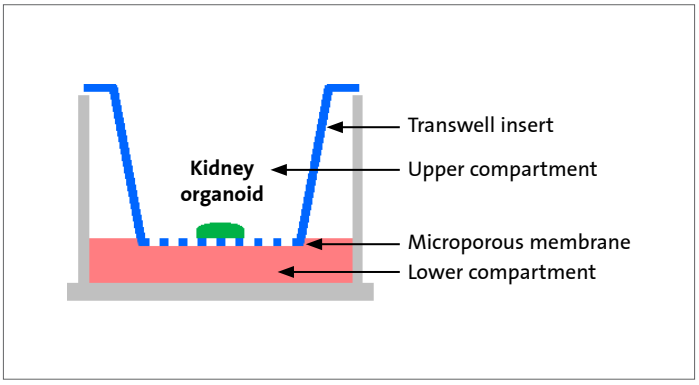

Figure 2. Sideview of kidney organoid on microporous membrane of Transwell insert.

References

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The protocol described herein may be similar to those published in patent applications WO2016094948 and WO2014197934 and Australian Patent No. 2014280843B2 and users are cautioned to review prior to practicing this protocol.

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